Contraception in Wildlife Management

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Letter of Transmittal

Recognizing that immunocontraception offers wildlife managers a new methodology for controlling the reproduction of certain wild animals of national and even worldwide significance, the Denver Wildlife Research Center—a unit of the U.S. Department of Agriculture, Animal and Plant Health Inspection Service's (APHIS) Animal Damage Control program—invited renowned specialists to address the scientific community at a symposium on this subject in October 1993. Speakers at that meeting assembled scientific articles on related topics for inclusion in this state-of-the-art compendium, revising content and bibliographic source material to cover information made available since the meeting.

APHIS is proud to produce this book because it demonstrates our commitment to research on nonlethal methods of suppressing wildlife damage to agricultural and human resources. Funding constraints necessarily limit how many books we can print. However, a limited number of individual copies are available from the library at the National Wildlife Research Center (NWRC), 1201 Oakridge Drive, Fort Collins, CO 80525, U.S.A. The U.S. Government Printing Office will make a single copy of this text available to the main library at all of the land-grant colleges and universities in the United States. Softbound copies and a microfiche version of the book will be available for purchase in perpetuity from the U.S. Department of Commerce's National Technical Information Service (NTIS), 5285 Port Royal Road, Springfield, VA 22161, U.S.A. Please write directly to NTIS for current pricing and ordering information.

For more details about ongoing investigations at the Denver Wildlife Research Center or information about the transfer of its research functions to the new APHIS NWRC in Fort Collins, you may contact the Director's Office at the NWRC address given above.

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We also acknowledge the excellent work and dedication the conference program planning committee provided, especially Barbara Rectenwald for her attention to detail, patience, and hard work. A special word of thanks is given to Terry J. Kreeger for his technical editing and oversight of the book’s scientific content and to Diana L. Dwyer for her leadership and dedication to publishing this volume. Finally, we acknowledge the excellent production support provided by editor Janet S. Wintermute, designer Heather Cooney, and printing specialist Anita McGrady from APHIS’ Legislative and Public Affairs unit.

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Contraception in Domestic and Wild Animal Populations Using Zona Pellucida Immunogens

Bonnie S. Dunbar

Introduction

The human population presently exceeds 6 billion and is continuing to expand at a startling rate. This population increase has resulted in the depletion of Earth's resources, which are essential for human survival. An unfortunate consequence of this expansion has been the destruction of wildlife habitats. As these habitats have diminished, numerous problems have arisen, including conflicts between wildlife and human populations. The threat of extinction of many plant and animal species has already become a reality; other wildlife populations have increased due to reductions in predator populations. While the increase in the human population must ultimately be checked, there is a need for effective and humane methods to regulate certain animal populations as well. Another factor relevant to animal overpopulation is regional distribution. Widespread overpopulation of such animals as white-tailed deer in North America and rabbits in Australia has caused environmental as well as health problems for humans. In Asia and Africa, the populations of some wildlife species, such as elephants, have been dramatically reduced. Often these animals have frequently been relegated to small areas of land that do not have sufficient resources to sustain them. There is a vicinal distribution of elephants in Asia and Africa whose localized high populations threaten the destruction of their own restricted habitats.

The rising domestic pet population also continues to be a problem, as more than 27 million dogs and cats are impounded annually in the United States, with more than 17 million of them being euthanized (Carter 1990). The extent of domestic pet overpopulation poses ethical as well as health-related dilemmas (Flowers 1979, Carter 1990). Countless dollars are spent each year to house and dispose of impounded animals and to combat diseases transmitted by the fleas and ticks that use the dogs and cats as hosts. As these problems continue to intensify, it is apparent that a serious need exists for effective and inexpensive methods of population control.

Conventional Methods of Animal Population Control

Despite the need for effective animal population control, few methods are available which are practical or cost effective for large-scale administration. The major methods of current methods are summarized below.

Surgical Sterilization

Of the surgical sterilization methods recommended for dogs and cats, the surgical removal of the uterus and cervix (where possible) as well as the ovary (ovario-hysterectomy) has several advantages over partial removal of reproductive organs.

While neutering of male animals by castration is also common, this procedure may not ultimately have a dramatic impact on population reduction in many animal populations where one virile male can mate with numerous females. It is also apparent that surgical procedures are not practical for large-scale sterilization of major populations of mammalian wildlife.

Endocrine Regulation of Fertility

Numerous steroid treatments have been tested for their ability to regulate fertility in animals, including treatment with progestogens or androgens. In female dogs, these hormones have been shown to suppress normal ovarian cyclicity. Many of the treatment regimens that include progestins have been found to promote the development of cystic endometrial hyperplasia and subsequent uterine infection, mammary development, and posttherapy lactation, while androgens may induce external masculinization. Alternatively, endocrine administration for the inhibition of implantation is possible. Because these methods have adverse effects in some animal species and because they are expensive and require regular administration, these methods are not generally considered to be practical for regulation of large populations of wildlife; for these reasons, they will not be dealt with in more detail.

Contraceptive Vaccines for Animal Fertility Regulation

The theory that immunization could be used as a method of contraception has led to numerous investigations into the development of safe and effective contraceptive vaccines. These vaccines have proposed the use of a variety of antigens as targets that would be specific for hormones, gametes, or other reproductive tissues. These vaccines use a variety of antigens as targets which would be specific for hormones, gametes, or other reproductive tissues (see reviews by Alexander et al. [1990] and Dunbar and O'Rand [1992]). This brief overview is not intended to provide a detailed outline of all research in this area.

The development of contraceptive vaccines is distinct from that of vaccines to control infectious diseases. Therefore, many of the approaches used in this area are unique. First, the contraceptive vaccines will be administered to normal, healthy individuals and not to infected or ailing individuals. Second, most traditional vaccines are directed against foreign organisms such as viruses or bacteria; contraceptive vaccines must elicit an immune response against "self" molecules that would not normally be recognized as foreign. This means that the "self" molecule to be used in the vaccine must be presented to the body in a "foreign" or "nonself" form in order for the immune system to respond effectively. These unique features have provided a significant challenge to investigators in the field as described in more detail below.

A second major factor relating to the development of contraceptive vaccines is that the immune system interacts with the male gonads in a different way than it does with the female system. It is well established that there is a blood–testis barrier in the male that partially protects the developing sperm in the testis from the immune system. The developing spermatozoa are formed at a stage long after the immune system has developed the capability to distinguish self from nonself antigens. Because ovaries initiate oocytes before birth in the human and many other animal species, the same immunological barriers are not developed. These fundamental differences have required that vaccines targeted toward the male be developed in a different manner than those for the female.

Although the ideal vaccine has yet to be formulated, research has provided a great deal of information relating to the fundamental mechanisms of hormone action, gamete interaction, fertilization, and implantation. These detailed studies have been important not only in laying the foundation for future development of contraceptive methods but also in developing a better understanding of reproductive systems of many mammalian species.

While the development of a contraceptive vaccine for humans has the criteria that it must be safe, effective, and potentially reversible, the criteria for wildlife management are different. In some animal populations, it is desired (if not required) that animals be permanently sterilized. In other populations, such as some captive animals in zoo or farms, it is desirable that the vaccine be reversible. This is an essential requirement where it is important not to deplete a potential gene pool even though there is temporary abundance of a species or where there is a temporary lack of space for housing animals. It is likely, therefore, that several strategies will be necessary to develop optimal vaccines for different animal species.

Hormone-Based Contraceptive Vaccines

To date, many of the more applied studies have been carried out using peptide hormones as immunogens. These include the peptide hormones, human chorionic gonadotrophin (hCG), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and the gonadotropin-releasing hormones (see review by Stevens [1988] and articles by Thau et al. [1987] and Mougdal [1990]). Of these potential vaccines, the most extensive studies conducted to date have been on the hCG (see reviews by Gupta and Koothan [1990] and Griffin [1991a,b]). The hCG vaccine, which is most advanced in clinical studies for human contraception, is limited to efficacy in primates and is not applicable to the majority of wildlife species. Because it interrupts

pregnancy rather than preventing it, this vaccine may not be a universally accepted alternative to contraception. Although some of these vaccines have promise for human contraception, their modes of action make them less likely to be effective for large-scale animal populations.

Gamete-Based Contraceptive Vaccines

Sperm Antigens

The onset of spermatogenesis and the appearance of mature sperm in the male reproductive tract occur during puberty at a time well beyond the establishment of immunological competence and tolerance to autoantigens (see review by Simon and Alexander [1988]). The further development of the male gamete occurs elsewhere in that it must mature in the epididymis (Dacheux et al. 1990, Cameo et al. 1990) and pass through the male reproductive tract, during which time the sperm surface antigens are modified (Isojima and Koyama 1988). Finally, the sperm are further modified in the female reproductive tract, where sperm capacitation occurs (Yanagimachi 1989, Bedford 1991). It is therefore possible to interfere with sperm function at several points in the reproductive process.

Because antibodies are produced against spermatozoa, Rosenfeld (1926) suggested that women repeatedly injected with human semen would become infertile. Since these early investigations, numerous studies by many laboratories have concentrated on identifying sperm antigens that would be (1) specific for sperm and not cross-react with any other cell types so they would not elicit antibodies with deleterious side effects on other tissues, and (2) present on the sperm surface or involved in the fertilization process so that antibodies would be able to inhibit fertility. These studies have been reviewed in detail elsewhere (O'Rand and Fisher 1988, Simon and Alexander 1988). Again, although these vaccines hold great promise for human vaccine development, they may not be practical for large-scale animal populations unless temporary infertility is desired and continuous and long-term antibodies can be sustained.

Oocyte and Zona Pellucida (ZP) Antigens

Studies on the identification of oocyte-specific antigens have been less numerous than those of sperm antigens for the simple reason that large numbers of mammalian eggs are not readily available to carry out many of the biochemical studies necessary for such research. The development of procedures to isolate large numbers of oocytes with their surrounding egg coat, the zona pellucida (ZP), has made it possible to study the study the glycoprotein antigens of the ZP in great detail (see review by Timmons and Dunbar [1988]).

The antigens of the ZP have long been considered to be an attractive target for immunological contraceptive or sterilization vaccines for several reasons. For human contraceptive vaccines, antibodies directed against the ZP antigens expressed at later stages of oocyte development should inhibit fertilization (i.e., this would be a nonabortive method). Alternatively, if antibodies are directed against ZP antigens expressed early in oocyte growth and follicular development, permanent sterilization may result because all oocytes are ultimately destroyed and no steroids will be produced. This latter method would be preferable for use in animal sterilization.

Because there are a limited number of glycoproteins associated with the ZP, the major proteins of several species have been studied in great detail. To date, three major glycoproteins have been identified in most species (see review by Timmons and Dunbar [1988]). Many studies have demonstrated that both the immunogenicity and antigenicity of the ZP glycoproteins is extremely complex (see discussion below on general aspects of immunogenicity and antigenicity). Antibodies can be identified which recognize amino acid and carbohydrate epitopes as well as conformational or structural epitopes of the ZP (Maresh and Dunbar 1987). Further, it is not possible to predict the immune response of a given animal when immunized with ZP antigens of a different species. Because isoimmunization with the ZP glycoproteins of the same species does not elicit a significant response, it has become apparent that immunogenicity of ZP is primarily

due to the foreign epitopes associated with the ZP of different species. These observations have further been demonstrated by the necessity to conjugate the A mouse peptide to the foreign molecule, keyhole limpet hemocyanin, to elicit an immune response. The distinct species differences in the antigens of ZP of different mammalian species and the need to define antigenic epitopes which will elicit antibodies that prevent fertilization but do not alter ovarian function foster the necessity to dissect the antigenic domains of the ZP glycoproteins.

Studies have also demonstrated that immunization with some ZP antigens can elicit an immune response which interferes with development of ovarian follicles (Skinner et al. 1984, Dunbar et al. 1989, Rhim et al. 1992, and Lee and Dunbar 1992). These effects vary among different mammals and may mimic such clinical conditions as premature ovarian failure, and polycystic ovarian disease. In the development of animal sterilization vaccines, it is preferable to eliminate growing oocytes along with the hormone-secreting granulosa and theca cells, which are responsible for estrous behavior. These side effects are not acceptable for the development of a human contraceptive vaccine.

In view of these observations and the need to develop different vaccines which may cause temporary or permanent infertility, it has been necessary to study the formation and development of the ZP during ovarian follicular development. These studies have demonstrated that the ZP proteins are produced in follicle stage-specific sequence (Wolgemuth et al. 1984, Lee and Dunbar 1992) and that the distinct ZP antigens are synthesized and secreted at different stages of development. It should therefore be possible to identify distinct antigenic epitopes that are associated with the ZP matrix following the differentiation of the steroid-producing granulosa cells.

Molecular biology techniques have allowed for the further study of ZP proteins' antigenic domains. Initial studies of molecular cloning and characterization of complimentary deoxyribonucleic acids (cDNA's) encoding the ZP glycoproteins of mouse and rabbit have been described (Ringuette et al. 1986, Liang et al. 1990, and Schwoebel et al. 1991). These studies have demonstrated the rabbit ZP 55 Kd antigen has a distinct amino acid sequence (Schwoebel et al. 1991) from the two sequences of mouse ZP proteins (Ringuette et al. 1986, Liang et al. 1990). Furthermore, the 55 Kd protein does not appear to be present in the mouse genome. A rabbit 75 Kd ZP protein has demonstrated significant similarity (70 percent) with the amino acid sequence of the mouse ZP2 protein, and cDNA isolated against the 55 Kd rabbit ZP protein has demonstrated that the pig has a homologue of this rabbit protein.

The complete amino acid sequences of ZP proteins for several species will ultimately be needed to determine their species similarities and determine vaccine efficacy. Results of these studies could explain why immunization of mice or rats with the ZP of pig ZP antigens does not cause infertility (Drell et al. 1984, Sacco et al. 1981), while immunization of other mammalian species including nonhuman primates with pig ZP proteins effectively reduces fertility (Drell et al. 1984, Skinner et al. 1984 and 1989, Dunbar et al. 1989).

Recently, studies have been carried out using the expression of ZP proteins produced using recombinant DNA techniques to begin to define specific epitopes that may be effectively used to develop a contraceptive vaccine. This vaccine would inhibit fertilization but not affect the early stages of ovarian follicular development (Schwoebel et al. 1991). These studies are the first to demonstrate that ZP proteins produced using bacterially expressed recombinant DNA techniques can elicit in monkeys antibodies that recognize the native ZP antigen. Furthermore, cynomolgus monkeys immunized with 55 Kd rabbit ZP protein show no alteration of ovarian follicular development, but antibodies to this protein inhibit monkey sperm from binding to monkey ZP. This is in contrast to monkeys immunized with a portion of the rabbit 75 Kd rabbit ZP protein, which does alter ovarian follicular development. Thus, antigenic domains of ZP proteins can also be dissected to define which ZP proteins will elicit specific antibodies that either (1) inhibit sperm binding to the ZP without affecting ovarian follicular development or (2) reduce the number of ovarian follicles and ovulations.

It is apparent that many studies need to be carried out to define specific ZP antigens which can effectively produce an immune response that results in reversible infertility or permanent sterilization. The advances in researchers' understanding of the development of the ovary, as well as the advances in molecular cloning techniques should allow investigators to identity specific antigens rapidly.

Trophoblast Antigens

The principal precursor of the fetal placenta is the trophoblast, which is composed of a group of cells that surround the developing embryo at the blastocyst stage. Research to identify and characterize specific trophoblast antigens has been important because they have provided the basis for studying reproductive failure. The research into the structure and function of these antigens is important in studies on the maternal–fetal interactions that can be used to devise strategies for improving reproductive success (see review by Faulk and Hunt [1990]). Although the antigens of the trophoblast could be used as targets for immunocontraception, the probability that many of antibodies directed against these antigens would result in abortive mechanism has made the development of this type of vaccine more controversial.

Major Goals and Hurdles in Contraceptive Vaccine Development

Large-Scale Production of Vaccinogens

To date, development of protein-based contraceptive vaccines, particularly those using gamete antigens, has been hampered by the inability to purify sufficient quantities of protein. However, rapid advances in recombinant DNA technology and genetic engineering have now made it possible to generate sufficient quantities of any target protein antigen (see review by Chin [1990]). The development of vectors designed for vaccine delivery systems using virus expression systems to express large proteins—such as the vaccinia virus that has been devised for rabies vaccination of wild animals—may also prove useful.

Optimizing Immune Enhancement for Development of Contraceptive Vaccines

The successful development of contraceptive vaccines, like other vaccines, will ultimately depend on the production of an immune response which is sufficient to elicit antibodies which will neutralize either the hormone or specific gamete antigens (see review by Woodrow and Levine [1990]). It is therefore necessary to understand the potential problems in generating a desired immune response using such vaccines. In general, *antigens* contain multiple regions (antigenic determinants) that are capable of being recognized by an antibody. These antigenic determinants may be associated with almost any molecular structure and may be associated with the peptide backbone of a protein or a carbohydrate of structure conformation. *Immunogens* have been defined as those chemical substances that are capable of inducing a specific immune response (see general discussions by Benaceraf and Unanue [1979] and Berzofsky and Berkower [1989]). Many molecules that are antigenic are not always immunogenic. In general, immunogenicity can often be achieved by covalently attaching defined molecules to a larger molecule called a carrier. Although many such studies have been carried out to study the immune response experimentally, fewer studies have been carried out to define specific molecules that can be effectively used in vaccines.

Another critical factor in the development of vaccines has been the identification of molecules that enhance the immune response. The use of *adjuvants,* which are agents that potentiate the immune response, has therefore become common. The term "adjuvant" has been defined as an agent that augments a specific immune response to antigens (Allison and Byars 1990). The adjuvant most commonly used is Freund's complete adjuvant, which contains a bacteria suspension in an oil vehicle. When it is inoculated with other specific antigens, this adjuvant induces a heightened immune response. Although Freund's is useful as an enhancing agent, it may also cause a variety of adverse reactions and is therefore not generally acceptable for use in clinical trials.

Recent studies using highly purified antigens alone have demonstrated the need for adequate adjuvants that can be used to enhance the immunogenicity molecules that are to be used in vaccines. In response, a variety of adjuvants have been introduced (see reviews by Allison and Byars [1990], Anderson and Capetola [1990], and Alam et al. [1991]), but their efficacy has yet to be proven in extensive clinical trials. It is apparent that, until such immune enhancement agents are readily available, many of the immunogens targeted for contraceptive vaccines cannot effectively be evaluated. The advances being made in other areas of vaccine development will therefore be critical for the future development of effective contraceptive vaccines.

Strategy for Development of Contraceptive Vaccines for Wildlife Populations

Based on observations that all nonrodent mammalian species tested to date have multiple ZP proteins which share antigenic determinants with other mammalian species, it is likely that the ZP proteins of elephant ZP will also contain similar antigenic determinants. It is possible to identify such antigens using as few as 20–30 zonae pellucidae using immunoblot analysis of ZP glycoproteins separated by one- and two-dimensional polyacrylamide gel electrophoresis. In this manner, it is possible to rapidly identify candidate ZP antigens. These procedures have been successful for identification of horse and deer antigens. Specific polyclonal as well as monoclonal antibodies are now available to ZP proteins of numerous mammalian species, and analyses can easily be used to define specific ZP antigens of the zonae pellucidae of different wildlife species. Furthermore, it has been possible to use cDNA's from the rabbit (Schwoebel et al. 1991) to isolate a cDNA from the pig's zonae pellucidae and to use mice ZP cDNA's to isolate human cDNA's (Chamberlain and Dean 1990, Liang and Dean 1993).

Despite the availability of cDNA sequences for ZP proteins of numerous species, it will ultimately be necessary to express sufficient quantities of proteins to use in fertility studies in target animal species. Initially, it will be essential to determine if immunization of individual species with ZP proteins will elicit a humoral immune response resulting in the production of antibodies to self ZP antigens. Secondly, it will be important to establish the stage of ovarian development during which the zonae pellucidae are formed, as well as the time of transition of oocyte recruitment from meiotic prophase to the time of ovulation. This timeframe is estimated to be 2 weeks in mice and up to 6 months in humans (Gougeon 1982). Because the time of exposure of oocytes in the developing follicle to antibodies is critical, it is important to understand the basic development of the ovary in each mammalian species for which contraceptive vaccines are being evaluated. The stage-specific expression of ZP proteins during ovarian development can easily be carried out using established immunocytochemistry methods or in situ hybridization methods. Once these studies are carried out for a target species, it should be possible to rapidly evaluate the potential for efficacy, safety, and reversibility of immunocontraception using these procedures. In some instances, small numbers of animals (e.g., elephants and some zoo animals) will need to be vaccinated for contraception or sterilization as compared to other large animal or human populations. It may be necessary, however, to identify the "species-specific" ZP antigenic domains for different target animals. Although this identification could pose a significant challenge, such species-specific vaccines will be essential if large-scale oral vaccine delivery systems are to be utilized.

Considerations for the Development of Vaccines for Wildlife Management

In order to design an effective contraceptive or sterilization vaccine for any wildlife species, it is necessary to take into consideration the long-term effects on the animal populations. For example, it may be desirable to develop methods for large-scale sterilization in animal species that are clearly not in danger of becoming extinct but whose population has become so excessive that starvation or other problems arise.

If extinction or reduction of the gene pool is, in fact, a consideration in an animal species, it will be important to consider contraception rather than sterilization. Because the zonae pellucidae are composed of distinct antigens that occur at different stages in oocyte growth and ovarian follicular development, they provide target antigens that can be developed to cause permanent sterilization instead of temporary contraception. However, the dissection of these antigenic domains can be carried out only by using advanced techniques such as recombinant DNA or peptide chemistry.

The efficacy of these methods is dependent upon the development of the ovary and the nature of the ovarian cycle, and it is important to develop a better understanding of the reproductive cycles of the females of any animal.

Summary

Development of most of the contraceptive vaccines, particularly those using gamete antigens, has been hampered by the inability to purify sufficient quantities of protein for development and use. The rapid advances in recombinant DNA technology and genetic engineering have now made it possible to generate sufficient quantities of any target protein antigen that has been identified. The use of these techniques for development of vaccines will ultimately depend on the production of an immune response sufficient to elicit antibodies that will neutralize either the hormones or the specific gamete antigens. This aspect of vaccine development is greatly dependent upon the production of more effective and safe adjuvant.

Although the "ideal" vaccine has yet to be formulated, research in this area has provided a great deal of information relating to the fundamental mechanisms of hormone action, gamete interaction, fertilization, and implantation. These detailed studies have been important not only in laying the foundation for the future development of contraceptive and sterilization methods but also in developing a better understanding of reproductive systems that may in turn shed light on reproductive dysfunction, disease, and infertility.

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Gonadotropin-Releasing Hormone (GnRH) Analogs or Active Immunization Against GnRH To Control Fertility in Wildlife

Susan E. Becker and Larry S. Katz

Abstract: The administration of analogs, both agonists and antagonists, of GnRH and immunization against GnRH have been investigated for their ability to control reproductive function in domestic species. These methods can be used to inhibit the secretion of gonadotropins, the necessary stimulants for steroidogenesis and gametogenesis, thereby potentially preventing ovulation and inhibiting spermatogenesis. Induction of infertility in this manner could be used for nonlethal population control of wildlife species. Relatively little research has been done in this area. This chapter reviews relevant studies with domestic species and discusses results from studies with wildlife species.

Keywords: gonadotropin-releasing hormone, GnRH agonist, GnRH antagonist, GnRH immunoneutralization, wildlife contraception

Introduction

Because hunting and natural mortality cannot control wildlife populations everywhere, there is increasing demand for the development of nonlethal methods for population control of both free-roaming and captive wildlife. Therefore, fertility control through administration of contraceptive agents is being investigated. The ideal contraceptive agent should be (1) reversible (for some species), (2) suitable for remote delivery, (3) effective with only a single administration, (4) unable to contaminate the food chain, (5) without harmful side effects, and (6) without effect on social behavior. Although steroid hormone treatments have been used successfully for fertility control in nondomestic animals (see review by Kirkpatrick and Turner [1991]), the possibility exists for steroids to enter the food chain. A nonsteroidal hormone such as gonadotropin-releasing hormone (GnRH), a small peptide, would not pass through the food chain because when ingested it would be cleaved to its constituent amino acids. Relatively little work has been done to investigate the effectiveness of GnRH as a contraceptive agent in nondomestic species.

Gonadotropin-releasing hormone, synthesized in the hypothalamus of both males and females, is a key regulator of reproduction in mammals. Released from the hypothalamus in a pulsatile pattern, it travels via the portal vasculature to the anterior pituitary, where it stimulates release of the gonadotropins, luteinizing hormone (LH), and follicle-stimulating hormone (FSH). These gonadotropins enter the circulation and regulate both steroidogenesis and gamete maturation in the gonads (Conn 1994). More specifically, in the female, FSH stimulates follicular growth and maturation, and LH induces ovulation and corpus luteum formation. In the male, the direct role for FSH in spermatogenesis is uncertain, and LH causes the Leydig cells of the testis to produce testosterone which is necessary for gametogenesis. FSH, in the presence of LH, stimulates estradiol production from both the ovary and the testis. The steroids secreted from the gonads feed back to the hypothalamus and pituitary to regulate GnRH and gonadotropin synthesis and release (see fig. 1).

It is possible to make the pituitary refractory to GnRH by administering GnRH, or an agonist of GnRH, in a continuous manner, rather than in the physiological pattern of pulses. Prolonged, continuous infusion of GnRH, especially at high concentrations, inhibits gonadotropin secretion (Belchetz et al. 1978), and that results in loss of gonadal function. Initially, pituitary desensitization is thought to result from loss of pituitary cell-surface receptors for GnRH by internalization of occupied receptors (Conn and Crowley 1991). Later, as receptor numbers recover due to recycling (Hazum and Conn 1988) and homologous upregulation (Conn et al. 1984, Braden and Conn 1990), desensitization may be maintained because the receptors become dissociated from their second messenger system (Conn and Crowley 1991).

Controlling the amount and pattern of GnRH stimulation to the pituitary affects gonadotropin synthesis and secretion, thereby affording a potential method of controlling fertility in both males and females. Administration of GnRH agonists or antago-

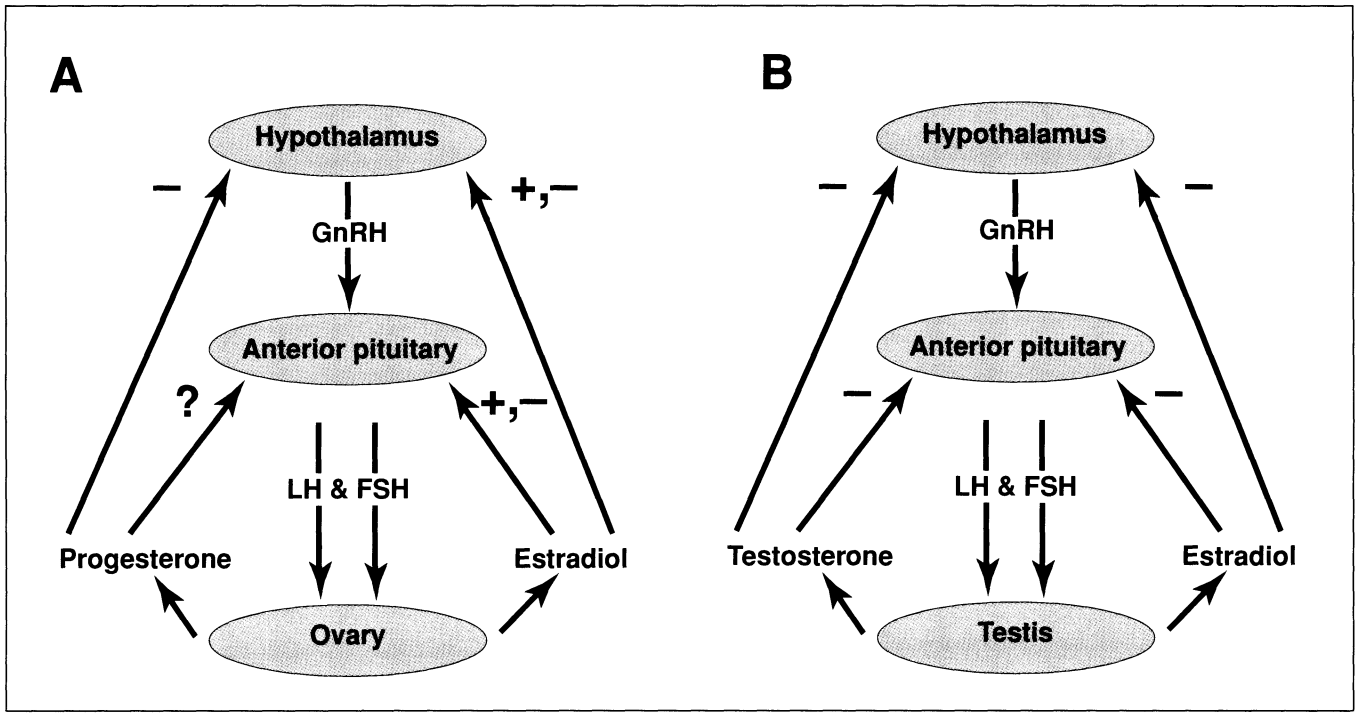

Figure 1. Pathways of positive (+) and negative (–) feedback of gonadal steroids on the hypothalamic–pituitary axis in (A) the female and (B) the male. The effects of estradiol in the female are positive or negative, depending upon the stage of the estrous cycle. Question mark (?) indicates relatively insignificant effects.

nists, as well as immunization against GnRH, have been tested for their ability to suppress reproductive function in humans and domestic animals, yet little work has been done in this area with wildlife.

GnRH and GnRH Agonists

Large doses or chronic administration of GnRH or GnRH agonists can inhibit gonadotropin secretion by pituitary desensitization. Agonists are often preferred, both clinically and experimentally, over GnRH itself due to their increased potency. In general, they have a higher binding affinity for the GnRH receptors, are more resistant to enzymatic degradation, and/or have a longer half-life in the circulation. Following commencement of treatment with GnRH or its agonists, there is a transient period of increased gonadotropin secretion before the suppressive effects of pituitary desensitization are realized (Conn and Crowley 1991). This results in a delay of effect in both sexes. In females, this initial increase in gonadotropin secretion may induce estrus and ovulation, depending upon the reproductive status of the animal when treatment is begun. However, if a female were bred during this induced estrus, the continued administration of GnRH would likely terminate the pregnancy.

In males, there seem to be species differences in the degree of desensitization possible in response to GnRH agonists (see review by Vickery [1986]). Depending upon the species, pituitary desensitization is not necessarily accompanied by a decline in testosterone secretion and a suppression of spermatogenesis. However, when it is, testosterone supplementation may be necessary if maintenance of normal sexual behavior in males is desired (Vickery et al. 1984). Given these shortcomings, GnRH agonists may not be useful as male contraceptives. Nevertheless, they may have some application in control of androgen-stimulated aggressive behavior.

In Hawaiian monk seals (Atkinson et al. 1993) and free-ranging African elephants (Brown et al. 1993), single injections of GnRH agonists have been tested for their ability to suppress testicular function, i.e. testosterone production, thereby controlling aggressive behavior. Male Hawaiian monk seals may exhibit a breeding behavior called "mobbing" when their numbers exceed those of the females by more than 2:1. The "mobbed" female or immature seal is severely injured and often dies (Atkinson et al. 1993). Atkinson and coworkers found that after a transient increase, serum testosterone concentrations were reduced to castrate levels for approximately 2 months in male monk seals following a single injection of a GnRH agonist. Effects on sexual and aggressive behavior could not be measured because no female seals were available.

In the case of African elephants, males go into musth once or twice a year, during which time they are dangerously aggressive. Captive elephants in musth have injured and killed handlers (Brown et al. 1993). A single injection of a GnRH agonist caused an initial increase in serum LH and testosterone concentrations followed by a decline to baseline values. The one bull which was in musth at the time of treatment did not appear to be in musth after the decline in serum testosterone levels. Subsequent challenge with an intravenous injection of GnRH resulted in an attenuated LH response, suggesting partial desensitization of the pituitary. However, testosterone secretion was increased compared with controls, indicating a hypersensitivity to increases in GnRH-induced LH concentrations (Brown et al. 1993).

From these studies it appears that GnRH agonists show promise as agents that may decrease aggressive behavior by reducing serum concentrations of testosterone. This may be very useful in captive populations such as those in zoos. Yet it is important to note that some species, such as cattle, may respond to chronic treatment with GnRH agonists with an increase in testicular function, despite depressed pituitary function, as evidenced by elevated serum testosterone concentrations (Melson et al. 1986).

Another possible outcome of prolonged administration of GnRH agonists is the stimulation of both pituitary and testicular function, as described by Lincoln (1987). In that study, red deer stags received continuous infusion of a GnRH agonist for 72 days beginning after the rut in winter, a time when the testes are still secreting significant amounts of testosterone. It was expected that testicular activity would be suppressed, causing the stags to cast their antlers prematurely. In fact, treatment with the agonist resulted in increases in plasma LH and testosterone concentrations, testes growth, and aggressive behavior, and did not affect time of antler casting. The wide variation in response of the hypothalamic–pituitary–gonadal axis to exogenous GnRH may be due to several factors, including (1) choice of agonist, (2) dose, (3) treatment regimen, (4) reproductive status of the animal, and (5) species. Clearly more research is needed to determine the usefulness of this approach.

It has been well documented that continuous treatment with GnRH will suppress gonadotropin secretion in females (Nett et al. 1981, Adams et al. 1986, Khalid et al. 1989). Inhibition of ovulation caused by chronic administration of GnRH agonists has been successful in several species, including dogs (Vickery et al. 1989), cattle (Herschler and Vickery 1981), sheep (McNeilly and Fraser 1987), horses (Montovan et al. 1990), stumptailed monkeys (Fraser et al. 1980, Fraser 1983), and macaques (Fraser et al. 1987). We recently attempted to inhibit secretion of LH in white-tailed deer does *(Odocoileus virginianus)* by continually infusing a GnRH analog (Histrelin™), with the goal of preventing ovulation (Becker and Katz 1995).

Briefly, four does received Histrelin at 8.3 µg/hour subcutaneously via osmotic minipump, for 14 days during the breeding season. Controls were administered continuous saline infusions ($n = 3$). On Day 1 (Day 0 = day of minipump insertion), the Histrelin-infused group had a higher mean serum LH concentration than the control group (16.0 ± 5.3 *v.* 0.9 ± 0.4 ng/mL, respectively). By Day 2, mean LH concentrations did not differ between the groups and remained at baseline for the duration of infusion (fig. 2). On Day 10, both groups received a subcutaneous

injection of 100 µg Histrelin to test the ability of the pituitary to respond to additional stimulation. At 4 hours after injection, the mean serum LH concentration for controls was 17.8 ± 3.3 ng/mL and was still elevated at 10 hours. In contrast, serum LH concentrations in the Histrelin-infused group remained at baseline (0.5 ± 0 ng/mL) (fig. 3).

Apparently, continuous infusion of Histrelin caused pituitary desensitization. It was not possible to monitor the ovaries ultrasonically; however, serum progesterone concentrations did not indicate that any of the four does infused with Histrelin ovulated in response to the initial rise in serum LH concentrations. Further research is needed to determine if reproductive status influences whether or not ovulation is induced (an undesirable side effect) during the transitory increase in serum gonadotropin concentrations. The practicality of this approach is dependent upon development of a long-acting, slow-release preparation of agonist that can also be remotely delivered.

GnRH Antagonists

Pituitary suppression may be achieved by administration of antagonists of GnRH, which exert their effects by competing with endogenous GnRH, preventing sufficient GnRH occupation of receptors to stimulate gonadotropin secretion (Conn and Crowley 1991). The main advantage to using GnRH antagonists rather than agonists is that pituitary suppression is immediate. There is no initial increase in gonadotro-

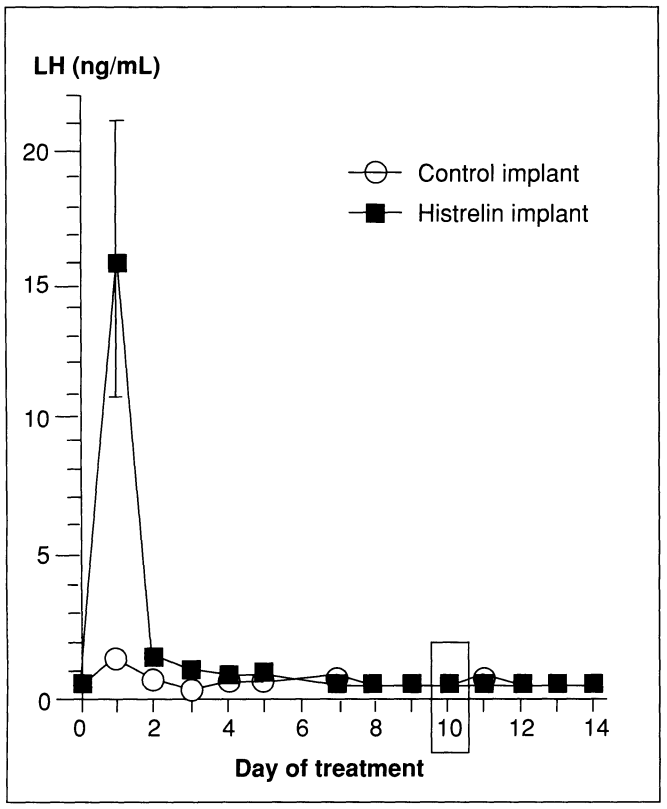

Figure 2. Effects of continuous administration of Histrelin (8.3 µg/hour, subcutaneously; $n = 4$) or saline (control; $n = 3$) on daily mean serum LH concentrations. Box indicates day of Histrelin challenge.

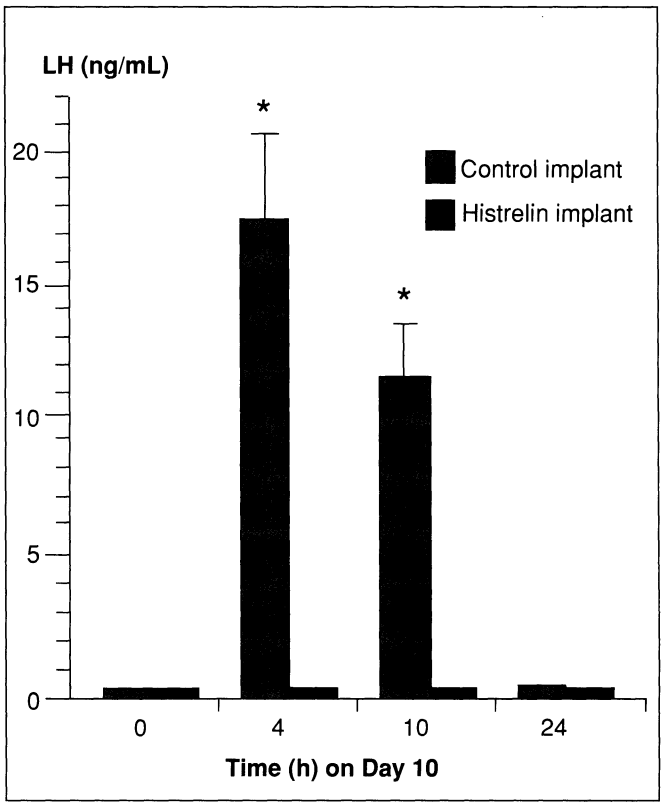

Figure 3. The LH response to a subcutaneous injection of 100 µg of Histrelin on Day 10 of the continuous Histrelin ($n = 4$) or continuous saline ($n = 3$) infusion period (Day 0 = day of implant insertion). * denotes treatment difference ($P < 0.05$).

pin secretion, which may stimulate the gonads. Unfortunately, these drugs are more expensive and require a higher dosage than the agonists, so they are best used for short-term treatment or instances where agonists are not effective (Vickery 1986). In males of several species, including rats, dogs, and monkeys, treatment with GnRH antagonists results in a decrease in serum LH and testosterone concentrations within hours, and that ultimately halts spermatogenesis (Vickery 1986). Choice of antagonist may be important, as evidenced by the work of Brown et al. (1993). They gave a single intramuscular injection of an antagonist to African elephant bulls that resulted in reduced basal and GnRH-stimulated serum LH and testosterone concentrations on Day 2 after injection. One of the bulls was in musth at the time of treatment but was no longer in musth by Day 2. In contrast, treatment of elephant bulls with a different antagonist of similar structure did not affect pituitary–testicular function, despite a higher dosage.

Antagonists of GnRH have successfully inhibited LH secretion and prevented ovulation in several species, including cattle (Rieger et al. 1989), rats, dogs, monkeys, and humans (see review by Vickery [1986]). For example, weekly subcutaneous injections for 20 weeks beginning during the midluteal phase of the estrous cycle resulted in suppression of circulating LH concentrations (compared with controls), and inhibition of ovulation throughout the treatment period in marmoset monkeys (Hodges et al. 1992). This effect proved to be reversible. Despite these successes, fertility control for wildlife often requires long-term treatment, for which GnRH agonists are better suited.

Immunoneutralization of GnRH

Another approach to inhibit gonadotropin secretion from the pituitary involves active immunization of an animal against endogenous GnRH. Because GnRH is a low-molecular weight, naturally occurring peptide, it is a weak immunogen. It must be adsorbed to a large, inert particle, such as charcoal, or covalently bound to a carrier protein, such as a serum albumin, to enhance immunogenicity. The latter seems to provide more consistent responses and higher antibody titers (see review by Jeffcoate and Keeling [1984]). Development of detectable antibody titers in the serum requires many weeks following primary immunization.

Although booster immunizations are not essential for the production of high antibody titers (Adams and Adams 1992), boosters almost always raise the existing antibody titers (see review by Schanbacher [1984]). Once titers are raised, circulating GnRH is recognized and bound by the anti-GnRH immunoglobulins before it reaches the pituitary, thereby suppressing LH secretion and usually FSH secretion (although not always to the same degree) and leading to an impairment of reproductive function. The degree of dysfunction appears to be correlated to the GnRH antibody titer; that is, the higher the titer, the greater the suppressive effects on reproduction (Lincoln et al. 1982, Safir et al. 1987, Bailie et al. 1989). Unfortunately, immediate inhibition of reproductive function is not possible unless immunization against GnRH is passive (administration of GnRH antiserum rather than a GnRH conjugate functioning as an antigen). For example, injection of ewes with ovine GnRH antiserum approximately 10 hours prior to the LH surge prevented the surge and blocked ovulation (Fraser and McNeilly 1982). Yet passive immunization against GnRH is not a practical method of fertility control because the effects are not long-lasting (Fraser et al. 1984). Frequent injections of GnRH antisera are not only impractical but also pose a health threat to the animal (Schanbacher 1984).

Active immunization against GnRH has successfully suppressed gonadotropin secretion and gonadal function in a variety of species, including rats and rabbits (Ladd et al. 1988), pigs (Esbenshade and Britt 1985, Awoniyi et al. 1987), sheep (Clarke et al. 1978, Adams and Adams 1986), horses (Garza et al. 1986, Safir et al. 1987), and cattle (Robertson et al. 1982, Adams and Adams 1990, Adams et al. 1993). However, little work has been done to test the effectiveness of this approach for wildlife. Studies in which red deer stags were actively immunized against GnRH met with varying degrees of success (Lincoln et al. 1982, Ataja et al. 1992, Freudenberger et al. 1993).

Effects on reproductive parameters ranged from a slight suppression of plasma LH concentrations compared with controls but no significant reduction of plasma testosterone concentrations (Ataja et al. 1992) to a significant decrease in testosterone levels compared with controls, testicular atrophy, and premature casting of antlers (Lincoln et al. 1982). Differences in the carrier protein used and the timing of the primary immunization with respect to reproductive season may account for this variability. When male and female wild Norway rats were actively immunized against GnRH, 100-percent sterility was attained for both sexes. In the males, testosterone was nondetectable, and testes were approximately 90-percent atrophied up to 11 months after vaccination (see Miller, this volume). Although these results are promising and immunoneutralizing GnRH is less costly than treatment with either GnRH agonists or antagonists, there can be large variation in response due to individual differences in the development of antibody titers.

Conclusion

None of the GnRH-related fertility control methods described herein meet all of the criteria of the ideal contraceptive agent outlined previously. One problem that may apply to any method of contraception in wildlife is the lack of consensus on the percentage of animals that must be rendered infertile to bring about the desired reduction in herd growth rate. Also, logistical and economic issues pertaining to delivery systems must be addressed. Perhaps the greatest problem with GnRH contraception is the resulting suppression of sexual behavior, which may affect social behavior and, consequently, social structure. This problem can be overcome by steroid supplementation using implants, but then food-chain contamination and the need to capture the animals to administer the treatment become issues that must be considered. However, inhibition of androgen-stimulated aggressive behavior may be desired in certain venues, such as zoos. In addition, care must be taken to ensure that the contraceptive activity of GnRH analog treatment lasts throughout the breeding season to avoid young being born when environmental conditions are unsuitable for offspring survival. Treatment must abolish, not merely delay, the breeding season. Targeting GnRH function for contraception of wildlife meets four of the six criteria mentioned earlier for the ideal contraceptive agent. Treatment is reversible, suitable for remote delivery, and unable to contaminate the food chain. Additionally, single administration is possible for active immunization against GnRH (and will be possible for GnRH agonists following the development of long-lasting, injectable microcapsules). Gonadotropin-releasing hormone contraception should be further investigated for potential applications in wildlife management.

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Surgical Sterilization: An Underutilized Procedure for Evaluating the Merits of Induced Sterility

By James J. Kennelly and Kathryn A. Converse

Abstract: Despite more than 4 decades of effort, development of effective wildlife damage control programs based on sterilization of target species has met with limited success. This is partly due to the fact that investigators have assumed, rather than empirically tested, whether the reproductive strategies of the target populations were vulnerable to the planned treatment. Equally important, methods selected to induce sterility usually involve a chemical agent that can affect sociosexual behaviors of the nuisance population. In this report, we illustrate how surgically induced sterility circumvents both problems—how it enables one to assess the feasibility and applicability of the concept without the potentially confounding secondary effects of a chemical. We assessed the merits of initiating research to develop a male chemosterilant for Norway rats, red-winged blackbirds, beaver, and Canada geese by inducing sterility surgically. The infidelity of many red-winged females to their polygynous territorial male was surprising and argued against searching for a male sterilant. On the other hand, beaver and Canada goose studies confirmed previous reports that both form pair-bonds and are monogamous. Both should be vulnerable to a male chemosterilant approach, and research toward this goal is justified.

Keywords: vertebrate pest, beaver, rodent, Canada goose, blackbird, surgical sterilization

Introduction

The concept of alleviating animal damage problems by reducing nuisance populations to acceptable numbers using induced sexual sterility has been researched for more than 40 years. Initially proposed for control of the screw-worm fly *Callitroga hominivorax,* the concept's validity was demonstrated by the eradication of this species from the island of Curaçao (Bushland and Hopkins 1951, Knipling 1955). The potential benefits of the concept compared to alternative pest control methods were recognized immediately (Knipling 1959).

Most attempts to induce sterility of vertebrate pest species have relied on use of a chemosterilant or antifertility agent. The most notable of these is the work of Elder (1964) and Wofford and Elder (1967), which resulted in the development and marketing of Ornitrol® (20,25-diazocholesterol dihydrochloride), an antifertility agent for feral pigeon control.

Success of induced sterility programs has been somewhat less than expected due to failure to understand mating strategies and related sociosexual behavior patterns of species targeted for reproductive control. Investigators often assumed, rather than empirically assessed, the feasibility of this approach for each problem situation. The question of whether or not a particular nuisance species may be vulnerable to fertility control often can be answered readily by inducing sterility surgically.

A few studies that utilized surgical procedures deserve mention. Neville (1983) and Neville and Remfry (1984) maintained several discrete populations of feral cats at acceptable levels by capture and surgical sterilization of all healthy adults. While the initial cost of castration was high, the authors estimated the long-term expense, including periodic castration of subsequent newcomers, was about half the cost of alternative eradication programs. Bailey (1992) described successful introduction of surgically sterilized red foxes *(Vulpes vulpes)* to two Alaska islands where native avifauna were adversely impacted by Arctic foxes *(Alopex lagopus)*. Because the two species were not sympatric on islands in Alaska, Bailey achieved the desired results of biological control. Nine years after introduction of sterile red foxes, Arctic foxes had disappeared and only one island had a few remaining red foxes. Another study demonstrates successful use of surgical sterilization to provide an indirect treatment effect. Five wolves *(Canis lupus)* were vasectomized before release in northern Minnesota to assess whether such males could keep mates and maintain territories (Mech and Fritts 1993). The rationale from a control perspective was that the absence of pups to feed might reduce livestock depredations. The results indicated that vasectomized wolves maintained pair bonds and territories and suggested that sterilization might be the technique of choice around farms experiencing livestock losses.

Proceeding on the premise that the polygynous mating strategy of feral horses *(Equus equus)* (i.e., dominant males maintain a harem throughout the year) would render this species particularly vulnerable to sterilization, Eagle et al. (1993) vasectomized 20 dominant males in each of 2 separate populations. Foaling was reduced for 2 years in bands containing a sterile male, and the treatment was considered efficacious. However, due possibly to movement of females between bands and breeding by subordinate or bachelor males, the reduced fecundity was insufficient to lower the population to an acceptable level.

Any discussion of advantages and disadvantages associated with utilization of surgically induced sterility depends largely on whether the procedure is intended to resolve research questions as proposed here or if it is intended to directly resolve a wildlife damage problem. Primary research advantages are permanency of the procedure, which allows long-term effects to be assessed for target species, and absence of behavioral or secondary effects that may accompany other methods of induced sterility. The advantages of irreversibility of the technique extend to situations where it is intended to be the method of choice for ameliorating a damage problem. The main disadvantages of surgical sterilization, the need to have the animal in hand and the expense of the procedure, limit its use. However, the value of surgical sterilization to answer important biological questions can often justify the time and expense. This report describes studies that illustrate use of surgical sterilization to better understand mating strategies and associated behavioral patterns and in some instances provide a definitive answer to damage problems.

Mating Strategies

The classification of the four mating systems pertinent to the topic at hand follow those reported by Wittenberger (1979). For the purpose of this report only the definitions for the general classification of each are given:

(1) Monogamy: "prolonged association and essentially exclusive mating relationship between one male and one female at a time."

(2) Polygyny: "prolonged association and essentially exclusive mating relationship between one male and two or more females at a time."

(3) Polyandry: "prolonged association and essentially exclusive mating relationship between one female and two or more males at a time."

(4) Promiscuity: "no prolonged association between the sexes and multiple matings by members of at least one sex."

Norway Rats (Rattus norvegicus)

The discovery of U-5897 (3-chloro-1, 2-propanediol) in the late 1960's as an effective sterilant for male laboratory rats (Ericsson 1970) stimulated interest in evaluating U-5897 as a potential reproductive inhibitor for control of free-ranging Norway rats. This interest occurred despite knowledge that Norway rats, with their promiscuous mating system, were considered to be unlikely candidates for male reproductive control (Knipling 1959, Marsh and Howard 1970). In fact, the latter authors postulated that in a polygamous mating system "relatively few non-sterile males can compete successfully for females against an overwhelming number of males." Since this premise was never tested empirically and since several other promising male chemosterilants appeared at that time, a study was initiated to address efficacy of male sterilization for Norway rats.

Kennelly et al. (1972) induced sterility in 85 percent of the adult males in one of two similar populations of Norway rats and compared fecundity and related parameters after 105 days by collecting and examining all juvenile and adult animals in each population. The results confirmed the postulate of Marsh and Howard (1970) and others (Davis 1961, Knipling and McGuire 1972) that development of a male chemosterilant for polygamous vertebrate pests offers little if any promise as a population control technique.

Although fecundity was reduced somewhat in the treated colony (table 1), the treatment appeared to be essentially ineffectual from a population perspective. This conclusion was based on the fact that the total number of pregnancies of the original adult population was almost equal and that the total progeny produced, 110 *v.* 130 in the treated and control group, respectively, differed by only 15 percent. The similarity in number of pregnancies between the two colonies is noteworthy. Based on necropsies at study termination, juveniles were capable of breeding by day 70 of the study. Although this is much sooner than was previously reported by Calhoun (1962), it indicates the majority of original females in the sterile colony conceived when only three original fertile males were available. Although one can justifiably argue that population density established at the outset was partially responsible for the fecundity level observed and that a larger enclosure might have reduced the number of fertile encounters, the study design did not permit this factor to be evaluated. Significant reduction ($P<0.05$) in first litter size for the treated colony was attributed to the large number of vasectomized males. Adler and Zoloth (1970) reported inhibition of sperm transport and reduced litter size following multiple vaginal or cervical stimulations of female rats within 15 minutes of copulation.

Table 1. Reproductive comparisons between a control and male sterility-induced colonies of Norway rats 105 days after treatment (from Kennelly et al. 1972)

Reproductive parameter	Control (100% of males fertile)	Treated (15% of males fertile)
Adult females		
No. pregnancies	38	39
Size first litter	10.8	8.6
Progeny		
Total produced	130	110
No. pregnancies	11	4

Table 2. Sterility treatments of breeding adults

Sterilization method	Number of colonies Female	Male
Ligation[1]	5	5
Castration	2	2
Control (fertile)[2]	2	2

[1] Female = oviduct; male = vas deferens.
[2] Sham operated.

Beaver (Castor canadensis)

Once virtually eliminated from much of its range in North America, the beaver has made such a remarkable recovery that it is now considered a serious nuisance species. The continual encroachment of humans into areas considered suitable beaver habitat has resulted in an ever-increasing number of beaver–human conflict situations. This circumstance, together with society's increasing reluctance to trap and kill offending animals, has generated considerable interest in developing induced sterility as a nonlethal alternative.

Nuisance beavers appear to be an ideal target for developing reproductive control procedures. Beavers breed once yearly regardless of whether the litter is successfully reared. They exhibit a long reproductive life, sometimes exceeding a decade (Larson 1967). They are reported to be monogamous (Seton 1928, Wilsson 1971, Boyce 1974), and once paired, they maintain the pair bond indefinitely barring death of one beaver or disruption of colony integrity by external factors. Other colony members do not breed despite the fact that they are sexually mature by 1.5 years of age. However, should one of the pair-bonded adults die or disappear, the remaining mate will generally pair-bond with one of the sexually mature progeny.

In 1980–83, two studies were conducted to determine the effect that induced sterility of the breeding adults might have on sexually mature but nonbreeding colony members. The objective was to assess whether sterilization would promote mating between the fertile adult and a sexually capable offspring and whether colony integrity would persist after all pretreatment offspring dispersed (Brooks et al. 1980, Kennelly and Lyons 1983). A total of 18 beaver colonies with at least 3 age-classes (adults, 1- to 2-year-olds, and <1-year-olds) were selected and assigned to treatments as shown in table 2.

Assessment of colony fecundity was on the basis of annual breeding cycles, i.e., whether or not reproduction occurred each year a colony was monitored. Therefore by definition, the maximum number of breeding cycles per colony was 3 and the total for all sterilized colonies was 42 (14 × 3) and control was 12 (4 × 3). There was no evidence of breeding outside the pair bond existing at the time of sterilization for all colonies remaining intact. Reproduction was successfully inhibited in 21 of 42 "sterile" colony breeding cycles (table 3). Reproduction in the remaining 21 was either undetermined because the colonies migrated to undiscovered sites (13), or external factors disrupted family associations to the extent that only some members could be relocated. It is noteworthy that castration adversely affected colony behavior, integrity, and fecundity in three (2 male, 1 female) of the four colonies treated in this manner. The fourth, a female-castrate, maintained the adult pair-bond throughout the study and did not produce any kits. We concluded that:

- The beaver monogamous mating system was reaffirmed.
- Adult × progeny breeding does not occur following induced sterility of one of the breeding pair.
- Tubal ligation does not affect colony sociosexual behavior, but castration does.
- Sterilization of either sex is equally efficacious.

Table 3. Beaver colony reproduction: 3-year summary[1]

Sterilization technique	No. of colonies	Potential no. of breeding cycles[2]	No. fertile breeding cycles Fertile	Sterile	Unknown
Male ligate	5	15	4	7	4
Female ligate	5	15	0	7	8
Male castrate	2	6	1	4	1
Female castrate	2	6	3	3	0
Subtotal	—	42	8	21	13
Control	4	12	9	1	2

[1] From Kennelly and Lyons (1983).
[2] Breeding cycle = one breeding season.

Table 4. Clutch fertility on territories of vasectomized red-wing males[1]

Treatment	No. of territorial males	Number of clutches Total	Fertile	Sterile	Percent fertile
Vasectomy	15	32	8	24	25
Control[2]	17	24	24	0	100
Totals	32	56	32	24	

[1] From table 2 in Bray et al. (1975).
[2] Sham vasectomy.

Red-Winged Blackbirds (Agelaius phoeniceus)

The combined gregarious and granivorous behavior of red-winged blackbirds have been a continual problem for humans despite repeated attempts to reduce, if not eliminate, the damage that they cause. When the concept of chemosterilization was initially conceived, the reported polygynous mating system of red-wings (Allen 1914, Beer and Tibbitts 1950, Nero 1956) appeared to present a point of vulnerability: sterilization of a territorial male should inhibit reproduction in all females nesting therein. Although all these researchers reported incidents of promiscuity, they were considered exceptions rather than the rule. Clearly, however, the probability of ever successfully developing an effective male red-wing sterilization program would be compromised if the incidence of female promiscuity proved to be significantly more frequent than reported. Thus, we conducted several studies to evaluate whether red-wing females were promiscuous and, if so, to what extent. The results have been published (Bray et al. 1975, Roberts and Kennelly 1977 and 1980), and the findings germane to the current discussion follow.

The percentage (25 percent) of fertile clutches observed on territories of vasectomized males (table 4) appeared to be correlated with proximity to fertile-male territories; the more distant females were from fertile-male territories, the greater the number of sterile clutches (Bray et al. 1975). Female red-wing infidelity was confirmed in subsequent studies (Roberts and Kennelly 1977 and 1980). The latter two studies

showed that there was no significant difference between fertile and vasectomized males with regard to the sociosexual behavior patterns observed: promiscuity occurred while the female was off territory, and fertile clutches on territories of vasectomized males accounted for 70 percent of the total number observed (21/30). The results indicated that red-wings are considerably more promiscuous than previously concluded, and their polygynous mating system classification should be modified accordingly.

It should be noted that a subsequent behavioral study by Monnett et al. (1984) refuted the above conclusions regarding red-wing promiscuity. Monnett's team concluded that the extra-pair copulations (EPC's) implied by our studies do not regularly occur and proposed that this major disagreement on an important aspect of red-wing mating behavior be resolved by "paternity determinations using various electrophoretic techniques as advocated by Sherman (1981)." However, Gibbs et al. (1990) conducted an indepth parentage study of red-winged blackbirds along the lines proposed by Monnett et al. (1984), utilizing DNA marker techniques. The Gibbs team concluded that extra-pair fertilizations "due to male cuckoldry are frequent in this species." In another study supporting our results, Westneat (1993) reported that 55 of 232 red-winged blackbird nestlings' offspring tested by DNA fingerprinting were sired by EPC's.

Apparently, red-wing breeding behavior is more opportunistic than previously thought, and the cost–benefit ratio of population control by means of male sterilization needs to be reassessed. The perceived potential advantages of this approach are not as promising as once believed.

Canada Geese (Branta canadensis)

Nonmigratory or resident populations of Canada geese have essentially defied all efforts to effectively reduce the nuisance problems that they generate. Because Canada geese are reported to be monogamous and quite territorial during the breeding and nesting season (Akesson and Raveling 1982), the possibility exists that one or both of these characteristics might render this species vulnerable to induced sterility. To test this, we vasectomized ganders and observed them and their mates for up to three subsequent breeding seasons.

The results reaffirmed the conclusion that Canada geese are monogamous (Converse and Kennelly 1994). Of 72 vasectomized males, 33 paired with a female for either 1 year (n=15), 2 years (n=13), or 3 years (n=5). These 33 breeding pairs represent 56 nesting attempts, 47 of which (84 percent) were reproductively unsuccessful. Goslings were observed with the remaining nine treated pairs. Behavioral observations suggested that in seven instances adoption was the likely explanation for presence of goslings. Probable reasons for goslings with the other two pair are unknown because behavioral observations suggest adoption was unlikely and the surgical procedure allowed virtually no room for error. We could only speculate that gosling production might be due to EPC's, which are rarely reported in Canada geese (Kossack 1950, Klopman 1962).

With one exception, the maintenance of pair bonds for 2 years and the fidelity of treated pairs to a nest site from 1 year to the next imply that sociosexual behavior patterns were not noticeably altered due to sterility treatment. The exception concerned clutch incubation time: treated pairs incubated clutches for 35 to 120 days before deserting the nest. During this extended incubation period, aggressive territorial behaviors slowly subsided.

Nuisance Abatement via Vasectomy

The results of the above studies suggested that there are some special circumstances where surgically induced sterility might prove to be a cost-effective control technique for beaver and Canada geese.

Aside from the fact that beaver colonies provide excellent material for instructional, conservational, and environmental purposes, there are few urban situations where beaver provide beneficial effects (Willging and Sramek 1989). However, beaver activity in rural and semirural areas presents some nuisance opportunities where surgical sterilization may be an effective and practical approach. If a beaver colony is creating immediate damage, nothing short of complete removal

of the offending animals will alleviate the problem. Nuisance situations that arise year after year when dispersing 2-year-olds establish colonies where none previously existed in the recent past may be alleviated in the long term by surgically induced sterility. Assuming that a small number of colonies could be tolerated in an area, sterilization of one or both breeding adults in these colonies and the removal of all other colony members should offer two desirable results. First, the beneficial effects of the colony would be maintained and the life of the colony extended due to reduced utilization of the food base. Second, the annual contribution of dispersing offspring to the population at large would be eliminated (Payne 1989), and the number of new colonies becoming established each year would be reduced proportionately. The fact that at least 50 percent of sterile colonies were intact and not producing offspring 3 years after one adult was sterilized (Kennelly and Lyons 1983) suggests that this approach has some merit. Fortunately, the extent of the value of sterilization for reducing beaver problems can be readily assessed by an appropriately designed study.

Canada geese attracted annually to the same small suburban ponds capable of holding 2–5 pairs of breeding adults often attain nuisance status upon the production of goslings. Adult geese may be tolerated, but, together with their offspring, they are usually a problem. Our findings that 17 of 18 sterilized pairs maintained pair bonds ≥ 2 years and returned to the same nest site each year suggest that the benefits of sterilization may extend for several years. Research is needed, however, to determine whether nesting sterile pairs would repel the ingress of fertile pairs. If so, the managing of geese by sterilization could be substantial.

Summary

In summary, we have discussed studies which represent four widely different mammalian and avian species and their mating strategies to illustrate use of surgical sterilization as an answer to biological questions and as an experimental technique for animal damage management. With Norway rats and red-winged blackbirds, it is apparent that male sterility is not an effective approach, although much was learned about their mating strategies. However, successful control of reproduction in beaver and Canada geese provides impetus for further infertility studies. In select situations, surgical sterilization may be the most appropriate means of achieving that desired infertility.

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Overview of Delivery Systems for the Administration of Contraceptives to Wildlife

Terry J. Kreeger

Abstract: Successful contraception in wildlife requires both an efficacious and safe contraceptive agent and an efficacious and safe method of delivering that agent to the animal. Remote delivery systems (RDS)—mechanical devices capable of administering a single dose to an unrestrained animal, usually by means of a ballistic projectile—can target specific animals and facilitate the administration of contraceptives on a body weight basis. Liquid, solid, and semisolid formulations can be delivered via RDS, and sometimes treatment costs can go down with this methodology. Disadvantages of RDS include the fact that many of them can be used only on larger animals and RDS' inherent complexity increases the probability of administration failure. Most RDS use a powered gun to deliver either a dart or biobullet containing the contraceptive product. Biobullet RDS are capable of treating many animals rapidly. Both darts and biobullets can be designed to deliver different formulations to provide controlled release of contraceptives at a predetermined rate for a given period. The four general classes of controlled release systems (mechanical pumps, osmotic pumps, chemically controlled systems, and diffusional systems) are discussed. Chemically controlled and diffusional systems comprised of biodegradable polymers offer the most promise for single-dose, prolonged contraceptive release that can be remotely delivered to wildlife.

Keywords: controlled release, drug delivery, wildlife contraception, polymers

Introduction

There are two fundamental components required for the successful use of contraceptives in wildlife: (1) an efficacious and safe contraceptive agent and (2) an efficacious and safe method of delivering that agent to the animal. Many delivery systems are available to administer contraceptives to wildlife, ranging from surgically implanting devices into individual animals (Bell and Peterle 1975, Matschke 1980, Plotka et al. 1992) to dispersing oral baits over a wide area to an entire population (Matschke 1977, Roughton 1979).

Traditionally, the term "drug delivery system" has resided in the domain of human medicine, where it refers to mechanical or chemical methods to protect drugs from immediate degradation (e.g., in the stomach) or to prolong or control their release. The efficacy of these systems does not depend on first getting one's hands on the subject; the patient is seen as a willing partner in the process. Obviously, wild animals cannot be counted on to cooperate with biologists. So drug delivery systems take on a different meaning when applied to wildlife.

There are at least two facets of drug delivery of importance relative to wildlife contraception: (1) getting the contraceptive agent into the animal and (2) controlling the release of the drug in a manner that either maximizes or prolongs its efficacy. This chapter will describe devices for remotely delivering contraceptives to individual, unrestrained animals. Technologies for drug release *into* the animal will also be reviewed as they must work in concert with any primary delivery device.

Remote Delivery Systems

For purposes of this discussion, remote delivery systems (RDS) will be defined as mechanical devices capable of administering a single dose to an unrestrained animal, usually by means of a ballistic projectile. In their most elemental form, RDS consist of a gun and a dart containing a product. Although the bulk of this discussion will focus on these ballistic systems, other technologies will be reviewed because they may contribute to the development or administration of wildlife contraceptives.

Remote drug delivery dates to pre-Columbian times, when aboriginal natives of Africa and South America dipped arrows, spears, and blow darts in preparations of muscle-paralyzing drugs derived from plant and animal sources (Bush 1992). Modern delivery systems have their genesis in the 1950's, when the first projectile dart capable of delivering a liquid drug was reported (Crockford et al. 1957). This dart became the predecessor of darts still used today.

Many types of delivery systems were developed in the following 3 decades, but only a few proved reliable and versatile enough to survive competition in a limited market (Harthoorn 1976, Jones 1976, Kock 1987).

The operational definition of RDS implies administration to an individual animal. This may appear to be antithetical to wildlife *population* management; however, RDS can solve many wild animal population problems. In many situations, wildlife populations functionally exist as if they were confined to islands. Such populations have limited opportunities for immigration/emigration and are usually not subject to the population-control factors of predation and hunting. In these situations, populations usually thrive and increase until the forage base is depleted, and then disease and starvation lead to population reduction. Many of these populations are also generally visible and accessible by road or trail systems. Examples include natural areas within urban settings, airports, military arsenals, parks, and zoos.

Use of RDS need not be limited to such confined settings, however. Many species are accessible because they inhabit open environments such as deserts, prairies, or tundra. Such species can usually be approached from the air so that selected individuals or entire herds can be treated. Examples include feral horses, mountain goats, and polar bears.

Advantages and Disadvantages

Using RDS to administer contraceptives offers at least six advantages:

1. *Specific animals can be targeted.* Animals can selected and treated based on sex, size, age, or status.

2. *Contraceptives can be administered on a body weight basis.* Biologists familiar with a species can often estimate body weights of free-ranging animals quite accurately. Fairly precise doses can then be administered under field conditions if necessary for research purposes or efficacy.

3. *Different formulations can be employed.* Solid, semisolid, or liquid formulations can be delivered by RDS.

4. *A wide range of volumes can be delivered.* Depending on the projectile type and volume, liquid doses ranging from a few microliters to as much as 25 mL and solid doses up to 300 mg can be delivered.

5. *Some RDS can both treat and mark individual animals.* Some projectiles can be equipped with marking dyes and others can deliver electronic identification devices along with the contraceptive.

6. *The treatment cost per animal can be low.* When compared to contraceptive delivery methods requiring capture of the animal (e.g., implants), RDS greatly reduce the cost of treating each animal (but see #1 below). Some RDS can treat large numbers of animals rapidly, reducing costs as much as 60 percent when compared to capturing and treating individuals.

At least six disadvantages of using RDS to administer contraceptives merit consideration:

1. *The treatment cost per animal can be high.* Depending on the circumstances and taking into account all costs, such as labor hours and helicopter time, it can cost several hundred dollars to treat one animal using RDS.

2. *The target animal must be first located and then approached closely.* Under most circumstances, animals must be within 75 m of the shooter for projectile-RDS to be effective. Many species are secretive and extremely difficult to locate, let alone approach closely.

3. *Many RDS can be used only on larger animals.* Those RDS using projectiles are not terribly accurate, and the preferred target area on smaller animals may only be a few square centimeters. If the shot is misplaced, it may injure or kill the animal outright. Even if placed correctly, the impact energy or penetration depth could be injurious or lethal to smaller animals. As a working rule, only animals weighing > 15 kg (33 lb) should be targeted when powered (e.g., CO_2 or .22-cal. systems) RDS are used.

4. *RDS are inherently complex.* Many system variables can fail or affect successful delivery. A working maxim could well be, "Everything that can possibly go wrong with RDS eventually will!"

5. *Many RDS are noisy.* Some RDS may spook other animals after the first shot is fired, rendering subsequent shots at other animals difficult or impossible.

6. *Training and experience are necessary.* RDS should not be used without some degree of formal instruction by experienced practitioners of remote delivery techniques, and RDS should never be used without fairly intense practice by the user in order to assess the performance of the device prior to using it on an animal.

Longbows/Crossbows

Projectiles containing drugs or biologics have successfully been delivered using blowpipes, longbows, crossbows, pistols, shotguns, and rifles. Arrows or crossbow bolts can be modified to administer a liquid product up to 5 mL upon impact (Anderson 1961, Short and King 1964, Hawkins et al. 1967). Longbows and crossbows, though, have generally fallen out of favor because of impact trauma. If used at all, they are usually limited to larger animals shot at long ranges. I believe there are no commercial manufacturers of longbow or crossbow RDS in North America.

Blowpipes

There are several makes of blowpipes on the market today. Most of them consist of one- or two-piece aluminum tubes measuring up to 2 m. Most propel 10-mm darts (measured by their diameter) having a maximum capacity of 3 mL. Blowpipes are silent and fairly accurate, but their effective range is limited (< 20 m). Darts propelled by blowpipe cause very little impact trauma to the animal, so they are generally safe for use on smaller species. With the appropriate equipment, animals as small as 3 kg (6.6 lb) can be treated. Blowpipes are used primarily on captive animals but can be used effectively on free-ranging animals under the right circumstances, such as treed animals or animals approached closely by vehicle (Brockelman and Kobayashi 1971, Haigh and Hopf 1976). Prices range from $75 to $160 (all monetary figures in this chapter are expressed in 1995 U.S. dollars).

Powered Blowpipes

Powered blowpipes or "blowpipe guns" are blowpipes modified to use compressed air to extend their effective range. Blowpipe guns consist of the blowpipe aluminum tube connected to a pistol grip containing a metering device. Air is compressed by a foot pump connected by a hose to the pistol grip. After the desired pressure has been built up in the reservoir, the hose can be disconnected. When the trigger is pulled, the compressed air is released, propelling the dart. Similarly, some powered blowpipes use CO_2 cartridges that feed into a reservoir that can be adjusted to either increase or decrease the amount of pressure. Because the dart flight distance is proportional to the pressure built up in the reservoir, these devices have a wide effective range (from 1 to 40 m). Blowpipe guns propel the same type of lightweight darts (10–11 mm in diameter and 1–3 mL in volume) as do blowpipes, and these guns are silent and safe for use on smaller animals. Prices range from $225 to $375.

Dart Guns

The most widely used RDS are dart-shooting guns. Some dart guns have been constructed by modifying existing shotguns, rifles, pistols, pellet rifles, or pellet pistols; other guns are almost entirely custom designed and manufactured for this purpose. Dart guns propel darts by either the gas generated from a .22 caliber blank cartridge, compressed CO_2, or compressed atmospheric air. Dart-firing guns are the most versatile of the RDS. Effective ranges can reach 100 m for larger animals having larger target areas. Dart volumes can be as much as 25 mL, although these larger, heavier darts drop rapidly after leaving the barrel, making longrange, accurate shots difficult. All darts, of course, begin falling as soon as they leave the barrel, but small darts (1–2 mL) traveling at higher velocities shoot flatter and go farther than large darts. Guns can be equipped with a variety of sights, including adjustable iron sights, rifle scopes, laser aiming devices, and light-intensifying scopes (night vision or starlight scopes). Prices range from $300 to $1,650.

Table 1. Characteristics of powered remote delivery systems

Category	.22-caliber Blank	CO_2	Compressed air
Maximum effective range (m)	75	50	50
Volumes (mL)	1–25	1–10	1–10
Availability of propellant	High	Medium[1]	Low[2]
Temperature sensitivity	None	Medium	None
Impact injury	High[3]	Medium	Medium–Low
Report	Medium–High	Medium–High	Medium–High
Maintenance	High	Low	Low
Performance reliability	Medium	High[4]	High
Ease of use	High	High	Low
Overall versatility	High	Medium	Low

[1] There are two general types of CO_2 cartridges: threaded and unthreaded. Most sporting goods stores carry the smaller, unthreaded $C0_2$ cartridge, but the larger, threaded $C0_2$ cartridge may be very difficult to procure when working in rural areas.

[2] This rating refers to systems using compressed air tanks only and does not apply to systems using foot pumps. Most fire departments can fill air tanks but are reluctant to do so because of liability concerns. Welding shops may have compressed air, but not always. Scuba shops have air compressors, but they usually do not have the necessary fittings required for the tanks used with dart guns.

[3] Twenty-two-caliber blanks come in a variety of strengths. Charge strengths are coded by different colors, usually brown, green, yellow, or red, with red being the most powerful. Darts propelled with either yellow or red charges are capable of causing significant injury or death.

[4] $C0_2$ cartridges generally provide consistent performance except when the propellant runs low. There is only a subtle drop in performance between the last acceptable shot and the next shot where the dart drops precipitously due to a rapid drop in pressure. Experienced shooters often allow only a fixed number of shots per cartridge before changing cartridges even though some shots remain.

Table 1 lists the advantages and disadvantages of the three types of dart-gun propulsion systems. Ten criteria have been analyzed.

Maximum Effective Range—This is the maximum distance at which the dart can be safely and effectively delivered. The range of most guns can be decreased from this maximum either by using a built-in metering device which directs little or all of the gas to the dart, by using different strengths of propellant (i.e., different sizes of .22 blanks), or by pushing the dart farther down the barrel to reduce its velocity and thus its range.

Volumes—Dart volumes range from 1 to 25 mL; however, not all systems are capable of delivering this full range of dart sizes.

Availability of Propellant—This category rates the ease of obtaining the propellant from local suppliers.

Temperature Sensitivity—The vapor pressure of some gases (e.g., $C0_2$) is temperature dependent. At cold temperatures, darts travel less far due to decreased vapor pressure. In extremely cold conditions, some guns may barely function without some means of warming the gas.

Impact Injury—The impact energy of the dart striking the animal is a function of its mass and velocity ($KE = 1/2\ MV^2$). Table 2 compares the relative muzzle kinetic energy of three darts of the same volume but from different manufacturers. Even on a large animal struck correctly, the dart can cause hemorrhage and hematoma. Misplaced shots can break bones or even kill the animal (Thomas and Marburger 1964).

Report—Muzzle report can cause problems in darting either captive or free-ranging animals. In captive situations, the noise can be more disturbing to animals than getting struck with a dart. Disturbed animals are then more difficult to approach, or the entire group of animals may run away.

Maintenance—Some systems need to be cleaned frequently in order to remain operable.

Performance Reliability—Systems are classified regarding consistency of shot-to-shot performance.

Ease of Use—Systems are classified relative to their simplicity of operation or ease of use under field conditions.

Overall Versatility—The above categories are evaluated to arrive at a subjective opinion on the overall versatility of the propulsion system.

Table 2. Comparison of muzzle velocity and kinetic energy of 2-mL darts representing three different brands

Brand	Weight	Muzzle velocity	Kinetic energy
	(g)	*(ft/sec)*	*(ft-lb)*
Pneu Dart®	9.8	284.2	27.7
Aeroject®	13.3	256.9	30.1
Cap-Chur®	17.3	249.7	37.1

Data represent the mean value of three firings. All darts were fired from a CO_2-powered gun using fresh charges between dart types. Muzzle velocities were measured by chronograph 0.5 m from the muzzle. Muzzle energy was calculated by standard formula. Nonmetric values are reported in order to compare with other ballistic data. All darts contained 2 mL (2 g) saline.

Darts

Most projectile RDS use a dart to deliver liquid or viscous products. Darts can be thought of as "flying syringes" consisting essentially of a needle, body, plunger, and tailpiece. They differ in the manner in which the plunger is pushed forward to inject the dart's contents and in the materials of construction. Darts have also been used to implant small, solid devices, such as electronic transponders (Kreeger, unpubl. data). Theoretically, darts equipped with large-bore needles could also deliver semisolid or solid implants required for controlled drug release (see In Vivo Drug Delivery Systems).

Darts discharge their contents either by expanding gas from an explosive powder charge, compressed air, vaporized gas (butane), chemical reaction (acid–base), or compressed spring (fig. 1). The mechanisms that enable the dart to discharge its contents upon impact range from moderately simple systems having few parts to complex systems of intricate design and operation.

Dart bodies can be made of aluminum or synthetic polymer (polypropylene, polycarbonate, etc.). Dart tail designs range from elaborate fins molded from synthetic polymers to simple strands of yarn stuffed into the back of the dart (Corson et al. 1984).

Dart needles can be as large as 75 mm long and 2.16 mm in inside diameter. Darts using explosive charges expel their contents in <0.001 second and thus require large-bore needles to allow the rapid expulsion of liquid. Needles are designed to either expel contents from the standard front opening (end port) or through a side port with the front opening occluded. End-port needles expel their contents more rapidly than do side-port needles, but large-bore needles can become plugged with a core of tissue when they penetrate hide and muscle (Henwood and Keep 1989).

Needle shafts can be smooth, or they can be equipped with a variety of barbs or collars to retain the dart in the animal. Smooth-shafted needles are used to deliver the drug and then fall out on their own, eliminating the need to capture the animal to remove the dart. If the dart contents are under high pressure, however, smooth-shafted needles can "rocket" back out of the animal due to the expulsion of the liquid and therefore not fully inject the substance.

Some needles are equipped with small collars that barely secure the dart in the animal but eventually fall out on their own. One company (Pneu-Dart) manufactures a gelatin collar that is rigid when dry but dissolves when it comes into contact with tissue fluids. These collared darts stay in the animal long enough to ensure complete expulsion of the contents but still fall out on their own later.

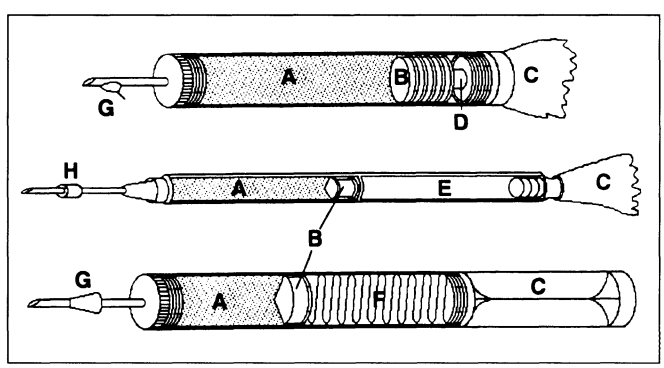

Figure 1. Schematic drawing of typical construction used in darts. (A) drug chamber, (B) movable plunger, (C) tail piece, (D) explosive charge, (E) compressed air chamber, (F) spring, (G) barb, (H) needle collar (slides back to discharge drug after dart penetrates skin).

To retain the dart in the animal securely, either spring barbs or metal collars are used. These darts require manual removal from the animal. Experiments with retractable barbs have been successful, but these are not commercially available (Van Rooyen and De Beer 1973, Smuts 1973). Barbed darts usually create a greater wound upon removal than do collared or barbless darts. Some barbs are so tenacious that they can be removed only with a scalpel.

Darts can be modified to mark as well as treat the animals that they hit. Darts can be equipped with dye-filled bladders fixed to the base of the needle that burst upon impact to mark the treated animal (Bush 1992). These bladders also serve as cushions to decrease the impact trauma of the dart. Another dart (Pneu-Dart) utilizes a "piggy-back" tailpiece containing the dye or paint that breaks loose from the dart body upon impact to spray the area.

Darts can also be equipped with small radio transmitters enabling location of animals that have run off after being darted with immobilizing drugs (Nielsen 1982, Lawson and Melton 1989). The effective transmitter range of these darts is usually <300 m, but the technology of small transmitters that can withstand impact energy holds promise of extended ranges. The price for such darts complete with reusable transmitter is $100 to $150.

The advantages and disadvantages of each dart injection system are listed in table 3. The following criteria were analyzed.

Injection Speed—If injection speed is rapid (e.g., <0.001 second), tissue can be injured and absorption slowed. However, if injection speed is slow, the animal (e.g., carnivores) may have time to remove the dart before all the contents have injected.

Weight—Lightweight darts may cause less impact when they strike the animal (table 2), but lightweight darts traveling at high speeds may be more subject to wind drift and prop wash.

Volume—This category lists the volumes capable of being delivered by each system.

Table 3. Characteristics of dart types

Category	Powder	Compressed air	Gas[1]	Spring
Injection speed	Rapid	Slow	Moderate	Moderate
Weight	Light–Heavy	Light	Light	Medium
Volume (mL)	1–25	1–10	1–6	2–3
Reliability	High	Medium	Medium	High
Contents under pressure	No	Yes	Yes/No	Yes

[1] Gas can be from either butane or acid–base mixture. Gas darts may be pressurized prior to firing or develop gas pressure after striking the target.

Reliability—Dart systems are rated based on consistency of injecting the entire dart contents.

Contents Under Pressure—This is a Yes/No rating only. The contents of some dart systems are pressurized when they are initially loaded. This type of dart is more prone to leaking or spraying contents than are darts that do not develop any expulsion pressure until they strike the animal.

Biobullets

A .25-caliber, biodegradable implant (biobullet) was developed to remotely administer biologics and pharmaceuticals to domestic and wild animals. Biobullets have been used successfully to treat elk *(Cervus elaphus),* bighorn sheep *(Ovis canadensis,)* bison *(Bison bison),* gray wolves *(Canis lupus),* fallow deer *(Dama dama),* roan antelope *(Hippotragus equinus),* impala *(Aepyceros melampus),* waterbuck *(Kobus lece),* greater kudu *(Tragelaphus strepsiceros),* wildebeest *(Connachaetes gnou),* zebra *(Equus burchelli),* and eland *(Taurotragus oryx)* (Jessup 1993, Kreeger unpubl. data).

There has been increasing interest in the potential of biobullets to deliver contraceptive products. Immunocontraceptives have been administered to white-tailed deer *(Odocoileus virginianus)* and feral horses *(Equus caballus)* using biobullets (Warren et al., this volume).

A biobullet is comprised of an outer, biodegradable casing and either a solid, semisolid, or liquid payload (fig. 2). Hydroxypropylcellulose (a food additive) and calcium carbonate are the primary components of the casing, which when injection-molded under high temperature and pressure, becomes a hard, plastic-like material. Upon entry into the animal and contact with tissue fluids, the casing immediately begins to dissolve and is entirely liquefied within 24 hours. The 10-sided bullet mates with a decagon-rifled barrel. This construction prevents the barrel fouling encountered with conventional land-and-groove rifling and allows for hundreds of rounds to be fired without cleaning.

The desired drug is inserted into the hollow base of the casing and can dissolve immediately upon contact with tissue fluids, if so designed. Freeze-dried vaccine pellets, for instance, dissolve completely within 3 hours, and concentrations of pharmaceuticals are detectable in the blood 30 minutes after administration (Kreeger, unpubl. data). Because the casing dissolves upon contact with a solvent, liquid formulations need to be first placed into a gelatin capsule then the capsule inserted into the casing. Semisolid formulations, such as silicone rubber, can be dispensed directly into the casing.

Currently, the casing is manufactured to deliver a 125- to 300-mg payload. The exact specifications of each casing are presented in table 4. Both casings are .25 caliber (6.43 mm diameter), but .20-caliber (5.08-mm) biobullets have been developed and used successfully.

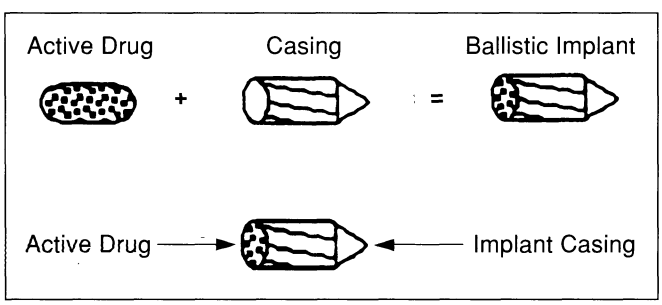

Figure 2. Biodegradable, 0.25-caliber biobullet showing position of payload and decagon rifling.

Table 4. Specifications of biobullet casing

Casing	Weight empty	External length	External width	depth	Cavity width	volume
	(mg)	*(mm)*	*(mm)*	*(mm)*	*(mm)*	*(μL)*
"Short"	481.0	14.66	6.43	6.60	4.06	85.44
"Long"	556.0	20.95	6.43	14.22	4.85	262.68

The maximum effective range is approximately 25 m. Longer ranges can be achieved by increasing the velocity and/or by formulating a heavier casing. Faster or heavier biobullets, though, would then have a *minimum safe* range because such projectiles could penetrate thin-skinned or small animals too deeply if shot at close distances. The average penetration distance in the hindquarter muscle mass of cattle is from 5 to 7.5 cm. Small-caliber or lighter weight bullets could be developed to decrease penetration, if necessary.

Biobullets are currently delivered by a clip-fed, pump-operated, compressed air-powered rifle. The compressed air is delivered by either a 1.44- or 2.78-L air tank. The larger tank can fire 300–350 biobullets before refilling. The biobullet is propelled at approximately 900 ft/sec. A single-shot, compressed-air rifle has also been developed that eliminates the need for an external air tank (Kreeger, unpubl. data).

The multiple shot capacity of the biobullet remote delivery system provides significant advantages over dart RDS for treating herds of animals. The preloaded biobullets eliminate loading time, spills, and accidental human exposure while ensuring complete dosage delivery. Another benefit of biobullets over darts is that if the animal is missed, the biobullet will completely degrade within a few days, reducing the possibility of human exposure.

The disadvantages of the biobullet RDS are the limited payload (300 mg), limited range (25 m), possible difficulty in refilling the air tank, and cumbersome system of air tank, regulator, hose and gun.

Biobullets have been used to administer electronic identification transponders (Trovan®) to cattle; but the operation of the transponder after delivery was variable, and this technique requires further development (Kreeger, unpubl. data). If transponders could be developed to withstand the impact of ballistic delivery, both a contraceptive and a transponder could be administered simultaneously. Thus, treated animals would be permanently marked which could aid field data collection and efficacy testing.

Theoretically, a polymeric (see below) biobullet could be manufactured so that the entire biobullet becomes a controlled drug delivery device. This technique could provide even greater flexibility in payload and dissolution rates.

Other Drug Delivery Systems

Although probably not a true remote delivery system in the context of this discussion, the remote capture collar (RCC) is a device that could aid researchers in the field evaluation of contraceptive safety and efficacy. The RCC is essentially a radiotelemetry collar that not only provides a location signal from the animal but also allows the researcher to remotely inject either an immobilization drug or a contraceptive product at the push of a button. The RCC allows multiple recaptures of the same animal providing long-term opportunities for pregnancy diagnosis, blood and urine sampling, contraceptive readministration, physical evaluation, and the collection of other data that require animal sampling.

The RCC consists of a transceiver that emits a location signal and also signals animal activity, battery life, ambient temperature, and dart status. The general sequence of its use is as follows: an animal must be initially captured by some means and the RCC fitted and the darts loaded with an anesthetic or other product. Currently, the darts can deliver 1.5 mL of a liquid product per dart. Usually, a single anesthetic dose is concentrated in each dart, allowing a backup dart should the first one fail to completely anesthetize the animal. However, a dart could also contain a liquid contraceptive which could be administered sometime after the animal was initially captured and treated. This feature may be useful for immunocontraceptives requiring repeated doses. At some later date, the researcher relocates the animal via the radio signal and moves to within 3.2 km (2 miles) and transmits a signal to fire one of the darts. The RCC then signals back if the dart successfully fired. The researcher can then monitor the animal's activity via the activity signal; when that signal indicates no activity, the animal is assumed to be immobilized and the researcher can close in on it using the radio signal.

Once the anesthetized animal has been located, new darts and batteries can be attached to the collar, samples taken, and data collected. If the batteries fall below a certain voltage, or both darts are triggered without the animal becoming immobilized, or simply at the command of the researcher, the RCC will disengage from the animal and emit a low-level signal allowing recovery by the researcher without the need to recapture the animal. Again, this feature could allow revaccination or a second (or third) contraceptive treatment with recovery of the collar without the necessity of handling the animal.

The RCC has been successfully used on gray wolves, white-tailed deer, and black bear *(Ursus americanus)* (Mech et al. 1990). The collar sells for $1,495 and the triggering transmitter for $2,395.

Implant Guns

Implant guns are devices that insert implants either intramuscularly or subcutaneously and require capture and restraint (either chemical or physical) of the animal. Implant guns use belts or clips capable of holding up to 20 doses that are inserted via a large-bore needle. Implant guns are being used to administer progesterone, testosterone, etsradiol, norgestomet, or other substances. Drug substances can be in the form of pellets or polymers. One product combines an injectable solution with a controlled-release hydrophilic polymer to provide an immediate as well as a delayed effect with a single administration. Most products are intended as growth promotants for production animals, but some are used to synchronize estrus in cattle.

Implant guns thus provide a means of inserting a variety of formulations without the need for a surgical incision and implantation. Animals can be treated quite rapidly and released immediately after treatment if manually restrained. Nonbiodegradable implants can be inserted into the ear, a desirable site because it will not be eaten if the animals are intended for human consumption. It may be possible to obtain implant guns and empty clips for those wishing to manufacture their own formulations for experimental use in wild animals.

In Vivo Delivery Systems

Very few drugs provide effective contraception after only a single administration. Immunocontraceptives, such as zona pellucida (ZP) vaccines, invariably require multiple administrations to create an anamestic response to develop and maintain effective titers. Steroid contraceptives and gonadotropin-releasing hormone (GnRH) agonists must be continually administered in order to remain effective over time. I have previously discussed how to get contraceptives *to* the animal, but it is equally important to review technologies that provide controlled release of the contraceptive *within* the animal.

Controlled-release systems (CRS) deliver a drug at a predetermined rate for a given period. The active ingredient in CRS differs from those in sustained-release preparations, which do not dissolve in the stomach yet do dissolve in the intestine. Generally, sustained-release systems release drugs in less than a day and are characterized by a drug concentration peak followed by a decline (Langer 1990). Multiple administrations of sustained-release preparations result in oscillations between these peaks and valleys. Sustained-release preparation are thus not uniform or "controlled." Controlled-release preparations are designed to reach and then maintain the drug within a desired therapeutic range following a single administration. The release rate of CRS should ideally be "zero order" in which the amount of drug released to the absorption site remains constant over time. Controlled-release preparations can also be designed to preserve drugs that normally would be rapidly metabolized and destroyed.

Although the bulk of the following discussion will emphasize CRS that can be delivered remotely, it should not be forgotten that such systems can be administered to captured animals by a variety of means. Surgical implants, transdermal patches, and vaginal rings are all viable delivery systems that can be employed as determined by efficacy, economic, and animal safety considerations.

Classes of Controlled Release Systems

There are five general classes of CRS appropriate for wildlife contraception: mechanical pumps, osmotic pumps, chemically controlled systems, diffusional systems, and liposomes.

Mechanical Pumps—Implantable mechanical pumps have been tested and proven in human medicine for the delivery of insulin, heparin, and other agents. Some mechanical pumps are powered by hermetically sealed, compressible fluorocarbon pushing against a septum that separates the gas from the drug compartment. The vapor pressure exerted by the propellant forces the drug solution through a filter and flow regulator at a constant rate. Mechanical pumps have to be surgically implanted and are relatively expensive, but they can be refilled and are capable of precise drug control. Their use for wildlife contraceptives is probably limited to research applications.

Osmotic Pumps—Osmotic pumps are devices consisting essentially of a liquid drug reservoir surrounded by an osmotically active polymer ("energy source") which, in turn, is surrounded by a water-permeable membrane (fig. 3). The osmotically active polymer maintains a constant water gradient across the rate-controlling membrane. The polymer acts as an energy source to create hydrostatic pressure on the reservoir. The reservoir consists of a soft, low modulus drug-impermeable elastomer that releases a water-soluble drug through a small opening to the body when "squeezed" by hydrostatic pressure. At steady-state, these pumps follow zero-order kinetics (Eckenhoff and Yum 1981). More simply, an osmotic pump or "osmotic tablet" can consist of a drug sub-

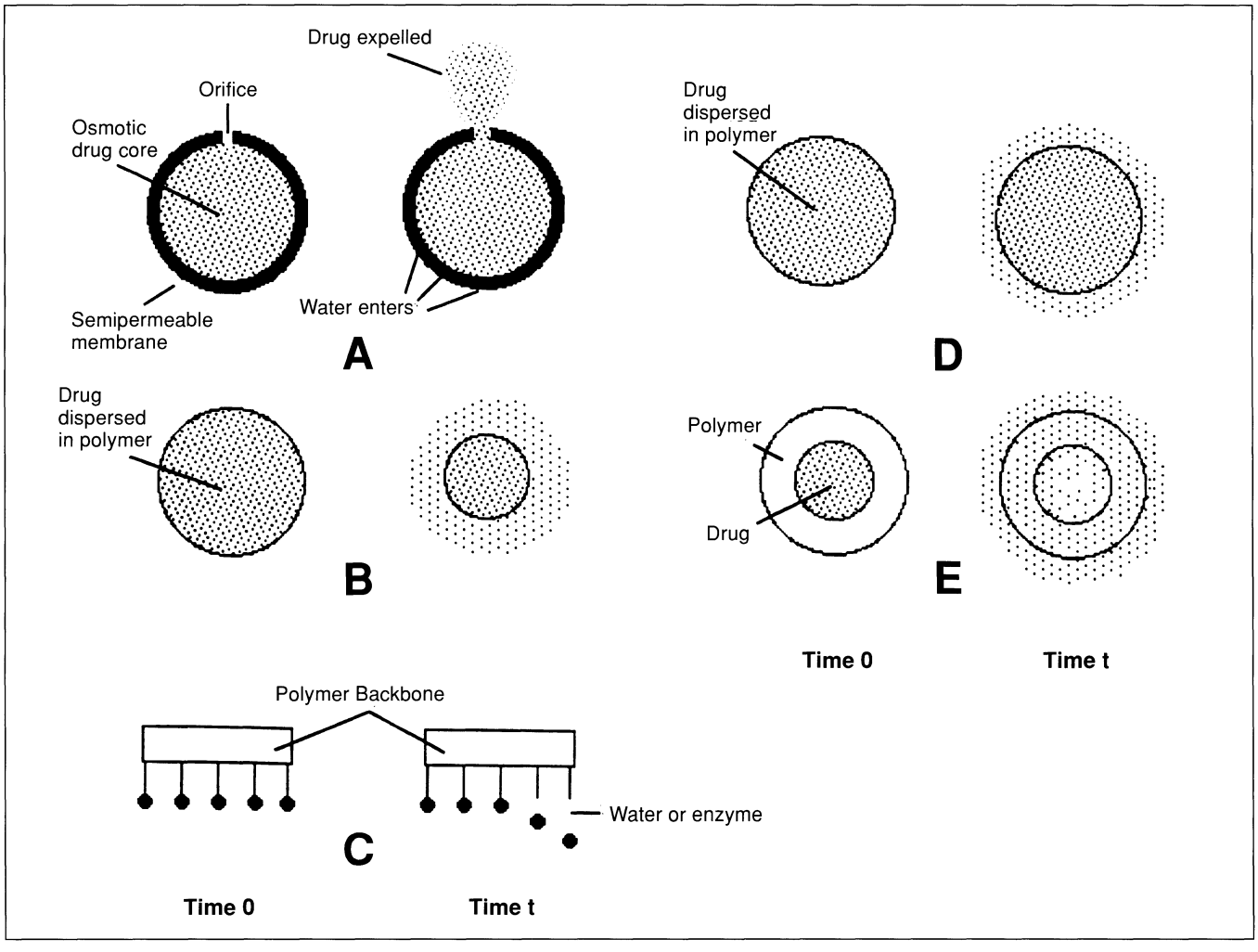

Figure 3. Polymer release mechanisms: (A) osmotic pump, (B) polymer degradation, (C) backbone cleavage, (D) diffusional matrix, (E) diffusional reservoir (after Langer 1990).

stance and an osmotic polymer or simple salt all surrounded by a semipermeable membrane. Osmotic hydration drives the drug out of a laser-drilled orifice (Squire and Lees 1992). Osmotic pumps need not be expensive, but they require animal capture and surgical implantation unless delivered by biobullet. However, depending on the potency of the drug substance, micro-osmotic pumps or osmotic tablets theoretically could be designed and delivered remotely.

Chemically Controlled Systems—The advantage of chemically controlled drug delivery systems over mechanical or osmotic pumps is that they need not be surgically implanted and they are biodegradable. Thus, no residues are left in the animal, which is not an unimportant concern in food-producing species. Release of the drug takes place by the following mechanisms:

1. Gradual biodegradation of a drug-containing polymer matrix. The drug substance can either by dispersed in the polymer matrix or encapsulated in it. The drug is released into the tissues at controlled

rates; the particular kinetics depend on the chemical composition of the polymer, the solubility of the drug in the polymer, and how the polymer matrix was prepared (fig. 3).

2. Cleavage of unstable bonds coupling a drug to a polymer backbone (fig. 3).

Diffusional Systems—Like chemically controlled systems, diffusional systems need not be surgically implanted nor removed if they are biodegradable. Drugs diffuse through polymers, leaving the polymer intact, or the polymer may biodegrade after the drug has been exhausted. There are two types of diffusional systems: reservoirs and matrices (fig. 3). Reservoirs can be surrounded by either a porous or nonporous membrane. In porous membrane reservoirs, the drug passes through liquid-filled pores of the polymer membrane rather than through the polymer itself. Thus, drug solubility within the liquid medium of the pores is more important than drug solubility in the polymer.

In matrix systems, the drug is distributed throughout the polymeric system. Such systems normally do not provide zero-order release because the drug is initially released from the outer layers and then released from sequentially deeper layers of the matrix.

Liposomes—Liposomes are vesicular structures built of one or more lipid bilayers surrounding an aqueous core. The backbone of the bilayer consists of phospholipids. Size, number of bilayers, bilayer charge, and bilayer rigidity determine in vivo performance. Liposomes deliver their contents through macrophage phagocytosis, membrane fusion, surface adsorption, or lipid exchange (Nässander et al. 1990).

Probably the earliest and certainly the most widely used controlled-release system for the delivery of contraceptives to wildlife employed silicone rubber (polydimethylsiloxane) implants (i.e., a diffusional system). In 1964, Folkman and Long determined that Silastic™ implants could deliver drugs for an extended period in dogs. Subsequently, silicone implants were devised to deliver steroid contraceptives to white-tailed deer and other species (Bell and Peterle 1975, Seal et al. 1976, Matschke 1977, 1980). Silicone implants containing melengesterol acetate have been used to control fertility in dozens of species representing hundreds of individuals held in zoos. Silicone implants are not biodegradable and generally require surgical administration. More potent agents, however, may be delivered remotely via biobullets containing small, silicone implants (see Kesler, this volume).

In the 1980's and 1990's, research on the use of polymers as excipients for controlled drug release has virtually exploded. Polymers can be used to form microspheres, microcapsules, implants, coatings, and fibers. Polymeric CRS are biodegradable and offer versatility in terms of release rates and duration. Although research on the use of polymeric CRS for wildlife contraception is in its infancy, this technology probably offers the most promise to the wildlife biologist in the future.

Polymers

Polymers are high molecular weight substances, made up of a chain of identical, repeated base units. Many polymers used in CRS are polyesters, an ester being an organic compound formed by the elimination of H_2O between the ^{-}OH of an acid group and the ^{-}OH of an alcohol group. Thus when implanted in vivo, polyesters are usually biodegraded by simple hydrolysis as opposed to requiring enzymatic action.

It is possible to design polymeric implants or microspheres that could be remotely delivered by a dart or biobullet. Once implanted, the polymer would consistently release a contraceptive drug or vaccine over an extended period of time (Aguado 1993, Morris et al. 1994). For substances such as zona pellucida vaccines, polymers could be used to coat pellets or form microspheres of a lyophilized vaccine which would degrade at specific intervals to provide one or more boosters. Additionally, polymers have been developed that not only provide for the controlled release of antigen but do so from a biodegradable antigen delivery device which degrades into material with adjuvant properties (Kohn et al. 1986, Morris et al. 1994).

There are virtually hundreds of candidate polymers being studied for controlled release (Chasin and Langer 1990), and a discussion of their specifics is beyond the scope of this chapter. Three principal biodegradable polymers developed for controlled contraceptive steroid release are copolymers of lactic and glycolic acid (Beck and Tice 1983), poly–ε–caprolactone (Ory et al. 1983), and poly(ortho esters) (Heller et al. 1984). A brief discussion of these and other polymers is included below to familiarize the reader with this subject.

Lactide/Glycolide Polymers—Lactide/glycolide polymers are some of the most widely investigated biodegradable excipients for controlled drug delivery. Their advantage is versatility in polymer properties and performance characteristics. For wide applications in controlled drug delivery, it is imperative that a range of rates and duration of drug release be achievable (Lewis 1990).

Homopolymers and copolymers of lactic and glycolic acids are synthesized by ring-opening and melt condensation of the cyclic dimers, lactide and glycolide (Kulkarni et al. 1971). Additionally, lactic acid exists as either D or L stereoisomers; thus, D, L, or racemic DL polymers can be synthesized. Performance versatility is achieved through the various combinations of the stereoisomers of lactic acid and/or glycolic acid. Because biodegradation is achieved through hydrolysis of ester linkages, crystallinity and water uptake are key factors in determining the rates of in vivo degradation (Lewis 1990). For example, water uptake increases as the glycolide ratio in the copolymer increases (Gildling and Reed 1979, Rosen et al. 1988) so that copolymers having a high glycolide component degrade sooner than do lactide polymers (table 5).

Lactide/glycolide polymers also provide fabrication versatility. At least three types of CRS based on these polymers have been investigated: microcapsules or microspheres, implants, and fibers. Microspheres have been used to deliver a variety of steroids and steroid contraceptives, such as norethisterone, levonorgesterel, testosterone, testosterone propionate, progesterone, norgestimate, and estradiol benzoate (Beck et al. 1979, 1980, 1981, 1983, 1985). A virtually infinite variety of lactide/glycolide polymer implants can be made by injection molding, compression molding, or screw extrusion. Rods comprised of 50:50 molar poly(D,L–lactide–co–glycolide) were successful in the extended, controlled release of a potent GnRH agonist in rats (Furr and Hutchinson 1992). Hollow fibers spun from poly(L–lactide) have been used for delivery of levonorgesterel (Eenink et al. 1987).

Table 5. Biodegredation of lactide/glycolide polymers (after Lewis 1990)

Polymer	Approximate biodegredation time
	(Months)
Poly(L-lactide)	18–24
Poly(D,L-lactide)	12–16
Poly(glycolide)	2–4
85:15 (D,L-lactide-co-glycolide)	5
50:50 (D,L-lactide-co-glycolide)	2
90:10 (D,L-lactide-co-caprolactone)	2

The rate and duration of steroid release is affected by (1) polymer composition, (2) drug:polymer ratio, (3) microsphere size distribution, and (4) microsphere quality (Lewis and Tice 1984). The smaller the microsphere, the higher the drug concentration and the shorter the duration of release due to the relatively greater surface area (Lewis 1990).

Lactide/glycolide polymers have also been used for controlled release of vaccines to provide initial and repeated antigen exposure in order to stimulate the desired anamestic response. Such technology could be useful for one-time administration of zona pellucida vaccines. A human contraceptive vaccine based on lactide/glycolide polymers is in development using a 37-amino acid peptide of beta–human chorionic gonadotropin (β–HCG as the antigen conjugated to diphtheria toxoid. The antigen is administered with microencapsulated muramyl dipeptide as an adjuvant

to provide 9–12 months of elevated antibody titers in rabbits after a single injection (Lewis 1990).

Over the last 2 decades, lactide/glycolide polymers as excipients for the controlled release of bioactive agents have proven to be both safe and efficacious in animal and human trials. The ready availability of these polymers from reputable firms, plus their versatility offer promise to biologists developing contraceptive delivery systems for wildlife.

Poly–ε–caprolactone—Poly–ε–caprolactone (PCL) was initially evaluated as a biodegradable packaging material to reduce environmental pollution due to its degradation by micro-organisms (Potts et al. 1973). The success of other polyesters such as poly(lactide) and poly(glycolide) as drug delivery systems led to the evaluation of the degradability of PCL in vivo (Schindler et al. 1977). The PCL homopolymer degrades very slowly compared to poly(glycolide) and appears to be quite suitable for long-term drug delivery, including contraceptives (Pitt and Schindler 1984). If desired, biodegradation of PCL can be enhanced by copolymerization with poly(DL–lactide) (table 5), and PCL has shown an exceptional ability to form compatible blends with a variety of other polymers as well (Koleske 1978, Pitt 1990).

PCL and its copolymers are highly permeable to low-molecular- weight (<400 daltons) drugs (Pitt et al. 1979a). As a comparison, the diffusion coefficient of PCL for several steroids is two orders of magnitude less than that of silicone rubber, but drug solubility is greater in PCL. Thus, the permeabilities (the product of the diffusion coefficient and solubility) of PCL and of silicone rubber are not greatly different (0.6×10^{-10} *v.* 2.2×10^{-10} g/cm·sec, respectively) (Pitt 1990). This high permeability of PCL and its copolymers coupled with controlled biodegradation lends PCL to the development of delivery devices that are based on diffusion-controlled drug delivery during an induction period prior to weight loss of the matrix. Subsequent biodegradation of the polymer eliminates the need for removal of the spent device (Pitt 1990).

Biodegradation of PCL begins with random hydrolytic chain scission of the ester linkages, manifested by a reduction in the viscosity and molecular weight of the polymer. This rate does not change despite 10-fold changes in the surface-to-volume ratio, indicative of a bulk process. Implant weight loss is not observed until polymer molecular weight has decreased to approximately 5,000 daltons, at which time there is a decrease in the rate of chain scission. Weight loss is then attributed to an increased probability the production of excised fragments that are small enough to diffuse out of the polymer bulk and to the breakup of the polymer mass to produce particles small enough to be phagocytized (Pitt 1990).

PCL can be formed into films, rods, microcapsules, or reservoir devices. Reservoir devices for the delivery of steroid contraceptives have been developed where drugs are surrounded by a PCL capsule that biodegrades after the drug is exhausted. Improved zero-order kinetics could be obtained by suspending the drug (levonorgestrel) in an oil within the PCL capsule (Pitt et al. 1979b). Increased permeability of reservoir devices can be obtained through copolymerization of PCL (Pitt et al. 1980).

Poly(ortho esters)—Although polymer diffusion systems have been developed to deliver contraceptive steroids, there is a need to develop systems where drug release is predominately controlled by polymer hydrolysis. Such polymers could be an important means of polypeptide delivery for those polypeptides that do not diffuse from polymers at useful rates, particularly as molecular weight increases (Heller et al. 1990).

Poly(ortho esters) are polymers containing acid-labile linkages in their backbones. Hydrolysis rates of poly(ortho esters) can be manipulated by incorporation of acidic or basic excipients into the matrix. Under certain conditions, the hydrolysis of such polymers could also be confined predominantly to the outer surface so that the resultant surface erosion allows excellent control of the release kinetics of incorporated therapeutic agents (Heller et al. 1990).

Two methods of controlling erosion rates of poly(ortho esters) are (1) using an acidic excipient to accelerate the rate of hydrolysis and (2) using a basic excipient to stabilize the interior of the device. When a hydrophilic polymer with a physically dispersed

acidic excipient is placed into an aqueous environment, water will diffuse into the polymer, dissolving the acidic excipient. That dissolution lowers the pH to accelerate hydrolysis of the ortho ester bonds (Heller 1985). Conversely, when long-term surface erosion is desired, the addition of a basic excipient, such as $Mg(OH)_2$, stabilizes the interior of the device so that water penetration into the matrix does not lead to hydrolysis. Theoretically, erosion can only then occur at the surface where the base has been eluted or neutralized. This is thought to occur by water intrusion into—and diffusion of the slightly water-soluble basic excipient out of—the matrix. Polymer erosion then occurs in the base-depleted layer (Heller et al. 1990).

The use of basic excipients to control and prolong release of contraceptive steroids was demonstrated by Heller (1985 and 1986) and Heller et al. 1990. Polymer rods containing 30 percent levonorgestrel by weight and 7.1 percent $Mg(OH)_2$ by molecular weight were implanted subcutaneously in rabbits. Polymer erosion and drug release appeared to occur concomitantly, and bulk erosion was not evident, indicating surface erosion. Blood concentrations of levonorgestrel were reasonably constant once the initial burst subsided.

Polyanhydrides—Aromatic polyanhydrides were first synthesized in 1909 but did not receive much attention until they were investigated as replacements for polyester fiber. The major deficiency of polyanhydrides in this role was their hydrolytic instability; however, this same instability rendered polyanhydrides attractive as biodegradable drug-carrier matrices (Rosen et al. 1988). Generally, it is desirable to have a polymeric system that degrades only from the surface. To achieve such heterogeneous degradation, the rate of hydrolytic degradation at the surface must be faster than the rate of water penetration into the bulk of the matrix. This characteristic would also aid in the delivery of water-labile drugs by making it more difficult for water to interact with these substances until they are released (Chasin et al. 1990).

Polyanhyride homopolymer implants generally erode completely, leaving no insoluble residue. Throughout erosion, implants decrease in size while retaining physical integrity, suggesting surface erosion (Rosen et al. 1988). Erosion and drug release profiles are approximately zero order, and complete release of drug substance correlates with complete matrix erosion. Copolymers of bis *(p*–carboxyphenoxy) propane (PCCP) and sebacic acid (SA) can be formulated to achieve degradation rates between 1 day and 3 years depending on the PCCP–SA ratio; the erosion rate increasing with an increasing proportion of the hydrophilic SA (Leong et al. 1985).

Polyanhydride microspheres have been developed for the controlled release of proteins. In a recent study, when trypsin was placed inside polyanhyride microspheres, the activity loss was <10 percent at 37 °C for 12 hours compared to an 80-percent activity loss for unprotected trypsin. The protein-loaded microspheres displayed near zero-order erosion kinetics without any large initial burst (Tabata et al. 1993).

Polyphosphazenes—Polyphosphazenes are a class of polymers that can serve two quite different functions: they can form inert, long-term structural components, or they can be made hydrolytically unstable so as to function as bioerodible materials. The hydrolytic stability or instability is determined not by changes in the backbone structure but by changes in the side groups attached to a long-chain backbone of alternating phosphorus and nitrogen atoms. Side groups attach to each phosphorus molecule, and these groups can range from hydrophobic groups that confer water insolubility that protect the backbone against hydrolysis through groups that generate water solubility together with hydrolytic stability, to side groups that provide a facile pathway for hydrolytic breakdown of the polymer to innocuous, excretable, or metabolizable molecules (Allcock 1990).

Poly- and Pseudopoly(amino acids)—Although many biodegradable polymers have provided significant treatment advantages, there is a continual concern about potential toxicity associated with a polymer that degrades in vivo. To alleviate this problem, polymers have been derived using naturally occurring nutrients or metabolites. The development of poly(lactide) and poly(glycolide) polymers is a good

example of this approach. Poly(amino acids) have been extensively investigated as candidates for a material that does not give rise to toxic degradation products because these acids are derived from natural molecules. However, the number of promising materials has turned out to be quite limited. One of the major limitations of synthetic poly(amino acids) is the pronounced antigenicity of those poly(amino acids) containing three or more different amino acids. Another limitation is that synthetic poly(amino acids) may have undesirable material properties. For example, most synthetic poly(amino acids) derived from a single amino acid are insoluble, high-melting materials that cannot be processed into shaped objects by conventional fabrication techniques. Many poly(amino acids) also absorb a significant amount of water when in an aqueous environment (Kohn 1990). Nonetheless, natural poly(amino acids) have been developed that are nontoxic and biodegradable. Poly(g–glutamic acid) polymers, synthesized by *Bacillus lichenformi,* have successfully delivered porcine growth hormone over an extended period (Fan and Sevoian, unpubl. data).

To overcome these difficulties of synthetic poly(amino acids), pseudopoly(amino acids) have been developed. Pseudopoly(amino acids) replace the peptide bonds in the backbone of synthetic poly(amino acids) with a variety of nonamide linkages. In peptide chemistry, the term "pseudopeptide" often denotes a peptide in which some or all of the amino acids are linked by bonds other than peptide linkages. Thus far, few pseudopoly(amino acids) have been developed, but initial investigations support the theory that they tend to retain nontoxicity and good biocompatability often associated with conventional poly(amino acids) while at the same time exhibiting significantly improved material properties (Kohn 1990).

Conclusion

Whether contraceptives useful for wildlife population management will ever be developed, let alone employed, is currently unknown. Whatever technologies are ultimately devised, however, it will never be an easy task to administer contraceptives to wildlife. In the above discussion, readers have merely viewed the contraceptive iceberg from the surface. Because there are tremendous financial rewards in the field of delivery systems, an immense amount of research goes on unseen and unannounced by both public and private investigators.

Nonetheless, the future development of contraceptive delivery systems by both the human and veterinary medical communities will work in favor of the wildlife biologist. Many potential technologies were not even discussed in this review as they were deemed premature for wildlife applications. For example, it is possible that isolated cells, such as luteal cells, could be encapsulated and protected so as to continually elaborate progesterone to prevent estrus cycling (Sefton et al. 1992). Even viruses and bacteria could be drafted as contraceptive delivery systems to produce sperm or ZP antigens via recombinant DNA technology (Morell 1993).

Ultimately, methods of delivering contraceptives to wildlife may be as varied as the species targeted. No one technology is likely to satisfy all the concerns on efficacy, efficiency, and animal and human safety. Also, the exigencies of wildlife overpopulation occurring in so many locations and circumstances will require the efficient and selfless collaboration of all concerned scientists. Thus, technologies from many disciplines will have to be combined to provide biologists with the extensive and sophisticated armamentarium required to confront the task of wildlife population control.

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List of Manufacturers

Advanced Telemetry Systems
470 First Ave. N.
Isanti, MN 55040
(Wildlink™ Data Acquisition and Recapture System)

Palmer Chemical & Equipment Co., Inc.
P.O. Box 867
Palmer Village
Douglasville, GA 30133 USA
(Cap-Chur® RDS)

Paxarms Limited
P.O. Box 317
Tomaru, New Zealand
(Paxarms RDS)

Peter Ott AG
Vet. Med. Gerate und Pharmazeutica
Postfach, CH 4007
Basel, Switzerland
(Dist-Inject® RDS)

Pitman–Moore Inc.
421 East Hawley Street
Mundelein, IL 60060
(Ralgro® implants)

Pneu Dart, Inc.
P.O. Box 1415
Williamsport, PA 17703
(Pneu-Dart® RDS)

Sanofi Animal Health, Inc.
7101 College Blvd.
Overland Park, KS 66210
(Syncro-Mate–B® implants)

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Delivery of Immunocontraceptive Vaccines for Wildlife Management

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Abstract: Immunocontraceptive technology appears to be a viable approach for population control of nuisance species of wildlife. The administration of immunocontraceptive vaccines is presently performed by syringe injection or by remote delivery via darts or biobullets. In order for immunocontraception to be successful in wide application to free-roaming animals, the vaccine must be delivered in an oral form. Recent advances in molecular biology, immunology, and pathology of mucosal infections give us tools to develop effective oral vaccines. Oral vaccines encapsulated in either biodegradable microspheres, synthetic adhesive liposomes, or nonvirulent live vectors hold promise as a practical approach for immunocontraception of free-roaming wildlife. Issues of safety, species specificity, and field application of the vaccine will need to be addressed.

Keywords: Wildlife vaccines, immunocontraception, vaccine vectors, oral vaccine delivery

A growing need for nonlethal methodology for population control of nuisance or damaging species of wildlife has fostered research in immunocontraceptive vaccine technology. Kirkpatrick et al. (1990) demonstrated that reproductive rates of feral horses can be reduced by vaccinating these animals with native porcine zona pellucida (PZP). Turner et al. (1992) demonstrated that PZP was effective as an immunocontraceptive in the white-tailed deer *(Odocoileus virginianus)*. Recent advancements in immunology and molecular biology have made it possible to produce and administer genetically engineered contraceptive vaccines, thus making reproductive control a very promising alternative in wildlife management.

In a previous study by Turner and Kirkpatrick (1991), the vaccine was delivered by darting or biobullet. This remote delivery is valuable for special applications. However, in order for this technology to have wide application, one must have a mode of application that can disseminate the vaccine to a large segment of a wildlife population at a reasonable cost (Garrott et al. 1992).

The most logical means of vaccine application to free-roaming animals is by oral delivery. Oral vaccination, however, is not without its problems (Bloom 1989). Because vaccines are proteins, they need a protective mechanism to prevent digestion in the gastrointestinal tract. Baiting with vaccines should be as species specific as possible and yet be designed to reach a large proportion of the selected wildlife population. Oral delivery of immunocontraceptive vaccines is an untested area of technology that will need several years of developmental research before the first vaccines are available for entry into the registration process.

The purpose of this paper is to review the immunological concepts of vaccination and how they may apply to immunocontraception, review the current technology of oral immunization, and propose some applications for oral immunocontraception in free-roaming pest vertebrates.

Reproduction and Immunology

Mammalian and avian reproduction involves interaction of spermatozoa and oocytes contributed by the male and female, respectively. Both these gametes have unique surface glycoprotein receptors against which an immune response can be elicited. The development of these gametes and corresponding hormones is under the control of follicle-stimulating hormone (FSH) and luteinizing hormone (LH), gonadotropins secreted from the pituitary and flowing through the bloodstream to the gonads. Secretion of the gonadotropins is in turn regulated by gonadotropin-releasing hormone (GnRH), which also has a role in sexual receptivity that is in addition to its regulation of FSH and LH release and the stimulation of ovulation. Immunocontraception involves producing antibodies against these reproductive hormones and gamete proteins that will interfere with their biological activity.

The power and efficiency of vaccines in combating infectious diseases is well recognized and accepted.

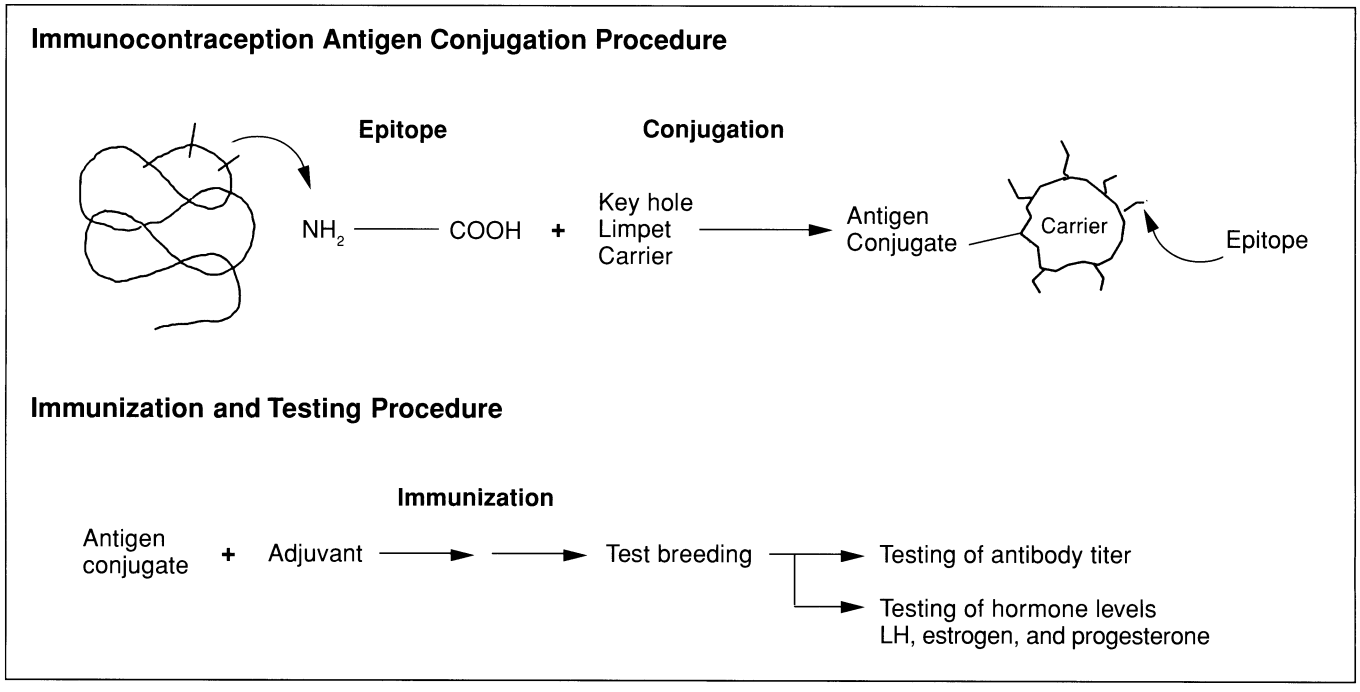

Figure 1. Reproductive "self" antigens are made "foreign" by coupling them (conjugation) to a protein foreign to the animal. Injecting the conjugate into the animal produces antibodies to the self antigen as well as the foreign protein.

The vast majority of vaccine research is concerned with the development of new and improved vaccines against viral and bacterial diseases. Antidisease vaccines are based on using immunologically foreign antigens, such as surface glycoproteins of viruses and bacteria, to stimulate the immune system to form antibodies that attack live viruses and bacteria just as they would the glycoproteins.

In order to understand the concepts of immunocontraception, one must understand how the immune system defends itself against outside organisms (Silverstein 1989). Development of infections and resulting immune responses are constantly in process because people live in a world filled with micro-organisms. Every facet of our existence brings us into contact with bacteria, fungi, viruses and a diversity of parasitic or potentially parasitic life forms. Yet we possess a rich, harmless, natural microflora on all body surfaces, within all body orifices, and throughout most of the gastrointestinal tract. Even vital digestive functions are mediated partly by the gastrointestinal flora. The body is able to differentiate normal flora and self-proteins from pathogens through a process called immune tolerance.

Antifertility vaccines are directed against self-reproductive antigens, either hormones or proteins, to which the recipient is normally immunologically tolerant (Jones 1983). These antigens are made "foreign" by coupling them to a protein foreign to the animal. The resultant vaccine induces immunity which interferes with the biological activity of that particular antigen. The result can be infertility (fig. 1).

An immunological approach to contraception is attractive because it requires only periodic vaccination. The approach is physiologically sound in the sense that antibodies induced in the target animal interfere with reproduction without the constant medication.

Immunocontraception occurs when fertility is reduced by means of antibodies attaching to and interfering with the biological activity of hormones or reproductive tract proteins. Immunization against most reproductive antigens generally gives rise to a

reversible response. Antibodies decline in the course of time, and animals regain fertility (Dunbar and Schwoebel 1988).

Systemic Vaccination

Dose amount, frequency and timing, immunogenicity of the antigen vaccine preparation, and mode of immunization all influence the immune response. The nature of the immune response required for an anti-fertility vaccine is equivalent to the response obtained by immunization. Rendering reproductive self-antigens immunogenic involves conjugating these self-molecules with foreign substances in order to break the state of tolerance associated with these molecules. These vaccines must be designed to react with macrophages (the antigen presenting cells) as well as with the two immune-processing cells (T and B). For example, the T cells receive the antigen from the macrophage and present the foreign material to the B cells. Enhancement of B-cell activity is essential to the production of high levels of antibodies as well as creation of B memory cells to that specific antigen.

Traditional immunization has always been associated with adjuvants (nonspecific immune stimulants). The most common adjuvant is Freund's complete adjuvant (FCA). This substance is a mixture of mineral oil and killed bacteria cells. Booster injection is performed with Freund's incomplete adjuvant (FIA) (minus the killed bacteria) to prevent abscesses at the injection site. The protein to be injected is dissolved in water and mixed with the oily adjuvant to form a water-in-oil emulsion. This emulsion provides a depot at the injection site allowing a slow release of the immunogen to the immune system. The optimal length of antigen presence for maximum antibody production is unknown; however, if antigen presence is too short, the antibody quantity is suboptimal. Chronic presence of antigen leads to antigen tolerance and a lack of antibody production response.

The immune system, both systemic and mucosal, seems to respond best by giving a priming dose followed in several weeks by a booster dose. A single dose produces a short-lived antibody response and does not result in a long-lasting memory response. Many times, the best response is observed when the animal is boosted several months after the original antigen exposure. Continued presence of the antigen for several weeks is important for a long-lasting immune response. Slow-release vehicles such as microspheres or liposomes can provide this effect.

The standard form of vaccination involves 50–100 μg of antigen for small animals (mice to rabbits) and 200–400 μg for larger animals. The antigen is mixed with FCA to produce a thick water-in-oil emulsion. This emulsion is injected into the animal using multiple subcutaneous, intradermal, or intramuscular sites. Booster doses use the same or slightly less antigen in FIA. When incomplete Freund's is used for boosters, abscesses at the site of injection generally do not form. Highly immunogenic antigens can produce sufficient antibody with doses of 5 to 10 μg.

Scientists at the Denver Wildlife Research Center (DWRC) have demonstrated that the hypothalamic hormone GnRH, made foreign by coupling to keyhole limpet hemocyanin (KLH), can sterilize both sexes of wild Norway rats *(Rattus norvegicus)* for up to a year. White-tailed deer immunized with a porcine glycoprotein (PZP), the zona pellucida that surrounds all mammalian oocytes, remained sterile for at least two breeding seasons.

Oral Vaccination

Mucosal Immune System

The pharyngeal and intestinal mucosae represent a major interface with the external environment and come in contact with food and products of food digestion, ingested micro-organisms, drugs, and the vast quantity of resident flora that populate the distal small intestine and colon (Mestecky 1987).

The intestine is the body's largest immunologic organ. It comprises 70–80 percent of all of the body's immunoglobulin (Ig) (antibody)-producing cells and produces more secretory Ig (SIgA) than the total production of serum Ig in the body. The primacy of the intestine in making Ig is not surprising because the

majority of infectious disease organisms are first encountered through the intestinal mucosal membranes. The main antibody produced by the mucosal immune system is SIgA. Intestinal SIgA response is of relatively short duration, lasting from 2 to 4 weeks. The SIgA system exhibits potent immunologic memory and can be repeatedly stimulated by renewed contact with antigen. Systemic IgG production may also be stimulated by oral vaccination, and the presence of IgG as a result of vaccination may be detected in serum years later. It is the serum IgG that provides the long-term interference with the biological activity of reproductive hormones and proteins.

Immune follicles, including tonsils, are located in the pharyngeal area at the entrance to both the respiratory and digestive tracts. The pharyngeal area of the throat may be considered the first line of mucosal defense and immune response. As a second line of defense, thousands of lymphoid follicles are located in the distal portion of the small intestine. Aggregates of these follicles called Peyer's patches (PP) are also found throughout the small intestine. The lumenal surface of PP are covered by an epithelium which contains a unique cell type termed the M cell (Childers et al. 1990). Intact viruses and micro-organisms and particulate antigens up to 10 μm in size are taken up by M cells for antigen delivery to the underlying lymphoid cells. This uptake of micro-organisms enhances the ability of the host to respond immunologically to a microbial challenge and fight off an infection. These antigens activate T and B cells and, along with macrophages, soon migrate out of the PP to the mesenteric lymph nodes and into the bloodstream via the thoracic duct, thereby presenting the antigen to the systemic immune system (fig. 2).

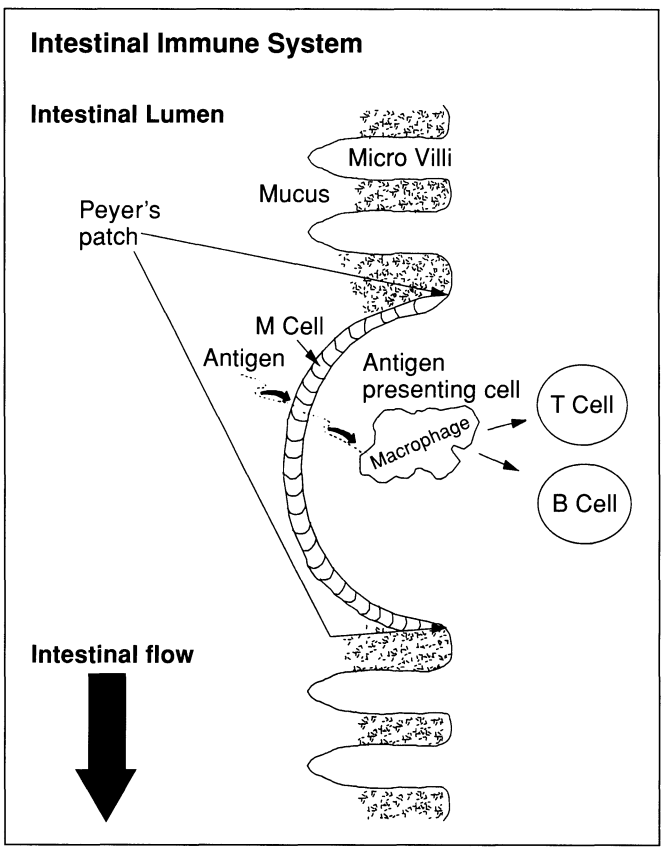

Figure 2. The small intestine contains thousands of immune follicles. Aggregates of these follicles are called Peyer's patches (PP). Their surface contains a unique cell type, the M cell, which takes up intact viruses, bacteria, and particulate antigens up to 10 μm in size. Once inside the PP, the antigens are processed by the macrophage and presented to the immune system.

Oral Delivery of Antigen to the Intestine

Many factors can influence the expression of mucosal immunity to a specific antigen. Most proteins are rather poor immunogens when given orally. This is the reason so few vaccines are currently administered by this route.

Effective mucosal immunogens appear to have certain characteristics: (1) They are not degraded in the mucosal environment (e.g., the intestine); (2) they can bind to and penetrate into the mucosal epithelium (thus allowing efficient uptake in the PP, as typified by cholera toxin (CT), one of the most effective mucosal immunogens known; and (3) they may also have adjuvant immunostimulating activity (Holmgren et al. 1992, McGhee and Kiyono 1994).

Live micro-organisms with mucosal adhesive properties are highly effective mucosal immunogens; killed and inert antigens without mucosal binding properties are poor mucosal immunogens. Most food antigens are poor mucosal immunogens because they are rapidly degraded into nonimmunogenic fragments in the mucosal environment. Food antigens generally do not bind to epithelial receptors.

As pointed out previously, immune lymphoid follicles are located in the pharyngeal area as well as distal portion of the small intestine. Most oral immunization studies use the gavage technique, which means the antigen was delivered into the stomach through a blunted needle. Lavage delivery, in which the antigen is delivered in the pharyngeal area, can stimulate the immune follicles in this area as well as in the small intestine. Delivery of unencapsulated protein antigens to the pharyngeal area may be an effective means of oral immunization since it precedes the stomach's digestive enzymes.

Enteric-coated capsules are commonly used for delivery of drugs to the small intestine. Enteric capsules are resistant to acid but are soluble in the alkaline solution of the small intestine. They provide only one-half of the formula of effective antigen delivery (i.e., protection from the stomach) because they generally cannot be made small enough to be taken up by the PP. Also, enteric-coated vaccines can get the protein past the stomach, dissolve, and release the antigen in the small intestine, but proteolytic enzymes in the small intestine may digest these proteins into nonimmunogenic peptides before they are absorbed by the immune cells. The safest way to deliver the antigen orally is to protect it until it is taken up by the PP and delivered to macrophages.

Combining two approaches—(1) enteric coating or using delivery vehicles that slow the intestinal degradation of the antigen and (2) targeting the vaccine design to attach to the immune follicles with M cell binding—could lead to an effective antigen uptake and potentiation of mucosal immune response.

The quantity of antigen used in oral immunization depends on how well the antigen is protected from degradation and how immunogenic it is. The antigen dose may vary from 12.5 µg to 1 g per dose, with larger animals receiving the larger quantities of antigen. Most studies indicate that two doses given 3–4 weeks apart are needed to produce a long-lasting immune response. Ahren et al. (1993) found that a third dose given within 3 weeks was counterproductive, probably because SIgA stimulated from the second dose interfered with the uptake of the antigen.

Two oral doses of a live salmonella vector produced IgG responses similar to the response of a systemic vaccination of the killed form of the same vaccine (Morona et al. 1994). Oral boosting after 42 days was needed for this response. A single dose or boosting after 14 days gave a much lower antibody titer.

Scientists at DWRC have demonstrated that white-tailed deer can be successfully vaccinated using a genetically engineered *Bacillus Calmette Guerin* (BCG). These bacteria were designed to deliver an outer surface protein A (Osp A) antigen onto the surface of the bacteria. A good IgG response to the Osp A antigen was demonstrated after two oral doses of bacteria. DWRC is also testing different oral immunocontraceptive vaccines in wild Norway rats.

Immune Tolerance

The constant systemic presence of antigen can induce a state of immune tolerance in which antibody production is reduced (Ernst et al. 1988). This process is probably a protective mechanism to prevent the animal from an excessive immune response. What is excessive depends on the antigen. However, gram quantities of antigen are generally considered excessive. The mucosal immune system seems to have a built-in limitation in terms of the magnitude of response to any single immunogen (oral immune tolerance). This limitation is in contrast to the systemic immune system, which responds vigorously to nonself-antigens. It would be impossible and perhaps even harmful for the intestine to mount a vigorous immune response to each of the thousand foreign antigens it encounters each day. The term "oral tolerance" is used when the animal's immune system ceases to respond to a given antigen. Oral tolerance is commonly found when a large dose of an antigen is given or when the antigen is highly immunogenic and therefore likely to cause the animal harm due to a severe immunologic reaction. An example of this second type of antigen is a bacterial surface lipoprotein, lipopolysaccharide (LPS).

Stok et al. (1994) discovered that conjugating cholera toxin (CT) to ovalbumin and revaccinating with the conjugate orally could reverse an earlier ovalbumin-induced oral immune tolerance.

Oral Vaccine Delivery Vehicles

Synthesized Vectors

Microspheres—Biodegradable microspheres have been used as a slow-release antigen-delivery system. These spheres are copolymers of DL-lactide and glycolide that are synthesized to contain trapped antigen. When these spheres are injected into the host animal, they dissolve, slowly releasing the antigen. The microsphere can be designed to deliver the antigen for from 1 week to several months, depending on the size and the polymer ratio of lactide to glycolide. In most applications, microspheres have been given systemically; however, they can be given orally (Eldridge et al. 1989 and 1990). Microspheres of 1–10 μm are taken up by the PP; however, the efficiency of the uptake is only 1–2 percent. The remaining microspheres pass out of the intestine. The microspheres taken up by the PP dissolve, releasing the antigen directly to the immune system.

Liposomes—Liposomes are spherical, artificial biological membranes made up of phospholipids and cholesterol (Alving et al. 1991). Liposomes contain lipids, chosen for their stability in the gastrointestinal tract. These lipids can protect the antigen from gastrointestinal degradation. Cholesterol in the liposome stabilizes the membrane and makes it attractive to the macrophage because of its lipophilic nature. The phospholipids in liposomes are amphipathic, i.e., they possess a hydrophilic (polar) head and hydrophobic (hydrocarbon) tail. In an aqueous medium, phospholipids exist as micelles or bilayers; the polar heads are at the outer layer due to their affinity to water (fig. 3).

Because of the nature of the membrane, the liposome mimics the microbial cell when the liposome is presented to the immune system. During the synthesis of the liposome, antigen is trapped inside, providing a protective vehicle for delivery of the protein antigen. The liposome acts as an antigen microcarrier and an adjuvant, capable of targeting the antigen directly to the PP. Liposomes have been used to deliver the antigen systemically or orally. When given orally, liposomes with a diameter less than 10 μm are preferentially taken up by the PP and may persist there for up to several weeks (Alving et al. 1991). Liposomes, especially small ones (1–2 μm), can be expected to reach the blood circulation rapidly through the intestinal lymphatics.

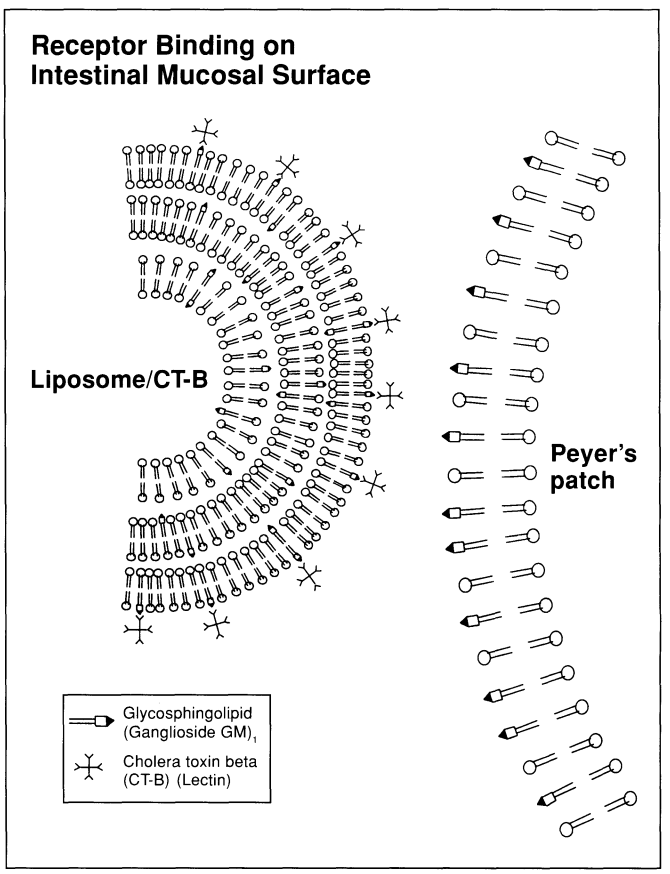

Figure 3. Liposomes are spherical artificial biological membranes. During the synthesis of the liposome, antigen is trapped inside the sphere. The result is a protective vehicle for oral delivery of the antigen. Cholera toxin B is attached to the outer surface of the liposome to provide intestinal adhesive properties. This adhesion to the intestine enhances immune response to the liposome and its antigen contents.

Liposomes Enhanced With Cholera Toxin B—Until recently, based on the relatively poor mucosal immunogenicity of soluble antigens, it was widely assumed that only live vaccines would effectively stimulate a mucosal immune response (Nedrud and Lamm 1991). Recent understanding of the mechanisms by which pathogenic viruses and bacteria colonize and infect the intestinal tract gives researchers new tools to develop successful oral vaccines. For example, a bacterium must survive the presence of the stomach's acid and proteolytic enzymes in order to infect the small intestine successfully. After surviving the stomach, the bacterium must have surface adhesive properties allowing it to adhere to and colonize the intestinal wall, resulting in an infection. Bacteria without these adhesive properties will be carried out of the gut with undigested food material.

Because of their lipophilic nature, liposomes are avidly taken up by the macrophages (Rooijen 1990). However, the liposome must bind to the mucosal surface of the intestine before it can be taken up. This mucosal adhesive property increases the mucosal uptake resulting in greater efficiency and allowing one to use a smaller oral vaccine dose. The most common liposome adhesive is the bacterial lectin CT, a member of a family of enterotoxins produced by several strains of enteropathogenic bacteria (Ahren et al. 1993, McGhee 1992, Mestecky and McGhee 1989). Lectins have multiple binding sites and can bind to receptors on the liposome as well as to intestinal receptors.

CT consists of two subunits—alpha (CTA), which has the toxic properties, and beta (CTB), which has the adhesive or mucosal binding properties. CTB bound to the liposome provides the adhesive properties without the toxicity associated with CT. The CTB bound to the ganglioside GM_1 receptor inserted in the liposome also binds to the ganglioside GM_1 receptors present on the surface of intestinal epithelial cells, thus providing the binding activity needed for mucosal antigens (fig. 3).

Heat-labile toxin (LTB) from pathogenic *Escherichia coli* bacteria represents another adhesive lectin that can be attached to liposomes to provide an intestinal mucosal binding.

Live Vectors

The common forms of existing vaccines are killed bacteria or modified live viruses that, when injected into the host animal, produce immunity by producing antibodies against surface proteins of these organisms. New techniques in molecular biology have introduced the concept of delivering the vaccine surface proteins in harmless live bacteria or viruses that act as a delivery system and therefore are called vectors. Vectors can be used to deliver the vaccine proteins systemically or orally. Vectors that are effective orally must have the ability to attach or adhere to mucosal surfaces. After attachment, these vectors are taken in by the mucosal immune system and thereby deliver the vaccine proteins directly to the immune system. Nonattaching vectors would be carried out of the intestine with the food bulk.

The ideal immunocontraceptive vaccine should be species specific; however, at the present time, species specificity is difficult to achieve. Live vectors can help provide species specificity by employing species-specific viruses or bacteria, such as swinepox, which was used to develop a vaccine carrier for the control of the feral hog.

Viral Vectors—The DNA representing several vaccines has been inserted into harmless viruses. The inserted DNA synthesizes the vaccine protein as the virus multiplies in the host animal, thereby vaccinating the animal. The most noted viral vector has been the vaccinia virus a member of the poxvirus family (Moss 1991). This virus has been genetically engineered to deliver a rabies vaccine. Given orally, the harmless vaccinia virus multiplies in the body and synthesizes a surface rabies protein. Antibodies produced against this protein protect the animal against a future rabies virus infection. This viral construct has been used successfully in eliminating most of the rabies in foxes and raccoons in Europe (U.S. Department of Agriculture, Animal and Plant Health Inspection Service 1991). The viral vectors can also be designed to contain immunocontraceptive proteins (Morell 1993).

Bacterial Vectors—As in viral vectors, bacteria can be genetically rendered harmless (nonpathogenic) and have immunocontraceptive vaccine DNA inserted into

them. This recombinant bacteria can deliver a immunocontraceptive protein, coded by the inserted DNA, to an animal host. The two bacterial vectors in use today are an attenuated BCG and a double gene-deleted *Salmonella typhi* bacillus. Both bacteria vectors are considered safe and have been used in many vaccine delivery applications. *S. typhi* strains, with deletion of two genes, are avirulent in animals, birds, and humans. These strains retain the intestinal adherence property found in unmodified *Salmonella* spp. and are absorbed by the intestinal immune cells. It appears to be safe and effective as a live vector for oral delivery of immunocontraceptive vaccines.

Morona et al. (1994) found that two oral doses of a live *Salmonella* construct elicited serum IgG responses that were comparable to intramuscular vaccination with formalin-killed *Salmonella*. Therefore, it appears that—even with live vectors—one needs at least two presentations of the antigen.

Live bacterial and viral vectors would be more economical to produce than the synthetic vectors; however, the public acceptance and safety issues have to be addressed.

Field Applications of Oral Immunocontraceptive Vaccines

Oral immunocontraceptive protein vaccines are untested. The protein vaccine must be mixed into liquid or solid baits that require some protection from the environment for at least several weeks. The bait must be attractive to a large segment of the target animal population. Present vaccine designs would require baiting an animal population twice about one month apart. Vaccine application should start about 2 months before the start of the breeding season. If the vaccine itself is not species specific, the delivery system should be. Problems of multiple visits to the bait and repeat baiting of dominant animals need to be understood in the practical application of population control. With the exception of the vaccinia virus rabies vaccine, safe use of recombinant bacterial and viral vectors has yet to be proven in a field application.

Summary

Immunocontraceptive vaccines delivered by injection or by darting have been shown to be a viable technique in preventing conception when used in confined or limited field applications. However, in order for immunocontraception to have widespread success against free-roaming animal populations, the vaccine must be delivered in an oral form in a designed bait. Because oral vaccines are proteins, they are subject to digestion by stomach gastric contents; therefore, the oral vaccine must be protected by some form of encapsulation. Inconsistent antibody responses to multiple oral doses may be due to the presence of intestinal IgA antibodies, which may prevent uptake of the antigen by the intestinal immune system.

Recent understanding of the mechanisms by which pathogenic viruses and bacteria colonize and infect the intestinal tract give us new tools to develop successful oral vaccines. Synthesized vectors, such as biodegradable microspheres and liposomes, can protect the protein vaccines and deliver them to the mucosal immune cells. Liposomes can be designed to contain lectin receptors that mimic the adhesive properties of intestinal pathogens, thereby enhancing their mucosal uptake and immunogenic properties.

Understanding the molecular genetics of oral pathogenic bacteria and viruses allows one to attenuate these virulent strains and insert the DNA of the vaccine to be expressed. Because these vaccine proteins are "self," they need to be linked to the DNA of a more immunogenic protein and be expressed together by the live vector. The use of these attenuated live vectors to deliver immunocontraceptive vaccines can provide economical vaccines of a consistent nature. These new tools should provide the basis for successful oral immunocontraceptive vaccines of the future. Successful field application of these vaccines needs careful study and is yet to be attempted.

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Development of a Bait for the Oral Delivery of Pharmaceuticals to White-Tailed Deer *(Odocoileus virginianus)*

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Abstract: Solid and liquid baits were tested for the delivery of drugs to white-tailed deer. The solid bait was comprised of a mineral block paired with apple, peanut butter, or acorn extract. The liquid bait was comprised of water, apple juice, glycerine, salt, and either peanut butter or apple odor. Although both solid and liquid baits were attractive to deer, the latter may be more useful because consumption can be measured directly, ingestion by nontarget animals is minimized, and bait degradation by weathering is reduced.

Keywords: bait, deer, *Odocoileus virginianus,* odor, taste

Deer *(Odocoileus* sp.) cause more agricultural damage than any other vertebrate species in the United States (Conover and Decker 1991). In New Jersey alone, white-tailed deer *(O. virginianus)* damaged more than $20 million of various food and nonfood crops in 1990 and were involved in 8,000 collisions with automobiles (New Jersey Farm Bureau 1990). Burgeoning deer populations also pose a growing threat to human and animal health and safety because deer are important in the life cycle of *Ixodes damini,* the tick that is the primary vector for transmission of the Lyme disease spirochete (Anderson 1988).

To date, deer control activities have focused on increasing hunter access to private lands (Atwill 1991, New Jersey Agricultural Statistics Service 1990), manipulating hunting seasons (Conover and Decker 1991), erecting deer fences (Boggess 1982), and developing repellants to protect localized areas from severe browsing damage (Conover 1984 and 1987, Scott and Townsend 1985). These techniques are effective, but lethal control is not considered safe, practical, and/or politically acceptable in many suburban and urban areas. Deer herds have grown so large in many areas that repellants now have little effect (Milunas et al. 1994).

Nonlethal methods to reduce deer numbers and/or slow population growth in suburban and urban areas are being sought (Kirkpatrick and Turner 1985). Chemical sterilants and immunosterilants may become available within the next decade (Turner et al. 1990), but the problem of inoculating large numbers of deer remains. Silastic™ implants (e.g., Plotka and Seal 1989) and direct intramuscular injections (Harder and Peterle 1974) are neither economical nor efficient. Although oral vaccines would be both inexpensive and relatively easy to use, no bait formulations are currently available. The investigations described herein address this need.

Materials and Methods

Three experiments were performed during the winter of 1991 and spring of 1992. Experiment 1 evaluated the relative attractiveness of apple, acorn, sweet corn, and peanut butter extract. Experiment 2 explored the attractiveness of salt blocks, mineral blocks, molasses blocks, and molasses–mineral blocks. All block types were presented in combination with apple extract. Experiment 3 assessed whether synergisms might occur among the food extracts.

Two additional experiments were performed during the spring and summer of 1993. The first (experiment 4) compared the relative attractiveness of mineral blocks with the attractiveness of a solution comprised of water, apple juice, glycerine, and salt. Apple and peanut butter extracts were evaluated as components of both bait types. The second (experiment 5) measured the consumption of the liquid bait adulterated with fluorescein. Feces were collected to determine whether they contained fluorescent particles.

1991–92

Study Sites—Four 25-ha sites near Poughkeepsie, NY, were selected, each representing a different habitat type. The first site was an annually mowed field; the second, an old field with scattered apple trees and clumps of honeysuckle; the third, a woodlot dominated by maple *(Acer* sp.); and the fourth, a

bottomland dominated by sweetgum *(Liquidambar styraciflua)* and sycamore *(Platanus occidentalis)*. Between 10 and 20 deer were regularly observed at all sites during the 4 weeks prior to the experiment. For all of the experiments below, transect grids were established within each site. Transect intersect points served as potential testing locations (see below).

Experiment 1—Apple odor extract was obtained from International Flavors and Fragrances (Union Beach, NJ). Peanut butter extract was donated by Hercules Flavor Co. (Middletown, NY). Acorn and sweet-corn odor extracts were purchased from the M & M Fur Co. (Bridgewater, SD). Each stimulus liquid was diluted in propylene glycol to produce 2-percent (mass/mass) stimulus concentrations.

We chose to examine extracts and not foods, per se, for two reasons. First, we expected that the extracts would be more durable in the field. Second, there is evidence that extracts of preferred foods are attractive to captive deer and increase ingestion even when presented "out of context." For example, food odors in solution enhance drinking (Rice and Church 1974).

Likewise, we chose to examine food odors instead of semiochemical odors (e.g., urine, glandular secretions) because we wanted to promote ingestion and not merely investigation. Semiochemicals typically result in the latter but only rarely the former (R. A. Mugford and D. Passe, pers. comm.) Also, we wanted to attract both males and females, and we surmised that food odors would be superior to social odors in this respect.

A priori, we chose salt as the principal ingredient in our formulations for the following reasons. First, we infer that it is relatively simple to incorporate a variety of pharmaceutical chemicals into a salt matrix. Second, as herbivores, deer are chronically sodium deficient (Belovsky 1981), and, therefore, they avidly consume salt (Jones and Hanson 1985). Third, the use of salt provided a measure of species specificity. Unlike herbivores, carnivores and many omnivores consume diets that are sodium replete. Meat eaters rarely show strong salt preferences and often are indifferent to this tastant (Beauchamp and Mason 1991).

Testing occurred during November and December 1991. At each of the four experimental sites, eight testing locations were randomly selected, with one qualification. That qualification was that no location could be situated within 20 m of an existing deer trail (operationally defined as clear paths with deer tracks). Four of these locations were randomly assigned to the odor condition (one odor/location). The remaining locations were assigned to the control condition.

On Mondays of each of the next 4 weeks, one odor location and one control location were randomly selected (sampling without replacement) at each experimental site. At odor locations, a 1.8-kg salt block (Cargill, Inc., Minneapolis, MN) was suspended 1 m above the ground in a holder attached to a metal stake or a large tree. A metal deflector was attached to the stake above the block to shield it against precipitation. Stakes were positioned so that there was a large tree immediately behind them to block extraneous activity records (see below). Next, one of the odor stimuli was randomly selected (sampling without replacement), and 10 mL was poured into a glass scintillation vial (5 cm in length, 1 cm in diameter). The vial was attached to the stake just below the salt block. At control locations, a vial containing 10 mL of propylene glycol was attached to the stake, as described above. Braided cotton wicks were inserted through holes in the lids of the vials; half of each (1.5 cm) was exposed to the environment. These wicks controlled the escape of stimulus odors.

Infrared motion detectors (Trailmaster, Inc., St. Paul, MN) were mounted 1 m above the ground and 3–4 m from the lure block on trees at each test and control location. The units were tuned to record the time and date of visits by deer. Each unit only detected activity by moving objects at least 60 cm in diameter within 0.5 m of the salt block. Accordingly, the units were insensitive to smaller animals and to activity on either side of the trees against which the lure blocks were placed (Mason et al. 1993). In addition to visit data, the weights of the salt blocks and animal tracks within 2 m of the blocks were recorded weekly. To record weight changes of the blocks, the salt was returned to the laboratory and placed in a drying oven at 40 °C for 48 hours. The block was then weighed,

and this weight was subtracted from the dry weight of the block prior to testing. Fresh oven-dried blocks were used for each 7-day test session.

Experiment 2—Salt blocks, mineral blocks, molasses–mineral blocks, and molasses blocks served as stimuli (table 1). All were presented in combination with apple extract, a generally attractive scent (see experiment 1 results, below).

Testing occurred during December 1991 and January 1992 at eight new locations that were randomly selected. The procedures followed were identical to those described for experiment 1.

Experiment 3—Mineral blocks were presented in combination with apple extract only, or paired extracts (apple–acorn, apple–peanut butter, or acorn–peanut butter). Testing occurred during January and February 1992 at eight new, randomly selected locations. The procedures followed were identical to those described for experiments 1 and 2.

Analyses—Visit data were heterogeneous (Bartlett's test, cited in von Eye 1990), and thus were transformed to their natural logs. Difference scores were created from the transformed data set by subtracting control location values from test location values. Because the baits were serially exposed, the data were evaluated in a two-way repeated measures analysis of variance (ANOVA). The within-subjects (repeated measure) was attractant. The between-subjects effect was habitat type. Tukey post-hoc tests (Winer 1962) were used to isolate significant differences among means, with the significance level set at $P < 0.05$.

Although stimulus weights were collected in all experiments, lengthy periods of severe wet weather eroded blocks and precluded accurate measurement of consumption. For this reason, these data are not reported.

1993

Study Sites—For experiment 4, three of the four 25-ha sites (old field, woodlot, bottomland) used in experiments 1–3 were selected. Deer were regularly observed at all sites prior to the experiment.

Table 1. Contents of stimulus blocks used in experiment 2

Type	Manufacturer	Contents
Salt	Cargill, Minneapolis, MN	Sodium chloride, white mineral oil
Mineral	Cargill, Minneapolis, MN	Sodium chloride, white mineral oil, 0.2% manganese, 0.1% iron, 0.1% magnesium, 0.05% sulfur, 0.025% copper, 0.01% cobalt, 0.008% zinc, 0.007% iodine
Molasses	Trophy Feeds, Walled Lake, MN	Corn syrup, sucrose molasses, peanuts, cracked corn, hydrogenated vegetable oil, lecithin
Molasses–mineral	PM Ag Products, San Francisco, CA	52% sodium chloride, 1.5% calcium, 0.4% phosphorus, 2.75% magnesium, 1.0% potassium, 0.0003% iodine, 0.03% iron, 0.00025% selenium, cane molasses, cottonseed meal

For experiment 5, five locations were selected at the Morris Arboretum of The University of Pennsylvania. The 40.5-ha arboretum has a resident deer herd of more than 50 animals. Each of the five locations was separated from the others by at least 200 m.

Experiment 4—Two locations were randomly selected at each test site. At one, a 1.8-kg mineral block was suspended in a metal holder about 1 m above the ground, as previously described. At the other, a liquid dispenser was suspended on a metal stake at the same height. The dispenser consisted of a 1-L polyethylene bottle with a metal, 15-mm-in-diameter single-ball sipper tube attached. The bottle contained a solution of 20 percent apple juice, 18 percent glycerine, 60 percent water, and 2 percent sodium chloride. Each bottle was encased inside a section of polyvinyl chloride (PVC) pipe 32 cm high and 14 cm in diameter. A PVC end cap was permanently attached

to one end. Extracts of apple or peanut butter (see below) were presented in glass scintillation vials, as previously described, and passive infrared motion detectors were used to record visits to bait locations.

A 2-week pretest period began March 1, 1993. During this period, both mineral-block and liquid baits were present, and the infrared units were used to obtain a baseline measure of activity. Eight weeks of testing immediately followed the pretest period. Testing consisted of four 2-week trials. During the first trial, the mineral blocks and liquid bait dispensers were presented in the same locations as in the pretest period. The scintillation vials contained apple extract. After 14 days, the number of visits to each location, and the amount of liquid bait consumed were recorded. An attempt was made to record consumption of the mineral block.

During the second 2-week trial, conditions were reversed. Freshly prepared liquid bait was presented at what was formerly the mineral-block location, and vice-versa. Visit and consumption data were recorded after 14 days, as described above.

The third 2-week trial immediately followed the second. Fresh solid and liquid baits were again presented in their original locations, and the only difference from the first trial was that peanut butter extract replaced apple extract in the scintillation vials. At the end of 2 weeks, fresh liquid bait was presented at what was formerly the mineral-block location, and vice-versa.

Experiment 5—Baiting locations were randomly situated (1 station/8.1-ha area) on the grounds of the Morris Arboretum. At each location, a dispenser identical to that described above was attached to a tree, approximately 1 m above the ground. Each dispenser was filled with the liquid bait, and a scintillation vial containing apple extract was taped to the PVC pipe. The bait formulation was identical to that previously described, except that it also contained 30 p/m of fluorescein. Laboratory studies had suggested that this was the minimum detectable concentration.

At each bait location, a 10-m^2 sampling plot around the bait dispenser was marked with stakes and orange spray paint. During the 12 days prior to bait presentation on June 22, 1993, each site was visited every 4 days, and all feces within the sampling plot were collected.

On day 13, 1 L of liquid bait was poured into each bait station. Over the next 3 weeks, each location was visited at 4-day intervals. Feces within sampling plots were collected, fluid losses from the bait dispensers were recorded, and the bait solution was replaced. In addition, any feces encountered as the observers walked from one bait location to the next were collected and their location was recorded.

Feces were placed in a drying oven (37.8 °C) for 72 hours. Dried feces were weighed, pulverized, and then examined under ultraviolet illumination for fluorescence. The number of fluorescein particles observed in each sample was recorded by two observers.

Analyses—Experiment 4 visit data were transformed to their natural logs. These log scores were evaluated in a three-factor (habitat, extract, bait type) ANOVA. As in 1991–92, weathering precluded measurement of solid bait consumption. For the liquid bait only, a repeated measures *t*-test was used to compare drinking in the

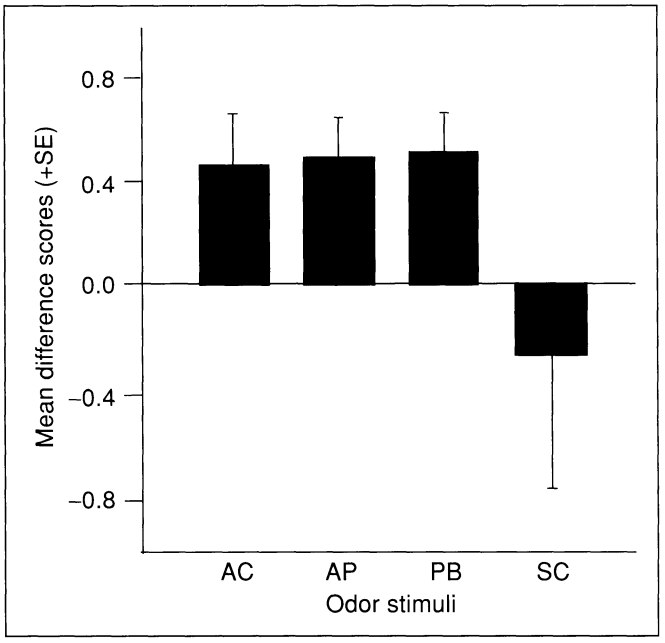

Figure 1. Mean difference scores for log visits to each odor presented in experiment 1. Capped vertical bars represent standard errors of the means. Abbreviations: AC = acorn, AP = apple, PB = peanut butter, SC = sweet corn (from Mason et al. 1993).

presence of apple extract *v.* drinking in the presence of peanut butter extract.

Experiment 5 consumption over days was evaluated in a single-factor repeated-measures ANOVA. Although the presence of fluorescein was not formally assessed, a descriptive summary of these results is presented below.

When appropriate, Tukey tests were used to isolate significant differences among means ($P < 0.05$).

Results

1991–92

Experiment 1—Deer tracks were observed at all experimental and control sites. The only other tracks observed were those of raccoons and mice.

There were significant differences among odor stimuli ($F = 3.6$; 3,72 df; $P < 0.02$), and an interaction between odor stimuli and habitat type ($F = 2.2$; 9,72 df; $P < 0.03$). Post hoc tests showed that, relative to controls, deer visited locations scented with apple, acorn, or peanut butter extract more frequently than they visited locations scented with sweet-corn extract (fig. 1).

Post hoc examination of the interaction term revealed the following pattern of effects. Apple extract had more visits than any other extract in the old-field habitat ($P < 0.05$). Peanut butter and acorn were more attractive than the other extracts in the bottomland ($P < 0.05$, fig. 2). Acorn, apple, and peanut butter were equally effective in the annually mowed field or the woodlot.

Experiment 2—Numerous deer tracks were observed at all experimental and control sites. The tracks of small birds and raccoons also were present occasionally. Other tracks were not observed.

There were significant differences among stimuli ($F = 4.9$; 3,72 df; $P < 0.004$). Post hoc tests showed that mineral blocks were significantly more attractive than salt blocks or molasses–mineral blocks ($P < 0.05$). The least attractive stimulus was molasses block (fig. 3). Otherwise, there were no significant effects.

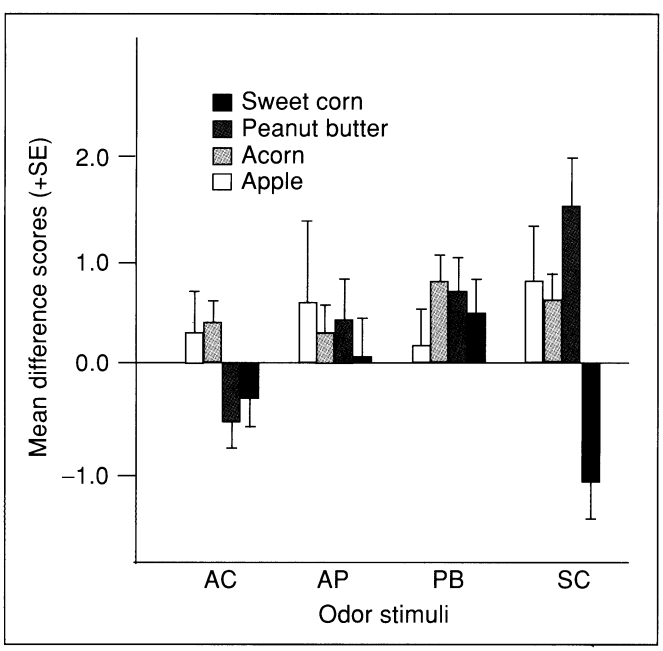

Figure 2. Mean difference scores for visits to each odor in annually mowed field (MF), old field (OF), woodlot (W), and bottomland (WET). Capped vertical bars represent standard errors of the means (from Mason et al. 1993).

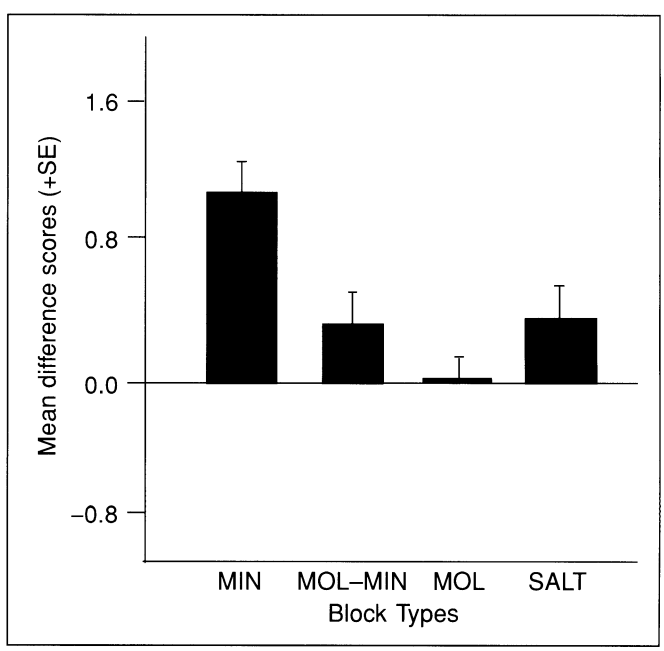

Figure 3. Mean difference scores for log visits to block stimuli presented in experiment 2. Capped vertical bars represent standard errors of the means. Abbreviations: MIN = mineral blocks, MOL–MIN = molasses–mineral blocks, MOL = molasses blocks, SALT = salt blocks (from Mason et al. 1993).

Experiment 3—Numerous deer tracks were observed at all sites. Raccoon tracks and the tracks of small birds were also occasionally present. Other tracks were not observed.

There were no significant differences among extract combinations ($P > 0.35$) or habitat types ($P > 0.45$) (fig. 4), and no significant interactions ($P > 0.25$).

1993

Experiment 4—There were significant differences among habitat types ($F = 12.8$; 2,3 df; $P < 0.034$). Deer visited old-field locations most often and wetland locations least often. Also, liquid bait locations were visited significantly more often than mineral-block bait locations ($F = 23.16$; 1,2 df; $P < 0.015$).

There were significant interactions between habitat type and extract ($F = 10.18$; 2,3 df; $P < 0.046$), and among habitat type, extract, and bait type ($F = 19.59$; 2,3 df; $P < 0.02$). Post hoc analyses of these interactions revealed the following pattern of effects. First, the number of visits to locations baited with apple or peanut butter extracts were equivalent at the woodlot and bottomland sites. Peanut butter was relatively more effective (i.e., attracted significantly more visits) in the old-field setting. Second, the liquid bait locations were visited more often than mineral-block locations (fig. 5). The one exception was when mineral blocks were paired with peanut butter extract in the old-field or bottomland sites. Under these circumstances, mineral blocks were visited significantly more often.

When consumption of liquid bait alone was examined, overall drinking of bait paired with apple extract (780 ± 60 mL) was significantly higher than drinking of bait paired with peanut butter extract (490 ± 80 mL). Nonetheless, preferences varied considerably from one habitat type to another (fig. 5).

Experiment 5—There were significant differences among measurement periods ($F = 6.56$; 6,24 df; $P < 0.0005$). Post hoc evaluation of this effect showed that liquid bait consumption increased steadily throughout the course of the experiment (fig. 6).

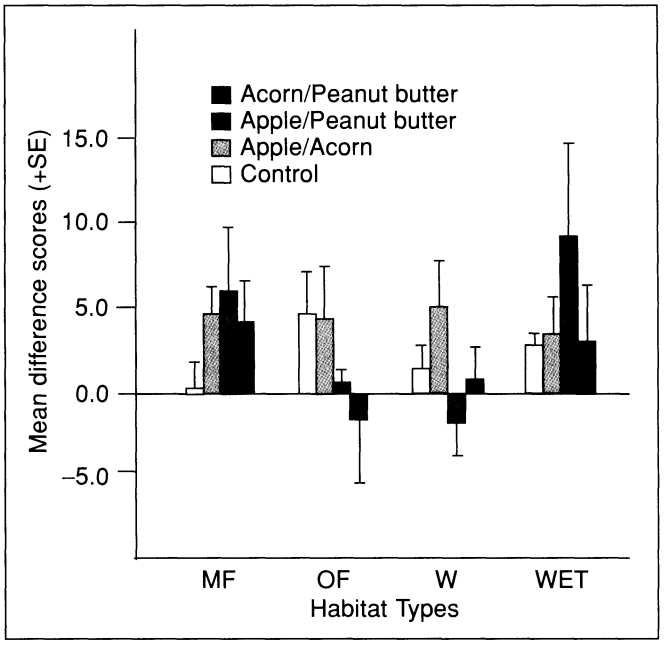

Figure 4. Mean differences scores for visits to odor pairs presented in combination with mineral block in experiment 3. Capped vertical bars represent standard errors of the means. Abbreviations: MF = annually mowed field, OF = old field, W = woodlot, WET = bottomland (from Mason et al. 1993).

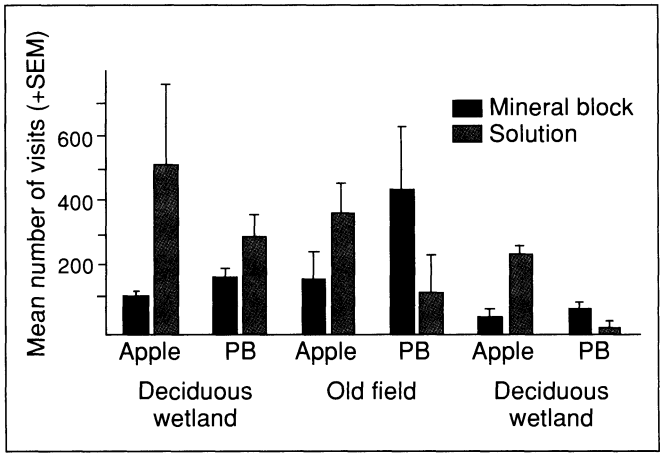

Figure 5. Mean number of visits to solid and liquid baits in experiment 4. *Bottom:* Mean consumption of liquid bait paired with either apple or peanut butter odors. Capped vertical bars represent standard errors of the means. Abbreviation: PB = peanut butter odor.

Deer droppings were found at baiting locations throughout the pretest period. Not surprisingly, no fluorescein was detected in these droppings. However, fluorescein was observed as soon as the dispensers were filled with liquid bait. Overall, fluorescent particles were observed in 75 percent of the deer droppings collected during the test period. The dye was also detected in two of the three samples of rabbit droppings collected during the experiment. All rabbit feces were collected outside of the 10-m^2 sampling grids. No fluorescence was detected in the droppings of other vertebrates (raccoon, five samples; fox, three samples).

Discussion

With the exception of sweet-corn, all food extracts evaluated in experiment 1 enhanced the attractiveness of salt blocks relative to blocks paired with propylene glycol. Attractiveness appeared to be habitat specific, however, and the reasons for this are unclear. Recent feeding histories, novelty, or differences in odor dispersion in different habitat types are all plausible explanations. There may also have been concentration differences in volatiles emanating from the stimuli. Although better documented for taste stimuli (e.g., Sclafani 1991), such stimuli undoubtedly influence the attractiveness of odors as well.

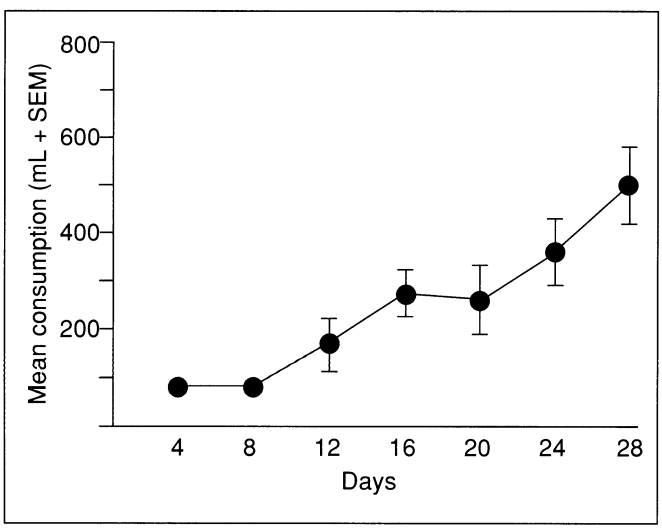

Figure 6. Mean consumption of liquid bait over measurement sessions in experiment 5. Capped bars represent standard errors of the means.

In experiment 2, mineral blocks were the most attractive stimuli and molasses blocks, the least attractive. Salt blocks and molasses–mineral blocks were moderately attractive. Habitat differences appeared to be unimportant. These data suggest that molasses may not be attractive to deer and that the presence of molasses in a formulation could decrease its attractiveness.

In experiment 3, combinations of extracts were no more effective than apple extract alone. This result was surprising because we had expected some synergy in the stimulus combinations. One possible explanation for this lack of effect is that the deer had considerable experience with each of the odor types at lure stations. Conceivably, naive deer would respond differently.

In experiment 4, the liquid bait was significantly more attractive (i.e., was associated with significantly more visits) than the mineral block–apple extract bait. As expected on the basis of experiment 1, apple and peanut butter extracts appeared to be equally effective stimuli. Although mean consumption was relatively higher in the presence of the former, presentations of the extracts were confounded with time. As such, visits might have continued to increase regardless of the extract presented. This possibility is consistent with the steady increases in drinking observed in experiment 5.

Although deer were the principal bait consumers in experiment 5, fluorescent particles were present in two of the three samples of rabbit droppings collected during the experiment. We speculate that one explanation for this observation may be that the rabbits consumed spillage under the liquid dispensers. In pilot testing, small amounts of spillage (15 mL/dispenser over 4 days) were observed. An alternative explanation is that rabbits may have eaten deer pellets containing fluorescein. Evidence indicates that rabbits will ingest the fecal pellets of conspecifics as well as those of other herbivores, including deer (Hill 1964). In this regard, it may be important that the contami-

nated feces were collected between baiting locations and outside the sampling grids. Whatever the explanation, the presence of fluorescein in rabbit droppings is evidence that nontarget exposures to pharmaceutical substances presented in a liquid bait can occur. Of course, solid baits (and possibly, even injected substances) present the same likelihood of nontarget exposure via coprophagia or consumption of spillage. Experiments are needed to address this issue.

Management Implications

We are cautious about extrapolating from a series of small field experiments. Nevertheless, our results have testable, practical implications. Foremost among these is the possibility that mineral blocks paired with apple, acorn, or peanut butter extracts, or liquid bait (water, apple juice, glycerine, salt) paired with apple or peanut butter extracts could provide effective, economical, and relatively selective baits for the delivery of contraceptives or other chemicals (e.g., vaccines against Lyme disease) to white-tailed deer.

A number of important research issues remain. First, we still do not know how many different deer actually contact baits, nor do we know the frequency of contacts by individual deer. Second, we suspect that the number of deer in an area could affect lure effectiveness. Finally, it would not be surprising if the attractiveness of lures was seasonally and/or geographically variable (Schultz and Johnson 1992). Although experiments 4 and 5 suggest that the liquid bait is as effective in summer as it is in late winter and early spring, there is evidence that deer are more likely to use mineral licks during the spring and early summer than during the winter (Weeks and Kirkpatrick 1976, Weeks 1978, Jones and Hanson 1985). This seasonal effect may be more pronounced in southern latitudes (e.g., Louisiana) than in the north (Schultz and Johnson 1992).

At present, liquid baits appear to be more useful than solid baits for several reasons. First, the addition of pharmaceutical substances to the formulation appears to be simpler. Second, problems with the direct ingestion of the bait by nontarget animals are reduced (though secondary nontarget hazards may still exist). Third, bait degradation by weathering is minimized, and the measurement of bait consumption is enhanced.

Acknowledgments

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A Review of Baits and Bait Delivery Systems for Free-Ranging Carnivores and Ungulates

By Samuel B. Linhart, Andreas Kappeler, and Lamar A. Windberg

Abstract: Baits and bait delivery systems have been described for orally administering a variety of chemicals and biologicals to selected carnivores and ungulates. Development has varied from species for which bait preferences and means of distributing oral contraceptives have not yet been determined even on a limited scale (e.g., white-tailed deer) to cooperative, multicountry programs involving the annual distribution of millions of mass-produced oral rabies vaccine baits (e.g., red foxes in Europe). Much of the technical literature on the subject has appeared in sometimes obscure sources encompassing such fields as medical epidemiology, wildlife diseases, animal behavior, applied ecology, flavor chemistry, furbearer trapping techniques (lures and baits), and wildlife management. To date, there has been no unified summary of the available information for the various species, whether the objectives were the application of contraceptives, toxicants, or vaccines. Techniques employed both in the past and at present will be of interest to wildlife biologists, public health officials, and other scientists seeking to develop contraception as a management technique for wildlife.

Keywords: baits, bait delivery techniques, baiting efficacy, carnivores, oral contraceptives, oral rabies vaccines, toxicants, ungulates

Interest in controlling wildlife populations with oral contraceptives began in the early 1960's, but the approach was abandoned due to a lack of safe, effective, long-lasting drugs and efficacious means of delivery. In the United States, such efforts were directed primarily at the coyote *(Canis latrans)* in the West because of its depredations upon livestock, and at the red fox *(Vulpes vulpes)* in the Northeast because, at the time, this species was the principal vector of rabies. Limited effort was later directed at the raccoon *(Procyon lotor)* and at the striped skunk *(Mephitis mephitis)* in the upper Midwest because they also were widely implicated as carriers of the disease. Since these early efforts, social, regulatory, and technological changes have been profound. As a result, investigators studying wildlife contraception are employing much more sophisticated approaches to the problem. Nonetheless, then as now, developing methods for delivering orally effective compounds to intended species by safe and selective techniques at a reasonable cost remains a major challenge.

Oral delivery of contraceptives is essential for free-ranging wild species because, unlike domestic animals, capture and restraint for parenteral administration of drugs are both impractical and costly. Moreover, delivery of biologically active compounds affecting reproduction is more complicated than was true for the bait-delivered, stable, and relatively inert chemical toxicants so widely used in the past. For example, oral contraceptives and immunogens may lose potency when exposed over time to ambient temperatures or when placed in direct contact with organic bait materials. If this approach is to succeed, such constraints—along with public concern about possible adverse environmental impacts and the need for definitive data to support claims of efficacy, safety, and licensure—will require those implementing it to have expertise in a number of diverse disciplines.

Developing contraception for wildlife will require a series of steps depending in large part upon what is currently known about delivery techniques for a given species. Wildlife professionals need baits, grains, or pelleted formulations that will be readily and consistently ingested by the intended species. For some animals, success may require the use of olfactory attractants to enhance bait discovery or determination of the best means of concentrating animals by pre-treatment supplemental feeding. Contraceptives must be incorporated into baits such that their stability and delivery to the site(s) of uptake within the animal is assured. It will be necessary to determine how well target animals discover and consume baits, the extent to which baits are removed by nontarget species, and if the latter are adversely affected. Delivery system development will require information about the feeding behavior, reproductive characteristics, ecology, and population dynamics of target species, and how baiting efficacy may change seasonally, geographically, or with the availability of naturally occurring foods. Field trials must determine selective bait-delivery methods and the minimum density of treated bait or grain (i.e., quantity/km^2) required to suppress

reproduction to desired levels. Efficient and cost-effective methods for mass production of treated baits or grains must be devised; and, finally, cost–benefit studies will be needed to justify widespread application.

Literature describing the field delivery of oral contraceptives is almost completely lacking. However, many published reports deal with baits and bait delivery systems for distributing toxicants (e.g., Debbie 1991) and oral rabies vaccines (e.g., World Health Organization 1990a), and for this reason they have been extensively reviewed and summarized. We used Wildlife Review (U.S. Department of the Interior, National Biological Service, Fort Collins, CO 80525–5589, U.S.A.), the National Wildlife Research Center Predator Literature Database (U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Animal Damage Control, National Wildlife Research Center, Fort Collins, CO 80525, U.S.A.), Wildlife Worldwide (National Information Service Corporation, Baltimore, MD 21218, U.S.A.), and Current Contents (Institute for Science Information, Philadelphia, PA 19104, U.S.A.) as literature sources. In addition, we have collectively investigated delivery methodology for many years, have accumulated the literature on the subject, and have contact with colleagues and collaborators doing such work. Coauthor Kappeler also contacted bait manufacturers for information about their laboratory and field tests, the physical properties of baits, and the numbers produced commercially. The individual species accounts that follow vary in length because they reflect the current status or "state of the art" for each species and associated reports and publications. The taxonomic source for scientific names was Wilson and Reeder (1993).

Red Fox *(Vulpes vulpes)*

Baits distributed for red foxes have served two major purposes: population control through either poisoning or inhibition of reproduction and oral vaccination against rabies. Although both poison baits and baits containing antifertility agents received some attention in the 1950's and 1960's, and again in recent years, their impact was minor compared to the effects of the European and Canadian oral rabies vaccination programs on the prevalence and distribution of the disease. In Europe alone, 74 million baits containing oral rabies vaccine had been distributed by the end of 1994, a few smaller trials in former member states of the Soviet Union not included (K. Stöhr, pers. comm.). The Canadian program in Ontario added another 4.5 million baits (1989–94). Because baits and baiting strategies for red foxes have varied widely, we elected to divide the red fox section in this review according to objectives, with additional subsections within the discussion on oral immunization.

Poisoning

Poisoning has been used primarily in North America during the 1950's and 1960's to reduce wildlife rabies vectors (for a review, see Debbie 1991). The most massive campaign was carried out in Alberta, Canada, prompted by an outbreak of rabies in 1952 (Ballantyne and O'Donoghue 1954). Fat from various sources (beef, pork, sheep, horse, bear) was melted, supplemented with paraffin and beeswax for summer use (higher melting temperature), and poured into small paper cups. Strychnine cubes or cyanide-filled capsules were inserted before the fat (15–20 g) solidified. Boiled eggs inoculated with strychnine or canned dog food rolled in birch bark also served as baits. These baits were distributed by trappers who used an unidentified scent to increase bait attractiveness, put out "draw baits" such as rabbit carcasses or pieces of meat, or inserted a feather into baits to make them more visible. It was estimated that more than 90,000 carnivores, of which 50,000 were foxes, succumbed to the 429,000 poison baits distributed in forest areas alone between October 1952 and March 1954 (Ballantyne and O'Donoghue 1954). The depopulation program may be one of the reasons why only two cases of rabies were recorded in Alberta between summer 1956 and 1971, when rabies in striped skunks became apparent (Rosatte et al. 1986).

Similarly, in Tennessee, U.S.A., more than 9,000 bait stations were operated at a density of 0.86/km^2 and replenished daily for 5 to 11 consecutive nights (Lewis 1968). Beef suet baits containing strychnine

were used, and 38 percent of them were removed. Foxes accounted for 43 percent of the baits taken, dogs *(Canis lupus familiaris)* for 29 percent, domestic cats *(Felis catus)* and scavenging birds for 4 percent each, and various other animals for 7 percent. Thirteen percent of the baits had been removed by humans. Nontarget species' bait uptake or removal was reduced when baits were distributed by landowners on their own properties. However, Lewis (1968) questioned the effectiveness of the program. Carcass searches were not conducted, and bait disappearance rates did not usually diminish over the baiting period, suggesting that the effort had only a small effect on local populations of potential bait consumers. An evaluation of the number of rabies cases per county before and after baiting was inconclusive.

Denmark has repeatedly stopped the spread of rabies in foxes by applying strict control measures (gassing, shooting, poisoning) in a 60-km-wide zone along the country's southern border with Germany. Following the distribution of strychnine baits (chicken necks, piglets, eggs) in January to March of 1979 and 1980, the carcasses of 482 red foxes, 498 martens *(Martes* sp.), 12 European badgers *(Meles meles),* and 4 dogs were recovered (Westergaard 1982). The number of rabies cases sharply declined in 1980, and the country has remained free of terrestrial rabies since 1982 (Gaede 1992).

Although rabies in terrestrial mammals is absent from Great Britain, that Nation has developed control strategies in the event an outbreak should occur. The policy aims at containing the disease locally by destroying the vector population with poison baits. After prebaiting target areas for several days with untreated chicken-head baits, these would be replaced by day-old chickens containing 50 mg of strychnine sulphate (Meldrum 1988). Trewhella et al. (1991) tested the proposed control method in a well-described urban fox population in Bristol using untreated baits and baits containing iophenoxic acid (IA) as a biomarker (Larson et al. 1981). Baits of mechanically recovered chicken meat were stiffened with a 2.5-percent gelatin solution to make them sufficiently firm while keeping them friable enough to prevent foxes from carrying them away and caching them. Baits were buried 5 cm deep at a density of 1.6–3 per fox (32 baits/km^2) and checked and replenished daily for 7 days. Seasonal bait disappearance rates increased from 27 percent in February to 56 percent in fall, with considerable variation between habitat types. It was estimated that 85 percent of baits were taken by foxes and the remainder by dogs. When the number of baits was doubled, the disappearance rates remained constant, suggesting that either the same number of foxes were taking more baits, or that more foxes had access to baits. Given the comparatively low percentage of foxes positive for IA (35 percent of adults and 17 percent of cubs after a summer trial, 29 percent of adults and 23 percent of subadults after a winter trial), Trewhella et al. (1991) concluded that the control strategy was unlikely to contain an outbreak of rabies in a local fox population.

In Australia, aerial distribution of brisket fat baits containing strychnine started in the late 1940's and may have resulted in the local and temporary reduction of predators that prey on livestock and the native fauna (wild dogs, dingoes *[Canis lupus],* red foxes) (Tomlinson 1954).

Compound 1080 (sodium monofluoroacetate) has since replaced strychnine as the poison of choice and is injected into pieces of meat or offal. Foxes are known to remove a considerable proportion of such baits (McIlroy et al. 1986a), and fox control has received more attention in recent years. Thompson and Fleming (1994) evaluated the effect of poisoned meat baits (6 mg of 1080 in 100 g of fresh meat) placed at 120 tracking stations in each of 2 plots of 10 km^2. Even though only 8–10 percent of all poison baits were removed by foxes, population reduction of 66 and 73 percent was achieved in the 2 plots, respectively, as estimated from assessments before and after baiting with tracking stations containing a different, nontoxic bait. Prebaiting and daily replenishment of baits during 10 days may have maximized the effect of the poisoning campaign to an extent not commonly achieved.

D–K9, a new, storable fox bait, was tested in New South Wales in 1992. It consisted of a 35-mm-long, green, shelf-stable sausage containing meat, lure chemicals, and a tablet of 1080 (Fleming et al.

1992b). The new bait was removed by foxes at about the same rate (23 percent) as fowl heads (25 percent), but considerably better than meat (10 percent) and liver baits (12 percent).

Another Compound 1080-based bait became commercially available in 1992. Foxoff® was developed by a private company (Applied Biotechnologies Ltd., 52–60 Export Drive, Brooklyn, Victoria, 3025 Australia) and the Department of Conservation and Natural Resources, Victoria. The bait, a rectangular tablet of either 35 g or 60 g, is semisoft and meat based and contains stabilizers and binders as well as 3 mg of 1080 (L. D. Staples, pers. comm.). To minimize the risk of nontarget uptake, individual landholders bury the baits 6–10 cm deep, preferably every 50–200 m along tracks or fences. The recommended density is 5–10 baits/km^2 in two campaigns per year.

In eight different trials, Foxoff was compared with one or two other 1080 bait types that were made locally of beef meat, bovine liver (fresh and cooked), horsemeat, mutton, lamb tongue, and chicken heads (Applied Biotechnologies 1994). In most trials for which the consuming species could be identified (based on tracks on sand pads), more than 90 percent of all baits had been taken by canids, mainly foxes. Uptake by nontarget species was rare (dogs, cats, pigs, birds) and independent of bait type, suggesting that the method of bait distribution (burying) rather than the bait itself accounted for the species specificity. In five out of eight trials, all baits tested performed equally well. On one occasion Foxoff was superior to both chicken heads and mutton but was outperformed by cooked liver and by horsemeat in one trial each. Typically, the number of baits taken per night dropped to very low levels within 2–3 weeks, suggesting a substantial reduction of the local fox population (Applied Biotechnologies 1994).

Reproduction Control

The application of antifertility agents as a less invasive method of controlling rabies vectors received considerable attention in the early 1960's. Linhart (1964) tested eight different bait types to evaluate a suitable vehicle for the synthetic hormone diethylstilbestrol (DES): limburger cheese cubes, fried beef fat cubes, crackling mixture (mixture of fried cheese, fish, suet, bacon), raw venison cubes, and four types of rendered tallow baits (mutton, beef, pork) that were used either directly or topped with an attractant (cheese, commercial honey bait mixture that included musk, beaver *[Castor canadensis]* casters, muskrat *[Ondatra zibethicus]* anal glands, spices, honey). For field placement, a feather was inserted into each bait to make it more visible in the snow. Two bait types were put out at each of a series of bait stations that were maintained and regularly checked for 2–3 months in winter. Foxes consumed 45 percent of all baits, dogs took 30 percent, and crows *(Corvus brachyrhynchos),* 16 percent. These pairwise comparisons did not reveal a single bait type to be superior in attractivity or species specificity (Linhart 1964).

In North Dakota, U.S.A., a bait made of ground pork fat coated with granulated sugar and containing DES as well as tetracycline as a biomarker, was distributed to a population of red foxes (Allen 1982). Even though 70–75 percent of the foxes had taken bait, as determined by tetracycline marks in mandibular bones, DES had no effect on fox reproductive performance.

Goretzki et al. (1979) studied the potential of a 2- × 4-cm cylindrical tallow bait as a vehicle for antifertility agents and assessed bait uptake by incorporating radioactive iron (Fe-59) into each bait. Baits were placed in groups of four near dens and on fox crossings at an overall bait density of 3 baits/km^2. Measurement of radioactivity of liver, spleen, and kidney samples of killed foxes showed bait uptake to be 68 percent (n = 40).

Recently, the Commonwealth Scientific and Industrial Research Organization (known universally by its acronym, CSIRO) Division of Wildlife and Ecology in Australia started a project to develop an immunocontraceptive vaccine for fox control, whereby a fox sperm-specific antigen would either be vectored in a vaccinia recombinant vaccine or as a non-viral vectored antigen encapsulated in microsphere particles. In both instances, the vaccine would have to be delivered by bait; corresponding studies are currently being undertaken (World Health Organization 1993a).

Oral Vaccination Against Rabies

It became apparent in the early 1970's that red foxes could be immunized against rabies using live attenuated vaccine given by direct oral instillation (Baer et al. 1971, Debbie et al. 1972, Mayr et al. 1972, Black and Lawson 1973). This early work has resulted in widespread application of vaccine baits in Europe and North America. The various baits that have been developed, their field evaluation, and the different baiting strategies that have been utilized are reviewed below.

Baits

The first successful immunizations of captive foxes with baits were done with vaccine inoculated into eggs; vaccine-impregnated dog biscuits coated with a mixture of tallow, paraffin, and sardine oil; and a commercial smoked beef sausage containing a plastic tube filled with vaccine (Debbie 1974, Winkler et al. 1975, Winkler and Baer 1976). Winkler et al. (1975) also tested placebo dog biscuits in the field (table 1), while eggs were abandoned as a delivery device for oral vaccine out of concern about foxes caching rather than consuming them. In Europe, baits based on ground meat and chicken heads were field-tested with good results (Wandeler et al. 1975, Manz 1976), and the latter were used in many studies aimed at orally immunizing captive foxes (e.g., Dubreuil et al. 1979, Häfliger et al. 1982). In Canada, Black and Lawson (1980) immunized 44–80 percent of captive foxes with vaccine contained in plastic pouches covered with fish oil. In later trials, a polyurethane sponge, coated with several layers of a beef fat and wax mixture and injected with 14 mL of vaccine, successfully immunized a high proportion of captive foxes (Lawson et al. 1987). The above bait was developed by the Ontario Ministry of Natural Resources, which has tested numerous bait types in captivity and in the field (Johnston and Voigt 1982, Bachmann et al. 1990, Johnston et al. this volume). Unfortunately, as with many other bait tests conducted in Europe, the results of most tests were restricted to internal reports; only limited data were ever published (see Ruette [1993] for a review of French and Canadian internal reports and Winkler [1992] and Winkler and Bögel [1992] for reviews of the development of oral rabies vaccination techniques).

The majority of the early European studies that used vaccine-laden baits emphasized vaccine performance rather than bait efficacy even though a bait that effectively delivers the vaccine to the target organ is a prerequisite for successful oral immunization (Wandeler 1991). Nevertheless, with about 74 million baits distributed in Europe by the end of 1994 (K. Stöhr, pers. comm.), more data on bait development might have been expected. However, excepting the use of chicken heads, all other baits distributed in Europe have been manufactured by or in close collaboration with companies pursuing a commercial interest. The manner in which oral vaccination campaigns evolved may therefore account for both the low number of field tests conducted with placebo baits and the lack of published data on such trials.

Before the first field trial with vaccine baits was initiated in Switzerland, chicken heads were tested in the laboratory and field (Wandeler et al. 1975). A vaccine container made from a polyvinylchloride (PVC) film and aluminum foil was developed and, when fixed under the scalp of a chicken head, was proven to deliver vaccine into the oral cavity of a fox chewing this bait (Häfliger et al. 1982). In October 1978 in the Swiss Alps, this system underwent its first field test in a mountain valley in the Canton of Valais, which was threatened by an advancing front of rabies. Field application of vaccine baits was subsequently repeated twice a year. Following the first campaigns, about 60 percent of foxes collected in the vaccinated area were found positive for the biomarker tetracycline that had been injected into each bait, and within a year epidemiologic data suggested that the treatment was successful (Steck et al. 1982).

Given the obvious success in the Canton of Valais, there was little incentive to investigate alternative baits or baiting strategies when other cantons began using this new approach to rabies control. Chicken-head baits were subsequently used with success in Germany when campaigns were begun in that country in 1983. However, as Germany's rabies situation required that very large areas be treated, the

Table 1. Disappearance rates of baits used in or intended for use in oral vaccination campaigns of red foxes

Bait type	Location[1]	Time	Bait density (no./km²)	No. of baits placed	Percent baits disappeared after 1d	2d	3d	4d	6d	7d	8d	9d	10d	14d	15d	Reference
Dog biscuit	USA, Florida[2]	June 72	?	273	58											Winkler et al. 1975
Ground meat	SWI, Bern[2]	Sept 72	?	210	49	71	82	90	98	99	100					Wandeler et al. 1975
	SWI, Bern[2,3]	Winter 72/73	?	120	38	70	81	88	97	98		99	100			
Chicken head	SWI, Bern[2,3]	Winter 72/73	?	120	33	72	81	87	95	97	98	99	100			Wandeler et al. 1975
	GER[2]	73–74	16–20	[4]5,327			43–78		58–92				70–95			Manz 1976
	SWI, Valais	Oct 78	12.1	150		63										Steck et al. 1982
		Oct 81	14.7	103	18	53										Capt and Kappeler, unpubl.
		Oct 82	16.5	107	17	41										
	SWI, Bern	Sept 83	14.7	260	23	38										Capt and Kappeler, unpubl.
	SWI, Bern[2,5]	May–Jun 88	15	147		61		79					95			Antognoli 1988
	SWI, Bern[2,6]	Sept–Oct 90	13.3	220		38		55					76			Kappeler 1990
	GER, Bavaria	Autumn 83	15.6	492										80		Schneider 1984 unpubl.
	GER, Baden	Autumn 83	16.7	524										84		
	GER, Hesse	Spring 83–85	15	3×360			39				61			81		Wachendörfer et al. 1986
		Oct 83–84	15	2×360			28				62			85		
	ITA, Brescia	Nov 84	10.7	551			26				42			61		Balbo and Rossi 1988
		May 85	10.7	537			32				68			85		
	BEL	Oct 87	40–50	250			21				42				70	Pastoret et al. 1988
Tübingen	GER	85–88?	15	?				30			60			80		Schneider 1989 unpubl.
	ITA	May–Jul 86	11	1,095			21				41			60		Balbo and Rossi 1988
		Mar–Apr 87	13	1,041			18				35			51		
	BEL	Sep 86	11	185			29				47			65		Brochier et al. 1988
		Jun 87	11	312			30				50			57		
		Sept 87	11	292			42				61			72		
	LUX	Sept 86	15	639			28				43			58		Frisch et al. 1988
		May 87	15	754			21				45			64		
		Sept 87	15	584			30				52			66		
	CZE	Spring 89–91	15–16	34,627										65		Matouch 1994 unpubl.
		Autumn 89–91	15–16	34,538										74		
	FIN	Sept 88	15	240			12				31			51		Nyberg et al. 1992
SFE–tallow[7]	SWI, Aargau	Apr 87	11.6	295		6		5d: 27					11d: 51			Antognoli 1988
Wusterhausen[8]	GER[2]	Apr 89	?	280			35		73			86			92	Stöhr et al. 1990b
	GER	Oct 89	16	500+			37			72				82		Stöhr et al. 1990a
	GER	89–90	16–20	10,367			35			61				75		Müller et al. 1993a
	GER, Brandenburg[2]	Apr 91	?	100			18			39				48		Müller et al. 1993a
Raboral V–RG	BEL	Oct 88	15	238			27				54				69	Brochier et al. 1990b
	FRA, V.d'Oise	May 92	20	220						63				83		Ruette 1993
	FRA, Essone	Jun 92	18	95		42				74				84		
Virbac	SWI, Bern[2,6]	Sept–Oct 90	13.3	220		38		55					76			Kappeler 1990 unpubl.
Rabifox Oral	SWI, Solothurn	Apr 91	15	148	43	67		88					97			Matter et al. 1991 unpubl.
Rabifox "Dessau"/ Altrofox 91	GER, Brandenburg[2]	Apr 91	?	400			43		75					93		Müller et al. 1993a

Lysvulpen	CZE	Spring 93	15–16	38,412		82	Matouch 1994 unpubl.
	CZE	Autumn 93	15–16	43,780		82	
Blister pack	ONT, Huron[2,9]	87	800	405	25–87		Bachmann et al. 1990

[1] USA = United States of America, SWI = Switzerland, GER = Federal Republic of Germany, ITA = Italy, BEL = Belgium, LUX = Luxembourg, CZE = Czech Republic, FIN = Finland, FRA = France, ONT = Province of Ontario, Canada.

[2] Tests done without vaccine.

[3] Comparative tests with chicken heads and baits made from ground meat.

[4] Baits were put out in groups of 5 at 814 bait stations in a total of 13 trials.

[5] Two comparative tests with chicken heads and other bait types (sausage, tallow/fishmeal bait) distributed in same area but not at same bait stations.

[6] Three comparative tests with chicken heads and Virbac Rabifox Oral baits distributed in same area but not at same bait stations.

[7] Bait based on 41 percent beef tallow, 41 percent paraffin, 12 percent Witepsol (wax), 5.2 percent water, 0.5 percent synthetic fermented egg attractant (Bullard et al. 1978). Approximately 5,500 baits were distributed in one campaign in Switzerland as part of a project of the Swiss Rabies Center aimed at replacing chicken heads with a bait that allowed for semiautomated production.

[8] Precursor of Rabifox "Dessau" / Altrofox 91; see table 2.

[9] Baits without and with different types of perforated and unperforated plastic bags placed in parallel rows with 35 m between baits and rows on small plots. Bait disturbance, defined as biting, chewing, or moving the cube, was signficantly higher for baits without bag (87 percent *v.* 25–78 percent).

World Health Organization Collaborating Centre in Tübingen investigated other bait types as an alternative to the labor intense preparation of chicken-head baits. This effort led to the development of a bait based on vegetable fat and fishmeal suitable for semiautomated production and containing a blister pack similar to that used for the chicken-head bait.

Following placebo bait trials in the fall of 1985 in Bavaria, the so-called Tübingen fox bait was exclusively adopted for use not only in Germany but also in many other European countries for several years (Schneider et al. 1988, Wilhelm and Schneider 1990).

A new bait, originally developed for raccoons (Hanlon et al. 1989), was introduced in 1988 along with a vaccinia:rabies recombinant vaccine, Raboral V–RG®. It consisted of fish oil and fishmeal bound by a synthetic polymer, ethylene vinyl acetate (EVA), known as Aquabind® (E. I. DuPont de Nemours). The vaccine, sealed in a plastic pouch, was held within the bait by a wax mixture (table 2, fig. 1). First used in Belgium in the autumn of 1988, it was found to be efficient both in captive and free-ranging foxes (Brochier et al. 1990a and b).

All other commercial baits introduced in Europe between 1989 and 1992 have resembled the Tübingen bait and been based on animal or vegetable fat and fishmeal, or meat and bonemeal, containing a blister pack as a vaccine container (table 2). The blister pack bait developed in Ontario in 1987 is similar to the above design but contains chicken "essence" rather than fish as flavor (Bachmann et al. 1990).

Although all of the above baits have been tested in captive animals and in pilot field trials, a questionnaire survey of all manufacturers revealed that very little information is available from these tests, especially those that were conducted to develop a certain bait matrix (table 3). Of special note is an evaluation carried out by the French wherein all three bait types used in France (Tübingen, Virbac Rabifox Oral®, Raboral V–RG) were compared in captive foxes. Each of 36 foxes received a single bait, and all but 1 consumed it. Independent of the bait type, only half of the foxes were protected from a virulent challenge given 34 days after bait consumption (Artois et al. 1993).

These findings may underline both the importance of sufficiently high bait densities in field trials to increase the chances of multiple uptakes by the same individual, and baits that deliver a high-titered vaccine efficiently to the target organ. Video surveillance of the behavior of captive foxes confronted with the Virbac Rabifox Oral or Raboral V–RG baits revealed that baits, though rarely rejected, were often bitten to pieces that were then consumed one after the other. Such feeding behavior may so significantly restrict exposure to the vaccine as to reduce its efficacy (Ruette 1993). In this regard, properly prepared chicken-head baits may have been advantageous over the early variants of the artificial baits in that thorough chewing is required before the blister pack or its remains can be separated from the bait.

Field Evaluation of Baits

The fate of baits during field tests has been monitored in two ways: by checking individually marked baits for disappearance (table 1), and by searching for the tetracycline biomarker in target and, less frequently, nontarget animals collected in the vaccination zone (table 4). While disappearance rates may give some information on the overall attractivity of a bait, it is usually hard to assess the percentage of baits that is taken by foxes. Identification of tooth imprints in empty blister packs found at baiting sites (Antognoli 1988, Matter et al. 1991 unpubl.), fecal markers incorporated into baits (Wandeler et al. 1975), and small radio-transmitters implanted in baits (Rosatte et al. 1991) have been used with some success.

In a comparative trial in Germany, the identification of tracks found at the (unprepared) site of bait placement allowed Müller et al. (1993a) to identify wild hogs *(Sus scrofa)* as important competitors that took 6–15 percent of all baits, while foxes accounted for 13–24 percent of Altrofox and 34 percent of Wusterhausen baits that were removed. Using tracks, Matter et al. (1991 unpubl.) were able to identify the species for 25 percent of Virbac Rabifox Oral baits taken: 14 percent had been removed by foxes, 9 percent by cats and dogs, and 2 percent by other species.

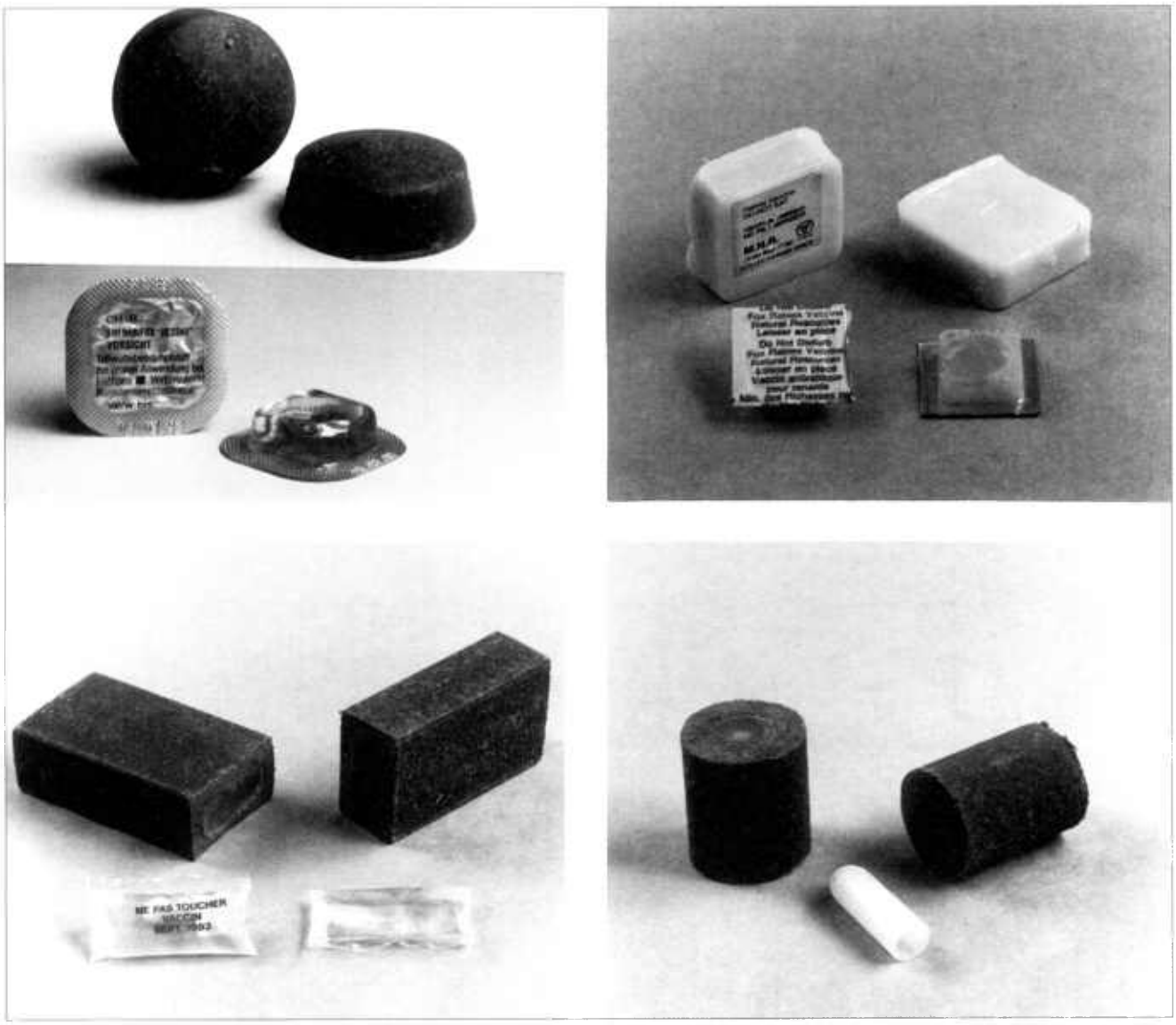

Figure 1. Examples of molded and extruded baits and vaccine containers used in oral vaccination campaigns of foxes and raccoons. Upper left: Rabifox 'Dessau' bait (41 × 15 mm); upper right: Ontario blister-pack bait (35 × 35 × 20 mm); lower left: Raboral V–RG bait as used for foxes in Europe (50 × 30 × 20 mm), lower right: cylindrical Raboral V–RG bait used for raccoons (28 × 35 mm).

In the Czech Republic, several thousand baits were checked in each campaign by the same hunters who had distributed them. Between 46 and 60 percent of the Tübingen baits and 68–71 percent of the Lysvulpen® baits that had disappeared were considered to have been taken by foxes (Matouch 1994 unpubl.). This is in sharp contrast to a French study, where 783 baits of three different types (Tübingen, Virbac Rabifox Oral, Raboral V–RG) were monitored with photo traps for 7 days. Foxes appeared on only 3 of 81 photos that were suitable for evaluation, 1 for each bait type (Ruette 1993). Other visiting species included mainly raptors, corvids, and cats for chicken heads, and hedgehogs *(Erinaceus europaeus)* for both artificial baits, which also received considerable attention from cattle.

Table 2. Characteristics of commercially produced baits used for the oral vaccination of foxes against rabies in Europe and Canada. Information was collected from manufacturers[1] by questionnaire survey in spring 1994[2].

	Chicken head[1]	Tübingen bait (Fuchsoral®)	Rabifox 'Dessau'/ Altrofox 91 bait	Raboral V–RG	Virbac Rabifox Oral
Shape	Chicken head	Rectangular tablet	Round tablet	Cube	Rectangular tablet
Dimensions (mm)	—	42 × 42 × 15	41 × 15	50 × 30 × 20	45 × 50 × 13
Weight (g)	30–80	17–20	14–17	35	27
Color	Meat	Dark brown	Dark brown	Brown	Dark brown
Bait matrix	Chicken head	Fishmeal coconut fat wax	Meat/bonemeal fish animal fat	Plant and animal protein, fish oil, aggregated by synthetic polymer	Fishmeal beef tallow
Manufacturing process	(Slaughter-house)	Molded, semiautomated	Molded, semiautomated	Extruded, automated	Molded, automated
Enhancers and additives	None	None	Animal fat	None	Fish flavor
Melting point of matrix	N/A	Approx. 42 °C	48–55 °C	250 °C	> 50 °C
Biomarker[3]	150 mg TC	150 mg TC	150 mg TC	150 mg TC	150 mg TC
Vaccine strain[4]	SAD_{BERN} SAD B19 (GER, ITA)	SAD B19	SAD P5/88	VVTGgRAB = V–RG (Vaccinia rec.)	SAG–1 SAG–2
Typical titre ($TCID_{50}$/dose)[5]	$10^{7.3}$	10^7, min. 10^6	10^7, min. $10^{6.2}$	$>10^{6.5}$	$>10^8$
Vaccine container, material[6]	Blister, A/PVC	Blister, A/PVC	Blister, A/PP	Pouch, P	Blister, A/PVC
Volume of vaccine (mL)	1.8	1.5	1.8	2.5	1.8
Storage requirements for bait	4 °C	–20 °C	–20 °C	4 °C or ambient	–20 °C
Shelf life	2–4 days	1(–2) years	2 years	18 months	6–12 months
Warning labels on bait on vaccine container	 No Yes	 No Yes	 No Yes	 Yes Yes	 No Yes
First field release with vaccine Country[7], number of baits	Autumn 1978 SWI, 5,800	Autumn 1985 GER, 180,000	Autumn 1989/91 GER, 45,000	Autumn 1988 BEL, 6,000	Autumn 1990 FRA, 201,000
Countries using bait[7], years, method of bait distribution[8], number of baits used[9,10]	SWI, 1978–90 MH 1,300,000 GER, 1983–85 M 476,000 ITA, 1984–85 M 15,000 FRA, 1987 M 3,200	GER, 1985–MF AUS, 1986–M BEL, 1986–92 MH FRA, 1986–92 MH ITA, 1986– M LUX, 1986–91 MH FIN, 1988– MF NET, 1988–? M SVN, 1988–M CZE, 1989–92 M HUN, 1992–F POL, 1993–F	GER, 1989–MF 12,000,000 (improved bait since 1991)[1]	BEL, 1988–FH 1,000,000 FRA, 1989–H 3,000,000 + LUX, 1992–FH 300,000	FRA, 1990–H 3,000,000 SWI, 1991–M 455,000
Total up to 1993	1,794,200	[10]34,646,000	12,000,000	4,300,000 +	3,455,000

	Lysvulpen[1]	Kamark	Meatball bait[11,12]	Sponge bait[12]	Ontario blister-pack bait
Shape	Half-oval tablet	Round tablet	Meatball	Cube	Cube
Dimensions (mm)	50 × 35 × 17	40 × 20	?	40 × 40 × 30	35 × 35 × 20
Weight (g)	18–20	36 ± 6	30	41 (+ attractant)	17–20
Color	Brown/grey	Brown/grey	Meat	Caramel	Pale yellow to beige
Bait matrix	Beef tallow Fishmeal	Beef tallow Fishmeal Dried milk	Ground meat, placed in polyethylene bag	Polyurethane sponge coated with mixure of paraffin, tallow, microbond wax	59% tallow 32% microbond wax 8% mineral oil 1% chicken flavor essence
Manufacturing process	Molded, semiautomated	Molded, semiautomated	Manual	Sponge dipped in mixture	Molded, automated
Enhancers and additives	Yes	None	?	Beef liver slurry or ground beef, added to cube in plastic bag	Platic bag in some early trials[1]
Melting point of matrix	40 °C	45 °C	N/A	?	60 °C
Biomarker[3]	150 mg TC	100 mg CTC	TC	75–140 mg TC	100–150 mg TC
Vaccine strain[4]	SAD_{BERN}	Vnukovo–32/107	No vaccine	$ERA–BHK_{21}$	$ERA–BHK_{21}$
Typical titre ($TCID_{50}$/dose)[5]	10^7	$>10^{5.6}$ $MICLD_{50}$[5]	N/A	?	$10^{7.0}$–$10^{7.5}$ $TCID_{50}$
Vaccine container, material[6]	Blister, A/PVC	Blister, PS	N/A	PUS	Blister, PVC/PF
Volume of vaccine (mL)	1.8	2.0	N/A	14.0	1.8–2.0
Storage requirements for bait	–20 °C	–18 °C	?	–20 °C	–30 °C
Shelf life	120 days	?	?	?	> 1 year
Warning labels on bait on vaccine container	 No Yes	 No Yes	 ? N/A	 On plastic bag No	 Yes Yes
First field release with vaccine country[7], number of baits	Spring 1992 CZE, 245,000	Spring 1992 SVK, 30,000	? ONT, ?	Autumn 1985 ONT, 10,700	Autumn 1987 ONT, 27,700
Countries using bait[7], years, method of bait distribution[8], number of baits used[9]	CZE, 1992–M 2,426,000	SVK, 1992–M 230,000	ONT, 1976–? F 150,000 ?	ONT, 1985–86 F 25,700	ONT, 1987–F 3,100,000
Total up to 1993	2,426,000	230,000	150,000 ?	[13]25,700	[13]3,100,000

[1] Manufacturers of baits and vaccines:

Chicken head baits were produced locally by veterinary and wildlife services. SAD_{Bern} was produced by the Swiss Rabies Centre, University of Bern, Laenggass-Str. 122, 3012 Bern, Switzerland. SAD B19 was produced by the WHO Collaborating Centre in Tübingen (see below).

Tübingen bait, marketed as 'Fuchsoral' since 1992, was manufactured by Klocke Pharma-Service GmbH, PSF 1140, Rudolf-Diesel-Str., 73356 Weingarten, Germany. SAD B19—originally produced by WHO Collaborating Centre for Rabies Surveillance and Research, Federal Research Centre for Virus Diseases of Animals, P.O.Box 1149, 72001 Tübingen, Germany—is currently produced by Impfstoffwerk Dessau–Tornau (see below).

Rabifox 'Dessau'/Altrofox 91 bait (introduced in 1991) was developed in a collaborative effort by Impfstoffwerk Dessau–Tournau GmbH, PSF 214, 06855 Rosslau, Germany, and Altromin/Sana–Service GmbH, Knobelsdorffallee 26, 06847 Dessau–Mosigkau, Germany. It is produced by Impfstoffwerk Dessau–Tornau. In 1989, the vaccine had been used in 45,000 "Wusterhausen" baits, which were based on fish oil, fishmeal, fat, paraffin, and propylene glycol (Müller 1993a).

Raboral V-RG is produced by Rhône–Mérieux SA, 29, Avenue Tony Garnier, BP 7123, 69348 Lyon Cedex 07, France.

Virbac Rabifox oral is produced by Laboratoires Virbac, BP 27, 06511 Carros Cedex, France.

Lysvulpen is produced by Bioveta, Komenského 212, 68323 Ivanovice na Hané, Czech Republic.

Kamark is produced by Mevak a.s., Biovetská 32 p.s.29/c, 94991 Nitra, Slovak Republic.

Meatball bait was produced by the Ontario Ministry of Natural Resources (OMNR), Wildlife Research, P.O. Box 5000, Maple, ON L6A 1S9, Canada.

Sponge bait: The bait was produced by OMNR (see above); the vaccine was produced by Connaught Laboratories Ltd, 1775 Steeles Ave. West, Willowdale, ON M2R 3T4, Canada.

Ontario blister-pack bait: The vaccine and the bait were originally produced by Connaught (see above). Since 1992, both vaccine and bait have been produced by Langford, Inc., 400 Mitchener Road, Guelph, ON N1K 1E4, Canada.

[2] Additional bait types are or were used in Belarus (fish, chicken heads, injected with Bye1NIIEV-VGNKI strain; Kovalev et al. 1992), in Lithuania since 1992 (pieces of fish, injected with EVHTI-VVMK71, a derivative of the Vnukovo strain; Petkevicius 1993), in Latvia since 1993 (Vnukovo strain, bait?), and in Russia (SIP-RB-71, derived from Pasteur strain, in chicken heads) (Matouch pers. comm.).

[3] CTC = chlorotetracyline, TC = tetracycline (hydrochloride salts).

[4] References for vaccine strains: SAD_{Bern}: Häfliger et al. (1982), SAD B19: Schneider and Cox (1983), SAD P5/88: Sinnecker et al. (1990), SAG-1: Leblois et al. (1990), SAG-2: Lafay et al. (1994), VVTgRAB: Kieny et al. (1984), ERA: Lawson et al. (1987, 1992).

[5] $TCID_{50}$ = 50-percent Tissue Culture Infective Dose; $MICLD_{50}$ = 50 percent Mouse Intracerebral Lethal Dose.

[6] Material used for blisters: A/PP = aluminum and polypropylene, A/PVC = aluminum and polyvinyl chloride, P = plastic sheet, PS = polystyrene, and PUS = polyurethane sponge, and PVC/PF = PVC and paper foil.

[7] Country abbreviations: AUS = Austria, BEL = Belgium, CZE = Czech Republic, GER = Federal Republic of Germany, HUN = Hungary, ITA = Italy, LUX = Luxembourg, NET = Netherlands, ONT = Province of Ontario, Canada, POL = Poland, SVK = Slovak Republic, SVN = Slovenia, SWI = Switzerland.

[8] F = by fixed-wing aircraft, H = by helicopter, M = manually by ground crew (usually with car).

[9] Total number of baits used, based on data provided by manufacturers. Estimates, indicating the minimum number used, are marked "+."

[10] The number of Tübingen baits used in individual countries: GER 20,946,000, AUS 4,100,000+, BEL 450,000, FRA 1,600,000, ITA 160,000+, LUX 450,000+, FIN 320,000+, NET 5,000+ ?, SVN 360,000+, CZE 1,435,000, HUN 320,000, POL 1,400,000. In addition, Tübingen baits with and without vaccine and with the anthelmintic Praziquantel were used in six field trials aimed at controlling *Echinococcus multilocularis* in fox populations in Bavaria, Germany, between 1989 and 1991 (Schelling et al. 1991).

[11] Meatball bait was never used with vaccine but was extensively tested in the field (Johnston and Voigt 1982).

[12] According to Bachmann et al. (1990) and Johnston and Voigt (1982). (No information was received through questionnaire.)

[13] Additional baits without vaccine were distributed in baiting trials in 1984 (sponge bait) and from 1987 onward with blister-pack bait (Bachmann et al. 1990).

Table 3. Evaluation of baits used for the oral vaccination of foxes against rabies in Europe and Canada. Information collected from manufacturers[1] by questionnaire survey in spring 1994.

	Chicken head[1]	Tübingen bait (Fuchsoral)	Rabifox 'Dessau'/ Altrofox 91 bait	Raboral V–RG	Virbac Rabifox Oral
Evaluation of baits: captive animals					
Captive animals in which baits were tested to determine attractivity (placebo or vaccine baits)	red fox	red fox	red fox dog wild boar	red fox dog	red fox
Test type: preference (A) or acceptance (B)	B	A, B	A, B	A, B	A, B
Bait matrix changed due to test	No	Yes	Yes	No	No
Vaccine container changed due to test	No	No	No	No	No
Captive animals in which final product was tested	Red fox	Red fox	Red fox dog	Red fox	Red fox dog
Responses measured[2]	VNA, CR, TC	VNA, CR, TC	VNA, CR, TC	VNA, CR, TC	VNA, CR
Evaluation of baits: field tests					
Pilot studies in field done prior to release of final product	Yes	Yes	Yes	Yes	Yes
Method of bait distribution	Ground	Ground, aerial	Ground, aerial	Ground	Ground, aerial
Responses measured[3]	DR,SI	DR,TC-T,TC-NT	DR,SI,TC-T	DR,SI,TC-T,TC-NT	DR,SI,TC-T,TC-NT
Bait or vaccine container modified due to tests	No	No	Yes, melting temp. of matrix	No	No
Suitability and conditions for aerial distribution tested	Yes	Yes	Yes	Yes	Yes
Altitude	<= 100 m	<= 100 m	<= 100 m	300 m	60–100 m
Groundspeed (km/h)	80–120	120–180	120–180	300	180–200
Losses, surface	< 10 %, prairie < 30 %, gravel	< 1 %, prairie	< 1 %, ?	0 %, tarmac	4 % (*n*=50), pavement
Bait or vaccine container modified after release of final product into the field	Yes, shape and size of blister	Yes, shape of blister	Yes, matrix and shape of bait	Yes	Yes, melting temp. of bait matrix
Reason for modification	Improve bait, manufacturing	Manufacturing process	Manufacturing process	Improve bait, manufacturing	Improve bait, manufacturing

Table 3—Continued

	Lysvulpen	Kamark	Meatball bait[4]	Sponge bait[5]	Ontario blister-pack bait
Evaluation of baits: captive animals					
Captive animals in which baits were tested to determine attractivity (placebo or vaccine baits)	Red fox	None	Yes	?	Red fox striped skunk raccoon
Test type: preference (A) or acceptance (B)	B	—	?	?	A; additional tests with same bait, different attractants
Bait matrix changed due to test	No	—	?	?	Yes
Vaccine container changed due to test	No	—	—	?	No
Captive animals in which final product was tested	Red fox	Red fox	—	Red fox striped skunk raccoon	Red fox
Responses measured[2]	VNA, CR, TC	TC	—	VNA	VNA, TC
Evaluation of baits: field tests					
Pilot studies in field done prior to release of final product	No	Yes	Yes	Yes	Yes
Method of bait distribution	—	Ground	Aerial	Aerial and ground	Aerial and ground
Responses measured[3]	—	DR, TC-T	TC-T	DR, TC-T, TC-NT	DR, TC-T, TC-NT, caching of bait
Bait or vaccine container modified due to tests	—	No	?	?	Yes, smaller bait of 17 instead of 20 g
Suitability and conditions for aerial distribution tested	Preliminary trials indicate suitability	No	Yes	Yes	Yes
Altitude (m)		—	?	100	[5]100
Groundspeed (km/h)		—	?	130	[5]130
Losses, surface		—	?	?	?
Bait or vaccine container modified after release of final product into the field	No	Yes	Bait never used with vaccine	Replaced by blister-pack bait	Yes, different oil (92) lower weight (93)
Reason for modification	—	Manufacturing process	—	Manufacturing process	Improve bait

[1] See table 2, footnote 1 for names of manufacturers.

[2] CR = challenge resistance, TC = biomarker as proof of bait uptake (tetracycline), VNA = virus neutralizing antibodies.

[3] DR = bait disappearence rate; SI = species identification based on tracks, tooth marks in blisters, etc.; TC-NT = biomarker (tetracycline) in nontarget species; TC-T = biomarker in target species.

[4] Meatball bait was never used with vaccine but was extensively tested in the field (Johnston and Voigt 1982).

[5] Information from Lawson et al. (1987) and Bachmann et al. (1990); no information received through questionnaire.

Table 4. Percentage of foxes positive for tetracycline (TC) after manual ground distribution or aerial distribution by helicopter or fixed-wing aircraft of baits containing oral rabies vaccine as well as TC as a biomarker

Bait type	Location[1]	Time	Bait density (no./km²)	Method of bait distribution	Sampling period[2]	Age[3]	*n*	% TC positive (range)	Reference
Chicken head	SWI, mountain valleys	1978–90	13.6	Ground	1–240, summer	Juv	[4]282	36	Kappeler 1991
						Ad	[4]222	64	
					1–240, winter	Subad	[41]1,592	59	
						Ad	[41]1,016	71	
	SWI, plateau and Jura hills		13.1	Ground	1–240, summer	Juv	[4]516	34	Kappeler 1991
						Ad	[4]420	76	
					1–240, winter	Subad	[42]2,024	63	
						Ad	[41]1,252	78	
	GER	1983–85	15	Ground	31–180, all year?	?	[42]2,231	75 (68–90)	Schneider et al. 1988
	ITA	Nov 84	10.7	Ground	30–170, winter	?	[5]46	63	Balbo and Rossi 1988
		May 85	10.7	Ground	30–170, summer	?	[4]21	43	
Tübingen	GER	1985–86	15(–20)	Ground	31–180, all year?	?	[41]1,278	73 (65–81)	Schneider et al. 1988
		Spring 87	15	Ground	?	?	[5]208	90	Schneider and Cox 1988
	ITA	Spring 86	11	Ground	30–170, summer	?	[5]199	42	Balbo and Rossi 1988
		Spring 87	13	Ground	30–170, summer	?	[4]107	36	
	BEL	Sept 89	15	Ground	15–240, winter	?	[4]97	61	Brochier et al. 1991
		June 90	14	Aerial	1–160, summer	Juv	[4]55	49	
						Ad	[4]31	80	
	AUS	1986–91	16	Ground	All year	?	[47]7,053	>70	Kissling and Gram 1992
	LUX	Autumn 86	15	Ground	24–90, winter	?	[5]157	62	Frisch et al. 1988
		Spring 87	15	Ground	22–65, summer	?	[4]114	71 (juv: 56)	
	CZE	Spring 89–91	15–16	Ground	Summer	?	[4]831	74 (56–83)	Matouch 1994 unpubl.
		Autumn 89–91	15–16	Ground	Winter	?	[41]1,106	68 (53–73)	
	FIN	1988–89	15	Ground/aerial	30–210, all year	?	[4]56	88	Nyberg et al. 1992
	FRA[6]	1989–92	13	Aerial	Summer	Juv	[4]26	46	Masson et al. 1993 unpubl.
						Ad	[4]31	90	
					Winter	Subad	[4]26	50	
						Ad	[4]18	67	
Wusterhausen	GER	Autumn 90	?	Ground	Winter	?	[5]949	78	Müller et al. 1993b
		Autumn 90	17.5	Aerial	Winter	?	[51]1,880	78	
		Spring 91	17.9	Aerial	Summer	Ad+Juv	[42]2,723	67	
Raboral V–RG	BEL	Oct 90	14	Aerial	1–200, winter	?	[4]153	76	Coppens et al. 1992
		May 91	15	Aerial	1–150, summer	?	[4]188	61	
		Oct 91	15	Aerial	1–70, winter	?	[4]66	71	
	FRA[6]	1990–92	13	Aerial	Summer	Juv	[4]114	33	Masson et al. 1993 unpubl.
						Ad	[4]83	81	
					Winter	Subad	[4]43	86	
						Ad	[4]53	85	
Virbac Rabifox Oral	FRA[6]	1991–92	13	Aerial	Summer	Juv	[4]28	32	Masson et al. 1993 unpubl.
						Ad	[4]40	75	
					Winter	Subad	[4]64	88	
						Ad	[4]96	94	

Table 4—Continued.

Bait type	Location[1]	Time	Bait density (no./km²)	Method of bait distribution	Sampling period[2]	Age[3]	*n*	% TC positive (range)	Reference
Rabifox 'Dessau'/ Altrofox 91[7]	GER, Brandenburg	Apr 91	18	Aerial	Summer	?	[5]79	67	Müller et al. 1993a
Lysvulpen	CZE	Autumn 92	15–16	Ground	Winter	?	[4]1,992	84	Matouch 1994 unpubl.
		Spring 93	15–16	Ground	Summer	?	[4]1,266	76	
Meatball[7]	ONT	Oct, Feb	35	Aerial	?	?	210	70	Johnston and Voigt 1982
		Oct 76/77/80	18–48	Aerial	1–28, Autumn	All	?	48–70	MacInnes 1988
Sponge	ONT	Sept 84–86	17–19	Aerial	1–28[8]	All	[5]275	63 (61–64)	Bachmann et al. 1990
Blister pack	ONT	Sept 87	17–25	Aerial	1–28[8]	All	[5]93	59 (50–67)	Bachmann et al. 1990
	ONT, Toronto[9]	Autumn 89–91	[9]50–70	Ground	?	All	[4]72	72	Rosatte et al. 1992
	ONT, Cambridge	Autumn 90	20	Ground	?	All	[5]180	35	Rabies Res. Unit 1991
	ONT	Oct 90	21.6	Aerial	20–, winter	All	[4]675	67	

[1] See table 1, footnote 1 for country abbreviations. AUS = Austria.

[2] Timeframe (days after bait distribution) and season in which animals were collected. Summer refers to sampling after campaigns in spring, winter to sampling after campaigns in autumn. Although not specified in all references, foxes positive for rabies were usually excluded from the samples.

[3] Age of foxes: Ad = adult (>1 year old), Juv = juvenile, Subad = subadult, All = all age classes.

[4] Sample comprises animals from areas that have been baited previously.

[5] Sample from area that has been treated only once.

[6] The French study compared the performance of three different bait types. The data selected for this table show the results from areas vaccinated three times with the same bait type.

[7] Without vaccine.

[8] Evaluation limited to foxes trapped within 28 days of bait drop because resident population was "diluted" by dispersal (small experimental plots).

[9] Baits distributed along creeks and rivers in metropolitan Toronto, 50–70 baits per kilometer of ravine, and at den sites (20 baits/den).

Gürtler and Zimen (1982) used the same method to evaluate chicken heads and found that 30–55 percent of the baits were removed by foxes. Conversely, Aubert (Ruette 1993) calculated that given a bait density of 13/km^2 and a fox home-range size of 3 km^2, only 3.5 percent of all baits would need to be consumed by foxes to reach 75 percent of the fox population, as determined by foxes positive for tetracycline.

Aside from the consumption by obvious competitors such as mustelids, wild hogs, dogs, and cats, a significant proportion of baits may be partially consumed by rodents, snails, and carnivorous insects. Kappeler (1990 unpubl.) found that 12–16 percent of chicken heads distributed in early autumn and checked for 10 days had signs of consumption by insects, while 24–38 percent of Virbac Rabifox Oral baits had been partially eaten by rodents and 23–29 percent, by snails. During an observation period of 14 days, about 30 percent of Tübingen baits (Brochier et al. 1988) and 15–67 percent of Raboral V–RG baits exposed in the field for 2 to 21 days were gnawed by rodents (Ruette 1993). However, partial consumption usually does not preclude a later uptake of the bait by carnivores.

For 2–8 days after their distribution, Bachmann et al. (1990) searched the ground for baits air-dropped in plastic bags. Between 42 and 53 percent of sponge baits (search effort of 8 days) and 11–36 percent of blister pack baits (2 days) had been contacted by animals. Crows had removed 63–87 percent and foxes 10–28 percent of contacted sponge baits. Blister-pack baits dropped without bags could not be located on the ground.

In some instances, only the bait matrix is consumed; an intact blister is left behind. In Swiss trials of chicken heads, 2 percent of blister packs were found intact (Capt and Kappeler, unpubl. data). In some early field trials, Schneider (1984 unpubl.) reported that 15–17 percent of the blister packs in chicken-head baits were found intact but attributed the high rate to poor bait quality. Intact blister packs were found for 7.5–16 percent of 639 Tübingen baits in Belgium (Brochier et al. 1988) and for 3.2 percent of Wusterhausen baits tested in Germany (Stöhr et al. 1990a).

The most widely used technique to monitor bait uptake by target and nontarget species has been the detection of tetracycline in thin sections of mandibular bones (most studies), femur (Capt 1981, Kappeler 1991), and calcified teeth (Bachmann et al. 1990). Tooth sections enable investigators to determine multiple bait uptake by juvenile and subadult animals, provided that ingestion occurred on different days. The method may also be used to determine the precise date of bait consumption provided the date of animal death is known (Johnston et al. 1987). While routinely used in Ontario, this technique has received only limited attention in Europe (Capt 1981, Hässig 1984, E. Masson, pers. comm.).

Tetracycline data are best used to compare samples collected and evaluated under identical conditions. However, direct comparisons between bait types and countries as shown in table 4 are difficult because such conditions may differ. Comparisons are further confounded because of missing information as to the time period over which animals were collected, the age composition of the sampled animals, and the number of previous baiting campaigns. Investigators who reported 90 percent tetracycline-positive animals (n = 208) following a single spring campaign (Schneider and Cox 1988) likely restricted their analysis to adult animals only or placed baits selectively at fox dens, or both. Using data from more than 13,000 foxes collected in areas treated with chicken-head baits in Switzerland between 1978 and 1990, Kappeler (1991) showed that repeated baiting campaigns, campaigns conducted in fall, use of high bait densities, and, with respect to juvenile foxes, campaigns carried out late in spring increased the percent of tetracycline-positive foxes. Adult foxes were more likely to have taken bait and so were animals from the Swiss Plateau rather than from less accessible mountain valleys.

Other species shown to compete for bait were stone marten *(Martes foina;* 46 percent marked), badgers (21 percent marked), and other small mustelids and domestic cats. However, the probable influence of these nontarget species on bait uptake by foxes could not be determined (Kappeler 1991). The above observations have been confirmed in part by

studies in France (Masson et al. 1993 unpubl.) and in Ontario, where the percentage of foxes positive for tetracycline increased with bait density in trials with 12.5 to 50 baits/km^2 (Rabies Research Unit 1991). Bait uptake by nontarget species was found in several other countries for various bait types as well, primarily in mustelids and wild hogs but also in a low percentage of sampled rodents and cervids (Schneider 1984 unpubl., Kalpers et al. 1987, Paquot et al. 1988, Stöhr et al. 1990b, Coppens et al. 1992, Müller et al. 1993a).

Baiting Strategies

Both temporal and spatial distribution strategies play an important role in maximizing the percentage of target animals that take baits. Foxes usually live in family territories within most of their geographic range and are opportunistic in their feeding behavior. Uniform distribution of baits is therefore more likely to give many individuals access to one or a few baits than would be the case with clusters of baits at bait stations (Hässig 1984), even when some habitat types are given preferential treatment. This concept was reflected from the onset of the bait distribution strategies used in Europe and Canada. The goal of 15 baits/km^2 set for the initial field trials in Switzerland served as a reference density for most European countries (tables 1 and 4). Higher densities of up to 25 baits/km^2 were sometimes used to control residual rabies foci and in areas where the fox population had increased significantly (Frost et al. 1985, U. Breitenmoser, pers. comm.).

Baits can be efficiently distributed by ground crews using vehicles, provided that an adequate road network is present, although bait placement (and camouflage) by hand rather than broadcasting baits from vehicles should be emphasized. In practice, manual distribution is usually carried out by game wardens, forestry personnel, and most often hunters, who distribute baits in their own hunting preserve at no or little cost to the state. The actual sites where baits are to be deposited are either predetermined and shown on a map, prepared by or in collaboration with local hunters, or by grids plotted on a map that serve as guidance. This distribution strategy was first employed in Switzerland (Steck et al. 1982, Kappeler 1991) and later adapted by Germany to meet specific local needs and hence was termed the "Bavarian model" (Schneider 1984 unpubl., Wilhelm and Schneider 1990). With slight modifications, it also served as a model for all the European countries where baits were distributed manually (Rigal 1987, Brochier et al. 1988, Frisch et al. 1988, Stöhr 1990a, Kissling and Gram 1992, Nyberg et al. 1992, Matouch 1994 unpubl.).

Ontario developed a system for the aerial distribution of potential vaccine baits long before embarking on oral vaccination campaigns (Johnston et al. 1988 and this volume); however, aerial bait distribution was uncommon in Europe until the late 1980's. From 1979 through 1984, the Swiss used helicopters in inaccessible mountain areas, but the vast majority of chicken head baits were distributed by ground crews (Kappeler 1991). In 1988, with increasingly larger areas to treat, France began using helicopters and soon completely abandoned ground distribution (Aubert et al. 1993). Residual foci of rabies in wetland areas first prompted the use of fixed-wing aircraft in Germany in 1988 (Schneider 1989 unpubl.). Because they provide the majority of field specimens for postvaccination surveillance, it was considered essential to keep hunters involved in bait distribution; thus aerial distribution was first considered a tool for emergency situations only. However, given the cost-effectiveness of aerial bait placement, both helicopter and fixed-wing aircraft have become increasingly popular in recent years in Germany as well as in other European countries (table 2). Baits are usually dropped from an altitude of 30–100 m at a groundspeed of 110–180 km/h. A helicopter or small aircraft can cover 750–1,000 km^2/d at a bait density of 13–25/km^2 and at 1–3 flight lines per km^2 (Brochier et al. 1991, Aubert et al. 1993, Müller et al. 1993b). Analysis of foxes from areas that were baited either by ground crews or by aircraft revealed no significant differences in the percentage of animals positive for tetracycline or rabies virus-neutralizing antibodies (table 4, Müller et al. 1993b).

Two baiting campaigns per year, usually in spring and fall, appear indispensable for the European rabies situation; some failures have been associated with a single campaign per year (Schneider 1989 unpubl., B. Brochier and R. Frisch, pers. comm.). Experiments were recently carried out in Germany in areas with a high incidence of rabies wherein baits were also dropped in summer to vaccinate young foxes before dispersal. Similarly, Switzerland is experimenting with an additional baiting campaign in early summer, where baits are placed specifically at fox dens (U. Breitenmoser, pers. comm.). Ontario currently carries out a single annual campaign, in the fall, with good success (Rabies Research Unit 1991).

We have not included in this review serologic or epidemiologic data used to evaluate bait efficacy because doing so inevitably involves at least one further component of oral vaccination programs, the vaccine. In the end, all baiting systems will be measured by the extent to which rabies is eliminated from a given area. Many insights can be expected from the French trials, where three different vaccination systems were used in the same country and evaluated by the same team (Masson et al. 1993 unpubl., Aubert et al. 1994). All the systems mentioned above can claim success in reducing or even eliminating rabies from areas of varying size, but many of them were also involved in cases where initial field trials failed to produce the expected result. Aside from baits and baiting systems, many other factors will exert an important influence on the outcome of oral vaccination campaigns. Thus, most likely it will always be difficult to determine which component(s) of oral vaccination determined the success or failure of efforts to control the disease in nature.

Arctic Fox *(Alopex lagopus)*

Population reduction of arctic foxes in Alaska, U.S.A., has been the primary focus of bait use for this species. This fox was introduced onto several islands in the Aleutian chain in about 1836, where it has been a serious threat to the indigenous avifauna (Bailey 1993). Between 1956 and 1986, poison baits were distributed on various islands to supplement other efforts directed at eliminating this unwanted predator. Bait types used included strychnine pellets imbedded in seal blubber, baits consisting of tallow, seal oil, beeswax, and the toxicant Compound 1080 (sodium monofluroacetate), as well as chicken eggs and fish, seal, and bird carcasses injected with Compound 1080. About 49,000 molded cone-shaped baits made of 90 percent beef tallow and 10 percent beeswax, each weighing 4.3 g and containing 4 mg of Compound 1080, were dropped on Kiska Island (324 km^2) in 1986, where a similar attempt at eliminating the arctic fox had failed in 1964. Within 6 days, 186 fox carcasses were recovered and many more animals were assumed to have died. Surveys in 1989 and 1992 revealed no sign of survivors (Bailey 1993).

Only recently has the baiting of arctic foxes received attention in the context of rabies control. Rabies, known in the north circumpolar region for more than a century, has often been reported in both sled dogs and arctic foxes (Crandell 1991). While dogs can usually be restrained for parenteral rabies vaccination, control of the disease in the arctic fox is much harder to achieve, given the enormous range that would have to be covered. However, oral vaccination of arctic foxes might provide at least some relief if applied in restricted areas around remote villages and industrial sites to protect humans from the disease. It was with this premise in mind that Follmann et al. (1988, 1992) successfully orally immunized captive arctic foxes against rabies. Six foxes were each given a 10-cm-long sausage bait inside of which was a sealed plastic straw with 1.4 mL of liquid SAD live attenuated rabies vaccine. All foxes accepted and consumed the bait within 1 hour. The size of the bait prevented foxes from swallowing it without thorough mastication and thus perforating the vaccine container. No other baits were tested; but according to E. H. Follmann (pers. comm.), arctic foxes will eat a variety of bait materials.

Rabies moved southward along the coast of Labrador and to the north of Newfoundland, Canada, in 1988. In response to this outbreak, 8,100 Ontario blister-pack baits (see red fox account) containing live attenuated ERA rabies vaccine were distributed by

helicopter (Johnston and Fong 1992). Both tetracycline and neutralizing antibodies against rabies were demonstrated in three arctic foxes recovered from one of the treated islands, proving uptake of baits by the target species (World Health Organization 1990b). However, before large-scale vaccination campaigns could be undertaken, a number of obstacles would have to be overcome, for example, the fact that freezing temperatures limit the time of year when a liquid vaccine can be used.

Jackals *(Canis adustus, Canis aureus,* and *Canis mesomelas)*

Livestock depredations have been the principal reason for using toxic baits to reduce jackal numbers. Technical literature describing specific baits and their application is sparse; however, Hey (1964) reported that, prior to World War II, glass capsules of "prussic acid" were placed in recently killed animals or in the wool about the neck region of live sheep. He also mentioned that strychnine was applied to the gut and intestines of freshly killed ungulates and stirred into the partly digested food and dung to kill black-backed jackals in South Africa. More recently, the humaneness and specificity of strychnine baits for jackal control have been questioned (Allan 1989, Brown 1988), and the use and abuse of strychnine in South Africa was the subject of a colloquium in 1986 (South African Veterinary Association 1986). Allan (1989) stated that 34–60 percent of livestock farmers regularly used poison baits to control mammalian predators. Brown (1988) believed that the widespread placement of poison baits and the poisoning of carcasses on farmlands were responsible for the decline of scavenging birds throughout southern Africa. However, the use of toxicants is more closely regulated at the present time. The effect of strychnine poisoning on wildlife in South Africa also has been discussed in some detail by Dobbs and Benson (1986).

Specific bait types and baiting techniques used in South Africa have been described. In the northern Transvaal, toxicants were delivered in 100-g meat cubes of beef, goat, or warthog *(Phacochoerus aethiopicus),* or in parts of their intestine. Such baits were placed around the carcasses of livestock which served as draw stations. A study of strychnine bait selectivity and efficacy for black-backed jackals was conducted in July 1985 and 1986 in the northern Transvaal wherein toxic meat pellets and coyote getters were placed every 100 m along 2- to 5-km transects. It was concluded that toxic baits were selective for jackals because civets *(Civettictis civetta)* were not susceptible to the dose levels used in jackal baits. Hazards to nontarget birds were eliminated by burying baits. Toxic baits were normally placed along farm roads and at watering holes at a density of about 10 baits/km. Widespread use and overuse of strychnine baits appeared to have reduced their efficacy (P.J.J. van Rensburg, pers. comm.).

Similarly in Natal, South Africa, sheep or cattle muscle meat and fat have been used to deliver strychnine and Compound 1080. Baits were placed along farm roads, tracks, and along fence rows. Toxicants were occasionally placed in sheep or lamb carcasses; the latter practice resulted in some nontarget species mortality. Legal restrictions in Natal control the type and use of toxicants. Their use is thought to be generally ineffective for reducing livestock losses, except perhaps for control of free-ranging domestic dogs (D. T. Rowe–Rowe, pers. comm.).

Foggin (1990) has briefly summarized the epidemiology of jackal rabies and the use of toxic baits for their control in Zimbabwe. Also in Zimbabwe, experimental baiting tests for black-backed jackals were undertaken in 1990 and 1991 with the objective of developing an oral rabies vaccine delivery system. Rotten offal was used as an olfactory attractant to increase bait discovery and uptake. Up to 70 percent of chicken-head baits were removed by jackals during the first night of bait exposure. Nontarget vertebrates removed about 10 percent of the baits, and bait consumption by beetles and millipedes was a problem in summer if baits were untouched by jackals during the first night of exposure. Captive jackals preferred meat and particularly chicken, but soft baits such as beef meat were swallowed intact.

Chicken heads containing a biomarker (tetracycline) were broadcast over a 250-km^2 area at an average density of 6.5 baits/km of roadway and about 6.3 baits/km^2. Baits were placed beneath vegetation or other cover to minimize their discovery by birds and exposure to direct sunlight. Sampling revealed that 72 percent of the jackals contained evidence of the biomarker. However, it was subsequently found that 37 percent of jackals collected countrywide contained a naturally occurring fluorescence in bones indistinguishable from that induced by tetracycline. Thus, the actual percent of the jackal population on the study area reached by baits was questionable (Bingham et al. 1992, 1993, and 1994; J. Bingham, pers. comm.).

Strychnine baits also have been used in some of the middle eastern countries to destroy jackals and foxes where rabies has been a problem, but documentation in the technical literature available to us is poor. Israeli scientists are currently developing oral rabies vaccine technology and baiting techniques for both golden jackals *(Canis aureus)* and red foxes (A. Shimshony, pers. comm.).

Coyote *(Canis latrans)*

The use of small baits to deliver toxicants for coyote control began after the introduction of strychnine in 1847 (Young and Jackson 1951), and continued in the United States until toxicant use was restricted by executive order (Nixon 1972). Baits were also used for delivery of single-dose toxicants (strychnine and Compound 1080) to reduce coyote depredations and to control rabies in Canada (Ballantyne and O'Donoghue 1954, Stewart 1972, Baril 1982, Dorrance 1992) and Mexico (Cocozza and Malaga Alba 1962, Brown 1983). Research to improve bait delivery to coyotes began with an interest in reproductive inhibition to reduce population densities and thereby livestock depredations. An initial field trial in 1963 to deliver baits containing the synthetic estrogen DES to female coyotes showed promise (Balser 1964). Linhart et al. (1968) explored various bait application procedures in subsequent field tests during 1964–67 but were unable to treat a sufficient segment of populations with the antifertility agent.

The executive order restricting toxicant use stimulated research during 1976–91 to evaluate and develop selective delivery of single-dose baits to coyotes. Those studies were directed toward increasing the proportion of coyotes that ingested baits by manipulating (1) the types of baits and associated odor attractants, (2) the distribution and placement of baits, and (3) the persistence of baits, which was largely related to consumption by nontarget animals.

Baits and Odor Attractants

Coyote preference for different types of small baits was first evaluated with captive animals by Tigner et al. (1981 unpubl.). Their pen tests with two sizes (6 g *v.* 12 g) of rendered beef tallow and lean horsemeat showed no effect for bait size but a 2× preference for tallow over horsemeat, as determined by order of consumption. Subsequent observations indicated that coyotes chewed three types of fat baits more than three meat baits before swallowing (Tigner et al. 1981 unpubl.), a finding that may have implications for delivery of certain contraceptives or vaccines.

Three field tests that compared coyote response, as measured by modifications of the scent-station technique (Linhart and Knowlton 1975), to several fat and meat baits showed a trend in preference for fat baits (table 5), although differences were generally not statistically significant. In a field test at Fort Bliss, TX–NM, U.S.A. (Feb. 1979) (table 5), coyotes consumed a greater percentage of the three fat baits (22 percent) than the five meat baits (14 percent) (G. E. Larson and J. R. Tigner, U.S. Department of the Interior, Denver Wildlife Research Center, unpubl. data). Consumption of baits by coyotes closely reflected visitation rates to bait stations and was relatively high in four studies (table 6). Tallow and lard baits used in field tests usually incorporated 10–25 percent beeswax to raise the melting point and hence increase bait persistence in warm conditions (Linhart et al. 1968, Servheen 1983, Knowlton et al. 1986).

The search for a more selective bait-delivery technique led to development of the Coyote Lure

Operative Device (CLOD), a passive mechanical delivery system based on coyote behavioral response to odors (Marsh et al. 1982). The CLOD is a ground-anchored, sealed plastic vial containing a sweet liquid solution (e.g., syrup and sugar), which can potentially serve as a carrier for vaccines, antifertility agents, toxicants, or physiologic markers (Ebbert and Fagre 1987, Scrivner et al. 1987, Stolzenburg and Howard 1989). Coyotes are attracted to CLOD's by a lure applied directly on top of the vial to elicit a biting response that exposes the liquid solution for ingestion. These three research teams also described and illustrated modifications of the CLOD. However, this device has not advanced beyond limited research use.

Odorous substances are routinely used to lure coyotes to foothold traps and sodium-cyanide ejectors (coyote-getter and M-44) (Young and Jackson 1951, Fagre et al. 1983). To improve efficacy and selectivity of coyote control techniques, several investigators have evaluated various odor attractants (table 7). One objective of these studies was to develop superior coyote attractants that could be formulated with consistent chemical and odor properties (Fagre et al. 1983, Turkowski et al. 1983). The studies resulted in development and field testing of several effective synthetic coyote attractants, such as CFA (synthetic monkey pheromone) (Linhart et al. 1977), SFE (synthetic fermented egg) (Bullard et al. 1978, Turkowski et al. 1983), FAS (fatty acid scent) (Roughton 1982), and TMAD (trimethylammonium decanoate) and WU-lure (TMAD plus sulfides) (Scrivner et al. 1987).

Odor attractants have been used to enhance delivery of small baits to coyotes by coating the baits, incorporation into baits, and placement near baits (Linhart et al. 1968, Tigner et al. 1981 unpubl., Servheen 1983, Guthery et al. 1984). Tigner's team used captive animals to compare acceptance of tallow baits with eight different odor attractants incorporated at concentrations of 1, 3, 5, 7, and 9 percent by volume, and found that the higher concentrations resulted in decreased palatability of baits. The Guthery team's field test suggested that coyotes preferred beef-tallow baits with 1 percent aldehydic, fruity, and scatologic fractions of SFE over unscented tallow (table 5). Most field tests of bait efficacy have used odor attractants placed adjacent to baits to increase their attractiveness to coyotes (tables 6 and 8). However, the relatively low percent of coyotes that ingested baits during many field tests (table 8) was likely related to the animals' failure to detect the baits. The development of more effective odor attractants for use with baits may be the key to enhanced delivery.

A complete review of the research aimed at developing synthetic coyote olfactory attractants is beyond our scope. Comprehensive summaries of the basis and criteria for development of coyote olfactory attractants were provided by Bullard (1982, 1985) and Fagre et al. (1983). Further review of studies that compare coyote attractants may begin with the references in table 7.

Recent field tests of the CLOD have evaluated the effectiveness of specific attractants to stimulate the key biting–licking response required to deliver the fluid solution. Ebbert and Fagre (1987) found that CLOD's scented with CDCL (Carman's Distant Call Lure) and WU-lure had greater visitation by coyotes than CLOD's with SFE and Mast's #6. Two other field tests had relatively low visitation by coyotes with no detectable differences among WU-lure (8 percent), SFE (7 percent), and a fetid lure (6 percent) in New Mexico (Stolzenburg and Howard 1989), and among CDCL (10 percent), FAS (8 percent), and WU-lure (7 percent) in Colorado (Hein 1992). The percentage of CLOD's activated by visiting coyotes was relatively low compared with consumption of baits (table 6) and differed among the three field tests. During Ebbert and Fagre's (1987) study in Texas, the percentage of visiting coyotes that ingested the contents of CLOD's averaged 42 (n = 81), and the rate was similar to the percentage activating M-44's, and also similar among the four attractants tested. Stolzenburg and Howard (1989) reported that 55 percent of visiting coyotes (n = 237) activated CLOD's in New Mexico, but the percent of activation was markedly less for CLOD's scented with SFE (26 percent) than with WU-lure (66 pecent) or a fetid lure (78 percent). In contrast, only three CLOD's were activated during 213 coyote visits in Hein's (1992) study in Colorado, a result the investigator attributed to seasonal effects.

Table 5. Rank of coyote preference for different baits based on consumption rate in field tests using modifications of scent-station technique (Linhart and Knowlton 1975)

	Reference, location (U.S.A.), time		
	G. E. Larson and J. R. Tigner, unpublished data Fort Bliss, TX–NM	Guthery et al. 1984 King and Knox counties, TX Oct–Apr 1980–82	Servheen 1983 Goliad and Refugio counties, TX May–June 1981
Beef abdominal fat	2		
Beef tallow (unscented)	1	5	[2]2
Beef tallow (aldehydic odor)[1]		2	
Beef tallow (fruity odor)[1]		1	
Beef tallow (scatologic odor)[1]		3	
Beef tallow (fishy odor)[1]		6	
Beef tallow (mink[3] musk odor)[1]		7	
Lard	3		[2]1
Lean beef meat	4		
Hamburger meat (30% fat)	6		
Horsemeat	5	9	[2]3
Sheep meat	8	4	
Jackrabbit *(Lepus* sp.) meat	7		
Angora goat meat		8	

[1] Odor fractions of SFE (synthetic fermented egg) were incorporated into tallow at 1 percent by volume.

[2] All baits were rolled in fishmeal.

[3] *Mustela vison.*

Table 6. Field tests of bait placement methods using modifications of scent-station technique (Linhart and Knowlton 1975)

Location (U.S.A.) and time	Type of attractant[1]	Bait placement method[2]	% baits visited by coyotes *(n)*	% baits consumed/ coyote-visit	Reference
Fort Bliss, TX–NM Feb 1980	None	Surface	26 (178)	83	G. E. Larson and J. R. Tigner, unpubl. data
	None	Elevated (30–40 cm)	11 (178)	58	
	SFE	Surface	30 (177)	87	
	SFE	Elevated (30–40 cm)	18 (176)	58	
	SFE	Buried (1–2 cm)	23 (177)	63	
Sevilleta National Wildlife Refuge, NM Jan–Feb 1981	None	Surface	17 (203)	94	G. E. Larson and J. R. Tigner, unpubl. data
	None	Elevated (30–40 cm)	10 (202)	84	
	SFE	Surface	21 (201)	93	
	SFE	Elevated (30–40 cm)	13 (200)	84	
	SFE	Buried (1–2 cm)	11 (201)	64	
Fergus Co., MT Sept–Nov 1981	SFE	Surface	8 (591)	94	Baril (1982)
	SFE	Elevated (45.7 cm)	5 (562)	90	
	SFE	Buried (1–2 cm)	6 (554)	84	
Goliad and Refugio counties, TX July 1981	CDCL	Surface	44 (233)	70	Servheen (1983)
	CDCL	Elevated (50.8 cm)	43 (225)	70	
	CDCL	Buried (5–7.5 cm)	37 (238)	43	
	CDCL	Covered[3]	46 (228)	70	

[1] Placed adjacent to bait to attract coyotes; SFE = synthetic fermented egg; CDCL = Carman's Distant Call Lure.

[2] Beef tallow (9.5 g) used a bait in all tests, except for lard (28.3 g) by Servheen (1983).

[3] Bait placed on surface and covered with indigenous materials.

Table 7. Some representative studies of the efficacy of odor attractants for coyotes

Location (U.S.A.) and time	Method of study	Types of attractants tested	No. of attractants compared	Attractants with greatest preference during study	Reference
4 Western States No date	Scent-station procedures	Synthetic, fetid, commercial	≥58	SFE, various others	Turkowski et al. 1979 Turkowski et al. 1983
Hopland, CA 1972–81	Observations of captives	Synthetic, urine	≥30	W-U lure, TMAD	Fagre et al. 1983 Scrivner et al. 1987
Colorado 1982–85	Capture devices	Synthetic, fetid, commercial, urine	45	W-U lure, sheep liver extract, CDCL	Graves and Boddicker 1987
Southern Texas 1982–86	Capture devices	Synthetic, fetid, commercial, urine	3	CDCL	Windberg and Knowlton 1990
Southern Texas 1984	Scent-station procedures	Synthetic, commercial	4	W-U lure, CDCL	Martin and Fagre 1988
Millville, UT 1988–90	Observations of captives	Synthetic	35	W-U lure, FAS	Phillips et al. 1990

Table 8. Field tests of the efficacy of delivery of nontoxic baits to coyotes during winter

Location (U.S.A.) and time	Size of area (km^2)	Initial bait density[1] (no./km^2)	Bait type and size (g)	Type of odor attractant used with bait (method)	Bait distribution method[2] (placement)	No. days of bait exposure (no. of bait applications)	% coyotes with physiologic marker *(n)*	Reference
Deming, NM Feb–Mar 1966	1,700	6.2	95% Beef tallow (9.5) and liver meal	Seal oil (incorporated)	Selective (surface)	28 (4)	28 [4](95)	Linhart et al. 1968
Arivaca, AZ Feb–Mar 1967	1,600	6.1	85% Beef tallow (9.5) and liver meal	Seal oil[3] (incorporated)	Selective (surface/elevated)	28 (4)	34 (119)	Linhart et al. 1968
Rawlins, WY Winter 1976–77	350		Lard (9.5) and fishmeal	None	24 Draw-stations (surface/elevated)	14 (2)	9 [4](55)	Tigner et al. 1981 unpubl.
Fort Sumner, NM Winter 1976–77	350		Lard (9.5) and fishmeal	None	19 Draw-stations (surface/covered)	14 (2)	27 [4](11)	Tigner et al. 1981 unpubl.
Monticello, UT Winter 1978	350		Lard (9.5) and fishmeal	SFE (adjacent)	19 Draw-stations (surface/elevated)	21 (2)	15 [4](26)	G. E. Larson and J. R. Tigner, unpubl. data
Goliad and Refugio counties, TX Jan–Feb 1982	174	0.5	80% Lard (28.3) and fishmeal	CDCL (adjacent)	Systematic (elevated)	30 (3)	18 [4](11)	Servheen 1983
Webb Co., TX (2 areas) March 1985	52	1.9	75% Beef tallow (4.3)	FAS (adjacent)	Systematic (surface)	14 (7)	34 [4](44)	Knowlton et al. 1986
Webb Co., TX (2 areas) March 1985	52		75% Beef tallow (4.3)	FAS (adjacent)	10 Draw-stations (surface)	10 (5)	23 [4](44)	Knowlton et al. 1986
Southern Idaho (2 areas) Jan–Feb 1989	127	0.8	90% Beef tallow (4.3)	FAS (adjacent)	Selective (covered)	21 (1)	60 [4](10) 45 [5](31)	R. D. Nass, unpubl. data
Southern Idaho (4 areas) Dec 1987—Jan–Feb 1989	127–260	1.9	90% Beef tallow (4.3)	FAS (adjacent)	Selective (covered)	21 (1)	34 [4](29) 63 [5](43)	R. D. Nass, unpubl. data
Southern Idaho (2 areas) Jan–Feb 1989	127	7.7	90% Beef tallow (4.3)	FAS (adjacent)	Selective (covered)	21 (1)	67 [4](12) 100 [5](22)	R. D. Nass, unpubl. data
Dona Ana Co., NM Jan–Feb 1991	104	1.9	90% Beef tallow (4.3)	FAS (adjacent)	Selective (covered)	20 (4)	29 [4](42) 32 [5](34) 59 [6](34)	F. F. Knowlton and R. D. Nass, unpubl. data

[1] Represents intended bait density, which was probably modified by differential loss of baits to nontarget animals and other factors.

[2] Selective distribution was placement of baits in relation to signs of coyote activity; systematic distribution was placement at specified spacing between baits without regard for coyote activity; draw-stations were carcasses of large animals with 10–40 baits placed nearby.

[3] Half of baits were also covered with fetid lure (commercial coyote-getter bait).

[4] Determined by physiologic marker in sample of coyotes from study area after bait exposure (sample for Knowlton et al. [1986] was prebaiting).

[5] Determined by particle marker (metallic flakes) in sample of coyote feces from study area after bait exposure.

[6] Determined by radioisotopes in sample of coyote feces from study area after bait exposure.

Baiting Strategies

Early field tests to deliver small nontoxic baits to coyotes were hindered by rapid disappearance of baits taken by nontarget animals, especially rodents and birds (corvids) (Linhart et al. 1968, Tigner et al. 1981 unpubl.). Subsequently, alternative methods of bait placement were compared to assess relative consumption by coyotes (table 6) and nontarget species. Baits placed on the soil surface tended to have greater visitation and consumption by coyotes than baits elevated above ground (30–51 cm) on steel wire and baits buried under soil (1–8 cm) in four comparisons (table 6). Servheen (1983) found similar visitation and consumption rates by coyotes for baits covered with indigenous materials (e.g., stones, cattle manure, dried mud) as for baits on the surface (table 6). Elevated baits tended to reduce consumption by rodents (Tigner et al. 1981 unpubl.) and insects (Servheen 1983). Buried and covered baits had less interference from birds but were still susceptible to rodents (Tigner et al. 1981 unpubl., Baril 1982; R. D. Nass, U.S. Department of Agriculture, Denver Wildlife Research Center, unpubl. data). The CLOD was affected significantly less by nontarget species: Stolzenburg and Howard (1989) reported that only 1 percent of 3,321 visiting nontarget animals activated the device.

Twelve field tests were conducted to measure delivery of nontoxic baits to coyote populations during winter on study areas of 52–1,700 km^2 (table 8). Because no coyotes were removed from the study areas during the bait exposure period, the tests simulate conditions for delivery of oral contraceptives or vaccines. A variety of baits and odor attractants, bait densities, and distribution, placement, and application procedures were employed (table 8). Various particle (metallic flake) and physiologic (tetracycline, mirex, IA, rhodamine B, radioisotope) markers (Savarie et al. 1992) were used to identify individual coyotes that ingested baits on the study areas. All baits were distributed on study areas from the ground either (1) selectively in relation to signs of coyote activity, (2) systematically spaced without regard for coyote activity, or (3) near draw-stations composed of large animal carcasses, which was a traditional method to attract coyotes for delivery of toxicants (Robinson 1948). Linhart et al. (1968) had made several bait applications with fixed-wing aircraft earlier but concluded that aerial distribution was not conducive for effective placement in relation to coyote activity.

The eight field tests during 1966–85 resulted in delivery of baits to only 9–34 percent of coyotes on study areas, based on presence of physiologic markers in samples of coyotes collected by trapping and aerial shooting (table 8). However, Knowlton et al. (1986) delivered baits to 50 percent of coyotes on their two study areas by a combination of three bait distribution methods (systematic, draw-stations, and near water). The four field tests that presented baits at carcass draw-stations resulted in relatively low delivery to coyotes (9–27 percent), a result likely related to territorial spacing patterns that restricted access to the draw-stations (Bowen 1981, Windberg and Knowlton 1988).

During 1987–91, field tests in winter on eight areas in southern Idaho, U.S.A., (R. D. Nass, unpubl. data) and one area in New Mexico (F. F. Knowlton and R. D. Nass, U.S. Department of Agriculture, Denver Wildlife Research Center, unpubl. data) achieved bait delivery to 29 to 67 percent of coyotes, based on presence of physiologic markers (table 8). All of those tests employed selective distribution of covered tallow baits with an adjacent odor attractant at three bait densities on relatively small areas. The greatest success in bait delivery was achieved at the highest density (7.7 baits/km^2). For the study in New Mexico (1991), estimates of coyotes that ingested baits based on presence of physiologic (29 percent) and particle (32 percent) markers were lower ($P < 0.03$) than the estimate based on presence of radioisotopes in feces (59 percent). Therefore, it appears that both types of estimates for percent coyotes that consumed baits during most of the preceding field tests may be biased low. The presence of particle markers probably underestimated coyotes that ingested baits because they were only present in fecal passages for a limited time whereas radioisotopes were detectable for several months (Savarie et al. 1992). The biases associated with the capture of coyotes (Windberg and Knowlton 1990) to examine for the presence of

physiologic markers may differ from biases in acceptance of baits.

The effect of multiple bait applications in improving bait delivery was difficult to assess among the field tests because of differences in other variables (table 8). However, the use of different radioisotopes in the initial versus three replacement bait applications on the New Mexico area (1991) provided evidence suggesting that coyotes were more prone to ingest baits after their initial acceptance (F. F. Knowlton and R. D. Nass, unpubl. data).

Two field tests provided data for bait acceptance in relation to age and social (territorial) classification of coyotes. In southern Texas (1985), 88 percent of eight juvenile (1-year-old) females ingested baits compared with 42 percent of 35 adults (F. F. Knowlton, unpubl. data). In New Mexico (1991), 50 percent of eight young (1- to 2-year-old) coyotes ingested bait compared with 24 percent of 34 older individuals (F. F. Knowlton and R. D. Nass, unpubl. data). In southern Texas (1985), 36 percent of 14 territorial females and 21 percent of 19 nonterritorial females had ingested baits from a systematic distribution (F. F. Knowlton, unpubl. data). Similarly, 31 percent of 13 territorial coyotes and 25 percent of 12 nonterritorial coyotes ingested baits from selective distribution at the same bait density (table 8) in New Mexico (1991) (F. F. Knowlton and R. D. Nass, unpubl. data).

The combined data from the two studies indicated greater vulnerability for young coyotes to ingest baits than adults ($P < 0.01$) but no difference in vulnerability between territorial and nonterritorial coyotes ($P = 0.36$). However, 29 percent of territorial females ingested baits placed near draw-stations in southern Texas compared with only 5 percent of nonterritorial females ($P = 0.07$) (F. F. Knowlton, unpubl. data). This result suggests that that bait-distribution method may offer greater selectivity for territorial coyotes.

The progression of research summarized above offers some guidelines for bait application procedures that can improve bait delivery to coyotes. In general, selective distribution of covered (hidden) fat-based baits with an odor attractant at relatively high densities (or application rates) in locales and seasons with minimal interference by nontarget animals should be most effective.

Interest in reproductive inhibition of coyote populations has waned owing to marginal success in delivery of baits (Linhart et al. 1968) and lack of a selective and effective antifertility agent (Stellflug and Gates 1987). Development of a selective immunocontraceptive for canids may overcome the latter obstacle. Till and Knowlton (1983) speculated that contraception may offer a selective method for reduction of coyote predation on livestock because it might remove a major motivation (i.e., feeding litters of young offspring) that often triggers depredations. Because breeding coyotes are primarily territorial adults (Knowlton et al. 1986, Crabtree 1988, Windberg 1995), baiting strategies for delivery of contraceptives must be selective for that cohort of populations in order to be effective. An epizootic of rabies in coyotes and domestic dogs in southern Texas that began in 1988 has stimulated interest in the delivery of an oral rabies vaccine to coyotes via baits (K. A. Clark, pers. comm.) and hence has renewed efforts to improve baits and baiting strategies for this species.

Dingo *(Canis lupus)*

Dingoes, along with wild dogs and their hybrids, are considered a significant economic threat to livestock enterprises in Australia. The application of single-dose toxic (strychnine and Compound 1080) baits has been one of the traditional methods for controlling dingo depredations and remains a contemporary cost-effective and publicly acceptable practice. The use of toxic baits for dingo control began during the mid-1800's in Australia (Rolls 1969). Guidelines for the preparation and ground placement of toxic baits were published in 1934 (Arnold and Herbert 1934). Aerial distribution of baits began in 1946 in Western Australia (Tomlinson 1954, Rolls 1969) and in the 1960's in New South Wales, Australia (Rolls 1969, Thompson et al. 1990).

Current procedures for dingo control vary with the relative risks to livestock and nontarget species, and State governmental agencies provide guidelines

for use of toxic baits (Hogstrom 1986, Begg and Davey 1987, Allen 1988, Downward and Bromell 1990, Thomson 1990). Generally, ground placement is used in more accessible areas and aerial application in the expansive or inaccessible regions of Australia.

Research to evaluate and improve the efficacy of bait delivery has been pursued (Hogstrom 1986). The implications for oral vaccination of dingoes for rabies control, if required, were explored by Thomson and Marsack (1992) and Fleming et al. (1992a). There has been no effort to deliver oral contraceptives to dingoes, and the comprehensive assessments of the potential for fertility control among various species in Australia by Bomford (1990) and Caughley et al. (1992) identified substantial limitations.

Baits and Odor Attractants

Two basic types of baits are used for delivery of toxicants to dingoes: fresh-meat baits and standard factory baits manufactured by the Agriculture Protection Board of Western Australia. Preparation of meat baits varies among States but, typically, fresh beef is cut into cubes of 100–250 g and injected with toxicant (Thomson 1986, Eastman and Calver 1988, Allen et al. 1989). In Western Australia, meat baits are partially dried in sunlight for 12 to 24 hours before injection to provide a firm dark skin that reduces attraction to insects (Thomson 1986, Eastman and Calver 1988). Factory baits are 6-g cubes (19 mm on a side) composed of 84 percent beef crackle (rendered fat) with glycerine (moisturizer), gelatin (binding agent), whale oil (odor attractant), a bactericide, fungicide, insect repellant, and the toxicant (Thomson 1986, Eastman and Calver 1988).

McIlroy et al. (1988) stated that fresh-meat baits were traditionally preferred over factory baits for wild-dog control in eastern Australia. Thomson (1986) incorporated plastic marker pellets into baits and documented dingoes' preference for meat baits over factory baits in Western Australia. Hogstrom (1986) suggested that the smaller size and unfamiliar odor of factory baits may make them less attractive than the larger and more familiar portions of fresh meat. Allen et al. (1989) compared the relative attractiveness and palatability of the two standard baits using modified scent-station procedures (Linhart and Knowlton 1975) in southern Queensland. Dingo visitation at their bait stations did not differ between fresh-meat and factory baits, but consumption was greater for meat baits. Allen's group also found that meat baits buried under 2 to 5 cm of soil were equally attractive and palatable to dingoes as those placed on the surface.

Several studies of baiting efficacy showed rapid disappearance of baits. Best et al. (1974) reported that 92 percent of 250 and 77 percent of 270 fresh-meat baits disappeared after 1 day on 2 study areas near Alice Springs, Northern Territory. Dingoes took most baits (58 percent); birds (37 percent) and foxes (5 percent) removed the others. McIlroy et al. (1986a) reported rapid disappearance of fresh-meat baits (69 percent of 275 in July 1980 and 87 percent of 304 in June 1981) after 4 days' exposure near Tumut, New South Wales, with foxes and birds removing most baits. In Kosciusko National Park, New South Wales, 92 percent of 160 fresh-meat baits were removed by nontarget animals (mainly foxes and birds) after 4 days in April 1982 (McIlroy et al. 1986b). In southern Queensland, nontarget animals removed more fresh-meat baits than factory baits (Allen et al. 1989). Allen's team also found that buried meat baits were lost to nontarget species significantly less than baits on the surface.

Interest in improving the efficacy of dingo control has recently stimulated research on odor attractants for use with baits. Mitchell and Kelly (1992) compared visitation rates and behavioral responses of dingoes to eight different attractants using scent-station procedures in southern and central Queensland. Jolly and Jolly (1991, 1992a) used captive dingoes to screen the attractiveness of 53 synthetic compounds and conducted field trials to validate data from the pen studies. Additionally, Jolly and Jolly (1992b) studied the food-finding ability of captive dingoes and suggested that acceptance may be greater for baits that are most recognizable as food.

Baiting Strategies

Ground distribution of dingo baits is typically by selective placement near signs of activity along unimproved roads and near sources of water (Thomson 1990). Aerial distribution is also selective by releases at regulated rates along identifiable dingo travel routes based on terrain features (Allen 1988, Thompson et al. 1990, Thomson 1990). A study of aerial placements using simulated baits (bags of lime) from a helicopter and fixed-wing aircraft was conducted to quantify baiting accuracy and provide guidelines for efficient application (Thompson et al. 1990). A subsequent study assessed the cost-effectiveness of aerial application of dingo baits (Thompson and Fleming 1991).

The early use of toxic baits to control dingoes was justified by circumstantial evidence suggesting its efficacy, such as perceived reduction of populations or declines in livestock losses and bounty payments (Rolls 1969, Newsome et al. 1972). Some initial attempts to assess baiting efficacy in reducing dingo populations were unsuccessful owing to complications with the bait or toxicant (Newsome et al. 1972, Best et al. 1974, McIlroy et al. 1986a). Fourteen field tests of baiting efficacy during 1971–93 resulted in variable dingo mortality (table 9). The greatest reductions in dingo abundance (78–100 percent) were achieved by aerial distribution of fresh-meat baits (Thomson 1986, Thomson and Marsack 1992, Fleming 1994; P. C. Thomson, pers. comm.). Reduction in dingo abundance was generally less for applications of factory baits (table 9), and Thomson and Marsack (1992) had greater reductions with applications of fresh-meat baits 4–5 weeks after distribution of factory baits on the same areas during two trials (44 percent *v.* 31 percent; 63 percent *v.* 6 percent).

Based on mortalities of radio-collared dingoes following bait distribution, Thomson (1986) determined that young and lone individuals were more vulnerable to bait consumption than adults or members of social groups. During his field tests of baiting efficacy in May and October 1980, all of 11 radio-marked dingoes <2 years old were killed compared with 6 of 13 that were ≥2 years. Thomson (1986) speculated that young and lone dingoes may have been most vulnerable because their poorer success in hunting large prey predisposes them to consume baits. No differential vulnerability to baiting between sexes was detected. Thomson (1986) considered the factors most influential in baiting efficacy to be the (1) number and distribution of baits, (2) bait type, and (3) age and social status of dingoes.

Domestic Dog *(Canis lupus)*

Large numbers of unvaccinated free-ranging dogs, both owned and unowned, are a major factor contributing to the lack of effective rabies control in many developing countries (World Health Organization 1988b, Joshi and Bögel 1988). Dog ecology studies conducted during the last 10–20 years (e.g., as summarized in World Health Organization 1984, 1988a, and 1994) have been helpful in furthering the development of oral vaccination for this species. A selective review of dog ecology in relation to rabies has been provided by Wandeler et al. (1993), while several recent dog studies in Africa also have added to our knowledge (DeBalogh et al. 1993, Kitala et al. 1993, Perry 1993). The World Health Organization (WHO) (1988b, 1989, 1991, 1992, 1993b, and 1994), and WHO and the World Society for the Protection of Animals (1990) have provided guidelines for developing oral vaccination of dogs that encompass, among other topics, development of vaccine baits, techniques for evaluating their efficacy, and baiting strategies to maximize bait ingestion by targeted dog populations. These reports also have summarized the results of WHO-coordinated studies and research by private industry.

Until recently, efforts to develop baits for dogs largely borrowed from earlier wildlife studies, and almost all the baits evaluated were those previously developed for red foxes and raccoons (Perry 1989). Using a fox bait described by Winkler and Baer (1976), Baer (1976) was apparently the first investigator to test a vaccine-laden bait with dogs. The bait consisted of a sealed plastic straw containing rabies vaccine that was inserted into a commercially avail-

Table 9. Field tests of efficacy of toxic baits in reduction of dingo abundance in Australia

Location and time	Size of area (km^2)	Type of bait	No. of baits	Bait distribution method	% reduction in abundance	*(n)*[1]	Reference
Alice Springs, NT Spring 1971	8,681	Fresh meat	520	Near water sources	[2]70		Best et al. 1974
Fortescue River site, WA May 1980	940	Factory bait	12,500	Fixed-wing aircraft	63	(19)	Thomson 1986
Fortescue River site, WA Oct 1980	940	Fresh meat	3,280	Fixed-wing aircraft	100	(18)	Thomson 1986
Fortescue River site, WA Oct 1981[3]	3,300	Factory bait	25,000	Fixed-wing aircraft	31	(13)	Thomson 1986
Fortescue River site, WA Sept 1984	1,500	Fresh meat	6,000	Fixed-wing aircraft	85	(13)	Thomson and Marsack 1992; P. C. Thomson, pers. comm.
Kosciusko National Park, NSW Apr 1982		Fresh meat	160	Selectively along roads	22	(9)	McIlroy et al. 1986a
Nullarbor Plain site, WA Oct 1984	1,600	Factory bait	10,000	Near water sources	29	(17)	Thomson and Marsack 1992; P. C. Thomson, pers. comm.
Nullarbor Plain site, WA May 1985[4]	1,600	Factory bait	25,000	Near water sources	6	(16)	Thomson and Marsack 1992; P. C. Thomson, pers. comm.
Nullarbor Plain site, WA Oct 1986	1,600	Fresh meat	6,200	Near water sources	80	(15)	Thomson and Marsack 1992; P. C. Thomson, pers. comm.
Nullarbor Plain site, WA Oct 1986	750	Factory bait	25,000	Near water sources	78	(9)	Thomson and Marsack 1992; P. C. Thomson, pers. comm.
Taunton Science Reserve, QU 1987	115	Fresh meat	441	Systematically along roads	[2]65		Tierney and Strong 1989
Northeastern New South Wales Apr 1991	151	Fresh meat	3,880	Helicopter	[2]90		Fleming 1994
Northeastern New South Wales Apr 1992	151	Fresh meat	3,040	Helicopter and ground[5]	[2]72		Fleming 1994
Northeastern New South Wales Apr 1993	151	Fresh meat	3,380	Helicopter	[2]78		Fleming 1994

[1] Determined by mortality of radio-collared dingoes following application of toxic baits (except as noted); number of marked individuals in parentheses.

[2] Determined by counts of dingo tracks on treated area before and after baiting.

[3] Aerial application of 4,000 fresh-meat baits on same area 4 weeks later resulted in 44-percent reduction of 9 radio-collared dingoes.

[4] Aerial application of 3,000 fresh-meat baits on same area 5 weeks later resulted in 63-percent reduction of 8 radio-collared dingoes.

[5] Seventy-four percent of baits were distributed by ground placement.

able sausage. In Zimbabwe, a bait that had been initially developed in Canada for red foxes was tested on dogs by Perry et al. (1988). It consisted of a polyurethane sponge cube saturated with a liquid placebo vaccine (egg yolk, molasses in water, and dye marker) that was distributed with a fermented odor attractant to enhance bait discovery. A preformed cigar-shaped bait of boiled and deep-fried cornmeal was used to administer liquid canine adenovirus vaccine to confined dogs (Baer et al. 1989). The adenoviruses are of interest as potential virus vectors for a recombinant rabies vaccine.

A chicken-head bait, originally used for red foxes, was tested with suburban dogs in Tunisia, while a German-manufactured bait ("Tübingen") and a bait of local sausage ("Köfte") have been tried in Turkey (World Health Organization 1991 and 1992). Also in Tunisia, household dogs were tested with four bait types: a sausage bait made of donkey meat and cooked rice, the DuPont polymer fishmeal bait (Hanlon et al. 1989), a chicken-head bait, and a polyurethane sponge bait inside a plastic packet (similar to the Canadian sponge bait) that also contained a fermented odor attractant (World Health Organization 1991, Kharmachi et al. 1992). Three of the above four baits were originally developed for vaccinating red foxes and raccoons. Four different candidate dog baits developed earlier for wildlife were tested in rural Mexico (World Health Organization 1991, Frontini et al. 1992). Two consisted of cylindrical corn, milk and egg batter-coated polyurethane sponge baits (Linhart et al. 1991), both deep-fried in either corn or fish oil and then air-dried. The other two baits were the DuPont polymer fishmeal bait and the Canadian tallow–wax chicken-flavored bait containing a blister pack (Bachmann et al. 1990). A commercially produced dog biscuit was used as a standard or control food item.

Development and field evaluation of baits and baiting strategies for dogs have recently received much more attention and although much of this work has not yet been published, summaries have been provided in World Health Organization documents. The chicken-head bait and polymer fishmeal bait have been compared at a waste-disposal site in northern Tunisia. As measured by visitation to tracking stations, the number of chicken-head baits picked up was estimated to be over seven times that for fishmeal baits (World Health Organization 1993b). An artificial bait made by Virbac Laboratories (Carros, France) for delivering SAG-2 vaccine has been described. It consisted of a solid core containing the vaccine in freeze-dried form. The core had hydrophilic properties and was coated with a protective envelope having hydrophobic properties and food substances attractive to dogs. The core was later modified to make it softer and more porous so as to enhance vaccine release. Several different prototypes of these baits containing biomarkers were tested using owned dogs in Tunisia where their acceptance was compared to that for chicken heads. Artificial bait acceptance rates varied from 24 percent to 60 percent; for the chicken-head bait, acceptance was 59 percent. One of the artificial bait types was compared with chicken heads at a waste disposal site in Tunisia. The same bait was evaluated by house-to-house trials in two semirural areas of Tunisia. Of >300 dogs offered the bait, 84.7 percent completely consumed it (World Health Organization 1994).

In Egypt, the bait preferences of farmer-owned dogs were determined following bait tests in the United States using confined laboratory beagles and mixed-breed dogs. In general, all three groups of dogs showed preferences for baits coated with either tallow, egg, cheese, or poultry products. Polymer fishmeal baits were less preferred by all three groups of dogs. Acceptance by Egyptian dogs of a polymer-bound commercial dogfood-meal bait coated with beef tallow and a dry cheese product was nearly identical to that of chicken-head baits (World Health Organization 1994, Linhart and Wlodkowski 1994).

Four bait types were evaluated in Nepal using both household and free-ranging dogs. Paired bait preference tests showed that a chicken-head bait was preferred over two Canadian blister-pack bait types (chicken or beef flavored) as well as a cylindrical dog-biscuit bait. A potential vaccination rate of 64 percent was estimated for chicken heads given to free-ranging dogs of unknown ownership (World Health Organization 1994).

In Turkey, tests of both the chicken-head bait and the Köfte bait (minced meat) were continued with baits targeted for free-ranging street dogs. A field test of an oral rabies vaccine was conduced wherein of 1,089 dogs that took baits, one-third took the chicken-head bait initially offered and the remaining two-thirds subsequently took the Köfte bait. As determined by dye marker, 28 percent of the vaccine capsules within baits were swallowed and 72 percent were punctured. Larger capsules were ruptured more frequently than smaller ones, and the latter were more often swallowed. Field trials also were conducted in Istanbul to determine the advantages and limitations of daytime *v.* nighttime baiting of free-ranging dogs (World Health Organization 1994).

The factors and requirements associated with delivering vaccine baits to dogs in Africa have been discussed and the African baiting trials summarized. Recommendations for the future distribution of baits and research still needed were presented by Perry and Wandeler (1993). Alternative methods for developing and evaluating dog baits and the various distribution techniques have been reviewed by Linhart (1993). Table 10 summarizes the results of earlier dog bait studies, but specific details of the more recent investigations have not yet been published.

Guidelines for evaluating bait delivery techniques have been compiled by the World Health Organization (1993b and 1994) and Matter (1993). These guidelines assume that candidate dog baits have been tested and shown to be well accepted by dogs under field conditions. The guidelines recommended that initial trials be conducted in towns or villages having 5,000–10,000 inhabitants and $\geq$ 500 dogs. The sequential field trials recommended were (1) a bait test using placebo vaccine (one or more systemic and/or topical markers) to determine dog acceptance and bait contact rates; (2) tests of baiting efficacy determined by providing dog owners with baits at central sites and having owners feed the baits to their dogs at home. Efficacy or probable vaccination rates would be determined by subsequent testing of these dogs for a systemic seromarker incorporated into baits; (3) door-to-door bait distribution to owned dogs in conjunction with estimating dog populations via "capture–mark–recapture" techniques; (4) overnight bait placement for free-ranging dogs; and (5) costs associated with both house-to-house and overnight bait distribution. The so-called handout method, that of offering baits to free-ranging street dogs as they are encountered, also has been mentioned as an alternative strategy.

Recent advances in bait testing and the results of vaccine safety and efficacy trials have led the World Health Organization to announce that field tests to vaccinate dogs orally will be initiated in Tunisia, Turkey, and southern Africa during 1995 (Anonymous 1994).

Raccoon Dog *(Nyctereutes procyonoides)*

Raccoon dogs, originally restricted in their distribution to eastern Asia, were introduced into the European and Asian parts of Russia in the first half of this century. This species subsequently spread over large parts of Eastern Europe and Finland (Anonymous 1984, Cherkasskiy 1988, Helle and Kauhala 1991). The species has accounted for a significant proportion of the rabies cases in eastern European countries since the 1960's. However, it was not until April 1988 that the first case was reported in Finland. At that time, Finland had been free of rabies for almost 30 years (Nyberg et al. 1992). Given the success of oral vaccination of foxes against rabies in Central Europe, Finnish authorities embarked on trials aimed at immunizing both raccoon dogs and red foxes. Because the Tübingen bait (see red fox account) was the only commercially available bait at that time, Tanskanen et al. (unpubl. data) tested these baits with various doses of SAD B19 live attenuated rabies vaccine in captive raccoon dogs. Test animals accepted the baits, and most developed neutralizing antibodies against rabies and survived a subsequent challenge with virulent rabies virus. Thus, there was little incentive to develop alternative bait types. Less than 6 months after the first rabies case was reported, 36,000 baits were distributed by some 800 hunters over an area of 2,400 km^2; an additional 4,500 baits were dropped by a fixed-wing aircraft in a less accessible area of

Table 10. Baits tested for delivery of oral rabies vaccines to domestic dogs (1975–92) (from Linhart 1993)

Bait types tested	Size	Type of test	% of baits bitten or chewed by dogs	% of baits completely ingested	% of dogs marked with placebo vaccine[1]	Reference (location of study)
Slim Jim® sausage	13 cm long	Laboratory dogs	—		—	Winkler and Baer 1976, Baer 1976
Polyurethane sponge cube in plastic sachet with fermented attractant[2] in outer bag	2 × 3.5 × 5 cm	Farms—free-ranging dogs	79 (65/82)	—	25 (138/553)	Perry et al. 1988 (Zimbabwe)
Cooked preformed cornmeal deep-fried in corn oil	"cigar-shaped" cylinder, 10 cm long	Farm-owned dogs	—	100 (11/11)	—	Baer et al. 1989 (Zimbabwe)
Chicken head	—	Suburban-owned dogs	—	78 (?)	78 (?)	World Health Organization 1991 (Tunisia)
		Unowned dogs	—	56 (10/18)	56 (?)	
Cylindrical polyurethane sponge containing cornmeal, egg, and milk, deep-fried in:		Rural towns—owned dogs				Frontini et al. 1992 (Mexico)
corn oil	1.5 × 5.5 cm		88 (45/51)	67 (34/51)	—	
fish oil	1.5 × 5.5 cm		85 (33/39)	69 (27/39)	—	
Dupont polymer fishmeal	2 × 3 × 5 cm		90 (43/48)	50 (24/48)	—	
Canadian blister pack (wax)	2 × 3.5 × 3.5 cm		44 (17/39)	10 (4/39)	—	
Small dog biscuit (control)	1 × 2.3 × 4.5 cm		97 (171/176)	88 (155/176)	—	
Sausage of minced donkey meat and cooked rice	7–10 cm long	Owned dogs	56 (28/50)	—	46 13/8)	Kharmachi et al. 1992 (Tunisia)
Dupont polymer fishmeal	2 × 3 × 5 cm		80 (40/50)	—	78 (31/40)	
Chicken head	—		96 (48/50)	—	98 (47/48)	
Polyurethane sponge cube in plastic sachet with fermented attractant[3]	?		66 (33/50)	—	30 (/10/33)	
1/2 Large dog biscuit	15 × 5 × 5.5 cm	Rural towns—owned dogs	—	81 (108/134)	—	Linhart et al, unpubl. data (Mexico)
Cylindrical polyurethane sponge containing cornmeal, egg, and milk, deep-fried in corn oil	1.5 × 5.5 cm		—	84 (111/133)	—	
As above but shorter length	1.5 × 3 cm		—	83 (108/130)	—	
Length of beef hotdog, dried and hardened	1.5 × 4.5 cm		—	77 (104/136)	—	

[1] Numerals in parentheses are dogs positive over total dogs checked.

[2] Attractant consisted of fermented meat, offal, fish, blood, cheese, and yeast; 5 mL placed in outer bag.

[3] Attractant consisted of fermented minced meat, eggs, yogurt, fish, and cheese.

225 km^2. A bait disappearance rate was obtained from 240 monitored baits. On days 4, 8, and 12, 12 percent, 31 percent, and 51 percent of the baits had disappeared, respectively. Tooth marks in empty blister packs recovered from the field suggested that raccoon dogs and foxes were the major consumers. This was later confirmed by demonstration of tetracycline in the lower jaws of 79 percent of 126 raccoon dogs and 88 percent of 56 foxes collected 1–7 months after the campaign, as well as with serologic data (Nyberg et al. 1992). As tetracycline marks were relatively faint in raccoon dogs, the dose per bait was adjusted to 300 mg in later campaigns (B. Westerling, pers. comm.). Finland has not reported any cases of rabies since February 1989, but it has continued its vaccination program along the border with Russia, where rabies is still endemic (Westerling 1993).

Raccoon *(Procyon lotor)*

Baits have been used to deliver toxicants, administer candidate oral contraceptives, and orally vaccinate raccoons against rabies. Fresh eggs were injected with a strychnine, honey, dye, and water mixture and used in past decades to destroy raccoons where rabies was a problem; two poisoned eggs were placed at each bait station (Lewis 1975). Eggs containing a tetracycline biomarker were distributed on a South Dakota study area in late summer to determine the percent of the population that might be reached by an oral contraceptive. Of the raccoons collected during the following 7 months, 87 percent were positive for the marker (Nelson and Linder 1972).

An epizootic of raccoon rabies that began in the mid-Atlantic States during the early 1980's, and European successes with oral rabies vaccination, provided the impetus for a number of laboratory and field baiting studies aimed at delivering oral vaccine to this species. C. E. Rupprecht and associates (Wistar Institute, Philadelphia, PA, U.S.A.), with the collaboration and expertise provided by D. H. Johnston (Ontario Ministry of Natural Resources, Maple, ON, Canada), offered captive raccoons more than 30 fruit, vegetable, and beef/poultry/fish oil extracts and slurries as candidate attractants using a "smorgasbord" testing protocol. Five attractants were then selected and field-tested in conjunction with a 3- × 3- × 4-cm polyurethane sponge bait covered with beef tallow and wax (Johnston and Lawson 1987, Bachmann et al. 1990). The above bait with placebo vaccine (tetracycline) and candidate attractant was placed inside a polyethylene bag and aerially dropped into five 4-km^2 Pennsylvania study sites at a density of 120 baits/km^2. Of the raccoons collected from the study sites, 36–76 percent had eaten one or more baits as indicated by tetracycline-induced fluorescence in the teeth (Rupprecht et al. 1987).

This type of bait also was used to administer candidate vaccine to captive raccoons (Rupprecht et al. 1986), and to subsequently assess experimental field delivery of placebo vaccine near Washington, DC, U.S.A. (Hadidian et al. 1989) and in Virginia, U.S.A. (Perry et al. 1989). The above bait with IA as a seromarker (Larson et al. 1981) was placed in a section (0.8 km^2) of Rock Creek Park, Washington, DC, in June 1986. The wax–tallow coating in which baits were dipped contained 150 mg of tetracycline hydrochloride (THC) per bait. A single bait and 10–20 mL of a ground mackerel–water slurry were placed in a 17- x 24-cm plastic bag and distributed by hand at 15-m intervals along 43 transects at a density of 1,240 baits/km^2. The mackerel–water slurry attractant was selected because it previously had been found to be effective; i.e., raccoons showed no preferences among mackerel, grape jelly, cod liver oil, feta cheese, fresh banana, and beef gravy attractants. Of the raccoons captured during a 3-week posttreatment period, 63 percent had eaten one or more baits (Hadidian et al. 1989).

The Virginia field trial was conducted following laboratory evaluation of 11 candidate bait or attractant combinations (hot dogs, marshmallows, doughnuts, gelatin, molasses, and apple butter). Polyurethane sponge baits were tested both with and without containment in plastic bags or aluminum foil. Candidate food attractants were field-tested using a series of 13 smorgasbord acceptance tests on 2 study areas, 1 located in the coastal plain and the second in the piedmont region of Virginia. Investigators formulated

sponge baits that contained a mixture of egg yolk, molasses diluted in water, and 200 mg of tetracycline per bait. They were used for a field trial in which baits and about 10 g of fish-based attractant (canned sardines and soybean oil used to deep-fry fish) contained in an outer bag were dropped from a fixed-wing aircraft on 2 4-km^2 sites at 120 and 450 baits/km^2. Analysis of captured raccoons for tetracycline revealed that between 30 and 73 percent of the animals had eaten one or more baits (Perry et al. 1989).

Field tests were subsequently conducted on Parramore Island, a barrier island in Virginia (Hanlon et al. 1989), where several candidate baits, including a commercially extruded cylindrical (3- × 4-cm) polymer fishmeal polymer bait (E. I. DuPont de Nemours Co., Orange, TX) were evaluated (fig. 1). Several tests of the fishmeal bait were conducted using different percentages of fishmeal, fish oil, and 0.5–10 percent of a water-proofing binder, an ethylene vinyl acetate copolymer sold as Elvax^R or Aquabind^{TM} by DuPont and patented by that company as a component of a long-lived, semiartificial, water-borne feed (Smith and Daigle 1988). The above study also compared raccoon acceptance of the polymer fishmeal bait to three other baits, the Canadian polyurethane sponge bait mentioned earlier, a Canadian sachet or blister-pack bait (Bachmann et al. 1990), and a bait designated as the "German sachet" or "Tübingen" bait (Schneider et al. 1988). The latter three baits had been originally developed for the red fox. Hanlon's team compared the above baits by placing each type 0.3 m apart in a smorgasbord grid pattern and randomly changing their positions following each trial. Raccoons were found to consume the polymer fishmeal baits more completely than the other three bait types. Paired bait tests also were conducted to compare removal of baits in plastic bags, baits without bags, and molasses-enriched baits. A final field trial involved placing baits in small plastic bags overlaid with a fish-based slurry (vegetable oil and salmon) every 30 m along transects (920 baits/km^2). Bait disturbance was high (80–100 percent), primarily by raccoons, as revealed by tracking stations. Biomarker analysis of raccoons captured on Parramore Island, VA, also was encouraging (Hanlon et al. 1989).

A subsequent Parramore Island test provided additional information. A candidate oral rabies vaccine (V–RG) was placed in a wax ampule that was inserted into a polymer fishmeal bait. The bait was then placed inside a plastic bag that contained approximately 50 mL of a slurry to enhance bait discovery and consumption. Baits were placed out on foot at 12- to 30-m intervals along linear transects, resulting in a density of about 1,000 baits/km^2. The presence of biomarkers in captured raccoons, tetracycline in teeth and bone, and sulfadimethoxine (SDM) in serum, revealed that 78 percent (38/49) to 84 percent (47/56) of raccoons from the two treatment areas had consumed one or more baits (Hanlon et al. 1993, Rupprecht et al. 1993a).

Field trials also were concurrently conducted on two barrier islands off the coast of South Carolina, U.S.A. (Murphy and South islands). Tracking stations placed at 0.1-km intervals (Linhart and Knowlton 1975) were used to compare raccoon visitation rates to different candidate attractants such as raccoon urine, synthetic fermented egg (Bullard et al. 1978), and commercially available essences or odor attractants such as persimmon, sweet corn, shellfish, and shrimp. A mixture of blue crab offal, synthetic shellfish oil, sucrose, vegetable oil, and raw eggs was selected for all subsequent baiting trials. The same four bait types Hanlon's team tested on Parramore Island were packaged in perforated plastic bags containing 10–20 mL of the above attractant into which was mixed 300 mg of biomarker, a rhodamine B dye powder. Baits at densities from 200 to 1,000/km^2 were placed by hand along transect lines and near known raccoon dens and their trails along waterways. Bait disturbance rates (all species) were reported as 93–100 percent by 7 days after bait deployment, with bait acceptance rates by raccoons at 49 to 85 percent.

The South Carolina workers (Hable et al. 1992) suggested that field crews use a minimum baiting density of 500 baits/km^2 in areas having average raccoon densities to achieve a 70-percent or higher acceptance rate but that 700–1,000 baits/km^2 or multiple baitings might be required under less favor-

able field conditions. Hable et al. stated that the relationship between raccoon density and the level of bait density required to reach 70 percent or more of the population had yet to be determined. They also concluded that the polymer fishmeal bait was superior to the others tested because of its durability, resistance to insect damage, its attractiveness to raccoons, and its potential for commercial production in different sizes and formulations.

The first mainland field trial to determine the safety of the V–RG vaccine was conducted in northern Pennsylvania, where field crews distributed 500 polymer fishmeal baits/km^2 by foot on a 10-km^2 study site that supported a wide variety of nontarget mammals and birds. Only 2 of 150 nontarget individuals comprising 9 different species trapped after baiting were found positive for the biomarker (tetracycline). Of raccoons, 70–85 percent had consumed one or more baits (Rupprecht et al. 1992; C. E. Rupprecht, pers. comm.).

Movement of raccoon rabies into New Jersey, U.S.A., in 1989 stimulated interest in determining the potential for oral vaccination in that State. Bait acceptance trials were conducted in the fall of 1990 using three different baiting densities on three study areas so as to estimate the minimum density of baits required to reach greater than 70 percent of the population. The polymer fishmeal baits used contained three different biomarkers: tetracycline in the bait matrix, IA in a paraffin wax ampule, and SDM in a blue-crab slurry. Baits and slurry were contained within plastic bags dropped by helicopter. Posttreatment raccoon capture revealed that 80 percent of the raccoons were positive for IA at 200 baits/km^2, 44 percent at 100 baits/km^2, and only 1 of 3 raccoons at 50 baits/km^2 (Diehl et al. 1991). Efforts were then initiated to create a barrier or zone of immune raccoons across the Cape May peninsula in southern New Jersey such that the southward spread of rabies would be excluded from the southern tip of the peninsula. More than 89,000 polymer fishmeal baits containing V–RG in wax ampules were dropped by helicopter and placed by hand along roads in three applications (spring–fall–spring) on 559 km^2 (160 baits/km^2) prior to rabies' reaching the vaccination zone. An additional 73,400 baits were subsequently distributed at 4 rabies "hot spots" and in 2 zonewide vaccinations. At tracking stations, 80–87 percent of baits were disturbed, primarily by raccoons, during the first 10 days after bait distribution in November 1993 and March 1994. The percentage of raccoons collected after treatment showing evidence of biomarker varied from 40 to 76 percent, depending upon when they were collected relative to time of bait distribution (Rupprecht et al. 1993b, Roscoe et al. 1993 and 1994). Unlike the Parramore Island, VA, and Pennsylvania tests, which sought to determine vaccine safety, the New Jersey trial was primarily concerned with oral vaccination efficacy, the first such test for the raccoon.

Modest efforts to develop raccoon baiting technology were made by the U.S. Department of Agriculture (Denver Wildlife Research Center, Animal Damage Control, Animal and Plant Health Inspection Service, Denver, CO 80225), during 1987–90. Captive raccoons were used in three-choice bait-preference tests to evaluate a variety of natural and synthetic food attractants. Investigators created a timing device that used analog clocks to record when individual baits were taken from bait trays. Preference could thereby be determined not only by the number of each type of bait consumed but also by the sequence in which baits were selected. These trials identified as preferred bait that consisted of a polyurethane foam sleeve dipped in a commercial food batter mixed with cornmeal, milk, and eggs. The bait was deep-fried in corn oil, and a 2-mL wax ampule for vaccine containment was inserted into the completed bait (Linhart et al. 1991). A field trial of this bait was conducted in spring 1990 on Sapelo Island, GA, where 2,300 baits were distributed from all-terrain vehicles or pickup trucks on 28 km^2 at a bait density of 82 baits/km^2. Subsequent capture of raccoons and evidence of biomarker (IA) indicated that 65 percent of the raccoon population had consumed one or more baits (Linhart et al. 1994).

Efforts by the Ontario Ministry of Natural Resources to develop oral rabies vaccination technology had, until recently, focused on the red fox (see red fox account). However, rabies in skunks, primarily in urban areas, and the movement of the epizootic of

rabies in raccoons northward through New York, U.S.A., redirected some research toward the latter two species.

The development of baits and an aerial baiting system evolved over a period of years. Baits developed for foxes and distributed at densities calculated to vaccinate this species also have been ingested by raccoons, a species normally found at much higher densities. In Ontario, aircraft-distributed wax- and tallow-coated sponge baits have reached 74 percent of the foxes and 43 percent of the raccoons. A small trial in Pennsylvania reached 76 percent of the raccoons (Johnston et al. 1988). Bachmann et al. (1990), working in Ontario, stated that bait acceptance by raccoons and skunks was also studied, but his team "did not design specific experiments to optimize acceptance by these species." However, Rosatte et al. (1990) has described a specific skunk and raccoon baiting study in an urban environment (Toronto) that compared two different attractants, chicken "essence" and cod oil, both in a tallow–wax–tetracycline mixture. These investigators found a positive correlation between bait density and raccoon bait acceptance (cod oil baits only) and showed that cod oil baits were better accepted by raccoons than those containing chicken essence. On one area, all blister packs within baits had been chewed and emptied of placebo vaccine, and only one partially eaten bait was retrieved. Sixty-eight percent (34/50) of the raccoons had eaten a bait at a density of 147 baits/0.04 km^2. More recently in Ontario, five attractants were exposed to captive raccoons and two were then field-tested. Both cheese powder and icing sugar plus marshmallow "essence" (ISM) were found to be highly acceptable. Baits containing ISM were aerially dropped on two 150-km^2 plots (50 and 100 baits/km^2) and also hand-placed (200 and 400 baits/km^2) on urban plots. Subsequent capture of raccoons and evidence of tetracycline indicated acceptance rates of 44 percent and 58 percent for 50 and 100 baits/km^2, respectively. However, samples obtained 2 weeks or more after baiting had bait acceptance rates up to 70 percent in the 100/km^2 plot. Additional tests of the above baits were continued in 1994 but results were pending at the time of this writing (Rosatte et al. 1994).

Tests of a new raccoon bait of unspecified ingredients that was used with a biomarker and distributed at a density of 200/km^2 on an urban–suburban site in central New York State have been described. Examination of raccoons after treatment revealed that 70 percent had eaten baits. A trial of the Ontario blister-pack bait, also distributed in similar type habitat, resulted in 91 percent of the raccoons consuming baits (Bigler et al. 1994). Oral rabies vaccination tests have been initiated in eastern New York State to determine if treatment will reduce the numbers of rabid raccoons detected in a baited *v.* unbaited area, both of which are experiencing an outbreak of the disease. The efficacy of two different vaccine containers will also be evaluated (Hanlon et al. 1994).

Recent efforts in Massachusetts, U.S.A., to establish a zone of orally immunized raccoons along the canal dividing the mainland from the Cape Cod peninsula have been summarized. A total of 16,500 polymer fishmeal V–RG vaccine baits were distributed by ground crews and helicopter over a 157-km^2 area. Three sites within the area were each treated differently; variables tested were baiting density and bait placement. Raccoons were collected after treatment and tested for virus neutralizing antibodies; it was found that the percent of antibody-positive raccoons varied significantly (19 percent, 39 percent, and 46 percent) among treatment sites. These data suggested that targeting preferred habitat would be a cost-effective way to vaccinate raccoons orally (Robbins et al. 1994). Table 11 provides a summary of raccoon baiting field trials.

Raccoons may learn to seek supplemental food at baiting sites (Dalgish and Anderson 1979), and a number of animals may visit such sites (Sharp and Sharp 1956, Dalgish and Anderson 1979, Slate 1985, Curran 1988). Competition at a raccoon feeding site has been documented by Nicolaus et al. (1982) and others, but how this might effect the delivery of nonlethal biologics and chemicals is not known. Several investigators have suggested using central baiting sites where raccoons could be concentrated by

Table 11. Summary of published raccoon biomarker uptake during bait efficacy or oral rabies vaccine safety field trials

Location and year	Bait type	Biomarker/ vaccine in bait and samples collected	Distribution method	No. of baits placed	Size of area (km²)	Bait density (no./km²)	% bait disturbance[1] raccoon	% bait disturbance[1] all spp.	No. of raccoons marked/ collected (%)	Reference
Central Pennsylvania (5 test areas) 1985	Sponge bait cubes	Oxytetracycline (100–200 mg) teeth	Aircraft	484 ? 484 ? 484 ?	4 4 4	120 120 120	NA[2] NA[2] NA[2]	NA[2] NA[2] NA[2]	5/14 (36) 6/9 (66) 13/17 (76)	Rupprecht et al. 1987
Rock Creek Park, DC 1986	Sponge bait cubes	Iophenoxic acid (5 mg) blood sera	Foot	1,000	0.8	1,240	—	80 (1) 97 (7)	33/52 (63)	Hadidian et al. 1989
Coastal plain of Virginia 1986	Rectangular sponge in plastic film	Tetracycline (200 mg) teeth & adj. bones	Aircraft	481 1,934	two 4-km² sites	107 483	— —	26 (3) 33 (3)	7/12 (58) 7/11 (64)	Perry et al. 1989
Piedmont plateau of Virginia 1986	Rectangular sponge in plastic film	Tetracycline (200 mg) teeth & adj. bones	Aircraft	500 2,078	two 4-km² sites	133 428	— —	57 (4) 21 (4)	5/8 (63) 11/21 (52)	Perry et al. 1989
Parramore Island,[3] VA (4 test areas)	Polymer fishmeal	Tetracycline (100 mg) & rhodamine B (100 mg) teeth, bones, & scats	Foot	117 26 140 25	0.13 0.02 0.51 0.07	870 1,300 270 340	89 (2) — — —	100 (2) 88 (2) 91 (2) 100 (2)	6/10 (60) 2/4 (50) 2/5 (40) 3/5 (60)	Hanlon et al. 1989
North Island, SC	Polymer fishmeal	Tetracycline (100 mg) bone	Foot	275	0.30	920	—	78 (2)	8/8 (100)	Hanlon et al. 1989
Murphy Island I, SC 1987–88	German fishmeal Canadian chicken	Tetracycline (150–200 mg) teeth & bones	Foot	400	2	200	—	59–99 (2) 93–100 (7)	12/17 (71)	Hable et al. 1992
Murphy Island II, SC 1987–88	Polymer fishmeal	Tetracycline (150–200 mg) teeth & bones	Foot	500	0.5	1,000	—		17/20 (85)	Hable et al. 1992
South Island I, SC 1987–88	Tallow/sponge German fishmeal	Tetracycline (150–200 mg) teeth & bones	Foot	600	2	300	—		19/39 (49)	Hable et al. 1992
South Island II, SC 1987–88	Polymer fishmeal	Rhodamine B (300 mg) teeth & bones	Foot	250	0.5	500	—		11/24 (46)	Hable et al. 1992
Parramore Island, VA	Polymer fishmeal	Tetracycline (150 mg) & sulfadimethoxide (250 mg) bone & sera	Foot	3,120	3.12	1,000	76 (5)	92 (5)	42/53 (79)	Hanlon et al. 1993
Sapelo Island, GA 1990	Corn-flavored sleeve bait	Iophenoxic acid (10 mg) blood sera	Ground vehicles	2,300	28	82	44 (2)	74 (2)	35/54 (65)	Linhart et al. 1994

Table 11—Continued

Location and year	Bait type	Biomarker/ vaccine in bait and samples collected	Distribution method	No. of baits placed	Size of area (km²)	Bait density (no./km²)	% bait disturbance[1] raccoon	% bait disturbance[1] all spp.	No. of raccoons marked/ collected (%)	Reference
Toronto, ON, Canada 1989	Canadian blister, cod oil	Tetracycline (100 mg) teeth	Foot	147	0.04	3,675	—	—	34/50 (68)	Rosatte et al. 1990
Ontario, Canada 1993	ISM[4]	Tetracycline (180 mg) teeth & bones	Aircraft	7,500	150	50	NA[2]	NA[2]	? (44)	Rosatte et al. 1994
Ontario, Canada 1993	ISM[4]	Tetracycline (180 mg) teeth & bones	Aircraft	15,000	150	100	NA[2]	NA[2]	? (58)	Rosatte et al. 1994
Central New York State 1992	Cornell[5]	Iophenoxic acid (10 mg)	Foot	?	?	200	—	—	48/69 (70)	Bigler et al. 1994
Central New York State 1994	Canadian ISM[4] (?)	Iophenoxic acid (10 mg)	Foot	?	?	200 ?	—	—	42/46 (91)	Bigler et al. 1994
Cape Cod, MA 1994	Polymer fishmeal	V–RG blood sera (VNA[6] to rabies)	Foot / aircraft	468 ? 832 ? 988 ?	52 ? 52 ? 52 ?	90 160 190	— — —	— — —	? (39) ? (19) ? (46)	Robbins et al. 1994
Southeastern New Jersey (3 test areas) 1990	Polymer fishmeal	Tetracycline (100 mg) iophenoxic acid (10 mg) sulfadimethoxine (250 mg) bone?, blood sera	Aircraft	268 648 1,166	5.4 ? 6.5 ? 5.8 ?	50 100 200	NA[2] NA[2] NA[2]	NA[2] NA[2] NA[2]	[7]1/3 (33) [7]11/35 (44) [7]47/59 (80)	Diehl et al. 1991
Southern New Jersey 1992–94	Polymer fishmeal	Tetracycline (? mg)	Aircraft/ ground	162,700+ (5 applications)	559	112	?	80–87	17/39 (44) 99/130 (76) 15/23 (65)	Roscoe et al. 1994

[1] Values in parentheses are the number of nights following bait placement when bait disturbance rate was determined.

[2] Not available due to aerial bait distribution

[3] Because of poor tetracycline marking of teeth of Parramore Island raccoons, rhodamine B marking of scats was used to indicate percentage of raccoons eating baits.

[4] Variant of the Ontario blister-pack bait (table 2) made of tallow, microbond wax, mineral oil, icing sugar, and marshmallow essence.

[5] Bait of unspecified ingredients produced at Cornell University as reported by Bigler et al. (1994).

[6] VNA = virus neutralizing antibodies.

[7] Iophenoxic acid data only.

supplemental feeding and then be administered vaccine baits (Slate 1985, Hadidian et al. 1989, Rupprecht et al. 1992, Linhart et al. 1994). This strategy has not been tested so far, although Johnson and Rauber (1970) successfully used permanent feeding stations to administer an anticoagulant on whole shelled corn to raccoons preying upon shorebird and sea turtle nests. These investigators placed covered feeders 25–30 cm above the ground to enhance selectivity and documented reduced raccoon activity following placement of the poisoned corn. Frantz (1994) has described a protective bait station for delivering rabies vaccine baits.

Currently available information and the results of the ongoing investigations summarized above should soon provide a wealth of data as to how baits and baiting strategies can be used to deliver orally effective biologicals to raccoons. However, data are still lacking to demonstrate that oral vaccination of this species can effectively reduce or eliminate rabies over large geographic areas. Furthermore, to our knowledge, no one is presently investigating oral contraception for the raccoon.

Striped Skunk *(Mephitis mephitis)*

Toxic baits made of meat, tallow, or egg have been used in south central Canada and in the central region of the United States to reduce striped skunk populations where this species was a carrier of rabies (Gremillion–Smith and Woolf 1988). Although toxic baits were widely used in the past, only a few studies have critically assessed bait preferences or alternative baiting strategies and only limited efforts have been made to use baits as a vehicle for oral contraceptives or for orally effective rabies vaccines. However, some data on skunk bait acceptance has been acquired as a secondary objective to the application of baits for red foxes, as in Ontario, Canada, for example (Johnston and Voigt 1982, Johnston et al. 1988, Bachmann et al. 1990).

In California, U.S.A., pieces of raw wiener impregnated with strychnine were placed in short sections of 6-inch-diameter pipe to restrict bait uptake by larger carnivores. Bait stations were placed in culverts and hollow logs, beneath bridges, and along irrigated ditches or streambanks (Maynard 1965). Schnurrenberger et al. (1964) used den gassing and strychnine and honey-laced chicken eggs to control skunks in Ohio. In the United States, strychnine-treated eggs also have been used in Montana and elsewhere in the northern prairie region as an emergency measure to control rabies in skunks (Seyler and Niemeyer 1974, Nesse and Seyler 1977). Poisoned eggs were distributed within a 5-km radius of sites where rabies had been diagnosed in skunks. Eggs were placed at skunk dens and holes, dumps, culverts, junkpiles, and unoccupied buildings. Poisoned skunks were most often found within 6 m of baiting sites. Surveillance areas also were established to monitor the distribution of the disease. However, Nesse and Seyler (1977) were unable to determine how effectively such efforts eliminated diseased skunks from baited areas. Efforts were made by the U.S. States of Wyoming and Montana to seek a Section 3 registration for strychnine-treated eggs from the U.S. Environmental Protection Agency (EPA) for controlling rabies in skunks (Thomas 1986). However, registration has not been granted, presumably because the additional data requested by EPA to support the registration were never obtained.

By far the greatest use of baits for controlling skunks has been in Alberta, Canada, when rabies in the Province's fox population was first diagnosed and subsequently spread southward. A massive wildlife control program—aimed primarily at gray wolves *(Canis lupus),* foxes, and coyotes but also affecting skunks—relied heavily on large numbers of toxic baits made of various fats, tallow, paraffin, and waxes that were melted and poured into cups containing capsules or cubes of toxicant (Ballantyne and O'Donoghue 1954). Similar baits have been widely used in North America and elsewhere for other species. Control efforts in Alberta were later focused on the striped skunk as this species emerged as the primary rabies carrier in south central Canada (Gunson et al. 1978). Chicken eggs (1–5) and bait cubes (beef fat and parawax) were placed in skunk habitat, and uneaten baits were retrieved and destroyed after 10 to 14 days.

Control was concentrated within 5 km of a known rabid animal. Available data suggested that population reduction effectively controlled the disease (Rosatte 1986, Rosatte et al. 1986).

Pybus (1988) extensively reviewed rabies and its control in Alberta and Saskatchewan, Canada, and in Montana and concluded that control efforts (poisoning, den gassing, and trapping) ". . . contributed to limiting the spread and establishment of rabies in striped skunks within prairie habitats." This assessment was in agreement with earlier program reviews (Ballantyne 1958, Gunson et al. 1978, Rosatte et al. 1986).

The use of poisons to control rabies in skunks is currently much more restricted; and, in Canada at least, research efforts are now being directed toward development of recombinant oral rabies vaccines as an alternative technique (Charlton et al. 1992).

Skunk preference for egg *v.* tallow baits was evaluated by Roy and Dorrance (1992), who found higher selectivity for egg bait. There were no significant seasonal differences in consumption of the two baits by skunks and nontarget species, but eggs cannot be used when nighttime temperatures fall below freezing.

Tallow baits were used to deliver a candidate antifertility compound (DES) to a population of wild skunks on a 186-km^2 area in Illinois, U.S.A. Baits (48/km^2) were distributed annually in the spring of 1965 and 1966 near culverts and fence rows and along roadways. More than 88 percent of the baits were taken by all species within 10 days after placement. However, skunk reproductive rates in the treated area and a reference area were not significantly different (Storm and Sanderson 1969).

Another field baiting study in South Dakota used a biomarker (dimethylchlorotetracycline, now commonly known as demeclocycline) in baits to assess the feasibility of delivering antifertility agents to skunks and raccoons (Nelson and Linder 1972). Chicken eggs containing the biomarker were distributed in August and September on a 65-km^2 agricultural area with wetlands at a density of 28.2 eggs/km^2. Sampling of animals from the test area after treatment revealed that 29 percent of the skunks had consumed one or more eggs.

Although the striped skunk has been a major carrier of rabies over much of North America, the technical literature indicates that only limited efforts have been directed at systematically developing efficacious baits and delivery systems for this species.

Small Indian Mongoose *(Herpestes javanicus)*

Of the 37 mongoose species, bait development and use has been reported only for the small Indian mongoose, which was introduced into the Caribbean area during the 19th century. Strychnine-laden baits of smoked herring, salted pork fat, shrimp, fish entrails, "heads of fowl," and eggs were used to control mongooses because of rabies on the island of Trinidad (Urich 1914). On the island of Puerto Rico, bait stations made of open-ended cans containing toxic 57-g sun-dried fish baits were placed at densities of about 250 stations or less per km^2. Mongoose visitation to stations was 18–57 percent with mortality on two test areas estimated at 88–89 percent. All adults (but not young juveniles) were eliminated from an island (1.6 $\times$ 2.4 km) by using fish baits in bait stations placed at a density of 167/km^2 (Pimental 1955).

Toxic baits were used intermittently over a period of years (1950–60, 1973) on the island of Grenada, but Everard and Everard (1985 and 1988) stated that although mongoose numbers were reduced by toxic baits, results were temporary and did not provide a long-term solution to the rabies problem. C. Vargas (pers. comm.) used captive mongooses to test baits for delivering oral rabies vaccines and concluded that polyurethane sponge baits (Linhart et al. 1993) saturated with a 50:50 mixture of raw eggs and corn oil were preferred. Similarly, an egg/corn oil-flavored polyurethane sponge bait and DuPont polymer fishmeal baits (the latter containing different food additives) were all well accepted without apparent preference by mongooses on the island of Antigua. Tracking tiles and a short-term oral biomarker (DuPont oil blue A™ dye) were effective in recording mongoose and nontarget animal bait take and ingestion rates (Linhart et al. 1993). Also on Antigua, polyurethane

sponge baits and polymer fishmeal baits distributed by foot along transects on 2 1-km^2 study sites at rates of 400 and 2,000 baits/km^2 marked (THC and DuPont oil blue A™ dye) 42 percent and 60 percent of the mongooses at the low bait density levels and 91 percent at the high bait density. Polymer baits (500 baits/km^2) at central bait stations on a third 0.81-km^2 study site reached 69 percent of the mongooses (Creekmore 1992, Creekmore et al. 1994).

Toxic baits have been used to reduce mongoose numbers because of their predation on game birds and endangered ground-nesting birds. On St. Croix, U.S. Virgin Islands, fresh 8- to 15-cm-long baits using fish, canned fish, fresh beef, canned horsemeat, dehydrated fishmeal, or dogfood were compared for delivery of toxicants to mongooses. Fresh fish baits at a rate of 800–1,200/km^2 were placed early in the morning to reduce nocturnal bait removal by rodents. Ants were estimated to have destroyed 10–20 percent of baits, and incorporation of an insecticide (chlordane) into baits was recommended. A nonremovable ground-meat bait placed inside wooden bait stations to protect it from nontarget species was distributed at 0.2- to 0.4-km intervals (Spencer 1950 unpubl.). In Hawaii, U.S.A., 57-g ground-meat baits containing warfarin were placed in tube-shaped bait stations of hardware cloth located about 90 to 150 m apart (Woodworth and Woodside 1953 unpubl.). Also in Hawaii, diced-meat, ground-meat, and fresh fish baits were compared for administering thallium sulfate to captive mongooses (Kridler 1965 unpubl.). Concern about mongoose predation on the eggs and young of at least eight species of endangered Hawaiian birds led to additional studies (1984–88). Ground-meat baits containing an anticoagulant and protected by bait stations made of plastic pipe placed 250 m apart within 0.25- to 1-km^2 plots have been tested. A 100-percent mortality rate was estimated by monitoring radio-collared mongooses trapped and released within test areas prior to treatment (Keith et al. 1990). No efforts have been made to evaluate oral rabies vaccines or to determine the efficacy of vaccine bait delivery by air to immunize mongooses over large areas.

Feral Swine *(Sus scrofa)*

Toxic baits are extensively used in Australia to control feral swine damaging crops and pastures, fences and watering sites, waterfowl habitat, and native vegetation. Swine numbers are also reduced because they prey upon young lambs and indigenous wildlife species. Concern about the role of feral swine in the maintenance and spread of endemic or exotic diseases has resulted in contingency plans to control their numbers by poison baits (McIlroy 1983).

Grains, grain-based pellets, fruits, vegetables, and meat baits have been used to deliver toxicants to feral swine. Recovery of placed baits following nighttime exposure, the use of dyed baits to reduce scavenging by birds, and burying or covering baits have been suggested as ways to increase their selectivity (McIlroy 1983).

Pretreatment feeding is considered the most important single step for effective ingestion of poison bait. Pretreatment for 3 days or more may be needed to accustom feral swine to novel bait types and to concentrate them prior to poisoning.

Establishment of permanent or semipermanent feeding stations is considered a worthwhile strategy under some conditions. Dead animals (carrion) were found to concentrate feral swine for increased baiting efficacy, and burying fermented grain bait kept it attractive for a longer period than grain placed on the ground surface. Buried bait was also considered less available for nontarget species (Allen 1984).

Wheat, sorghum, and corn were equally accepted by penned feral swine (Kleba et al. 1985); however, another study (O'Brien and Lukins 1988) found that bait type significantly affected uptake rates by free-ranging feral swine and pelleted baits were preferred. Investigators placed bait stations in areas showing signs of feral swine activity, often near water, and used fencing to exclude livestock. Pretreatment feeding was carried out for 3 to 7 days, and the amount of toxic bait subsequently placed was approximately 75 percent of untreated bait uptake (O'Brien and Lukins 1988). Elsewhere in Australia, a poisoned wheat bait was placed at recently rooted areas and at creek crossings, holes in livestock fences, and along

animal and vehicle tracks. Bait trails and 1 kg piles of treated grain were laid for 15 days at sites where untreated grain had been previously consumed. Most treated bait was covered with vegetation or fallen branches or buried to reduce uptake by nontarget species (McIlroy et al. 1989). McIlroy's team have suggested that a 95-percent or greater reduction of feral swine in New South Wales would be required in order to eradicate foot-and-mouth disease within a 3-week period.

Hone (1986 and 1992) developed predictive models for poisoning vertebrate pests, especially feral swine, and applied his models to the results of an actual swine poisoning program. He also discussed the use of models both for planning and for evaluating population reduction programs.

Feral swine control in two national parks in Australia and United States was studied by using various indices such as the extent of rooting, track counts, swine feeding on plants, and deposition of feces to measure the success of population reduction programs. Control was implemented on both areas because of extensive damage to and alteration of native vegetation. Partial control was achieved in the Australian park with treated wheat grain following pretreatment feeding of bread, acorns, and wheat for several days. The investigators (Hone and Stone 1989) recommended methods for further reducing feral swine densities.

In another Australian study, water-soaked wheat was applied in piles along trails for 14 days followed by warfarin-treated wheat placed at 69 sites for 57 days on a 94-km^2 study site. A 98.9-percent reduction in swine numbers was achieved; however, an additional 38 animals were removed during the following 12 months (Saunders et al. 1990). Warfarin-treated grain was placed out on two test areas either ad libitum or intermittently over a 14-day period to evaluate baiting frequency. Control was estimated at 61 percent and 35 percent, respectively, with little or no reduction of feral swine on an unpoisoned reference area (Choquenot et al. 1990).

Choquenot et al. (1993) used proportional bait take (i.e., index–manipulate–index) of soaked wheat grain laid in piles and along trails between piles to estimate the percentage reduction of wild pigs by trapping. Proximity to nontoxic wheat bait and hunger appeared to be the primary factors responsible for seasonal differences in bait consumption by feral swine. Trail baiting in the hill country of southeastern Australia was more likely to be effective in late autumn than during other seasons. Bait types (wheat *v.* pellets) and whether or not baits were covered also affected bait uptake by swine and nontarget mammals and birds (McIlroy et al. 1993). Significant factors affecting bait uptake by swine in southeastern New South Wales, Australia, were the locality characteristics relative to vegetative cover, recent swine activity, and season (Saunders et al. 1993). A recent Australian document outlined the conduct of pest control in each State including that for feral swine and proposed national guidelines for future activities (Braysher 1993).

The status of feral swine (Brooks and Ahmad 1993) and use of several candidate toxicants and delivery methods have been evaluated in Pakistan. Dough baits made of wheat flour were left overnight at sites where swine activity had been observed. Bait of whole oats was placed in furrows and lightly covered with soil; another method involved surface placement of treated whole-wheat grain bait after 4 to 5 nights of pretreatment. Wheat grain baits in buried plastic bags and similar baits placed in modified wooden hog feeders were also tested. A cracked-wheat grain bait in sugar-flavored paraffin was placed in soil furrows and checked daily. The advantages and disadvantages of each type of treatment were summarized by these investigators (Brooks et al. 1990 unpubl.).

In the Galapagos Islands, Ecuador, Coblentz and Baber (1987) found that poisoning destructive feral swine that had been introduced onto the islands had the best potential and was the most cost-effective control method of several under consideration. Fifty-nine percent of placebo toxic baits (30 g of goat meat) were eaten following their placement for 2 to 4 days along trails and under vegetation.

Different food baits and olfactory attractants were tested using captive and free-ranging feral swine in the Great Smoky Mountains National Park (Southeastern United States), where swine were adversely

affecting native vegetation. Investigators tested 28 candidate attractants in pens and noted swine responses from adjacent observation blinds. Ten attractants and a control were later field-tested using free-ranging feral swine response to attractants placed at tracking stations. The study was flawed by procedural problems and low visitation rates to tracking stations. However, in general, results suggested that feral swine significantly preferred fermented corn mash to other baits, and that there were no significant preferences for the various olfactory attractants (Wathen et al. 1988 unpubl., Peine and Farmer 1990).

Polymer fishmeal baits containing biomarkers were hand-placed in a grid pattern on a 405-ha test site on Ossabaw Island, GA, and uptake by feral swine was found to be rapid: 88 percent of monitored baits were removed within 72 hours. Capture of feral swine after treatment showed that 95 percent had consumed one or more baits (Fletcher et al. 1990). More recently, aerial distribution of polymer fishmeal baits, also on Ossabaw Island, has shown potential (D. Kavanaugh, pers. comm.). These results suggested that delivery of oral vaccines for control of pseudorabies and swine brucellosis may be feasible.

Lastly, efforts are underway to develop baiting methods for delivering oral vaccines to control hog cholera (classic swine fever) in Germany (A. Neubert, pers. comm.) and in France (E. Masson, pers. comm.).

White-Tailed Deer *(Odocoileus virginianus)*

Treated feeds or baits containing vaccines, medications, contraceptives, or biomarkers have the potential for controlling diseases of white-tailed deer, reducing local populations where they are a nuisance or causing damage to agriculture, or for studying deer movement and activity patterns. Little information is currently available as to how these agents might be delivered. Furthermore, not much is known about how seasonal movements, habitat use, behavior, and food preferences of deer might affect acceptance of free-fed treated grains or food pellets. As recently as 1980, Hubert et al. stated that there was a lack of studies aimed at determining use of supplemental rations by high density free-ranging deer populations. Supplemental feeding of deer with a goal of improving hunting success is a common practice, but few data are available that relate such feeding to the oral administration of chemical or biologic agents. In theory at least, the various types of automatic deer feeders currently marketed would be suitable for dispensing treated grain or pellets.

Whole corn coated with a marker of metallic glitter was used to deliver invermectin to control ear mites in captive deer (Garris et al. 1991). A corn bait was also used to deliver an anthelmintic to treat fluke-infected deer (Qureshi et al. 1994). Shelled corn and apples were preferred over commercial pelleted feeds and were used to deliver an oral candidate contraceptive to confined deer within a 24.3-km^2 fenced enclosure. The corn was covered with molasses and mixed with alfalfa granules impregnated with DES. Prebaiting trials suggested that deer would consume 0.23–0.46 kg of treated corn per day. Quartered apples containing tablets of DES also were used to deliver the compound to deer (Harder and Peterle 1974). A commercial dairy cattle ration and later a mixture of shelled corn, oats, molasses, and a mineral–vitamin supplement were fed over time and compared with consumption of natural forage (Hubert et al. 1980). In a similar study, Ozoga and Verme (1982) compared seasonal consumption of a supplemental pelleted ration with natural forage and the response of the deer herd over a 5-year period. Seasonal differences in feeding activity (Ozoga and Verme 1970) and consumption of a pelleted ration (Wheaton and Brown 1983) have been reported for captive deer in Michigan and Texas, respectively, further suggesting that the success of administering chemical or biological agents will depend, in part, upon seasonal factors such as the availability of natural forages. Free-ranging deer were offered a pelleted supplement to determine how long they stayed at the feeding site (mean = 12.4 min), how long they fed (mean = 2.6 min), and how much they ate per visit (mean = 0.68 kg) (Zaiglin and DeYoung 1980). Free-ranging deer use and habituation to three supplemental feeds (two pelleted, one corn) were

evaluated; consumption increased nearly sevenfold over a 1-year period (Murphy et al. 1992). Confined ad libitum-fed deer consumed an average of 1.6 kg/day of a commercial deer feed (Warren et al. 1984).

White-tailed deer had no preference for protein-energy supplement blocks coated with extracts of cedar fronds, cloves, wintergreen, or a commercially sold attractant (Gamelur™) when compared with untreated blocks (Ullrey et al. 1975). Anderson et al. (1975) field-tested the above supplemental feed blocks by placing five blocks coated with the extracts mentioned above at each of four deer winter yarding sites in Michigan. Despite the feeding stations being placed where deer were active, consumption was noted at only two. Deer appeared to use those blocks located near main deer trails leaving the yarding area. Salt blocks in association with several olfactory lures have been evaluated for use as deer baits. Apple, peanut butter, acorn, and sweet corn extracts were tested, and the first three were found to enhance the effectiveness of salt blocks significantly. Mineral blocks followed by salt blocks and mineral–molasses blocks were preferred in that order. Mineral blocks with apple extract were as attractive to free-ranging deer as mineral blocks presented with mixtures of the above extracts (Mason et al. 1993). Solid baits comprised of mineral blocks with the above-mentioned extracts, and a liquid bait using similar substances (plus water, glycerine, and salt) are described elsewhere in this publication (Mason et al., this volume).

Several investigators have reported on the characteristics of naturally occurring mineral licks and their use by deer (Weeks and Kirkpatrick 1976, Weeks 1978, Jones and Weeks 1985). Most deer frequented licks that were located within or adjacent to their home ranges, but some deer also traveled to licks outside their home range. Artificial licks have been suggested, with salt blocks to be placed 1.5 km apart (Wiles and Weeks 1986). A supplemental mineral supplement was evaluated as a means of improving body mass and increasing antler size (Schultz and Johnson 1992). Such licks or salt blocks may be useful for dispensing agents to free-ranging deer. Rock salt has been used as a bait in live traps for deer (Mattfeld et al. 1972).

Radio-tracked deer were found to use point attractants (e.g., cultivated crops and feeders), but these sources of food did not appear to draw deer from long distances (Licht 1987). Similarly, a simulated test of illegal baiting sought to determine if radio-collared deer were attracted to shelled corn at feeding stations. It was concluded that deer having home ranges that included a feeder used it; however, deer were unlikely to change their movement patterns to visit feeders located outside their home range (Jacobson and Darrow 1992, Darrow 1993).

Orally administered THC has been evaluated as a biomarker in captive deer that were given known doses of the antibiotic (Van Brackle et al. 1994). Large quantities of whole corn overcoated with THC and using Rhoplex-B60A as a sticker were offered as a supplemental feed to free-ranging deer. A high percentage (63.9–90.8 percent) of deer were subsequently found to be marked (Van Brackle 1994). IA given orally to deer will bind to protein and persist in the blood serum and thus appears to be another suitable biomarker for this species (White et al. 1995). Such markers can be used to determine the percentage of local deer populations that ingest treated baits and feeds and also as a means to learn about the movement and harvest patterns of hunter-killed deer (Van Brackle et al. 1994).

Summary

A review of the literature describing baits and bait delivery techniques revealed that considerable information was available for some species (e.g., the red fox in Europe and Canada) but that very little is known about others (e.g., the arctic fox and white-tailed deer). Moreover, literature specifically describing the field delivery of oral contraceptives is limited or nonexistent for nearly all species of interest. Fortunately, much of what is known about delivery of toxicants and vaccines can be adapted for oral contraception. Such information includes bait formulation, knowledge of effective ingredients, bait acceptance rates, use of biomarkers to assess baiting efficacy, selective placement of baits to reduce removal

by incidental species, seasonal and geographic variables associated with baiting, and minimum bait densities needed to treat the desired percent of targeted populations. Furthermore, concepts or field techniques that have been devised for some species can be modified or adapted with judicious care for different animals and situations. For example, the polymer fishmeal bait originally developed in the United States for raccoons was subsequently found to be well accepted and is now widely used for red foxes in Europe. Similarly, a corn, milk, and egg batter-based bait developed for raccoons was accepted by a high percentage of dogs in two rural Mexico studies. However, use of the same bait for different species sometimes may be a poor choice, even for other canids. The raccoon and red fox polymer fishmeal bait was found to be poorly accepted by domestic dogs in the United States, Mexico, and Egypt. Moreover, even the efficacy of biomarkers may differ among species. Tests in captive animals have shown that iodine levels in blood sera induced by iophenoxic acid may persist for different lengths of time (e.g., white-tailed deer *v.* coyote) such that sampling of treated populations must be adjusted accordingly. Differences in masticatory or ingestive behavior among species may also lead to incorrect assumptions; a rabies vaccine container and bait that effectively immunized red foxes has reportedly performed poorly for jackals.

The reproductive physiology and behavior of a species will play an important role in the development of contraceptive delivery systems. For example, dominance and sexual activity of certain individuals within social groups doubtless will have an important influence on when and how contraceptives should be delivered and which sex and age classes should be targeted. Whereas seasonal influences usually have a limited effect on toxicant delivery, this factor may be crucial for most species that have a restricted reproductive season. An understanding of factors such as those mentioned above will help define the approaches needed for contraceptive development. In fact, research on contraceptives (laboratory) and delivery techniques (field) should proceed concurrently, not sequentially, so as to reduce the time required for research and development and because they are mutually dependent upon one another.

Finally, proponents of wildlife contraception should be prepared to respond to both legitimate concerns and the misinformed perceptions of the public and lay and professional organizations that oppose artificially limiting reproduction of species that are hunted, that provide income or economic benefit, or that have been popularized in the media.

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Research and Field Applications of Contraceptives in White-Tailed Deer, Feral Horses, and Mountain Goats

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Abstract: This paper reviews our applications of long-acting implantable steroids and immunocontraceptives in selected wild ungulates. We implanted captive white-tailed deer does with levonorgestrel but did not successfully prevent conception. We also evaluated antisperm immunocontraceptives delivered remotely via biobullet. Does immunized with plasma membrane proteins isolated from deer or porcine sperm showed persistence of antisperm antibody titers for 5 months, but these titers did not cause infertility. Our research also has included applications via biobullet of antigonadotropin-releasing hormone (GnRH), antisperm, and antiporcine zona pellucida (ZP) vaccines in female horses. Although the biobullet was effective in delivering immunocontraceptives at distances of ≤25 m, the anti-GnRH vaccine did not significantly reduce foal production in treated mares. In separate experiments, our treatment of captive mares with the antiporcine ZP vaccine resulted in high antibody titers and caused infertility in most mares for 2 years. Federal scientists have experimented with implanting melengestrol acetate (MA) in free–ranging, exotic mountain goats following capture by helicopter. Treated goats demonstrated lower reproductive rates than controls goats; however, this technique was time-consuming and expensive. Practicality and feasibility are of primary importance when considering the application of contraception in wildlife management. Ideally, these contraceptives should cause long-term infertility or sterility and should be remotely deliverable to improve their potential applicability in wildlife population management.

Keywords: Biobullet, contraception, feral horse, fertility control, immunocontraception, mountain goat, remote delivery, white-tailed deer

Introduction

Recently, wildlife managers have had to consider controlling populations of wild and feral ungulates by nonlethal means (Warren 1995). Public opposition, legislative mandates, human safety concerns, or budgetary limitations often make the application of traditional methods of hunting, controlled shooting, poisoning, or trapping-and-relocation programs unacceptable management alternatives. Therefore, interest and research in wildlife contraception has increased dramatically in the past decade.

This paper will review our research and other published applications of contraceptives in white-tailed deer *(Odocoileus virginianus),* feral horses *(Equus caballus),* and mountain goats *(Oreamnos americanus)* with the assumption that these methods must be practical, safe, and time and cost effective to be useful in wildlife management. We believe that two primary contraceptive tools have potential applicability in these species: long-acting, implantable steroids and immunocontraceptive vaccines. Most of our work in this area has focused on development and evaluation of these wildlife contraceptives and evaluation of delivery-method efficacy.

White-Tailed Deer

Need for Control

White-tailed deer have become so overabundant in many areas of the United States that they now represent a significant problem for natural resource managers. Warren (1991) reviewed the historical causes of this problem and presented an ecological justification for control of overabundant deer populations in national parks. In addition to ecological concerns, overpopulated deer herds also represent a public health and safety risk and cause significant economic losses in the form of crop damage, damage to landscape plantings, and damage from deer–vehicle collisions (Conover and Decker 1991, Curtis and Richmond 1992).

While regulated public hunting can effectively control deer populations (Behrend et al. 1970), it cannot be used as a management technique in some areas (e.g., national or State parks and urban or suburban areas). Hence, there has been great public interest in contraception as a technique for controlling deer populations in these areas. But before they will be accepted by wildlife managers for routine use in deer population control programs, contraceptives must

be safe, effective, easily administrable, and, ideally, capable of lasting the reproductive lifespan of the doe (Matschke 1980).

Contraceptive Steroids

Oral administration of the synthetic progesterone melengestrol acetate (MA) (Roughton 1979) or the synthetic estrogen diethylstilbestrol (DES) (Harder and Peterle 1974) can inhibit ovulation in female deer, but these contraceptives are not practical because they require daily oral exposure. Microencapsulation of DES can extend the effective treatment interval to 30 days, but these high doses are not readily eaten by deer (Matchke 1977a).

Subcutaneous steroid implants can increase treatment intervals to several months or years, but these techniques require costly trapping and handling of individual deer. Silicone tubing implants containing MA and DES have successfully reduced reproductive rates in deer for 1 to 2 years (Bell and Peterle 1975). Matschke examined fertility control in deer with Silastic® implants of DES and a synthetic progestin (DRC–6246). Calculated release times for DES were 1–2 years *v.* 3 years for DRC–6246 (Matschke 1977b); however, suppressed reproduction in the field lasted only 2 years before depletion of DRC–6246 occurred (Matschke 1980).

Implants containing MA have caused infertility in nonpregnant captive deer for 2 years (Plotka and Seal 1989). When the implants were applied to pregnant does during winter, however, pregnancy was not terminated, and the implants had to be removed. Plotka and Seal recommended that pregnant deer not be treated with MA implants unless pregnancy is terminated. It is unfortunate that these contraceptive steroid implants cannot be used in winter during pregnancy because at that time deer generally are easiest to bait, capture, and treat.

Levonorgestrel (LNG) is an implantable progestin that provides effective, long-term (>5-year) contraception in humans (Diaz et al. 1982). Contraception of deer for >5 years from one treatment may justify the time and cost associated with capturing and treating individual deer and hence increase the potential for providing a practical technique for contraceptive management of deer populations.

Despite the potential for this deer contraceptive, two studies with LNG implants in captive white-tailed deer have shown this device to be ineffective. In the first study, Plotka and Seal (1989) implanted five does with a single homogenous silicone rod containing 200 mg LNG, and three of the five does became pregnant. Plotka and Seal did not measure LNG concentrations, so the lack of contraception may have been related to the shape and matrix of the implant, both of which can affect steroid hormone release (Robertson et al. 1983).

White et al. (1994) also tested LNG implants in deer but used the technique as it is applied in humans (i.e., 216 mg of LNG sealed inside six small silicone tubes). White's team compared six *v.* nine LNG implants (containing a total of 216 *v.* 324 mg of LNG) in adult *v.* fawn does. Fawns were included to determine the effects of LNG implantation on puberty attainment. Despite significant release of LNG from both doses of implants, three of five implanted adults and one of two fawns that survived 2 years after implantation became pregnant.

Norgestomet (NGM) is a synthetic progestin that has been applied successfully as a contraceptive in white-tailed deer (Kesler, this volume) and black-tailed deer *(Odocoileus hemionus)* (Jacobsen et al. 1995). This hormone originally was marketed for synchronizing estrus in domestic livestock. Antech Laboratories, Inc. (Champaign, IL), has complexed 42 mg of NGM into silicone rods and loaded it into biobullets (Kreeger, this volume) for remote delivery (Kesler, this volume). In both species of deer, NGM was nearly 100-percent successful in preventing pregnancies but it was effective for only 1 year (Jacobsen et al. 1995; Kesler, this volume). Therefore, annual treatments would be required to maintain control over deer reproduction. This requirement would limit the applicability of this contraceptive technique primarily to smaller populations and small areas where there is substantial control over the deer herd.

Immunocontraceptives

The basic principle of immunocontraception is to produce endogenous antibodies against a particular protein or peptide involved in the reproductive process. Sufficiently high antibody titers disrupt the function of the protein and cause contraception. Infertility is maintained as long as antibody titer levels remain high. However, fertility can resume after exposure to the antigen has ceased and the antibody titers decrease (Primakoff et al. 1988). For white-tailed deer, the proteins that have been evaluated as immunocontraceptive antigens have been the oocyte ZP and spermatozoa plasma membrane proteins.

Immunocontraceptives have numerous advantages over contraceptive steroids that may make the former more effective and efficient for use in deer. Immunocontraceptives can be delivered remotely, which makes them more feasible for field application than methods that require capture and immobilization of individual deer. Also, a protein-based vaccine likely would be deactivated if ingested orally by nontarget organisms, in contrast to the persistent tissue residues that often characterize the synthetic steroids. Digestion of the vaccine after oral ingestion also probably would prevent unintentional transfer to carnivores or humans.

Turner et al. (1992) successfully used porcine zona pellucida (PZP) antigen in an immunocontraceptive for white-tailed deer. Turner's team vaccinated white-tailed does with 5,000 heat-solubilized porcine zonae pellucidae (64.3 μg protein) emulsified in 0.5 cm^3 Freund's Complete Adjuvant and delivered remotely in a 1-cm^3 self-injecting dart. Booster injections of PZP emulsified in Freund's Incomplete Adjuvant were given 3–6 weeks after the initial injection. Six months after onset of the injection scheme, the does were bred to a healthy buck of known fertility. None of the ZP-treated does but six of seven (86 percent) of the control does produced fawns.

The requirement of multiple booster injections limits the practicality of using this contraceptive vaccine in free-ranging deer populations. However, recent advances in research with PZP include microencapsulation of the booster vaccinations, which allows release of the booster over a period of weeks or months so that only 1 vaccination/year is required (J. F. Kirkpatrick, pers. comm.; Stevens et al. 1992).

Several different sperm proteins also have been considered for use in immunocontraceptive vaccines (Naz and Menge 1990). Antisperm vaccination may cause infertility in the male or female. In the male, antisperm antibodies may cause an autoimmune response to the sperm, thus resulting in infertility (Mathur et al. 1988). Treating bucks in a free-ranging deer population with an antisperm vaccine would have limited effect on the reproductive rate of the herd because deer are polygynous breeders. However, applying an antisperm vaccine may be more practical if males and females do not have to be distinguished prior to treatment.

In the female, antisperm antibodies may cause agglutination of sperm (reviewed in Shulman 1986), reduced penetration of sperm through the cervical mucus (Clarke 1988), or altered sperm binding to the zona pellucida (Naz et al. 1992). Antisperm vaccines also may be "self-boosted" (i.e., additional exposure and boosting of the immunity against sperm may occur with each insemination). Some women with spontaneous sperm-antibody titers have reduced titers following the use of condoms, which probably function to prevent boosting from sperm in the vagina (reviewed in Shulman 1986). Thus, if antisperm vaccines are self-boosting, they may be more practical for field application than multiple booster vaccinations of antiporcine ZP vaccines.

Very little research exists on the use of antisperm vaccines in deer. To date, our research laboratory has presented the only data available on antisperm vaccines for deer (White et al. 1993). We developed antisperm vaccines using anterior acrosomal sperm plasma membranes from deer, bull, and boar sperm. These vaccines were injected into adult does, from which blood samples were collected for antibody titer analysis. High antisperm antibody titers occurred in does injected with antisperm vaccines made from all species tested. However, antibody recognition of deer sperm was greatest in those does injected with either deer or boar sperm. Despite high antibody titers that

persisted for at least 11 months after immunization, the does treated in this preliminary trial became pregnant.

Feral Horses

Need for Control

Feral horses occupy extensive areas of public land in the United States. Historically, these herds were controlled by local citizens who captured wild horses for use as beasts of burden, in pet food, and as rodeo stock. In 1971, the Wild Free-Roaming Horse and Burro Act (Public Laws 86–234 and 92–195) specifically eliminated these uses and required Federal agencies to control feral horses through capture and adoption (Wagner 1983). The adoption program has been very costly and unsuccessful. The cost of capture and adoption of each feral horse ranges from $300 (Turner and Kirkpatrick 1986) to more than $1,800 (Godfrey and Lawson 1986). In 1985, the Federal Government spent more than $5 million to remove and maintain up to 18,000 feral horses and burros through the "Adopt-a-Horse" program (Boyles 1986). Many of the horses captured were older stallions and were not in demand by potential adopters (Slade and Godfrey 1982). Clearly, alternative techniques for managing feral horse populations are needed.

Public sentiment and interest in feral horses is extremely high. This fact has led to the tremendous amount of political support this species has received. Visitors to national wildlife refuges and national parks that contain feral horses are very interested in observing these animals. Yet the agencies charged with managing these herds are faced with a dilemma: how to control the feral horse populations so as to minimize the effects of overgrazing on native vegetative communities and still meet public interest needs and/or comply with the Wild Free-Roaming Horse and Burro Act in a manner that is logistically and fiscally feasible.

Garrott (1991) and Garrott et al. (1992) evaluated the potential and economic feasibility of fertility control for managing feral horse populations. Based on their simulation analyses, Garrott and coworkers concluded that contraceptives could reduce substantially the number of horses that would have to be removed from Federal lands each year. Thus, fertility control may represent an effective alternative for feral horse population control that may be more economically feasible than maintenance and placement of excess horses in the Adopt-a-Horse programs.

Contraceptive Steroids

The harem breeding structure of feral horses, in which a dominant stallion breeds most of the mares in a particular harem, permits the use of male-based wildlife contraception management programs for this species. Turner and Kirkpatrick (1982) administered a microencapsulated form of testosterone propionate (MTP, given intramuscularly) to feral stallions. Foal counts were reduced to 0.07 foal/mare for MTP-treated bands compared to 0.37 for mares in control bands. The investigators also observed no differences in stallion behavior parameters, such as scent marking in response to mare elimination marking, mounting, or copulation. The primary advantage to this type of treatment was that the fertility of an entire herd could be controlled through the treatment of a single stallion. Disadvantages included the fact that immobilization of the stallion and multiple injections were required, resulting in high cost and hard work.

In a subsequent study, Kirkpatrick and Turner (1987 unpubl.; also cited in Turner and Kirkpatrick 1991) used MTP to treat feral stallions in a different population, each with a harem of proven fertility. In this study, the stallions were treated remotely instead of being immobilized. During the 5 years prior to treatment, harem fertility rate ranged from 42 to 50 percent; the year after MTP treatment, the fertility rate averaged 28.9 percent, compared to 45.4 percent for control harems during the same year. This method of contraception would require annual treatments and probably would be less effective in bands having sexually mature subordinate stallions or a high degree of movement by mares between harems.

Feral mares also have been treated with chemical contraceptives. Plotka et al. (1988) used silicone implants with estradiol and/or progesterone to inhibit

ovulation. Plotka's team observed greater levels of serum progesterone and estradiol for at least 21 weeks in treated mares but no reduction in ovulation or conception. In a subsequent study, Plotka et al. (1992) used silicone implants containing ethinylestradiol, estradiol 17–beta, or progesterone. The higher dosages of ethinylestradiol resulted in contraceptive rates of at least 88 percent for 3 years, with an estimated efficacy period up to 5 years. Contraception occurred regardless of route of implant delivery (subcutaneous, intramuscular, or intraperitoneal).

In another study, microencapsulated norethisterone (MNET, a synthetic progestin) was administered by remote delivery to six feral mares of proven fertility (Kirkpatrick and Turner 1987 unpubl., Turner and Kirkpatrick 1991). No contraceptive effect from this treatment was detected, which supported the results of Plotka et al. (1988) for progesterone.

Applications of microencapsulated steroids have a number of practical limitations. The microencapsulated suspension tends to settle out and clump if not delivered within 10 minutes of mixing (Turner and Kirkpatrick 1991). In addition, remote delivery of the suspension via barbless or microbarbed darts, which ultimately fall out of the animal, represents a potential for environmental contamination from lost or unrecovered darts. The method also necessitates careful calculation of velocity and trajectory in order to prevent rebound of the dart (Turner and Kirkpatrick 1991).

Immunocontraceptives

For feral horses, the proteins that have been evaluated as immunocontraceptive antigens have been hormones, the oocyte zona pellucida, and spermatozoa plasma membrane proteins. The only hormone used as an antigen for equine immunocontraception thus far has been luteinizing hormone-releasing hormone (LHRH, also referred to as gonadotropin-releasing hormone or GnRH). This hormone is a decapeptide released from the hypothalamus. The immunogenicity of peptides generally is low; therefore, LHRH must be conjugated to a larger protein to increase the response by the immune system.

Safir et al. (1987) used LHRH conjugated to human serum albumin (HSA) and combined with Freund's Complete Adjuvant as an immunocontraceptive vaccine to inhibit ovulation in captive mares. After multiple booster injections, three of five mares immunized against LHRH failed to ovulate for at least 5 months (duration of the study), whereas five of five untreated control mares ovulated. Inhibition of folliculogenesis and ovulation was related directly to LHRH antibody titers.

Field application of an LHRH immunocontraceptive vaccine has been evaluated in feral horses. Goodloe (1991) used a vaccine composed of LHRH (15 mg immunogenic activity) conjugated to keyhole limpet hematocyanin (KLH) and mixed with one of two adjuvants, either alum (AP) or Ribi ImmunoChem's triple adjuvant (TA; 100 mg each monophosphoryl lipid A, trehalose dimycolate, and *Bacillus Calmette–Guerin* cell-wall skeleton). Initially, captive domestic mares were given the vaccine as a means of monitoring antibody titer levels. Vaccines were prepared in a microencapsulated form for the first year of study in captive mares; biobullet delivery was used for the second year. The microencapsulated vaccine was designed to release one dose immediately upon injection, one dose in 2–3 months, and a final dose in 6–9 months. The microencapsulated vaccines could not be delivered via biobullet because of its limited payload capacity (300 mg).

During the first year of the study using microencapsulation, the vaccine with the TA adjuvant produced higher antibody titers than did the vaccine with the AP adjuvant (Goodloe 1991). Three of the four mares treated with the TA form of the vaccine ovulated an average of 111 days after initial treatment, compared to a mean of 44 days for control mares. Thus, this treatment delayed but did not significantly inhibit ovulation. Microencapsulated vaccine also resulted in swelling or an abscess at the injection site of each mare.

During the second year of study, the same mares were boosted remotely with a single biobullet. Although it did not stimulate antibody production sufficient to inhibit ovulation, this mode of vacination did not result

in abscess formation. Overall, there were no significant differences between treatment and control mares in terms of date of first ovulation, number of ovulations, or percentage of mares that became pregnant.

Goodloe (1991) applied the same vaccine via biobullet followed by one booster injection 2–3 months later and a second booster injection an additional 8 months later to 21 feral mares on Cumberland Island, GA. The field study further substantiated the results from the captive mares: foal production did not differ between treated and untreated mares.

The differences in success between the studies by Goodloe (1991) and Safir et al. (1987) could be due to either the type of adjuvant or conjugate used, or to the number of booster vaccinations given. However, both studies indicate that LHRH-based immunocontraceptive vaccines may be expected to produce infertility for at most a single breeding season, which obviously limits the practicality of this method of contraception for routine use in feral horse population management programs.

The oocyte ZP also has been used as an immunogen for contraception in horses. Liu et al. (1989) used a series of four vaccinations (given at 2- to 4-week intervals) with heat- solubilized porcine ZP (64.3 μg) and either aluminum hydroxide gel or Freund's Complete Adjuvant. Ten feral mares were vaccinated and released into their natural territory, known to contain fertile stallions. Eight months later, the mares were recaptured, and their pregnancy status was determined by rectal palpation. Four domestic mares also were treated and monitored in captivity. Contraception occurred in 12 of the 14 (86 percent) fertile mares studied.

Subsequently, Kirkpatrick et al. (1990) evaluated the applicability of this porcine ZP vaccine to feral horse populations on Assateague Island, MD. The investigators selected sexually mature mares with known breeding histories; 26 mares were treated with the porcine ZP vaccine, six control mares were injected with phosphate buffer and adjuvant, and 11 mares served as untreated controls. The vaccine contained 5,000 heat-solubilized porcine zonae pellucidae (64.3 μg protein) emulsified with equal volumes of Freund's Complete or Incomplete Adjuvant. An initial and two booster vaccinations were delivered remotely using self-injecting plastic syringe darts tipped with barbless needles.

Of the 26 porcine ZP-treated mares, 3 developed abscesses at the injection site after the third vaccination (Kirkpatrick et al. 1990). At the time of initial injection, 14 of the 26 mares (57.6 percent) were pregnant, and all these gave birth to live foals 1–3 months after the final injection. Similarly, two of the six control mares produced live foals that same year. In the year after vaccination, the foaling rate for mares treated with porcine ZP was significantly lower the year after treatment (3.8 percent) compared to the 2 years prior to treatment (53.8 percent). In addition, foaling rates were significantly lower for the 26 mares treated with porcine ZP in the year after treatment (3.8 percent) when compared to foaling rates for the six control mares (50 percent) or the 11 untreated mares (45.4 percent).

Kirkpatrick et al. (1990) had difficultly in relocating all mares they initially injected. Of those that were relocated, some had become very wary and could not be given booster vaccinations. Subsequent work by this research team has focused on developing a one-shot injection in which a booster vaccination is microencapsulated (J. F. Kirkpatrick, pers. comm.).

Kirkpatrick et al. (1991) continued this study and gave booster vaccinations to 14 of the original 26 mares treated with porcine ZP approximately 1 year following the initial treatment. Foaling data collected in the subsequent year indicated that foals were produced by 1 of 14 (7 percent) boosted mares, 3 of 6 (50 percent) control mares, and 7 of 16 (44 percent) untreated mares. During a third year for the same study, Kirkpatrick et al. (1992) treated 10 of the mares boosted during the second year with another identical vaccination. None of the 10 mares boosted for a third consecutive year became pregnant, as opposed to 11 of 20 (55 percent) control mares. The researchers also used intensive visual observations and urinary analysis to monitor behavioral estrus and ovarian function for seven of the treated and four of the control mares. Based on these analyses, only two of the

seven treated mares demonstrated ovulatory cycles. The lack of cyclicity was thought to be due to altered ovarian function as has been shown to occur in other species given a similar vaccine (Skinner et al. 1984, Dunbar et al. 1989).

Thus, the porcine ZP vaccine has been shown to be very effective as an immunocontraceptive in feral horses. However, the formulation of porcine ZP vaccine used by Kirkpatrick et al. (1990, 1991, 1992) has certain characteristics that may limit its practical applicability in the field. Use of Freund's Complete Adjuvant in the vaccine is a distinct disadvantage. Most approved veterinary vaccination protocols do not permit the use of Freund's Complete Adjuvant in any applications other than small-scale, experimental trials. Therefore, a porcine ZP vaccine containing this adjuvant probably will not be approved for routine use in feral horse population-control programs. In addition, because Freund's Adjuvant cannot be lyophilized, a vaccine incorporating it must be administered as a liquid in a syringe dart as opposed to the biobullet.

Because of the above-mentioned concerns over the use of Freund's Adjuvant, Willis et al. (1994b) evaluated the use of an alternative formulation of the porcine ZP vaccine for horse contraception. The Willis team's formulation contained 200 μg of porcine ZP antigen and 500 μg of synthetic trehalose dicorynomycolate glycolipid (Ribi ImunoChem Research, Inc., Hamilton, MT) as an adjuvant. This porcine ZP vaccine formulation was lyophilized and packed into biobullets (Kreeger, this volume) for remote delivery to captive horses. Goodloe (1991) had previously documented the efficacy of this method for remote delivery of immunocontraceptives to free-ranging feral horses. Willis et al. (1994b) treated four mares with an initial and one booster vaccination separated by 1 month. Four control mares were injected with biobullets containing only the adjuvant. All mares were bled for antibody titer analysis and bred to a sexually mature stallion at each estrous period through two complete breeding seasons. The control mares had no titer levels and conceived during their first estrous period, in contrast to three of the four mares treated with porcine ZP, which had high antiporcine ZP titers and did not conceive. The fourth treated mare had lower titer levels and was fertile, but she too became infertile after administration of a subsequent booster vaccination.

Willis et al. (1994b) monitored antibody titer levels and infertility during the breeding season the year after initial treatment and, despite not having received a booster vaccination in the second year of this study, all of the treated mares continued to have high titer levels and were infertile. The greater potential duration of contraceptive effect apparent for this porcine ZP vaccine formulation may greatly improve its field applicability compared to other formulations that require annual booster vaccinations.

Sperm proteins also have potential for being used as antigens in immunocontraceptive vaccines, although relatively little research has been done in this area with horses. The most extensively characterized sperm immunocontraceptive thus far was developed by Naz et al. (1984). Naz and coworkers developed a monoclonal antibody (MA–24) in the mouse that specifically recognized a glycoprotein found only on the postacrosome, midpiece, and tail of sperm. This glycoprotein was termed fertilization antigen 1 (FA–1). FA–1 was determined to be nonspecies specific because the MA–24 antibody cross-reacted with sperm from mice, rabbits, rhesus monkeys, and bulls (Naz and Menge 1990). The MA–24 antibody apparently causes contraception by blocking sperm–egg binding. Treatment of sperm with either the MA–24 antibody or antiserum against FA–1 prior to insemination significantly reduced in vitro fertilization rates as compared to untreated sperm (Naz 1987). In addition, female rabbits immunized with purified FA–1 had significantly lower in vivo fertilization rates (14.7 percent) as compared to control rabbits (91.7 percent) (Naz et al. 1986).

The only research published to date in the area of sperm plasma-membrane protein immunocontraception in horses was done in our research laboratory (Willis 1993, Willis et al. 1994a). We developed antisperm vaccines using plasma membrane proteins from porcine and equine sperm. These vaccines were adjuvented with synthetic trehalose dicorynomycolate glycolipid, lyophilized, and administered via biobullet to two captive mares. Four mares served as controls

and received biobullets containing only the adjuvant. All mares were bred to a fertile stallion, and blood samples were collected for antibody titer analysis. High antisperm antibody titers were detected in the treated mares, but no significant differences occurred in conception: one of two treated mares became pregnant compared to three of four controls.

Mountain Goats

Need for Control

The mountain goat is a native species in some mountainous habitats in North America. However, there is debate over its status as a native species on the Olympic Peninsula of western Washington. National Park Service (NPS) scientists in Olympic National Park maintain that the mountain goats were artificially introduced into this park by humans in the 1920's (Moorhead and Stevens 1982). Conversely, Lyman (1988) argued that this species may have become locally extinct on the Olympic Peninsula prior to the first biological surveys in the late 19th and early 20th centuries. If Lyman is correct, mountain goats may not be exotics in the park.

The status (i.e., native or exotic) of mountain goats in this park is very important because NPS management policies encourage the elimination of exotic species that may threaten the survival of native species. Ecological research conducted in the park has shown that fragile alpine plant communities are being threatened by grazing pressure from mountain goats (Olympic National Park 1981 and 1987). Because of this concern, NPS began a capture-and-removal program in 1981 to reduce or eliminate mountain goats from the park (Houston and Stevens 1988, Carlquist 1990, Houston et al. 1991). This removal program has been very costly (as much as $900–1,000 per goat removed) (Houston et al. 1991). Therefore, NPS has experimented with fertility control as a more cost-effective means of controlling mountain goats in the park. Lethal removal of mountain goats, while acceptable and cost effective for control of feral goats in some countries (Parkes 1990), is very controversial in the United States.

Contraceptive Steroids

In deciding which contraceptive techniques to evaluate, NPS scientists considered a number of criteria: ease of application in the field, safety to goats and humans, potential for treating a large proportion of the population, duration of efficacy, and cost. In an experimental evaluation of contraceptive and sterilization methods to control goats in Olympic National Park, Hoffman and Wright (1990) captured and placed silicone implants containing MA in the neck region of 11 female mountain goats. The investigators also captured five male goats in a different portion of the park and injected their epididymis with a sclerosing agent containing lactic acid (Chemcast™, BioCeutic Laboratories, Inc., St. Joseph, MO). The females were monitored in the field for a period of 5 years, during which the rate of kid production averaged 10 percent as compared to 68 percent for a similar group of untreated females in the park during the same time period. Comparative examination of the treated males 2 years after treatment confirmed blockage of their epididymides and sterilization. However, visual monitoring of the area occupied by the males for 2 years after treatment revealed no change in population size or the rate of kid production by females. Male-based contraceptive techniques obviously have limited potential for success in a wild, polygynous species. Hoffman and Wright (1990) concluded that the techniques they tested might have potential for controlling the numbers of mountain goats in the park. However, the requirement that goats had to be captured for treatment made these techniques very expensive and limited their widespread operational application over a larger portion of the park. In addition, captures by helicopter, which were mandatory for capturing mountain goats in the park, were discouraged in 1990 by the U.S. Department of the Interior's Office of Aircraft Safety after a risk assessment revealed that the goat capture procedures represented a significant human safety hazard (Tuler et al. 1992).

Immunocontraceptives

No published research is available on immunocontraceptives in mountain goats. Immunocontraception may have potential for application in Olympic National Park. However, logistical and fiscal limitations associated with fieldwork in this park will necessitate long-acting (preferably permanent) immunocontraceptives that can be delivered remotely via a biobullet or syringe dart shot from a helicopter. Field application of remotely delivered immunocontraceptives from a helicopter in the rugged, mountainous terrain of Olympic National Park likely represents the most difficult situation for contraceptive management of a wildlife population.

Conclusions and Practical Applicability

The above literature review demonstrates that several available contraceptive techniques are effective in individually treated deer, horses, and mountain goats. Additional literature on the application of contraceptives in other species has been reviewed by Bomford (1990) and Kirkpatrick and Turner (1991). Despite the success of some contraceptives in individually treated animals or in captive situations, management of wildlife populations with contraceptives may be infeasible if they are not practically applicable in the field. More research is needed to document that contraceptives can be effective when applied as a management technique at the population level. Garrott (1995) published results of a study that used computer simulations to consider the prospects of controlling wildlife populations with contraceptive techniques. However, nobody will know the true potential for contraceptive management until these techniques are tested under real-world conditions where feasibility of field applicability, interactions among ecological factors and processes, the potential population-level efficacy of treatments, and considerations of time and cost efficiency become paramount to the success or failure of any program for wildlife contraception.

A number of important practical questions exist relative to the potential applicability of contraception in wildlife management. Aside from the questions about the efficacy of the particular contraceptive agent, its duration of effect, and its safety to treated animals are important questions relative to the potential effects of the agent on the ecological food chain. In this regard, contraceptive vaccines likely will be safer than contraceptive steroids. Nonetheless, research is needed to determine the extent to which contraceptive vaccines may be effective after ingestion by nontarget organisms, including humans. Immunocontraceptives will not be approved by Federal and State regulatory agencies for routine field implementation in wildlife management programs until these potential secondary effects are documented as being environmentally insignificant.

Interactions among ecological processes will likely exert a great effect on the success of wildlife contraceptive-management programs. Reduced reproduction by females treated with contraceptives may provide greater chances for survival of offspring born to other untreated females in the population. In other words, there may be a compensatory increase in juvenile survival. Additionally, reductions in a particular population because of reduced reproduction could be offset by immigration of individuals from areas surrounding the treatment area.

Time and cost efficiency are extremely important issues when considering wildlife contraceptive-management programs. The contraceptive agents themselves may be economical, but the personnel and operating expenses associated with delivering contraceptives to significant proportions of individuals in a wildlife population likely will be cost prohibitive. State wildlife agencies obtain much of their funding from sales of hunting and fishing licenses and Federal aid funds for wildlife restoration (e.g., the Pittman–Robertson tax). Therefore, it may be inappropriate for these agencies to use these revenues for contraceptive management programs. Alternate State, Federal, municipal, or private funds probably will be required to support contraceptive management programs.

The nature of the wildlife management profession has changed greatly in the United States during the past few decades. Societal changes have altered the opinions and expectations of the public we serve. Wildlife biologists and managers are entrusted by the public with the responsibility of managing our wildlife resources. Nonlethal techniques for wildlife population management are increasingly demanded by the public in some situations. The potential for contraception to become a successful wildlife population management technique in certain publicly sensitive situations is great. Wildlife biologists have a professional obligation to consider all possible techniques for use in wildlife population management.

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Immunocontraception in White-Tailed Deer

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Abstract: Immunocontraception may have management application for white-tailed deer populations in parks and preserves, where hunting is illegal or impractical. This study examines physiological aspects of immunocontraception with porcine zonae pellucidae in 53 fertile white-tailed does. In separate studies, we employed protocols of three and two porcine zona pellucida (PZP) injections as well as two different protocols using one injection. Each one-injection vaccination consisted of one dose of porcine zonae pellucidae as in other protocols plus a second controlled-release dose of the material delivered via an osmotic minipump implant or injected, biodegradable polymer microspheres. We monitored fawn production for 1 to 3 years in all does and measured serum PZP antibody titers at various times after treatment in 15 randomly selected PZP does and in 2 control does. All three-injection PZP does were given a single PZP booster inoculation after 1 year. None of the two- or three-injection does and none of the one-injection does with a minipump produced fawns the first year after treatment, whereas two of seven does given a single injection with controlled-release microspheres produced fawns. In 49 control-doe breeding seasons, the pooled incidence of fawn production was 93.8 percent. Regarding reversibility of infertility, the incidence of fawn production was 75 percent within 2 years after treatment was discontinued. Serum anti-PZP antibody titers were present only after PZP treatment, and highest titers occurred in does given two or three separate PZP injections. PZP-treated does showing >50 percent of maximal antibody titers at the onset of a breeding season did not produce fawns; those showing <33 percent of maximal titers did. These data demonstrate in white-tailed does that (1) multiple-injection PZP vaccination can produce complete contraception for at least one breeding season, (2) the contraceptive effect is reversible within 2 years in most does, (3) an elevated anti-PZP antibody titer occurs after PZP vaccination, and (4) multiple-injection PZP vaccination produces a sustained antibody response through at least one breeding season.

In separate field studies of wild, free-roaming deer in three locations, we successfully lured does to within darting range using bait stations. In two locations, we attempted and achieved remote delivery PZP vaccination of 60–90 percent of does. In the one location for which we have data on fawn production, the fawning incidence was 0/10, 6/9, and 8/9 for two-and one-injection porcine zonae pellucidae and untreated controls, respectively. In the same location, we monitored seasonal aspects of activity, behavior, and physical condition. We also determined that there is a good potential for remote assessment of pregnancy in does using measurement of pregnancy-specific elevations of steroid metabolites in fecal samples.

Keywords: antibody titers, fertility control, immunocontraception, *Odocoileus virginianus,* reproduction, vaccine, white-tailed deer

Introduction

The purpose of this paper is to present studies performed by our research team addressing immunocontraception in white-tailed deer *(Odocoileus virginianus)*. Our team participated in the 1987 and 1990 "Contraception in Wildlife" conferences in Philadelphia (U.S.A.) and Melbourne (Australia). At the 1987 conference, there were no papers addressing immunocontraception; our own work in this area was embryonic (Turner and Kirkpatrick 1991). At the 1993 conference in Denver, CO, there were 16 papers on immunocontraception! Since the original studies of immunocontraception in feral horses, we have treated 45 mammalian species with the same porcine zona pellucida (PZP) antifertility vaccine. To date, these treatments included 157 individuals across 28 species of captive, exotic ungulates, with 87.7-percent inhibition of fertility in 68 outcomes; and 172 free-roaming equids, with 92.5-percent inhibition of fertility in 133 outcomes. These numbers provide strong encouragement for the exploration of PZP vaccine use for contraception in wildlife.

The first study of immunocontraception in wildlife was in free-roaming feral horses (Kirkpatrick et al. 1990a). We used PZP vaccine, delivered remotely by dart, to produce greater than 95-percent infertility. The effects were reversible after 1 year, did not affect pregnancies in progress, and did not produce behavioral side effects (Liu et al. 1989, Kirkpatrick et al. 1990a and 1991a). Based on this success using PZP vaccine to inhibit fertility in feral horses, we began a series of studies to evaluate the potential for PZP immunocontraception in white-tailed deer.

The PZP vaccine appears to act by inhibiting fertilization (Sacco et al. 1984). In mammals, the egg is surrounded by a noncellular membrane named zona pellucida (ZP). The ZP is made up of three glycoproteins; one of these, ZP3, is the sperm receptor (Florman and Wassarman 1985). Injection of raw

porcine ZP protein plus adjuvant (to enhance the immune response) into other species causes the female to raise antibodies against the sperm receptor on the ovum. It is hypothesized that the antibodies bind receptors which prevent sperm binding and, hence, fertilization.

Let us now turn to white-tailed deer. In times past, white-tailed deer populations lived unrestricted in their habitat, regulated by predation, resource limitations, and human hunting. With increasing human settlement and urbanization, predators have been eliminated, and many deer populations and their habitat have become islands surrounded by human communities. While hunting has been the standard approach to controlling North American deer populations, it is illegal or impractical in many parks and preserves. Furthermore, current management practices in these places have not limited populations.

We have undertaken studies of immunocontraception in white-tailed deer in hope of developing an alternative tool for management of unhuntable populations (Kirkpatrick and Turner 1993). We will present data from our several studies of captive, unrestrained deer and from three ongoing studies of wild, free-roaming deer. Major issues which must be addressed in considering the potential for PZP vaccine in deer contraception essentially comprise the list of requirements for a management-useful contraceptive vaccine for deer: (1) accessibility to the deer, (2) reliable treatment-delivery technology, (3) high efficacy of treatment, (4) appropriate duration of treatment effect, (5) reversibility of treatment effect, (6) safety of treatment to deer and environment, (7) safety of treatment to deer consumer, (8) cost effectiveness of treatment, and (9) public acceptance of method and treatment.

In our studies, we have attempted to bring together the most important elements of successful wildlife contraception: scientifically sound basic research and successful application to wild animals in the field. This union has included collaborations with the Smithsonian Institution's Conservation and Research Center (Front Royal, VA) on deer behavior and social dynamics; Canada's Pt. Pelee National Park and the Ontario Ministry of Natural Resources on access to wild deer; Fire Island National Seashore, NY, on treatment in the field; and The Humane Society of the United States (Washington, DC) on education regarding urban deer issues.

Studies in Captive Deer

Materials and Methods

General—We examined the effects of a PZP vaccine on fertility and plasma antibody titers in studies consisting of four experiments testing various protocols of PZP contraceptive vaccine, each with its own controls. Assessment of contraceptive effectiveness and reversibility was determined by comparison of fawn counts in PZP-treated and control does. Serum anti-PZP antibody titers were measured by specific enzyme-linked immunosorbent assay (ELISA).

The PZP vaccine, an emulsion of porcine zonae pellucidae and adjuvant, was prepared from porcine ovaries as described (Liu et al. 1989). It was stored at –20 °C until used in the field. An emulsion of 0.5 cm^3 vaccine (equivalent to approximately 5,000 zonae or 64.3 μg of protein) in phosphate buffer and 0.5 cm^3 of Freund's Complete Adjuvant (FCA) was prepared and injected remotely (Turner et al. 1992). All inoculations subsequent to the first one contained Freund's Incomplete Adjuvant (FIA) in place of FCA. In a previous study, the use of incomplete adjuvant in subsequent injections produced effective contraception with no visible signs of abscess at the injection site (Turner et al. 1992).

The studies were conducted at a private, U.S. Department of Agriculture-approved deer facility in west-central Ohio (lat. 41° N., long. 84° W.). We used 53 does ranging in age from 2 to 7 years and in mass from 47 to 65 kg. All does had fawned previously. They were maintained in two adjoining 0.5-ha, grass-covered enclosures with a 3-m chain-link fence. The west end of the enclosures was a solid wooden wall with an overhang to provide the deer shelter from cold, wind, and precipitation. They were fed alfalfa, corn, oats, and commercial food pellets (Purina Lamb

Conditioner®, Purina Corp., St. Louis, MO). Food and water were provided ad libitum. We weighed the does and noted their condition when the study began. Health and condition of each doe were subjectively assessed monthly during the study in terms of vigor, demeanor, and physical appearance. Each doe was eartagged to facilitate remote identification and randomly assigned to a PZP or control group.

Effects of PZP Vaccine on Fawn Production—For each of four experiments, does were vaccinated prior to the breeding season, placed with a fertile buck, observed for breeding, and observed for fawn production the following spring and summer. Details of these activities are presented below.

Antifertility effectiveness in each experiment was determined by comparing fawn production in PZP-treated and control does. The PZP vaccine was given as two or three injections, 3 weeks apart, or as a single injection combined with a 4-week controlled-release delivery of porcine zonae pellucidae. A three-injection experiment (begun 1989), a two-injection experiment (begun 1990), and two one-injection experiments (begun 1991 and 1992) were performed separately, with respective controls. Untreated controls or a combination of does that received sham injections (saline and adjuvant) and untreated controls were used in each experiment.

Each year, injections were begun in October. In November or early December (3–4 weeks after the end of PZP exposure), PZP-treated and control does were placed together in groups of no more than 18. A single breeder buck was placed with each group. The bucks were healthy 3-year-olds that had previously sired fawns. We observed breeding within 3 days after placing the buck and does together, and the male was permitted to remain with the does thereafter.

Although details of estrus and breeding behavior were not recorded, increased activity and restlessness of a given doe accompanied by increased attention to the doe by the buck, indicated the onset of estrus. A breeding was considered to have occurred when the buck mounted a receptive doe and, after 7–15 seconds, the doe and buck separated vigorously (Halls 1984). Brief mountings (3–4 seconds) were not considered a successful breeding. During the breeding season, observations were usually made daily for 15–30 minutes during feed replenishment, around 6 A.M. and 5 P. M.

After the breeding season, deer were observed for health and physical condition as described previously. Fawning was usually complete by mid-June. Different incidences of fawning among groups in a given experiment were tested for significance using binomial probability distribution (Zar 1984).

In the three-injection experiment treated does (n=5) received three separate PZP treatments, three weeks apart, beginning October 1989. Control does (n=6) were untreated. In October 1990, the above PZP-treated does were given a booster inoculation (porcine zonae pellucidae + FIA), and the above control does were given a sham (saline + FIA) booster (n=4) or remained untreated (n=2). In order to determine reversibility of treatment effect, the above control and previously treated (1989; 1990 booster) does received no treatment in October 1991 but were bred as usual during November–December 1991.

In the two-injection experiment, does (n=8) received two injections, 3 weeks apart in November 1990. Control does (n=8) were given saline/adjuvant (n=4) or were untreated (n=4). The 16 does in the experiment were divided into 2 groups (4 receiving porcine zonae pellucidae, 2 receiving sham injections, and 2 left untreated in each group). Each group was placed in a separate pasture with a buck of proven fertility. Fawns were counted in the summer of 1991. In order to determine reversibility of treatment effect, the remaining PZP-treated and control does were bred without further treatment in November 1991 and November 1992, and fawns were counted in the summers of 1992 and 1993, respectively.

In the first single-injection, controlled-release experiment, six does were given one injection of porcine zonae pellucidae (65 µg) and FCA, and on the same day an osmotic minipump containing 65 µg of porcine zonae pellucidae was implanted subcutaneously in the neck of three of these does. The pump was designed to release PZP antigen continuously for 4 weeks at an approximate rate of 2.5 µg/day. The

three other does that received just the single PZP injection served as "active" controls: two of these does were implanted with a pump containing saline buffer, and one of these does was not implanted. Another six does served as untreated controls. Does were bred, and fawns were counted as described previously.

In the second single-injection, controlled-release experiment, seven does were each given one injection containing 130 µg of porcine zonae pellucidae emulsified in FCA. The pellucidae in the injection were present in one of two forms: 65 µg unsequestered or 65 µg sequestered in 10- to 50-µ microspheres of bioerodable lactide and glycolide polymers in a 1:1 ratio. The polymer microspheres were prepared by E. Schmitt, R. Linhardt, and D. Flanagan at the University of Iowa using a coacervation method described by Wang et al. (1991a). Release rates were projected to be continuous over approximately 4 weeks, with greater release in weeks 1 and 4 (Wang et al. 1990 and 1991a). Seven does served as untreated controls. Does were bred, and fawns were counted as previously described.

Effects of Porcine Zonae Pellucidae on Antibody Titers—Anti-PZP antibody titers were measured in serum obtained from randomly selected does in each of the above experiments. Blood collection was scheduled to provide antibody titer data for prevaccination, peak titer period, and after fawns were produced. Except for prevaccination, the timing of blood collections was partly dictated by accessibility to deer and availability of assistance for their handling. In total, 15 PZP-treated and two control does were sampled. Does were tranquilized with xylazine (1.5 mg/kg) delivered by darting. The tranquilizer induction time was 8–12 minutes. Samples (10 mL) were taken by jugular venipuncture and allowed to clot for 1 hour at approximately 30 °C. Serum obtained from these samples was frozen at –20 °C until assayed for anti-PZP antibody titers. These titers were determined using an ELISA based on the method described by Voller et al. (1986), with the modifications reported previously for horses (Liu et al. 1989) and additional modifications for deer. The assay is described below.

Fifty µL of 5 µg/mL zona antigen solution in 0.1 M glycine buffer (pH 9.5) were placed in each well of a flat-bottom ELISA microplate (Cat. #MS-3496, E & F Scientific Products, Saratoga, CA) and incubated overnight at 4 °C. The plate was washed with PBS-Tween and incubated for 1 hour each with 50 µL of the following PBS-Tween diluted reagents and subsequent washings: deer serum (1:500), biotinylated rabbit antideer immunoglobulin G (IgG) (1:250), or alkaline–phosphatase avidin (Zymed Laboratories, San Francisco, CA) (1:1,000). Finally, 50 µL substrate solution of 1 mg *p*-nitrophenyl phosphate per mL (Sigma Chemical Co., St. Louis, MO) in 10-percent diethanolamine buffer (pH 9.8) was distributed to each well. After incubation of approximately 20 minutes, the plate was scanned for absorbance at 405 nm using an MR 580 Microelisa Auto Reader (Dynatech Laboratories, Alexandria, VA). The rabbit antideer IgG (Kirkegaard & Perry Laboratories, Gaithersburg, MD) listed above was biotinylated according to the method described by Bayer et al. (1986) and Gretch et al. (1987).

The experimental sera were tested in duplicate, and their results were expressed as percentage of the positive reference serum, which consisted of a pool of sera that had demonstrated anti-PZP antibody titers in the high positive range. Maximal serum antibody titers usually occur 4–6 weeks after initial PZP injection (Liu et al. 1989). We therefore determined the average value of maximal titers (designated as 100 percent) achieved during this period in six successfully contracepted does. All other titers are reported as a percentage of this positive reference serum. Pooled pre-immunization sera served as negative control.

In the three-injection experiment, four PZP-treated does were blood-sampled for titer testing at 0, 6, and 36 weeks. Samples were also taken from three of these does at 92 weeks (approximately 1 year after a booster inoculation). In the two-injection experiment, four PZP-treated and two sham-treated does were bled at 0, 6, and 40 weeks. Two of the above PZP-treated does were also bled at 52 and 64 weeks. In the single-injection, osmotic-pump experiment, blood samples were taken at 0, 4, 12, and 32 weeks after vaccination from PZP-injected does in the PZP-

pump group (n=3) and in the saline-pump group (n=2) for anti-PZP antibody testing. Untreated and unimplanted does were not bled. In the second single-injection experiment, employing controlled-release microspheres, blood samples were taken at 0, 4, and 36 weeks after vaccination from four PZP-treated does.

Where appropriate, titer data are presented as $\mu \pm$ the standard error of the means (S.E.M.). Results are presented descriptively, without statistical analysis, because responses to treatment were marked and straightforward in most cases, and differences in sample intervals and the small group sizes limited possibilities for comparisons.

Results

General

None of the does exhibited a visibly draining abscess at the injection site, but a raised area of 16–24 mm in diameter developed at the final injection site on six does. This response disappeared after 8–10 days in three of the does, after 2 months in two other does, and after 6 months in the sixth doe. We observed 60 percent of the does being bred. Subjectively, we observed no differences in breeding behavior between treated and control does. During the breeding seasons monitored in these studies, none of the control does was observed breeding after January, while 26 percent of the PZP-treated does were observed breeding through February. Breeding of two treated does was observed during the first week of March.

Parturition began late in May each year and spanned a 4-week period, with the exception of two fawn births in July and one in August of 1992. All fawns appeared healthy and nursed normally.

Based on criteria described in Methods, does generally appeared to be in good health and physical condition throughout the study period from October 1989 to July 1993. However, during this period two treated and two control does died from causes unrelated to the study as determined by necropsy and bacterial culture. Deaths were associated with a jaw infection (n=1), a compound fracture (n=1), and *Clostridium* sp. (n=2). One treated and one control doe also died of undetermined causes within a 2-week period.

Effects of Porcine Zonae Pellucidae on Fawn Production—Across all experiments, involving 49 control-doe (no PZP) breeding seasons, 1 sham-treated doe and 2 untreated control does did not produce fawns. The fawning rate for sham (7 of 8) and untreated (39 of 41) control-doe breeding seasons was not different (p <0.01), and these control data were pooled. In contrast, the incidence of fawn production for the first breeding season was 0 percent (100-percent contraception) among does given multiple injections and 20 percent (80-percent contraception) among does given a single injection of porcine zonae pellucidae plus additional pellucidae in a controlled-release form of PZP vaccine. The annual incidence of fawning among control does ranged from 83 to 100 percent between 1990 and 1993, and the incidence of twinning was 38 percent. Over all years, 46 or 49 doe-breeding periods associated with control does resulted in production of fawns (reproduction success = 93.8 percent). Thus, the pooled pregnancy failure rate among control does was 6.2 percent.

The fawn-production data for all treatments are presented in table 1. Among does given a three-injection protocol (in October–November 1989), none of five PZP-treated does produced fawns in 1990 while all six control does did. After booster PZP treatment in October–November 1990, none of the above five PZP-treated does produced fawns in 1991. Of the above six control does, four received sham injections and two remained untreated in 1990; all six again produced fawns in 1991. One PZP-treated and one control doe in these groups died in 1991. In both 1990 and 1991, the differences in fawn production between PZP-treated and control groups were significant (p <0.01). The remaining PZP-treated (n=4) and control (n=5) does were bred in the 1991 rut without further manipulation and produced fawns in 1992 as follows: four of five controls and three of four PZP-treated does. There was no difference between groups (p >0.1).

Table 1. Fawn production among white-tailed deer given three, two, or one injections with porcine zonae pellucidae

Experiment	Year	Treatment (+ or –)	Years since treatment	Sample size (*n*)			Percent of does producing fawns[1]	
				PZP	Control[2]	Placebo[2]	PZP	Placebo & control[3]
3-injection	1989	+	0	5	6	0	[6]0	100
	1990	+[4]	0	5	2	4	[6]0	100
	1991	–	1	4	5	0	75	80
2-injection	1990	+	0	8	4	4	[6]0	100
	1991	–	1	7	7	0	[6]29	100
	1992	–	2	4	4	0	75	100
1-injection/pump[5]	1991	+	0	3	6	3	[6]0	83
1-injection/ microspheres	1992	+	0	7	7	0	[6]29	86

[1] Fawn production was determined by observation during May–August the year following PZP treatment.

[2] Control = untreated control; placebo = saline and adjuvant.

[3] Placebo and control does were pooled for comparisons with PZP-treated does because fawn production did not differ between placebo and control does (binomial test for equality of proportions, $p < 0.05$).

[4] Treatment was a single-injection booster of PZP + adjuvant.

[5] PZP = PZP injection + PZP pump, placebo = PZP injection + saline pump ($n = 2$) or no pump ($n = 1$).

[6] Different from pooled control, based on binomial test for equality of proportions, $p < 0.05$.

Among does in the two-injection experiment that were bred in the 1990 rut, none of eight PZP-treated and eight of eight controls became pregnant. The between-group difference was significant ($p < 0.01$). One treated and one control doe from these groups died late in March. Necropsy showed that the PZP-treated doe was not pregnant but the control doe was. In order to test possible multiple-year effectiveness of porcine zonae pellucidae, without further treatment, all 14 of the above does were bred in the 1991 rut. Two of seven previously treated with porcine zonae pellucidae and seven of seven control does produced fawns in 1992. The between-group difference was significant ($p < 0.05$). All control animals had fawned by June 25, while the two births to PZP-treated does were June 29 and August 15. Prior to the 1992 rut, three of seven previously PZP-treated (two-injection) and three of seven control does were dropped from the study for economic reasons. The remaining four PZP-treated does, which had not produced fawns since the study had begun, were bred in the 1992 rut and three of these four does produced fawns before June 30, 1993. The other doe did not produce a fawn. All four of the remaining controls produced fawns. The difference between groups was not significant ($p > 0.10$).

Data from the multiple-injection studies in table 1 address reversibility of PZP treatment. Of the treated does monitored for reversibility, 75 percent produced fawns within 2 years of cessation of treatment.

In the single-injection, osmotic-pump experiment, none of the does given a PZP injection produced fawns, regardless of the presence or absence of a pump or of PZP in the pump. Five of six untreated control does produced fawns. Statistical analysis was precluded by the small number of does in the respective PZP treatment groups.

In the single-injection, microsphere experiment, two of seven PZP-treated does and six of seven untreated control does produced fawns. The difference between groups was significant ($p < 0.05$). All control does fawned by June 30 except the one that fawned on July 8. Fawning dates for the two PZP-treated does were June 17 and July 14.

Effects of Porcine Zonae Pellucidae on Antibody Titers—Blood samples obtained from PZP-treated does in all cases showed anti-PZP antibody titers in baseline (prevaccination) which were <8 percent of the positive reference serum value, i.e., similar to titers found in negative reference serum. All of these

Table 2. Anti-PZP antibody titers during one breeding season in captive white-tailed does given PZP contraceptive vaccine[1]

		Anti-PZP antibody titers (% of positive reference serum)[2]				
			Postbreeding season[4] (weeks 32–40)		Prebreeding season (weeks 4–6)	
Experiment	No. of does titer-tested	Prevaccination baseline[5] (week 0)	$\bar{\chi}$	SE	$\bar{\chi}$	SE
3-injection	4	<8	102.1	1.2	72.7	15.7
2-injection	4	<8	100.0	0.0	92.0	5.2
1-injection/pump	3	<8	76.3	7.2	69.5	11.8
1-injection/microspheres	4	<8	53.7	15.5	19.1	4.3
Control[3]	2	<8	<8		<8	

[1] Does were treated in October–November; fawning occurred the following summer.

[2] Positive reference serum, designated as 100%, was the average value of maximal titers achieved in six PZP-treated does that did not produce fawns.

[3] Placebo-treated does from 2-injection experiment.

[4] All values were below the lower limit of detection.

[5] No differences between pre- and postbreeding season titers in any treatment group, based on analysis of variance with repeated measures, $p < 0.05$.

PZP-treated does exhibited titers >33 percent of the positive reference serum value after vaccination. No does with titers >50 percent at the onset of a given breeding season produced fawns in the following summer.

Specific antibody titer data are presented in table 2. In the three-injection experiment, average titers among the four tested does were 102.1 ± 1.2 percent by 6 weeks after the initial inoculation and were 72.7 ± 15.7 percent at 36 weeks. In the two-injection experiment, anti-PZP antibody titers in four treated does averaged 100.0 ± 0.0 percent of positive reference serum 6 weeks after vaccination; and 40 weeks after PZP vaccination, titers in these same does averaged 92.0 ± 5.2 percent.

Additional blood samples taken from two of these does showed titers of 70 percent and 57 percent at 52 weeks (prior to placement with a buck), and titers of 86 percent and 75 percent at 64 weeks. Neither of these does fawned. One doe that did produce a fawn had shown a 79-percent anti-PZP antibody titer 12 weeks prior to breeding, but there were no November or February antibody data for this doe. Titers were <8 percent at all times in samples from the two sham-treated does.

In the first single-injection study, prevaccination anti-PZP antibody titers were <8 percent of the positive reference serum titers. Titers in the PZP-pump group (n=3) averaged 76.3 ± 7.2 percent, 78.3 ± 9.3 percent, and 69.5 ± 11.8 percent at 4, 12, and 32 weeks, respectively. Titers in active (single PZP injection and saline pump) control does (n=2) averaged 41.5 ± 1.5 percent, 50.0 ± 5.5 percent, and 41.5 ± 12.5 percent at the above sampling times.

In the second single-injection study, employing controlled-release microspheres, average anti-PZP titers in the four treated does sampled were 53.7 ± 15.5 percent at 4 weeks and 19.1 ± 4.3% at 36 weeks. Anti-PZP antibody titers in the two PZP-treated does that produced fawns in this experiment were 32 percent and 27 percent at 4 weeks, i.e., prior to breeding.

Discussion

In the present study, 100 percent of the does given multiple injections of porcine zonae pellucidae and 80 percent of does given pellucidae as a single injection with additional pellucidae provided in a controlled-release form were effectively contracepted

for the first breeding season after treatment. In contrast, does in combined sham and untreated control groups had only a 6.2-percent average annual failure rate in producing fawns. In all cases in which it was applicable, statistical analysis of fawning data within studies showed that the proportion of does producing fawns was lower for PZP-treated does than for control does. These results confirm of our previous report on PZP contraception in white-tailed deer (Turner et al. 1992).

The mechanism of action of the PZP vaccine appears to be inhibition of fertilization (Sacco et al. 1984). Porcine zona pellucida is comprised of several glycoproteins, at least one of which, ZP3, functions as a receptor for sperm surface molecules (Florman and Wassarman 1985). In the PZP-treated mare, it appears that anti-PZP antibodies block sperm-binding sites on the ovum (Liu et al. 1989).

This study associates the presence of highly elevated, sustained anti-PZP titers with infertility in deer. Some does were monitored for antibody titers across one complete breeding season (6–9 months). Average titers appear to have remained highly elevated (73–92 percent of maximal) during this period in the does given multiple PZP injections while average titers among single-injection does never approached maximal. These results indicate that a minimum of two separate treatments with porcine zonae pellucidae may be necessary to maximize anti-PZP antibody titers across one breeding season.

In a previous study of porcine zonae pellucidae in the mare using similar dosages and protocol, the lowest pellucidae titer associated with contraception was 64 percent of the positive reference serum for mares. In the present study, 3 of 13 does sampled just before or during the breeding period had titers between 40 and 50 percent of positive reference serum for does. None of these three does produced fawns from that breeding season. On the other hand, five of six does with titers <33 percent at the onset of breeding produced fawns the following summer. Although the small number of samples and titer variability across the test animals preclude firm conclusions regarding the contraceptive threshold for anti-PZP titers in white-tailed deer, the present data suggest that the threshold may be in the range of 33 to 50 percent. One doe that had a 79-percent titer 12 weeks before breeding did produce a fawn the following June. Because this doe was not sampled immediately prior to the breeding season, it is possible that her titers were below 50 percent during breeding. In this regard, another doe sampled 12 weeks before breeding had a 93-percent titer at that time, and her titer was 57 percent in November.

The fawning data (table 2) also indicate that a single injection containing unsequestered porcine zonae pellucidae plus controlled-release PZP microspheres was less effective in providing contraception for one breeding season than were two or three separate exposures to the pellucidae (100-percent effective). The fact that the contraceptive effect was less in single-injection protocols than in multiple-injection protocols may reflect that the controlled-release component was not equivalent to a second PZP injection. First, the release pattern of porcine zonae pellucidae was continuous in the controlled-release preparations rather than discrete as in the bolus release provided by a second injection. Second, the multiple-injection protocol included adjuvant in each injection, while in the single-injection protocol, there was no adjuvant in the controlled-release component. Nonetheless, the partial effectiveness of the single-injection PZP preparations encourages further consideration of controlled release of porcine zonae pellucidae for use in immunocontraception (Eldridge et al. 1989, Wang et al. 1991b).

The potential value of controlled-release components is highlighted by the observation that anti-PZP antibody titers were numerically greater in does given a PZP injection plus an osmotic pump containing porcine zonae pellucidae than in does given a PZP injection plus an osmotic pump containing saline. However, the small number of does per group and the lack of fawn production in the saline-pump group make it impossible to draw conclusions regarding a possible role of controlled-release porcine zonae pellucidae in this experiment.

It appears that PZP-mediated infertility is reversible. Eight PZP-treated does in the multiple-injection experiments were monitored for up to 2 years after cessation of PZP treatment. Six of these does produced fawns within this 2-year period. Serum anti-PZP antibody titer data, where available, were consistent with reversibility: none of the titer-tested does that fawned after cessation of PZP treatment showed anti-PZP antibody titers >30 percent at the onset of the breeding season during which they conceived. However, investigation of the possibility of long-term infertility after several consecutive years of PZP treatment appears warranted, based on data which have demonstrated this in horses (Kirkpatrick et al. 1992).

None of the does given a single PZP booster injection prior to year-2 breeding produced fawns the following summer. While it may be that the booster maintained the infertility seen in year 2, it is also possible that the original vaccination effect would have lasted 2 years without the booster. The latter possibility is in part supported by the results of the second experiment. Seven does given a two-injection vaccination in year 1 were not treated in year 2. No fawns were born in the first summer after the vaccination, and only two of these seven does fawned in the second summer after the vaccination, despite the absence of a booster treatment. In any case, these results preclude conclusions regarding the contraceptive effectiveness of a single PZP booster injection.

The maintenance of infertility across two consecutive breeding seasons in five of seven does given the two-injection PZP protocol was an unexpected finding. However, the limited antibody titer data in these does corroborated the fawning data, with titers maintained above 70 percent for 12 to 15 months in both does that were titer-treated at these times. Interestingly, the same PZP preparation and dosage given to does in the present study was effective in the mare for 1 but not 2 years (Kirkpatrick et al. 1992). The fact that the dosage per kg of body weight was severalfold greater in the deer than in the horse may explain why the antibody titer elevations in the deer were sustained longer. Studies of other PZP preparations in various species, using dosages as much as tenfold greater than those used in the horse have reported the occurrence of long-term infertility (Mahi–Brown et al. 1985, Skinner et al. 1984, Dunbar et al. 1989).

In a previous PZP study in white-tailed deer, we reported that some PZP-treated does continued to be bred for at least 4 weeks longer than control does. The extension of estrus was also observed in the present studies in some PZP-treated does, with the time between end of breeding in control does and treated does ranging from 3 to 5 weeks. More than 90 percent of white-tailed fawns are born in May and June (Halls 1984). In the present studies of 53 does, 3 fawns were born after June 30. A control doe fawned on July 8, and two PZP-treated does fawned, on July 14 and August 15, respectively. While these results do not clearly demonstrate that incomplete contraception causes fawns to be born late in the season, the subject of breeding-period extension due to contraception warrants further study for potential influence on deer bioenergetics and fawn survival.

Studies in Wild Deer

Based on the encouraging results obtained in the studies with captive deer, we began to address the issues of accessibility to deer and deliverability. Toward this end, we entered into three field studies. The first study addressed accessibility to wild, free-roaming deer and it was carried out at Pt. Pelee National Park in Ontario, Canada, in collaboration with D. Voigt (Ontario Ministry of National Resources) and G. Mouland and D. Rieve (Park employees). This preliminary study examined the accessibility of deer to within darting range.

Point Pelee National Park is a small natural area (16 km^2, with only 30 percent dry land) located in the southwest corner of Ontario. It is isolated from other natural areas by urban development and an intense agricultural zone. In recent years, the park has been subject to white-tailed deer densities as high as 38 deer/km^2 of dry land. Two population reductions were carried out, in 1991 and 1993, to reduce and maintain the population density at about 7 deer/km^2.

Before immunocontraception via injection can be considered, investigators must make sure they have sufficient access to free-ranging does at low population density. To determine this, we established bait stations using apples and whole-kernel corn within 5 m of active deer trails at clearings before and after the 1993 population reduction. Prior to the population reduction (density = 12.5 deer/km^2 dry land), 14 bait stations were established throughout the park. Within 5 days, seven stations had daily deer activity. Over a period of 15 days, all bait stations were being used. Random observations showed 44 deer utilizing bait stations (23 does, 2 bucks, 13 deer of unknown sex, and 4 fawns). After the population reduction, bait stations were reestablished at five of the previous locations where deer were known to remain. Within 5 days, all 5 stations had daily deer activity, and over a period of 18 days, 23 deer were sighted (10 does, 6 bucks, and 7 of unknown status). Dusk and dawn were the predominant times of sighting. Because we found that the use of blinds gave us darting access to deer within 15 m, we believed the scenario adequate to attempt remote delivery of immunocontraception. The results of this study suggest that wild deer at Pt. Pelee can effectively be baited into darting range.

The Pt. Pelee deer are among the most wary and least visible of the numerous deer populations that we have observed on our invited visits to various parks during the 1990's. Our results at Pt. Pelee suggest that appropriately operated baiting programs can guarantee access to deer for vaccination in most parks. It is further encouraging that accessibility was only temporarily hindered by the occurrence of the cull. This is particularly important in light of the high probability that already excessive deer populations in many places would be culled before management by immunocontraception would be implemented.

In an effort to further our understanding of the vagaries of field delivery of porcine zonae pellucidae to deer, and in order to examine potential social–behavioral aspects of PZP contraception (such as continued estrus cycling and late fawning), we undertook a second field study. In collaboration with W. McShea and S. Monfort, we remotely vaccinated free-roaming does with porcine zonae pellucidae at the Smithsonian Institution's Conservation and Research Center in Front Royal, VA. The Front Royal field site was a 30-ha fenced area of mixed wood (80 percent) and fields (20 percent). The study population consisted of 28 adult, ear-tagged does, 4 bucks, and an unknown number of fawns.

The PZP vaccine treatment consisted of either two injections (3 weeks apart) of porcine zonae pellucidae plus Freund's adjuvant as described previously (n=10) or one injection of porcine zonae pellucidae plus Freund's adjuvant and a second dose of the pellucidae in controlled-release microspheres (n=9), as described previously. The nine remaining does, serving as controls, were injected with saline plus adjuvant. All injections were given in September. Access to the does was achieved by stalking, blinds, and bait stations. Most of the deer were extremely wary and nervous. Best success in darting was accomplished using blinds at bait stations. All does were vaccinated with a self-injecting 1-cm^3 dart fired from a 22-caliber rifle (Pneu-Dart, Inc., Williamsport, PA). Effectiveness of treatment was determined by counting fawns between June and August the following year. Does and bucks were also monitored for mating behavior and activity budgets between October and March. Details of behavioral and activity monitoring are presented in the paper by McShea et al. elsewhere in this book. Treatment effects on fertility, behavior, and activity budgets are presented below.

Regarding efficacy, none of the 10 does receiving 2 injections of porcine zonae pellucidae produced fawns; 67 percent of the one-injection does and 89 percent of the untreated control does produced fawns. All except one of the fawns produced by the one-injection does were born between mid-July and the end of August. The results demonstrate complete effectiveness of the two-injection PZP treatment. They also suggest that the one-injection PZP treatment inhibited fertility for most of the breeding season but did not prevent conception at the end of the season. Based on titer data from our previous studies and the fawning dates in the present study, it is likely that the anti-PZP antibody titers fell below the contraceptive threshold by the March following treatment.

Regarding behavior and activity budgets, 38 hours of observation on females revealed that contracepted does were more active and spent less time feeding than control does. However, the average body weight of treated does was greater than that of control does at the end of the winter. These results suggest that for does, the metabolic drain of pregnancy is greater than the metabolic demands of an extended breeding season with greater activity. During 84 hours of observations on males, mating occurred until March, but mating activity shifted from largest to smallest males as the winter progressed. Also, the intensity of the rut fell markedly after December. These conditions considerably moderated the energetic cost to bucks of an extended breeding seasons associated with PZP contraception. While these results suggest that there are no life-threatening behavioral or energetic consequences to a breeding season extended by PZP contraception, the details of PZP effects on these factors nonetheless deserve further exploration.

Remote Assessment of Reproductive Status

As a corollary to the development of PZP immunocontraception for wildlife management, we have been exploring the remote assessment of reproductive status in free-roaming wildlife via monitoring of sex-hormone metabolites in remotely collected urine and feces (Kirkpatrick et al. 1990b, 1991b, and 1992). Recently, we have begun to evaluate this technology for use in white-tailed deer. Using fecal samples collected from deer studied in the reports presented in this paper, we have performed a preliminary analysis of concentrations of pregnancy-specific steroid metabolites in pregnant and nonpregnant does. The metabolites, measured by specific ELISA, were estrone conjugates (E_1C) and pregnanediol-3α-glucuronide (PdG). In samples collected between April 25 and May 21 from does that produced fawns (n=9) and does that did not produce fawns, there was a 100-percent correlation between pregnancy and elevated E_1C, and/or elevated PdG. The E_1C and PdG concentrations averaged 10× and 4× greater, respectively, in pregnant does as compared to nonpregnant does. All does that produced fawns showed an E_1C concentrations >1.0 ng per g of feces, while all nonpregnant does had E_1C levels <0.7 ng/g. All but two does that fawned exhibited PdG >7 ng per g of feces, while only one doe that did not fawn had PdG >5 ng/g. In general, E_1C appears to be more reliable than PdG as an indicator of pregnancy, although it is useful to have both measures to maximize the probability of accurate assessment. These results are generally similar to those reported for equids, and suggest that this methodology can be useful for remote assessment of reproductive status in wild white-tailed deer.

Summary

For all treatments in which does were exposed to porcine zonae pellucidae plus adjuvant at least twice within 4 weeks, the contraceptive effectiveness was 100 percent. Our combined data for all one-injection protocols shows a 42.1-percent fertility rate. While this represents better than 50-percent reduction in fertility relative to controls, we consider the single-injection efforts to be a failure. Most of the fawns born to one-injection does were delivered in August. This late-fawning phenomenon, with the consequent probable high winter mortality of late-born fawns, makes it essential to complete the development of a single-injection vaccine with a guaranteed minimum of 1-year efficacy. In fact, without denying the importance of issues such as recombinant *v.* natural ZP and dart *v.* biobullet, it is clear that there can be no practical field application of this technology without a single-injection vaccine that is highly effective for at least 1 year. The bioengineering of this PZP preparation is under way.

Regarding our continuation of field studies of wild deer populations, we completed the PZP vaccination of 68 wild, free-roaming does on Fire Island National Seashore in New York State in October 1993. The 68 treated does represent >70 percent of the adult does in the discrete population that we targeted. We employed a two-injection protocol because the single-

injection, 1-yr vaccine was still in development. The results of this study should provide useful data toward the potential use of PZP immunocontraception for wild deer management in limited deer populations.

Conclusions

The studies presented in this paper have clearly demonstrated that PZP immunocontraception can prevent pregnancy in white-tailed deer. A brief reexamination of the requirements for management-useful contraceptive in wild, free-roaming deer reveals that our data also show feasibility of access to the deer, remote delivery of PZP vaccine, and remote assessment of pregnancy. When development of a single-injection capability is completed, management of many urban deer populations with PZP immunocontraception should be technologically realistic. In the meantime, other issues regarding immunocontraception must be addressed in the area of public attitudes and education, administration and policies, and long-term management strategies (Kellert 1991), all of which are influenced by politics, economics, and management goals as discussed in other papers from this conference.

Members of our research team have visited many locations where insular deer populations are presently so large that the deer have already severely compromised their habitat. Many species of plants and animals, not just deer, are now suffering the consequences of ballooning deer numbers. While contraception may be used to limit population growth, it will not reduce the numbers presently in excess. Therefore, initial population reductions by existing means may be necessary where indicated in order to protect habitat for the future and to establish a basal population of deer that thenceforth may be managed by contraception.

While our research team recognizes that wildlife population control is an important issue, the need for such control reflects a much larger and burning issue: expanding human population. We believe that as we continue our work, all of us in the growing field of wildlife contraception have an obligation to help focus public consciousness in this regard.

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Contraception of Wild and Feral Equids

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Abstract: Fertility control in wild horses has been attempted with both stallions and mares. Nonreversible surgical sterilization by means of vasectomy has been successful in inhibiting reproduction in wild horses in Montana and Nevada. Administration of a microencapsulated form of testosterone to wild stallions reduced sperm counts and motility and foal counts. In a third approach, intraperitoneal Silastic™ implants containing ethinylesdtradiol and progesterone blocked ovulation in wild mares for up to 3 years.

The first immunological fertility control of free-ranging wildlife was accomplished with wild horses. Initial experiments demonstrated that immunization with porcine zonae pellucidae was capable of causing contraception in domestic mares. Later, contraception was achieved with the vaccine in free-ranging horses. That study demonstrated that the vaccine (1) could be delivered remotely via darts, (2) was safe to administer to pregnant animals, and (3) did not alter social behavior. A followup study revealed that a single annual booster inoculation would extend the contraceptive effects for a second year, and the vaccine's effects are reversible after short-term use.

After 6 years of treating 52 different mares with porcine zonae pellucidae, contraceptive efficacy exceeded 95 percent. In more recent studies, investigators are studying the effects of long-term treatment (4 to 7 consecutive years) upon ovarian function. The porcine zona pellucida (PZP) vaccine has also proved to be effective in contracepting free-roaming feral donkeys in Virgin Islands National Park, captive Przewalski's horses, and onagers. Tests are currently under way on 150 feral horses in Nevada for the purpose of developing a one-inoculation form of the PZP vaccine that will deliver from 1 to 3 years of contraceptive protection. An initial field test of this vaccine indicated a high degree of success with a single inoculation over a single year, and a field test of a second-generation of microcapsules also indicated a high degree of contraceptive efficacy over a single year.

Keywords: equids, immunocontraception, porcine zonae pellucida, wildlife contraception

Introduction

Over the past 20 years, advances in wildlife contraception have been driven largely by concern over increases in wild horse *(Equus caballus)* populations. Prior to 1971 in North America, wild horse populations were controlled by the removal of horses from rangeland and their sale for various commercial purposes. The passage of the Wild Horse and Burro Act (P.L. 92–195) in 1971 by the U.S. Congress provided almost complete protection to wild and feral equids on public lands. At the time of the passage of this law, there were an estimated 17,000 wild horses on U.S. public lands. In an attempt to provide some form of population control, the U.S. Department of the Interior's Bureau of Land Management, the agency responsible for management of wild horses in the United States, initiated the Adopt-a-Horse program. Horses were gathered and adopted by people interested in acquiring a wild horse. However, the high cost of this program, its inability to remove sufficiently large numbers of horses, and increased reproductive success by the horses remaining on rangelands led to steady population increases between 1970 and 1980. By 1980, the estimated number of wild horses on poublic lands had increased to somewhere between 60,000 and 80,000. Clearly, alternative control methods were necessary.

Chemically or immunologically induced inhibition of fertility in equids has been little studied. Historically, equine fertility control has focused on castration of the domestic stallion. Most often, this common procedure is carried out not only to prevent reproduction but also to reduce testicular androgen production and accompanying aggressive behavior. Recently, interest in contraception of the equids has increased, largely because of uncontrolled populations of free-roaming wild horses and feral burros *(E. asinus)*.

Contraception of the Stallion

Initial attempts at chemical contraception of wild horses focused on the stallion and attempted to exploit the haremlike social structure common to the majority of wild equids. To test the concept, two adult wild stallions inhabiting the Pryor Mountain Wild Horse Refuge in Montana were vasectomized and permitted to return to their bands. After 2 years, no foals appeared among the mares accompanying the vasectomized

stallions (Kirkpatrick 1982). When this experiment was later repeated with larger numbers of wild stallions in Nevada, the results indicated that dominant stallions were responsible for the vast majority of successful breedings (Eagle et al. 1993).

Several potential contraceptive compounds, including testosterone cypionate, testosterone propionate, quinestrol (17α–ethinylestradiol 3–cyclopentyl ether), 17β estradiol, and α–chlorohydrin (3–chloro–1,2–propanediol) were tested in domestic pony stallions. The α–chlorohydrin led to neurological disorders, so tests were discontinued. Repeated intramuscular injections of the two androgens and quinestrol (1.7 g per 100 kg of body weight, monthly × 6 months) resulted in significant oligospermia and a significant reduction in sperm motility, but Silastic implants contraining estradiol failed to achieve significant reductions in sperm counts, probably because of poor release rates (Kirkpatrick 1982, Turner and Kirkpatrick 1982).

A microencapsulated form of testosterone propionate (mTP) was selected for field tests of contraceptive efficacy in wild horses in Challis, ID. The microencapsulation polymer (D,L–lactide) coating (Southern Research Institute, Birmingham, AL) permitted a sustained release for up to 6 months after intramuscular (i.m.) injection. On contact with intercellular water, the lactide coating erodes and releases the active steroid. The coating is converted to carbon dioxide and lactic acid.

Fifteen wild stallions—seven experimental subjects and eight controls—were located by helicopter and darted with approximately 300 mg succinylcholine. After immobilization, stallions received i.m. injections of 5, 7.5, or 10 g mTP in the hip. Stallion libido and quantitative aspects of sexual behavior, based on elimination marking behavior (Turner et al. 1981), were unaffected, and breeding took place. There was an 83-percent reduction in foal production compared with mares bred by control stallions (2 *v.* 13 foals, respectively), with no differences between fertility and the doses of mTP administered (Kirkpatrick et al. 1982, Turner and Kirkpatrick 1982).

Concerns for the safety of the stallions, dangers of immobilization, and high cost of opioid immobilizing drugs (approximately $50/dose of etorphine and reversal agent) led to an attempt to deliver 3 g mTP remotely to wild harem stallions on Assateague Island National Seashore, by means of barbless darts. In this study, the stallions were located and darted from the ground without capture or immobilization. The pharmacologic success of mTP contraception was evident; there was a 28.9-percent fertility rate for the mares accompanying treated stallions and an approximate 45-percent fertility rate among control mares. However, the difficulties of delivering 3 g mTP in four separate doses to each stallion were discouraging (Kirkpatrick and Turner 1987, Turner and Kirkpatrick 1991).

Contraception in the Mare

Steroids

The difficulty of darting a stallion up to four times plus concerns over band infidelity by mares turned the focus of wild horse contraception to the mare. Attempts were made to administer contraceptive doses of progestins to wild mares. Based on experience with persistent corpora lutea (Stabenfeldt et al. 1974) and data which indicated that plasma progesterone concentrations in excess of 0.5 to 1 ng/mL inhibited ovulation in the mare (Squires et al. 1974, Noden et al. 1978, Palmer and Jousset 1975), microencapsulated northisterone (norethindrone, mNET) was administered remotely, with barbless darts, to six wild mares on Assateague Island (Kirkpatrick and Turner 1987, Turner and Kirkpatrick 1991). This particular progestin, which had been used successfully to inhibit fertility in women, was given at a dose of approximately 2 g, in microcapsules similar to those used in previous studies with testosterone propionate. All six mares receiving the mNET produced a foal a year later—a highly improbable event among Assateague mares, where annual foaling rates seldom exceed 55 percent (Keiper and Houpt 1984).

In another experiment, groups of 30 captive wild mares in Nevada were each implanted with Silastic

rods containing 8 g estradiol (E), 24 g progesterone (P), 8 g E plus 8 g P, 4 g E plus 12 g P, 12 g E plus 12 g P, or no hormone (Vevea et al. 1987, Plotka et al. 1988). Fewer mares receiving 8 g E, 4 g E plus 12 g P, or 8 g E plus 8 g P displayed estrus, but all animals displaying estrus—treated or control—ovulated. These data indicated a rapid decline in plasma steroid concentrations within 5 weeks of receiving steroid implants and suggested increased metabolic clearance of the steroid, an event that would also explain the failure of the mNET treatments described above. Because of the rapid decline in E and P concentrations, Silastic implants containing the synthetic estrogen ethinylestradiol (EE2) or EE2 plus P were placed in captive wild mares (Plotka et al. 1989). Animals pregnant at the time of implantation delivered healthy foals. Contraceptive efficacy ranged from 88 percent to 100 percent through two breeding seasons and was approximately 75 percent for three seasons. Endocrine studies of these mares suggested that contraception occurred by blocking ovulation and/or implantation.

In a similar study, intraperitoneal implants of 1.5, 3, or 8 g EE2 alone also resulted in contraceptive efficacy of 75 percent to 100 percent through two breeding seasons. Rates of EE2 decline in the plasma suggested a contraceptive life of 16, 26, and 48–60 months for the 1.5-g, 3-g, and 8-g doses, respectively (Plotka and Vevea 1990, Eagle et al. 1992).

Results achieved with estradiol, progesterone, and ethinylestradiol in mares brought to focus advantages and disadvantages of natural versus synthetic steroids for contraception in equids. Steroids native to the mare, such as estradiol and progesterone, are recognized by the mare's metabolic enzymes and degraded so rapidly that contraceptive doses must be so large that they are difficult or impossible to administer. The use of some synthetic steroids, such as ethinylestradiol, may delay metabolic degradation and permit sustained contraceptive effects and provide useful fertility inhibition in certain instances. Any risk, however small, of the passage of these synthetic steroids to humans or other wildlife may make registration by regulatory agencies such as the Food and Drug Administration (FDA), the U.S. Department of Agriculture (USDA), or the Environmental Protection Agency (EPA) unlikely or difficult.

Immunocontraception

Attention has turned to immunocontraception because of (1) the difficulties associated with the delivery of large doses of microencapsulated steroids, (2) dangers associated with capture and restraint of horses, (3) surgical procedures associated with intraperitoneal implants, (4) concern over long-term effects of steroidal contraception, and (5) passage of synthetic steroids through the food chain. One immunologically based contraceptive strategy involves blocking the release of gonadotropin-releasing hormone (GnRH) from the hypothalamus, thereby preventing pituitary secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) and their subsequent tropic actions on the ovary or testes (Schanbacher 1984). Immunization of domestic mares against GnRH blocked ovulation in three of five mares for 4 months (Safir et al. 1987). Each mare was inoculated with 2 mg of GnRH conjugated to human serum albumin emulsified in Freund's Complete Adjuvant (FCA). Analysis of plasma LH revealed a lack of pulsatile secretion that was correlated with antibody titers. The high variability in the mares' response to the antigen and the subsequent variabilities in antibody titers suggested that this approach was unreliable.

In a study to immunize captive wild mares with GnRH conjugated to ketolymphohemocyanin (KLH), either aluminum hydroxide (alum) or monophosphoryl lipid A/trehalose 6,6–dimycolate/BCG cell wall skeleton (triple adjuvant, TA) was used as the adjuvant (Goodloe et al. 1988). Mares immunized with GnRH plus TA had higher antibody titers and significantly less ovarian follicular activity. The vaccine was field-tested in 29 wild mares on Cumberland Island National Seashore, in Georgia. The vaccine was freeze dried and administered as a solid biodegradable 0.25-caliber bullet by means of an air-powered gun. After imbedding in the tissue of the target mare, the compressed compound forming the biobullet degrades over 24 hours, releasing the antigen. A total of 25 treated mares survived (four died from natural

causes) and 17 (68 percent) produced foals, which was not significantly different from foaling rates of control mares.

Immunization against GnRH has also been attempted in the stallion (Dowsett et al. 1991). Four weanling colts were passively immunized, either intramuscularly or subcutaneously, with an anti-GnRH antibody (Peptide Technology, Ltd., Sydney, Australia) and given booster inoculations approximately 75 days later. Colts immunized intramuscularly maintained plasma testosterone concentrations of < 0.15 ng/mL (equivalent to concentrations in geldings) for 5 months following the booster inoculations. Thereafter, testosterone concentrations rose to control levels for yearlings. Colts immunized subcutaneously had slightly increased plasma testosterone concentrations (up to 0.37 ng/mL) between the two immunizations but decreased concentrations similar to those seen in the intramuscularly injected group after the second inoculation. Antibody titers were generally higher in the colts immunized intramuscularly, although sexual development was effectively delayed for 12 months in both groups of colts.

A second immunocontraception strategy for equids was based on the identification of antibodies directed against the zona pellucida of the ovum in naturally infertile mares (Liu and Shivers 1982) and immunological cross-reactivity of equine zona-positive antisera and porcine sperm binding (Shivers and Liu 1982). Liu et al. (1989) immunized 10 captive wild mares and 4 domestic mares with the protein equivalent of 2,000 to 5,000 porcine zonae pellucidae. FCA was used for the first inoculation and Freund's Incomplete Adjuvant (FIA) for the three monthly booster inoculations that followed. Of the 14 treated mares, 13 failed to conceive during the first year. The four domestic mares all conceived during the second year after antibody titers decreased.

A field test of the PZP vaccine was carried out on Assateague Island National Seashore (Kirkpatrick et al. 1990). For the test, 26 wild mares were remotely inoculated with approximately 5,000 porcine zonae pellucidae (65 μg protein) and FCA in March 1988 by means of barbless darts. The mares received a second inoculation with PZP + FIA 2 weeks later, and 18 of the mares received a third inoculation with that combination 1 month later. No foals were produced by the treated mares, whereas 50 percent of the 6 sham-injected mares (controls) produced foals, and 45 percent of 11 untreated mares produced foals. Post-treatment foaling rate for the PZP-treated mares was significantly different ($P < 0.002$) than that for the 2 pretreatment years, for sham-treated control mares and for untreated mares. Of the 26 PZP-treated mares, 14 were pregnant at the time of inoculation, and all 14 produced healthy foals 2–3 months following PZP treatment. Thus, the PZP vaccine had no effect on pregnancies in progress or the health of the foals born. Social behaviors and organization were also unaffected by treatment. Once antigen recognition had taken place, a single annual booster inoculation was sufficient to maintain contraception.

In March 1989, 14 of the previously treated mares were given a single booster inoculation. A year later, only one foal was produced. The 12 mares immunized in 1988 but not given a booster in 1989 produced 5 foals, demonstrating for a second time the reversibility of the vaccine's contraceptive effects (Kirkpatrick et al. 1991a). After 6 consecutive years of booster inoculations among the Assateague mares and 104 mare-years (the number of mares treated × the number of years of treatment), four foals have been produced, for an effectiveness of 96 percent. Over the 6-year period, the difference between foaling rates among treated and untreated mares was highly significant ($P < 0.001$).

Field trials were initiated in 1992 with feral donkeys in Virgin Islands National Park. Sixteen adult female donkeys received an initial inoculation of 65 μg of porcine zonae pellucidae + FCA, a second identical inoculation 3 weeks later, and a third 10–12 months later. Eleven untreated adult females were used as controls. Results were not calculated until 12 months after the initial inoculation in order to allow for any pregnancies already in progress at the time of inoculations. Based on observed foals and on fecal steroid analysis (Kirkpatrick et al. 1991b and c), of the treated females, only 1 of 16 (6.2 percent) produced a foal or was pregnant, while among the controls, 6 of

11 (54.5 percent) produced foals or were pregnant. The difference between foaling and pregnancy rates between the two groups was highly significant ($P < 0.01$).

Large-scale field trials with wild horses were initiated in December 1992 in Nevada. Slightly more than 500 wild horses were captured by driving them by helicopter into a trap and portable corrals. Adult mares (n = 131) between the ages of 4 and 12 were included in the study. All mares were freeze-branded with numbers for later identification. One group received two inoculations of PZP about 3 weeks apart with FCA used for the first inoculation and FIA for the second. A second group received only a single inoculation + FCA. A third group received a single inoculation of porcine zonae pellucidae + FCA that contained another 65 μg of pellucidae in lactide–glycolide microspheres. These microspheres were designed to release the antigen over a 4- to 6-week period.

During September 1993, fecal samples were collected from 78 treated or control animals and analyzed for steroid hormones and steroid hormone metabolites, which signal pregnancy (Kirkpatrick et al. 1991b and c). Of 27 untreated mares, 14 (52 percent) were pregnant. Of 17 sham-injected controls, 9 of 17 (53 percent) were pregnant. None of 14 mares receiving 2 inoculations of porcine zonae pellucidae were pregnant. Of 20 mares receiving only a single inoculation of raw pellucidae, 6 (30 percent) were pregnant. No samples were recovered from mares receiving the porcine zonae pellucidae in microspheres.

During June 1994, after aerial foal counts, a total of 65 experimental mares were observed, and foaling results were in general agreement with pregnancy data. From 32 mares that received 2 inoculations, only a single foal was observed. Of 16 sham-treated mares, 10 had foals (63 percent), and of 7 mares receiving only a single inoculation, 3 had foals (43 percent). Of 10 mares receiving a single inoculation of the microencapsulated porcine zonae pellucidae, none had foals. These data suggested a contraceptive effect of a single inoculation with microencapsulated porcine zonae pellucidae that lasted 8–9 months following treatment.

The PZP vaccine has also been tested in captive exotic equids, including 23 Przewalski's horse *(Equus przewalskii),* 1 onager *(E. hemionus),* and 26 zebra *(E. zebra grevyi)* (Kirkpatrick et al. 1992b, 1993). Thus far, results are available only for the Przewalski's horses and the onager, and the vaccine has been 100-percent effective in preventing pregnancies in these species (Kirkpatrick et al. 1993). It is better than 80-percent effective in zebras.

Two important issues are raised with regard to PZP immunocontraception of equids. The first is the possibility of long-term effects of the vaccine on ovarian function. PZP-induced contraception is thought to be due to a block to fertilization (Liu et al. 1989). At least two of the major glycopreoteins of the noncellular zona pellucida, ZP3 (Florman and Wassarman 1985) and ZP4 (Hasagawa et al. 1992), are necessary components of the receptor molecule for sperm surface molecules. The role of the ZP3 receptor in the horse has been confirmed in vitro as a zona pellucida-induced acrosome reaction with horse sperm (Arns et al. 1991). The initial study of PZP immunization of horses (Liu et al. 1989) and field tests with the Assateague wild horses (Kirkpatrick et al. 1990, 1991a) demonstrated that the contraceptive effects of PZP immunization were reversible after 1 year of treatment. Plasma progesterone concentrations during the year of treatment indicated a luteal phase and therefore evidence of ovulation. However, the long-term effects of continuous PZP immunization have not been described in either the horse or other species. Reversibility of the contraceptive effect has been demonstrated in several species but only after short-term application of the vaccine (Gulyas et al. 1983, Sacco et al. 1987, Liu et al. 1989).

In the dog and the rabbit, PZP immunization appeared to be very damaging to ovarian follicles and often led to cessation of ovarian function, with accompanying anovulation and depression of estrogen concentrations (Wood et al. 1981, Mahi–Brown et al. 1985). Unusually large doses (5,000 μg) caused anovulation in the baboon (Dunbar et al. 1989). These data suggested that the antibody response of the treated animal attacks not only the zona pellucida of the mature ovum but oocytes and possibly other

Table 1. Antibody titers in response to porcine zonae pellucidae in domestic mares after one inoculation using porcine zonae pellucidae plus PZP-containing microspheres or two inoculations of porcine zonae pellucidae given 3 weeks apart

		Dates of inoculation					
Treatment	**Horse**	**3/13**	**4/2**	**4/16**	**5/7**	**6/18**	**10/14**
		Antibody titers (% of positive reference serum)					
FCA–PZP bolus & PZP microspheres	Aldo	5	99	114	131	106	79
	Mia	4	70	107	123	82	28
	Imp	6	33	70	95	98	131
	Lab	5	38	76	120	114	39
	Dee	5	56	108	106	99	26
FCA–PZP bolus + FIA–PZP bolus 3 weeks later	Ant	4	90	130	131	152	115
	Con	6	66	145	135	144	58
	Len	8	58	118	123	91	35
	Rou	3	73	116	133	123	79
	Sou	4	67	147	126	136	94

FCA = Freund's Complete adjuvant. FIA = Freund's Incomplete adjuvant.

ovarian tissue with resulting changes in estradiol and progesterone secretion. These effects have not been demonstrated in the horse after short-term use, but there is evidence that these same effects may appear after long-term use.

After 3 consecutive years of PZP treatment, three of seven Assateague mares showed decreased urinary estrogen concentrations and no evidence of ovulation (Kirkpatrick et al. 1992a), and after 6 consecutive years of ovulation, five mares showed no evidence of ovulation. However, three of these five mares showed recurring rhythmic estrogen peaks, suggestive of developing follicular waves, and two of these five mares demonstrated classical estrous behavioral concurrent with an estrogen peak and permitted breeding (Kirkpatrick, unpubl. data). Another mare demonstrated a luteal-phase urinary progesterone metabolite pattern after 5 consecutive years of treatment and 1 year off, suggesting that normal ovarian function returned. The next 3–5 years will provide critical data regarding the effects of the PZP vaccine on ovarian function and reversibility after long-term vaccination with porcine zonae pellucidae.

The second critical issue is related to the number of initial inoculations necessary for contraception. While it has been clearly established that two inoculations, given 3–6 weeks apart, will provide contraception for about a year, the need to give two inoculations decreases the usefulness of the vaccine for wild and feral equids. Thus, the single most important direction for future research is the development of a one-inoculation form of the vaccine which provides at least a full year, and ideally 2–3 years, of contraception protection.

In an initial attempt to develop a one-inoculation PZP vaccine, the PZP antigen was incorporated into nontoxic, biodegradable, 50-μ, homogenous lactide–glycolide microspheres. Upon i.m. injection, the lactide–glycolide material erodes, releasing the anitgen over predetermined periods of time (Eldridge et al. 1989). The carrier itself is metabolized to lactic acid and carbon dioxide.

Five domestic mares were inoculated with an initial bolus of 65 μg of porcine zonae pellucidae + FCA plus another 65 μg of pellucidae contained in microspheres. Antibody titers were compared to titers in mares inoculated with two doses of pellucidae (65 μg PZP/FCA + 65 μg PZP/FIA) given 3 weeks apart. Contraceptive antibody titers lasted for approximately 200 days with the one-inoculation preparation (table 1).

This same preparation was administered to 14 wild mares on Assateague Island. One dart failed to inject. That mare, plus only one other mare, produced foals following treatment. The differences were significant ($P < 0.05$) comparing either 2 foals/14 mares or 1 foal/13 mares (to account for the failed dart) with untreated control mares. Similar research is currently under way to produce a one-inoculation vaccine using microcapsules. The microcapsules are made from the same lactide–glycolide material, but after injection they release the PZP antigen in pulses rather than continuously.

Conclusions

PZP-induced contraception of the mare may be useful to prevent unwanted pregnancies among wild and feral equids. In the case of captive exotic equids, such as the Przewalski's horse and the zebra, contraception may be useful to prevent the expression of undesirable genetic traits ("floppy mane" or the fox allele, for example) or merely to prevent the production of unwanted surplus animals without the need to remove animals and disrupt well-defined social groups. Anti-GnRH vaccines may be useful for the same purpose in the stallion, or simply to reduce aggression among stallion groups. Finally, contraception may represent a publicly acceptable approach to the management of wild and feral horses inhabiting public lands.

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Remotely Delivered Contraception With Needle-less Norgestomet Implants

Darrel J. Kesler

Abstract: A remotely delivered contraceptive was developed that suppressed estrus and prevented pregnancy in deer with 100-percent efficacy. This contraceptive utilized norgestomet, a potent progestin that is approved by the Food and Drug Administration (FDA) for use in cattle. Although the needle-less norgestomet implant is not FDA approved for use in deer, it is safe for treated animals, humans, and the environment. The remote delivery of this implant can be accomplished up to 40 m away and causes minimal tissue damage and stress if administered properly. Because of its ease, its simplicity of delivery, and the control it provides for proper drug handling, the needle-less norgestomet implant holds much promise for control of the overpopulation of deer in the United States. Further, no part of this product will remain to pollute the environment. Although this contraceptive was developed for female deer, preliminary studies suggest that the needle-less norgestomet implant may be effective in males. Widespread use of the needle-less norgestomet implant in deer requires further extensive (and costly) establishment of safety and efficacy as well as FDA approval.

Keywords: Remote delivery, needle-less implants, norgestomet, norethindrone acetate, wildlife contraception, black-tailed deer, white-tailed deer, controlled release, silicone, Food and Drug Administration

Introduction

Deer overpopulation has become a major problem in many areas of the United States. Warren (1991) has presented a detailed review of the historical causes of this problem, the ecological effects of deer overpopulation, and the need for controlling deer populations. Overpopulated deer herds are causing significant economic losses in the form of crop damage, damage to landscape plantings, transmission of diseases to livestock such as cattle (Forbes and Tessaro 1993), and damage to vehicles and humans (injury or death) in deer–vehicle collisions. In many areas, regulated public hunting has been proven to be an effective means of controlling deer populations (Behrend et al. 1970); however, this procedure has become very controversial and political. Contraception of deer may, therefore, be a logical alternative to control deer population.

The purpose of this article is not to provide an extensive review of the literature but rather to review a specific contraceptive (and its development) developed for deer. Because this contraceptive utilizes a steroidal compound, I will refer to other steroids that have been tested for deer contraception, but I will not attempt to provide an extensive review of other contraceptive compounds or procedures.

The selection of a deer contraceptive involves several criteria. The following is a selected list of essential criteria:

- Safety. This involves not only the animals being treated but also the human population and the environment.
- Cost. The product has to be cost effective relative to other methods of population control.
- Efficacy. The product has to be highly effective. Although 100-percent efficacy is not essential, like an equivalent product for humans, it still must be highly effective in preventing unwanted pregnancies.
- Ease of delivery. The product must be uncomplicated and easy to deliver. Even if a product meets the previous three criteria with 100-percent efficacy, it will not be routinely used unless it can be delivered with simplicity and ease.

Several contraceptive systems have been tested in deer and are reported in the literature. None of the developed contraceptives, however, have been accepted with enthusiasm either because of efficacy or because of the difficulty in their delivery. The contraceptive most widely tested is the steroidal compound melengestrol acetate (MGA®; 17α–hydroxy–6–methyl–16–methylenepregna–4,6–diene–3,20–dione; fig. 1) (Budavari 1989, Bell and Peterle 1975, Matschke 1980, Plotka and Seal 1989). MGA is approved by FDA for use in cattle (0.5 mg is orally administered daily; Zimbelman and Smith [1966]) for the suppression of estrus, increased rate of weight gain, improved feed efficiency (Bennett 1993), and, more recently, estrus synchronization in the United States. Another steroid tested is levenorgesterel (also referred to as norgestrel; 13β–ethyl–17α–ethynyl–17β–hydroxygon–4–en–3–one; fig. 1) (Budavari 1989, Plotka and Seal 1989, White et al. 1994). Levenorgesterel is

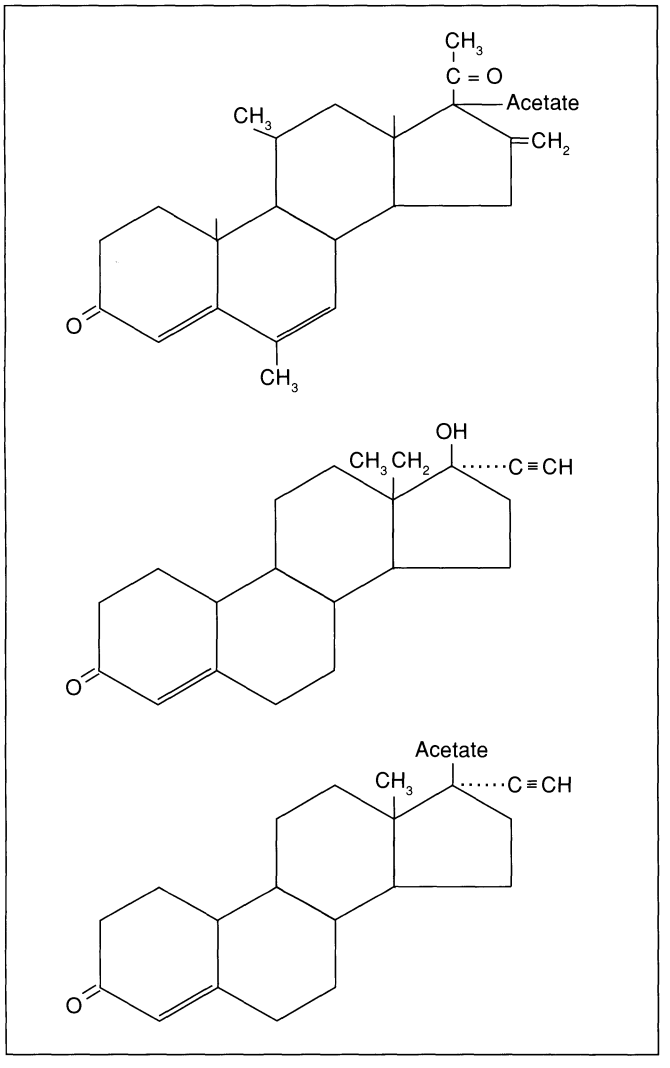

Figure 1. Chemical structures of melengestrol acetate (top), levenorgesterel (middle), and norethindrone acetate (bottom).

the active component of the Norplant® implant approved for human use as a contraceptive implant by FDA in the United States (McCauley and Geller 1992).

Although effective, MGA requires the implantation of a relatively large implant. These implants necessitate capturing the target animal and performing minor surgery for implantation (Plotka and Seal 1989). The implants have been demonstrated to be efficacious for several breeding seasons (Matschke 1980). Levenorgesterel also requires animal restraint for implant placement; however, the implants are smaller than the MGA implants. Unexpectedly, both studies that used levenorgesterel in deer reported that—administered at dosages similar to those used efficaciously in humans—levenorgesterel was not an effective contraceptive in deer (Plotka and Seal 1989, White et. al. 1994).

Both MGA and levenorgesterel were delivered via silicone (polydimethylsiloxane) (Roseman 1972). Because controlled chronic release of steroids in vivo (which is necessary for steroidal contraception) is obtained with silicone implants, and because they are biocompatable in mammals (Dziuk and Cook 1966), silicone proves to be an efficacious delivery system suitable for steroidal compounds in deer (Kesler 1989).

Norethindrone Acetate (NA)

The first compound selected for efficacy evaluation was norethindrone acetate (19–nor–17β–ethynyl–17ß–ol–3–one acetate; fig. 1) (Budavari 1989). Its chemical structure is very similar to that of levenorgesterel. NA is used in combination with ethynylestradiol in the United States (with FDA approval) as an oral contraceptive in humans. A human contraceptive was selected because investigators originally assumed that it would be reasonable to obtain FDA approval (for use in deer) for a compound already approved for a human use. NA was also selected because (1) the acetate provides longer in vivo half-life (Sinkula 1978), and (2) esterification enhances steroid secretion from silicone implants (Christensen and Kesler 1984 and 1986, Kesler et al. 1996). NA implants have been used efficaciously (as a contraceptive) in humans (McCauley and Geller 1992). The first, and last, study (as reported below) was in beef heifers; the compound norgestomet was then selected for evaluation as a deer contraceptive.

Fourteen beef heifers were selected for the study. Heifers were divided into two groups. All heifers had been previously synchronized with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; Kesler 1985a and b, Kesler and Favero 1989a) and observed for estrus. Twelve days after detected estrus, all heifers were bled, and

plasma was assayed by a validated enzyme-linked immunosorbent assay (ELISA) (Kesler et al. 1990) for progesterone concentrations. All 14 heifers had progesterone concentrations greater than 1.5 ng/mL, which suggests that they had corpora lutea that developed subsequent to the previously detected estrus (Kesler et al. 1981). Half (7) of the heifers were subcutaneously implanted with an NA matrix silicone implant. The cylindrical implants, each 3.5 mm in diameter and 2.5 cm in length, were implanted subdermally on the convex surface of the ear. Each treated heifer received one implant that contained 11.5 mg of NA (equivalent to 8.35 mg of norethindrone). At the time of implant insertion, all heifers were administered a luteolytic dose of $PGF_{2\alpha}$. Implants were left in situ for 4 days; after removal, total remaining NA was determined (Kesler et al. 1995 and 1989c). In vitro implant secretion over 4 days was also determined and corrected for in vivo secretion by the procedure reported by Machado (1994).

NA was released from the silicone implants in a typical linearly declining fashion ($r = -0.997$; $y = x(-0.21) + 1.15$) (Ferguson et al. 1988, Kesler and Favero 1989c, Kesler et al. 1995). Over the 4-day period, a total of 2.53 mg (22 percent of the total) was delivered in vivo. Three of the four control heifers (43 percent) were detected in estrus whereas all seven (100 percent) of the treated heifers were detected in estrus (table 1).

Estrus was detected at similar times after $PGF_{2\alpha}$ treatment for both groups. To verify $PGF_{2\alpha}$-induced luteolysis, all heifers were bled 2 days after $PGF_{2\alpha}$ treatment, and plasma was assayed for progesterone concentrations (Kesler et al. 1990). The progesterone concentrations in all heifers suggested that luteolysis was ensuing or had ensued.

In summary, NA did not suppress estrus. In fact, during a period of high NA secretion (2.53 mg over the 4-day period), there was a tendency for more ($P = 0.02$) NA-treated heifers than control heifers to display estrus. Therefore, NA was not considered further.

Table 1. Norethindrone acetate implant secretion and estrus suppression efficacy in beef heifers

Item	Control	Treated
Number	7	7
Number in estrus	3 (43%)	7 (100%)[1]
Mean interval to estrus	61 hours	59 hours
Norethindrone acetate secreted		
Day 1	0	947 µg
Day 2	0	738 µg
Day 3	0	501 µg
Day 4	0	341 µg

[1] Differed from the control group at the 0.02 level of significance.

Norgestomet Chemistry and Physiology

Chemistry

Norgestomet is approved by FDA for use in cattle for estrus synchronization (Darling 1993). The procedure, designated Syncro-Mate B®, includes a 9-day implant containing 6 mg of norgestomet and an intramuscular injection that consists of 3 mg of norgestomet and 5 mg of estradiol valerate that is administered at the time of implant insertion (Chien 1978, Kesler et al. 1995). The purpose of the implant is to suppress estrus. When it is used for estrus synchronization in cattle, subsequent timed breeding (cattle are bred 48–52 hours after implant removal) pregnancy rates range from 40 percent to 60 percent (Odde 1990, Kesler and Favero 1996). Norgestomet has also been successfully used for resynchronization in cattle (Favero et al. 1993 and 1995, Machado 1994, Kesler et al. 1994) and for estrus suppression and synchronization in sheep (Kesler and Favero 1989b and 1997).

Chemically, norgestomet (17α–acetoxy–11β–methyl–19–norpreg–4–ene–20,dione; SC 21009) is a modified 19–norprogesterone (fig. 2). Norprogesterone is identical to progesterone except that the methyl group at the 19 position is absent. Norgestomet has two other modifications: the presence of a methyl group at the 11 position and an acetate at the 17 position. Acetate has been added to provide longer half life in situ (Sinkula 1978). Norgestomet is me-

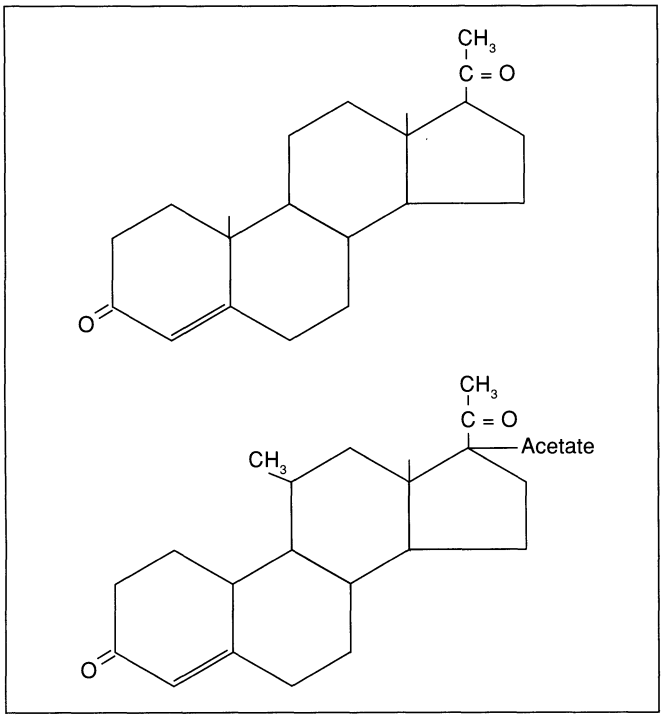

Figure 2. Chemical structures of progesterone (top) and norgestomet (bottom). Norgestomet is a norprogesterone (exactly like progesterone except the methyl group at the 19 position is absent). Two other differences from progesterone are that norgestomet has an acetate at the 17 position (in order to increase half-life in vivo), and a methyl group is included at the 11 position.

tabolized quickly (Moffatt et al. 1993) and is excreted in the urine and feces (Searle 1982). In both urine and bile, most of the excreted metabolites are highly polar materials demonstrated to have only about 4 percent of the progestational activity of norgestomet in the Clauberg assay (Searle 1982).

Norgestomet is a highly biologically active progestin. Gilbert et al. (1974) demonstrated that norgestomet is 15 times more biologically active than progesterone when orally administered to rabbits and 216 times more biologically active than progesterone when subcutaneously administered to estradiol–17β-treated mice. Wishart (1972) demonstrated that 140 µg of norgestomet and 45 mg of progesterone were required to suppress estrus in all treated heifers (which means that norgestomet is 321 times more potent than progesterone in this model). These data, combined with the data of Zimbelman and Smith (1966), would suggest that MGA is 90 times more potent than progesterone. This minimal dose of norgestomet required to suppress estrus in cattle was confirmed with silicone implant delivery of norgestomet by Machado (1994) and Machado and Kesler (1996). In their studies, 6-mg and 8-mg silicone implants were administered to cows for 16 days. None of the cows with 8-mg implants were detected in estrus with implants in situ. The smallest daily dose of norgestomet released by these implants was 136 µg, which occurred on day 16. However, in three cows with 6-mg implants, estrus was detected the first day after implant secretion dropped below 136 µg/day. Although this represents only 16 percent of the treated cows, 100-percent efficacy of estrus suppression was lost.

Norgestomet's principal mode of action for estrus synchronization is by suppressing estrus. Further, norgestomet has the progesterone biological activity to maintain pregnancy in ovariectomized heifers (Favero et al. 1990; Kesler, in press). Favero and coworkers demonstrated that norgestomet would maintain pregnancy from day 10 through parturition. Upon removal of the norgestomet implants, parturition (if the implants were removed at term) or abortion (if the implants were removed at midgestation or earlier) occurred within 52 hours. Therefore, norgestomet is as effective as progesterone (but at a substantially reduced dosage) for two of progesterone's main biological actions: estrus suppression and pregnancy maintenance.

Progesterone also has a role in regulating luteinizing hormone (LH) and subsequent follicular growth and maturation. Experiments utilizing the commercial hydron (polyethylene glycomethacrylate; Short 1975) norgestomet implant (6 mg) have demonstrated that, when it was implanted during pro-estrus, the dominant follicle present was maintained for the duration of the treatment, and there was no growth of medium or small follicles (Rajamahendran and Taylor 1991). Systemic estradiol concentrations were also elevated, and there was insufficient progestin activity to maintain a strong negative feedback on LH pulse frequency in a manner comparable to that of the luteal phase of a normal estrous cycle (Savio et al. 1993).

Rajamahendran and Taylor (1991) suggested that this implied that the norgestomet treatment given during pro-estrus mimics the actions of low concentrations of progesterone. This time period is, in fact, a time of low norgestomet secretion by the hydron implant (Kesler et al. 1995), and, therefore, obtaining a low progestin effect would be expected. In fact, when implants were changed during the persistence of the dominant follicle, LH pulse frequency decreased, estradiol concentrations decreased, and follicular atresia occurred (Savio et al. 1993). Therefore, when given in appropriate amounts, norgestomet was effective in provoking the progestinlike negative feedback on LH pulse frequency and on follicular atresia.

These conclusions were supported by Butcher et al. (1992), who reported that daily injections of 100 mg were was required to elevate systemic progesterone concentrations to levels of the luteal phase (5 to 7 ng/mL). In contrast, daily injections of only 45 mg were required to suppress estrus in all treated animals (Wishart 1972). The dosage selected for the norgestomet implant was based on the minimal quantity required to suppress estrus.

Administration of norgestomet on days 5–21 of the estrous cycle had no effect on progesterone secretion by corpora lutea (Domatob et al. 1994) and no negative effects on the establishment of pregnancy (Favero et al. 1993 and 1995, Machado 1994, Kesler et al. 1994). In order to assess the effect of norgestomet on early corpora lutea function and development in bovines, norgestomet was administered on days 1, 2, 3, and 4 after estrus (2 cows/day). The implants were left in situ for 12 days. In all eight cows, development of the corpora lutea, secretion of progesterone, and length of the estrous cycle were unaffected by norgestomet treatment. Therefore, negative feedback of norgestomet during met-estrus and di-estrus did not disrupt corpora lutea development or function (Kesler, unpubl. data).

It has been reported that norgestomet has a higher binding affinity to bovine uterine receptors than progesterone (Moffatt et al. 1993). Interestingly, however, although norgestomet has a higher binding affinity to bovine receptors, it did not bind (less than 0.1-percent cross-reactivity) to highly specific anti-progesterone immunoglobulin G developed in rabbits (Kesler et al. 1990). Norgestomet exhibits only a weak ability to competitively bind bovine endometrial glucocorticoid receptors (Moffatt et al. 1993). Although norgestomet does not interact with endometrial estrogen receptors, it exhibits weak estrogenic activity when tested in an estrogen-dependent stimulation of human breast cell test. However, to provoke estrogen stimulation, a dose of at least 100 mg of norgestomet given at one time would be required (Moffatt et al. 1993).

Norgestomet Safety

To obtain FDA approval for its use in cattle, investigators conducted numerous studies to establish norgestomet's safety in both the treated animals (cattle) and humans (Searle 1982). For cattle, studies were conducted with doses up to 60-fold excess to the recommended dose (6 mg implants). Daily observation of animals indicated no adverse reactions. Further, postmortem evaluation of the thoracic and abdominal viscera indicated that norgestomet caused no adverse effects.

To evaluate human safety, researchers conducted several studies in both monkeys and rats (Searle 1982). The study conducted in monkeys was designed to evaluate the human oral contraceptive effect of norgestomet. Oral treatment of 30 and 100 μg/kg (but not 0 and 10 μg/kg) per day increased the length of menstrual cycles, decreased the ovulation rate, and decreased the number of cycles during the 84-day treatment period. Throughout the treatment period, the only remarkable effect was amenorrhea, which was observed in five of six and three of six monkeys orally administered daily doses of 30 or 100 μg/kg, respectively. Further, when norgestomet was administered at these doses, the conception rate was depressed to zero. The 10 μg/kg of norgestomet per day had no significant effects on menstrual cycle length, ovulation rate, amenorrhea, or conception rate (Searle 1982).

For the rat studies, norgestomet was administered orally by gavage to two generations of rats at daily doses of 0, 0.0001, 0.001, 0.01, 0.1, or 1.0 mg/kg (Searle 1982). Administration of all doses produced no clinical signs indicative of toxicity. Weight gain was affected slightly only in the second-generation rats treated at the 1.0 mg/kg daily dosage. Also, in these same second-generation rats, fertility was slightly lower when compared to that of controls. There were no gross or histologic (adrenals, pituitary, and sex organs) changes that could be attributed to treatment with norgestomet. Absolute and relative organ weights from the treated groups were not different from the controls, although there was a slight decrease in liver weights in all treated animals.

In published resynchronization studies where norgestomet was administered during pregnancy, 158 pregnancies have resulted (Favero et al. 1993 and 1995, Machado 1994, Kesler et al. 1994, Domatob et al. 1997). No adverse effect of any kind has been observed. Therefore, the administration of norgestomet does not appear to affect embryonic or fetal development. However, as previously noted, norgestomet will inhibit parturition and therefore should not be inadvertently administered to pregnant animals where the implant is not going to be removed before parturition (Favero et al. 1990; Kesler, in press).

Contraceptive Efficacy

One study of norgestomet's contraceptive efficacy in deer was completed in 1995 (Jacobsen et al. 1995), and another was more recently published (DeNicola et al. 1997). Jacobsen's study was conducted in confined black-tailed deer. This study included 10 deer of which 7 were treated with 42-mg norgestomet implants approximately 1 month before the breeding season. In addition to the 10 female deer, 2 fertile males were included in the same confined area. Observations were collected over a 2-year period after treatment.

Subsequent to treatment, all of the treated female deer failed to exhibit estrous behavior. Further, males exhibited neither intentional pursuit, courting, nor tending bond behaviors toward treated females. After the first breeding season, all three control deer fawned, producing two sets of twins and one set of triplets. None of the seven treated deer fawned. All of the 10 female deer exhibited estrous behavior the next breeding season, and all 10 conceived.

Although this study utilized a small sample, additional studies with white-tailed deer (DeNicola et al. 1997) confirm the contraceptive effect of the 42-mg norgestomet implant. In addition, a contraceptive effect with similar efficacy to that of the 42-mg implant has been demonstrated with a 21-mg norgestomet implant (DeNicola et al. 1997).

The desired duration of contraception is controversial. Some groups encourage lifetime sterilization; others suggest that contraceptives should be reversible. The needle-less norgestomet implant was designed, as data confirmed, to be a 1-year contraceptive. Therefore, after 1 year of reducing the deer population, a decision can be made regarding how to control it in subsequent years.

Release from the 42-mg implant has been evaluated. This was accomplished by utilizing a validated in vitro system that mimics in vivo secretion (Kesler et al. 1995). Implants were evaluated daily over a 4-month period. The release of norgestomet from the implants was in a typical linear declining fashion (see figs. 3 and 4; Kesler et al. 1995). The best fit line was determined by correlating daily norgestomet released *v.* the log of day in vitro. This produced a correlation coefficient of –0.996. The maximal release of 638 μg was on the first day. During the first 3 months, more than 136 μg of norgestomet was released daily. This is a quantity that, as described earlier, suppresses estrus in cattle. The amount of norgestomet released daily thereafter decreased linearly. Based on the best fit release, norgestomet was released from the implant for 252 days.

For practical reasons, emphasis was placed on developing a contraceptive for the female deer. However, the contraceptive effects of progestins in males have been known for some time (Liskin and Quillin 1983). To assess the usefulness of norgestomet in male animals, researchers conducted a preliminary study to evaluate its effects on fertility-related factors in male rats.

This study included six males rats that were 12 weeks old at the onset of the experiment. Three rats served as controls and received no treatment. The other three rats were each administered one 6-mg silicone implant. At the end of 9 days, the implants were removed and replaced with new 6-mg silicone implants. This cycle continued for 63 days (7 implants/rat—9 days/implant). On day 63, all six rats were killed and trunk blood was collected. The plasma was analyzed for testosterone concentrations via a validated ELISA (Kesler et al. 1990). In addition, testes were collected and weighed. Mean individual testis weight of the norgestomet-treated rats was reduced ($P < 0.01$) and was only 37 percent of the control rats' mean testis weight (table 2). Mean testosterone concentrations in the plasma of norgestomet-treated rats were only 15 percent of the control rats' testosterone concentration. Although not highly significant ($P = 0.19$), norgestomet clearly had a biological effect on testosterone concentrations. A high level of significance ($P < 0.05$) was not achieved because the untreated rats demonstrated significant variability in their testosterone concentration and because so few animals were included in this prelimi-

Table 2. Mean testosterone concentrations and testes weights of rats treated with norgestomet.

Item	Control	Treated
Number	3	3
Mean individual testis weight	2.08 g	0.76 g[1]
Mean testosterone concentrations	4.54 ng/mL	0.66 ng/mL[2]

[1] Differs from the control group at the 0.01 level of significance.

[2] Differs from the control group at the 0.19 level of significance.

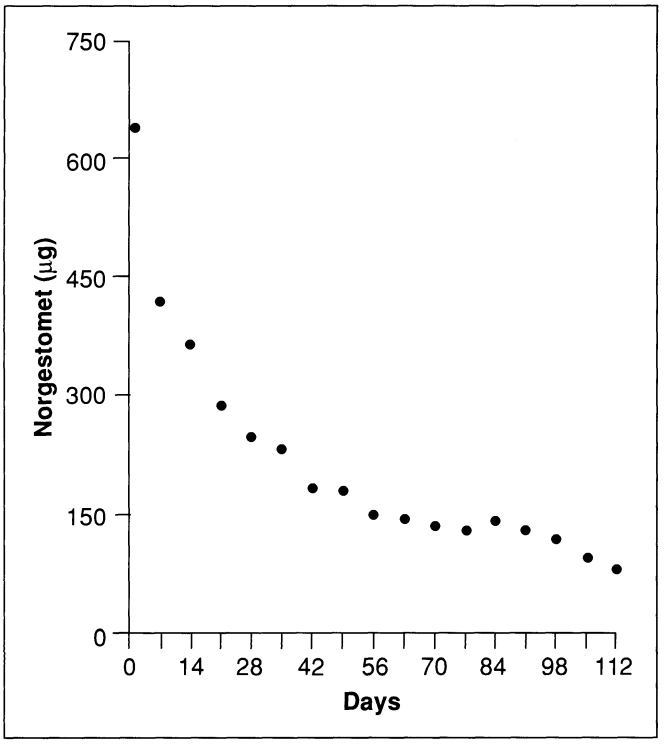

Figure 3. Actual daily in vitro release of norgestomet. Daily observations were collected; however, only weekly observations are illustrated.

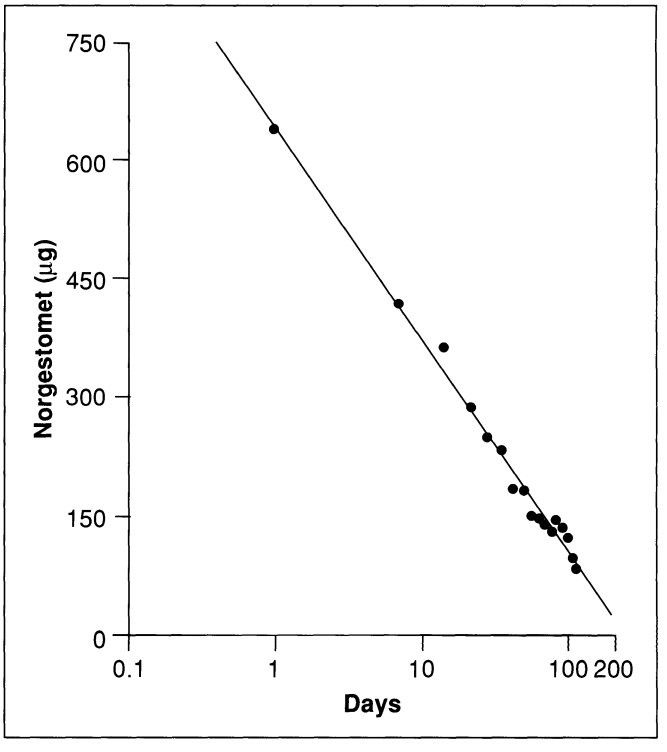

Figure 4. Daily release (with days converted to log of days) in vitro of norgestomet. Daily observations were collected; however, only weekly observations are illustrated. The regression equation is Y = X (–265.26) + 637.23 with Y = norgestomet concentration [µg] and X = log of days (r = –0.996; $P < 0.01$).

nary study. However, the three norgestomet-treated rats had the three lowest concentrations of testosterone in their plasma.

Collectively, these data suggest that norgestomet may have a contraceptive effect in males. However, these studies were conducted with high concentrations of norgestomet and not in deer. Further investigations evaluating sperm concentrations in the epididymis of male deer or in their ejaculate are needed.

Remote Needle-Less Delivery

Delivery of contraceptives to free-roaming animals is critical to successfully suppressing reproduction. The idea contraceptive should (1) be capable of being delivered remotely, (2) not pollute the environment, and (3) allow control such that only animals intended to be treated are treated and that the drug is handled and dispensed properly.

The norgestomet implants used in the deer efficacy studies were needle-less implants (fig. 5) that could be delivered at distances up to 40 m from the target animal (DeNicola et al. 1996). The needle-less implants have two major components. Their outer shell is manufactured from food-grade biodegradable and biocompatable chemicals. The components are already approved as food additives; even if all of the implant remained in place at the time of slaughter and was eaten by humans, that would not pose a hazard (U.S. Government 1993). The outer biodegradable shell is 0.635 cm in diameter and 2 cm long. The second component is the norgestomet manufactured in a matrix silicone implant. The silicone matrix is 0.42 cm in diameter and 1.4 cm long. It weighs 215 mg, of which 42 mg (19.5 percent) is norgestomet. The outer shell combined with the silicone/norgestomet weighs about 880 mg.

The needle-less implants are propelled via a compressed-air delivery system. For the 1995 study (Jacobsen et al. 1995), the needle-less implants were delivered at 26,152 cm/second (858 feet/second) producing 3.07×10^5 g-cm (22.15 foot-pounds) of

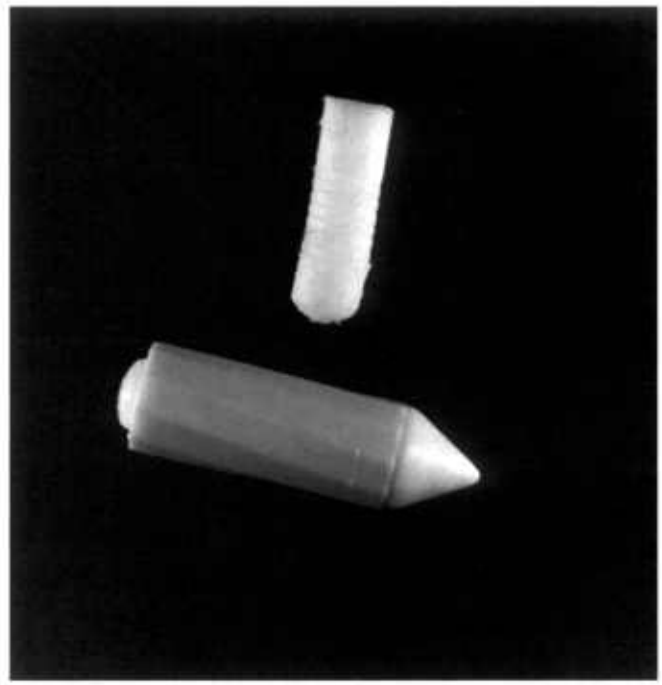

Figure 5. The needle-less norgestomet implant used in the deer studies. The photo shows the outer biodegradable shell (0.635 cm in diameter and 2 cm long) and the inner silicone matrix norgestomet implant (0.42 cm in diameter and 1.4 cm long).

kinetic energy. This system was designed for use in cattle, whose skin is far thicker than that of deer (Kesler and Favero 1997). Propelling the implants with that much kinetic energy caused trauma in deer (Jacobsen et al. 1995).

Jacobsen's coworkers administered the needle-less implants in biceps femoris or semitendinosus or semimembranosus muscular at a distance of 3–30 m. Upon contact, deer exhibited one of two reactions: fleeing response without any apparent change in gait, followed by standing and grooming of the administration site, or immediate carriage of the hindlimb and lack of attempted weight bearing for variable durations.

In subsequent studies, the needle-less implant has been delivered with far less kinetic energy. Using less kinetic energy does not compromise the accuracy but significantly reduces the trauma in deer (DeNicola et al. 1997). In fact, when needle-less implants can be delivered silently, deer have minimal reaction to

their delivery. In one study where cortisol concentrations were monitored to evaluate stress caused by the needle-less implant, they were not increased (Kesler, unpubl. data).

Upon contact with the skin, the needle-less implant first causes it to stretch (Gould 1984). After stretching, the implant penetrates the skin by producing a slit in it. After penetration has occurred, the skin then contracts back to almost its original form, with only a small slit left behind. The entry slit is shorter than the diameter of the projectile. Minimal, if any, bleeding occurs after penetration. Scab formation follows (Willis et al. 1994, DeNicola et al. 1996). The projectile does not carry a portion of the animal's hide into the wound but leaves behind only a small, raised welt on the skin at the point of projectile entry (Drake and Paul 1976, Kesler et al. 1989a).

Upon entry into living tissue, the outer shell dissolves in vivo in approximately 6 hours. I conducted both in vitro and in vivo studies to determine dissolution of the outer shell (table 3). The matrix silicone implant, although biocompatable and nonirritating, remains and delivers norgestomet by Fick's first law of diffusion as long as there is norgestomet contained within the silicone. By design, two deer that have been remotely treated with needle-less norgestomet implants were killed (about 2 months after treatment), and investigators examined the administration sites and musculature. In both cases, the norgestomet–silicone implant was recovered. Surrounding tissue was normal (DeNicola et al. 1996).

This remote delivery system is unique and has many advantages over all other delivery formats. Another remote delivery system utilizes syringe darts. Although syringe darts provide remote delivery, a nondegradable syringe and needle remain in the environment. Another remote delivery system being proposed utilizes genetically engineered viruses which provides no or very minimal control on its spread (Morell 1993, Wagner et al. 1994).

Table 3. In vitro and in vivo dissolution of the biodegradable shell of the needle-less norgestomet implant

Hour	Percent of implant dissolved	
	In vitro[1]	*In vivo*[2]
0	0	0
1	40	—[3]
2	70	—
3	90	—
4	95	—
5	98	[4]98.25
[5]6.39	100	—
24	—	[6]100

[1] In vitro conditions consisted of suspending the implant shell in 100 mL of phosphate buffered saline (pH 7.0) at 37 °C.

[2] In vivo conditions consisted of subcutaneously implanting the implant shell in rabbits. At 5 and 24 hours after implantation, eight rabbits (four each time) were killed to determine the amount of implant shell remaining.

[3] No observations were collected for times marked —.

[4] At 5 hours after implantation, approximately 2 percent, 0 percent, 3 percent, and 2 percent of the implant was remaining.

[5] The implant shell had completely dissolved at 6.08, 6.42, and 6.67 hours after placing the implants in solution.

[6] At 24 hours after implantation, no intact implants were present in any of the four treated rabbits.

Government Regulations

It is not the purpose of this article to review government regulations; however, it is important to make a few important comments. First and foremost, the norgestomet–silicone contraceptive reported herein is not approved for use by FDA. An Investigational New Animal Drug (INAD) authorization has to be granted to conduct the experiments reported. FDA has required that these studies be conducted only on confined animals and that they do not escape in such a way that they could enter the human food chain. Although approved in cattle, norgestomet is not approved for widespread use in deer. Before that approval is possible, a sponsor much accomplish numerous tasks (table 4) to ensure that the product is efficacious and safe not only to the treated animals but also to the humans that may consume treated animals. It is my opinion that this product can be approved by FDA.

Table 4. Information required to be submitted to the FDA's Center for Veterinary Medicine when requesting approval for the marketing of a new animal drug product (Center for Veterinary Medicine 1994)

1.	Identification
2.	Table of Contents and Summary i. Chemistry ii. Scientific rationale and purpose
3.	Labeling i. Label identification ii. Nonprescription labeling iii. Prescription labeling iv. Use restrictions v. Medicated feed labeling vi. Draft labeling
4.	Components and Composition i. Components ii. Composition iii. Fermentation of drug substance
5.	Manufacturing Methods, Facilities, and Controls i. Manufacturer ii. Personnel iii. Facilities/equipment iv. New drug substance synthesis v. Raw material control vi. Manufacturing instructions vii. Analytical controls viii. Lot control number xi. Container x. Stability xi. Additional procedures xii. GMP (good manufacturing practice) compliance
6.	Samples
7.	Analytical Methods for Residues
8.	Evidence to establish safety and effectiveness
9.	Good Laboratory Practice Compliance
10.	Environmental Assessment
11.	Freedom of Information Summary
12.	Confidentiality of Data and Information in a New Animal Drug Application

However, requirements for distribution have yet to be accomplished.

Since animals treated with norgestomet would have their implant in situ during the hunting season, a legitimate concern is finding the answer to the question, what will happen to the people who consume such an implant in a treated animal? First, tissue studies demonstrate that minimal norgestomet residue exists in all treated cattle tissues except liver and kidney (Searle 1982). Second, in regard to consumption of an implant, the silicone is exceptionally durable. When placed in vitro in concentrated hydrochloric acid over a 3-day period, the polymer is unaffected. Therefore, complete breakdown and absorption of all remaining norgestomet (like the effect on compressed pellets) is extremely unlikely (or impossible). Further, implants incubated in 250 mL of 1 N hydrochloric acid (at 37 °C), to mimic the acidic conditions of the stomach, released the same amount of norgestomet as in plasma in vitro conditions. New implants incubated for 24 hours in plasma and 1 N hydrochloric acid released 638 μg and 648 μg, respectively (within 1.5 percent of each other). Therefore, consumption of an implant a few weeks after implantation would release less than the safe 10 μg/kg daily dose previously discussed in monkeys.

Summary

Progesterone, produced by the corpus luteum, suppresses estrus in deer and cattle. Synthetic progestins (melengestrol acetate and norgestomet) that suppress estrus in cattle are also effective in deer. Synthetic progestins that are effective contraceptives in humans, however, do not suppress estrus and are not effective contraceptives in deer or cattle. Steroidal compounds are often viewed negatively because of the diethylstilbestrol (DES) scenario, even though they are widely used by humans. DES became implicated as a carcinogen because large doses (50 mg/day) of DES given to pregnant women caused an increased incidence of cervical cancer in their daughters (0.14 to 1.4 cases per thousand exposures [Cheeke 1993]). Norgestomet evokes all progesteronelike actions but at a much reduced dosage. Further, there are no data available to indicate that this steroid poses a risk. In addition to the data reported herein, norgestomet has been used for over a decade in cattle without any reported problems to either the cattle or to the human consumption of meat from treated animals. The only known progestin potent enough to be manufactured in a remotely delivered needle-less implant and still be efficacious as a contraceptive is norgestomet. This

contraceptive system was evaluated by a scientific committee for use in wild goats (Warren 1992). That committee gave the needle-less norgestomet the highest possible ratings for delivery, safety, and efficacy. All data support their conclusions. In fact, the committee rated the needle-less norgestomet implant as the best contraceptive for wild goats (Warren 1992). Based on all data available, the same conclusion can be reached for deer. I encourage further evaluation and support of the development of this contraceptive for use in deer.

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Considerations for Immunocontraception Among Free-Ranging Carnivores: The Rabies Paradigm

Cathleen A. Hanlon and Charles E. Rupprecht

The raging North American controversy over the reintroduction of wolves into the ecosystem of the greater Yellowstone National Park area exemplifies the emotive relationship between humankind and the Carnivora. What forces act in concert to portray this much maligned Order in unfavorable light? Control of free-ranging carnivore populations by *Homo sapiens* has been practiced for centuries as part of a pastoral lifestyle, with the intent of protecting one's own life and livelihood from becoming freshly killed prey in the onslaught from mammalian competitors. Traditionally, control is equated most commonly with population reduction through direct elimination of individuals (e.g., typically social canids or solitary large-bodied felids) via lethal means including shooting, poisoning, trapping, gassing of dens, and habitat modification (Lewis 1968, U.S. Department of Agriculture 1992). In addition to reducing direct predation upon domestic livestock (sheep and cattle losses alone in the United States are estimated in excess of $80,000,000 annually [U.S. Department of Agriculture, National Agricultural Statistics Service 1991]), other perceived beneficial aspects of free-ranging carnivore population reduction include conservation of endangered species, such as Australian marsupials, subject to predation by introduced European red foxes (Boyle 1994), conservation of otherwise "desirable" species (game fowl and wild ungulates), and the alleviation of objectionable human–carnivore interactions (Wynne–Edwards 1964). Today, such interactions range from local citizen complaints of seemingly frivolous or nuisance wildlife encounter—raccoon disruption of a backyard songbird feeder, bear vandalism at vacation homes, etc.—to significant global public health issues (such as animal bite from the stray dog) and related human mortality either directly from overt injury or indirectly from exposure to a plethora of zoonoses, such as rabies or echinococcosis (Beran 1994). Nevertheless, a "manageable" number of mammalian carnivores is clearly viewed as beneficial when they serve human desire for sport, pelts, companionship, etc. Moreover, sound ecological, economic, and ethical arguments weigh against sole reliance upon lethal mechanisms to resolve such conflicts. A comprehensive approach to conflict management, rather than a narrow focus only upon overt, uncompromising predator decimation, is a valid and potentially more sustainable strategy to manage human–wildlife conflicts. Can targeting and controlling carnivore proliferation resolve the dilemma and validate this premise of alternative, nonpernicious intervention?

Historically, control of mammalian reproduction has been primarily directed toward domestic companion animals and livestock. Contraception has typically consisted either of surgical neutering of individuals, hormonal manipulation of reproductive function, or simple physical separation of the sexes. While the neutering of feral cats has been suggested as an alternative to elimination (Zaunbrecher and Smith 1993), these techniques, which are suited for management of individual reproductive function, may only rarely be applicable to most free-ranging carnivore populations, given the constraints of diverse species distribution and abundance. In contrast, oral delivery of a contraceptive agent for reproductive control among wild carnivores may be more feasible; initial efforts were reported as early as three decades ago (Balser 1964).

The observation of naturally occurring antisperm antibodies in a small proportion of humans has generated interest in the recruitment of the immune system for reproductive modulation (Aitken et al. 1993). Some postulated advantages of immunologically mediated contraception may be (1) economical vaccine production by recombinant techniques, (2) ease of administration, (3) relatively few side effects, and (4) a higher degree of biological specificity than traditional chemical drug delivery, which may have a broader phylogenetic and physiological spectrum of activity. From an ecological perspective, one potential advantage of wildlife immunocontraception would be to minimize deleterious effects of free-ranging carnivores by reducing or stabilizing total numbers, while avoiding vacant niches inherent to lethal reduction. A nonreproductive adult would inhibit ingress of new, fully reproductive individuals from surrounding areas (Porter et al. 1991). Arguably, one

weakness of the immunocontraception prospectus is individual variation in the immune response, which may lead to unpredictability regarding the duration and magnitude of effect in a particular animal. However, if a measurable effect among a local population is achieved, some variation among individuals may be acceptable.

Typically, oocyte and sperm antigens are sufficiently compartmentalized so that an immune response is not normally elicited; however, these antigens are clearly immunogenic (Haimovici et al. 1992, Liu et al. 1990). In this regard, considerable research has focused on zona pellucida (ZP) antigens (Kirkpatrick et al. 1991 and 1992, Hasegawa et al. 1992, Jones et al. 1992). Despite a highly species-specific interaction between the sperm surface and a glycoprotein component of the ZP, antibodies to the ova of one species inhibit in vitro and in vivo fertilization of another species (Aitken et al. 1993). An unexpected finding from ZP immunization has been the delayed cessation of ovarian cycling from destruction of primordial follicles or essentially induced premature menopause in animal models (Hasegawa et al. 1992, Jones et al. 1992), another potential drawback in the implementation for wildlife.

Alternative approaches have focused upon inducing antibodies to the cumulus oophorus of the conceptus (Tesarik et al. 1990) or disrupting regulatory hormones such as human chorionic gonadotrophin, gonadotropin-releasing hormone, luteinizing hormone-releasing hormone, and follicle-stimulating hormone (Aitken et al. 1993). Additionally, while still the subject is still in the early stages of investigation, some promising results have also been obtained with disruption of spermatogenesis (Grubb 1991). Some of these methods raise complex medical or ethical issues, for human reproductive manipulation because the end result may be essentially abortifacient or complications related to immune-complex formation. Whether these matters would be equally as controversial when applied to a "nuisance" carnivore species has yet to be determined. However, it should be clear that absolute restriction to the species of interest would be optimal.

Other suggested interventions would target levels of reproductive hormone (testosterone or progesterone) directly (Linhart 1964, Linhart et al. 1968, Awoniyi et al. 1992, Moudgal et al. 1992, Talwar et al. 1992, Vanage et al. 1992, Deshmukh et al. 1993, Dowsett et al. 1993, Ladd 1993). The significant limitation of this approach in a free-ranging carnivore population is the potential for an undesirable effect upon sexual behavior, social interactions, and hierarchy (Awoniyi et al. 1992, Moudgal et al. 1992, Dowsett et al. 1993).

To date, no species-specific reproductive antigens have been identified, although unique contraceptive antigens for humans (Aitken et al. 1993), wolves (U.S. Department of Agriculture 1992), red foxes, rabbits, kangaroos (Morell 1993), deer (Porter et al. 1991), wild swine (Fletcher et al. 1990), and many others (Wynne–Edwards 1964), would be of great utility. The apparent conservation of many reproductive antigens among mammalian groups raises the undesirable, even detrimental, potential to unintentionally affect nontarget species, possibly including humans, valuable domestic animals, endangered or threatened wildlife, and nonnuisance carnivore species. In lieu of species-specific antigens, a species-specific vector (plasmid DNA, viral, bacterial, etc.) would be a potential strategy to limit the contraceptive effect solely to the target species. Unfortunately, such carnivore species-specific vectors have also yet to be identified.

The physical delivery of a desired contraceptive may consist of a variety of singly applied or combined approaches. For example, live-trapping of free-ranging carnivores and direct inoculation of a contraceptive may be of some value, particularly in areas where high human–carnivore interaction is problematic and necessitates a response, but complete elimination of carnivores is not desired by human residents, and lethal control is unacceptable. Except for under these limited conditions, the labor-intensive nature of this approach and the poor capture rates of some carnivore species may render this method largely impractical.

Conversely, injection of contraceptive agents may be achieved remotely via a blow gun, dart gun, or similar device. This is currently a procedure in progress for an insular population of feral horses off the eastern mid-

Table 1. Oral vaccination of carnivores with recombinant viruses

Agent	Species (common name)	Reference
Vaccinia-Rabies Glycoprotein Recombinant Virus		
Family Canidae	*Vulpes vulpes* (red fox)	Blancou et al. (1986)
	Canis lupus (domestic dog)	Blancou et al. (1989)
	C. latrans (coyote)	Artois et al. (1990)
	Alopex lagopus (arctic fox)	Chappuis and Kovalev (1991)
	Nyctereutes procyonoides (raccoon dog)	Chappuis and Kovalev (1991)
	Urocyon cinereoargenteus (grey fox)	Rupprecht et al. (1992a)
Family Felidae	*Felis domesticus* (domestic cat)	Blancou et al. (1989)
	Lynx rufus (bobcat)	Rupprecht et al. (1992a)
Family Mustelidae	*Mephitis mephitis* (striped skunk)	Tolson et al. (1987)
	Mustela putorius (ferret)	Brochier et al. (1988)
	Meles meles (European badger)	Brochier et al. (1989)
	Lutra canadensis (river otter)	Rupprecht et al. (1992a)
	Mustela vision (mink)	Rupprecht et al. (1992a)
Family Procyonidae	*Procyon lotor* (raccoon)	Wiktor et al. (1985)
Family Ursidae	*Ursus americanus* (black bear)	Rupprecht et al. (1992a)
Raccoonpox-Rabies Glycoprotein Recombinant Virus		
Family Procyonidae	*P. lotor*	Esposito et al. (1988)
Family Canidae	*C. lupus*	Esposito et al. (1992)
	U. cinereoargenteus	Esposito et al. (1992)
Family Felidae	*F. domesticus*	Esposito et al. (1992)
	L. rufus	Esposito et al. (1992)
Family Mustelidae	*M. mephitis*	Fekadu et al. (1991)
Human Adeno(5)-Rabies Glycoprotein Recombinant Virus		
Family Procyonidae	*P. lotor*	Charlton et al. (1992)
Family Canidae	*V. vulpes*	Charlton et al. (1992)
	C. lupus	Campbell (1994)
Family Mustelidae	*M. mephitis*	Charlton et al. (1992)
Baculo-Rabies Glycoprotein Recombinant Virus		
Family Procyonidae	*P. lotor*	Fu et al. (1993)

Atlantic shore (Kirkpatrick et al. 1991 and 1992). As above, this approach is also largely limited by species secretiveness, tolerance for humans, the accuracy of the operator, and the ability to identify previously inoculated individuals.

Given these limitations, additional methodologies may have to be considered for long-term, widespread carnivore reproductive control. For example, the effectiveness and relative ease of using baits to deliver a biological, rather than lethal chemicals as practiced historically, to wild carnivores has been demonstrated, principally through the wildlife rabies vaccination of several reservoir species in Europe and North America (Johnston et al. 1988, Bachmann et al. 1990, Brochier et al. 1990, Rupprecht et al. 1992a, Winkler and Bogel 1992, Campbell 1994). This example of wildlife rabies vaccination has often been cited over the last decade to document the degree of sophistication achieved in reaching free-ranging carnivore populations. To date, these field systems involve either modified live rabies viruses or recombinant orthopoxvirus vectors that undergo limited replication without perpetuation or apparent adverse effect (at least in the latter viral scenerio) in the targeted host. The advantages of a self-replicating entity are economy and the more reliable induction of an immune response without the need for

multiple doses or adjuvants. Moreover, vectors with wide carnivore host susceptibility (table 1) are advantageous with a disease such as rabies, in which the pathogen is not restricted to a single narrow host niche. For example, a single biologic may be useful for control of rabies in raccoons, red foxes, and coyotes in various geographic areas where rabies strains are perpetuated by different carnivore species. Yet this same precept of broad application may be counterproductive without species-specific expression products, when the effect is immunocontraception in co-occurring species, rather than simply rabies vaccination.

In addition to live virus vaccination, successful oral immunization of raccoons in captivity has also been demonstrated with a baculo–virus system, in which rabies glycoprotein expression in an insect cell culture resulted in sufficient quantities of antigen to immunize animals directly by mouth (Fu et al. 1993). Similarly, raccoons and other carnivores may be orally immunized with inactivated viral preparations (Rupprecht et al. 1992b). While the amount of antigen required may be economically prohibitive given current production limitations, the concept offers a choice avenue of investigation that departs from the traditionalist approach toward a replicative vector, if a restricted reproductive antigen were available.

A self-replicating biologic has an inherent potential for adverse effects that is influenced by host variables, such as species and individual age, immune status, concurrent infectious or metabolic conditions, etc. The latent risk for adverse effects may be nearly immeasurable under traditional laboratory or field conditions. These concerns are particularly relevant to an immunocontraceptive, self-replicating biologic destined for free-choice broadcasting and consumption. While the occurrence of immunocompromised hosts at risk for vaccine exposure may be remote, any self-replicating vector, even a highly attenuated virus, presents increased risks in such a host (Fenner et al. 1988, Hierholzer 1992).

The immunocompromised host scenerio has led to the development of functional animal models. Bosma and Carroll (1991) have identified a single gene mutation in mice that results in the inability to form functional B and T cells in homozygotes. Lacking the capacity for a specific immune response to pathogens and commensal organisms alike, severe combined immunodeficient (SCID) mice must be housed under aseptic conditions in a pathogen-free environment. An inheritable, functionally similar condition occurs in humans. Thus, the SCID model may be particularly useful in the elucidation of events during recombinant viral infection and may contribute toward the knowledge of the overall biosafety of these new biologics (Hanlon et al. 1997). Additionally, such studies may identify critical components of a prophylactic regimen, should adverse effects occur in an immunocompromised host. As a more sophisticated working knowledge of viral genetics is gained, genomic sequences crucial for replication in a particular host may be targeted and eliminated (Tartaglia et al. 1992a and b), increasing species specificity, as well as overall biological safety.

The synergism provided by vaccine vector, bait type, and distribution parameters (density, method, spatiotemporal factors) should ultimately maximize target species contact and minimize nontarget species uptake of a given biological. However, it is difficult to imagine total vaccine restriction to a single carnivore population even under ideal circumstances. Many bait studies have previously demonstrated an effect on species other than the target and implications for nontarget groups, such as domestic animals, humans and nonmammals, despite the original application and intention (Ballantyne and O'Donoghue 1954, Linhart 1964, Lewis 1968, Westergaard 1982, Bachmann et al. 1990, Fletcher et al. 1990, Trewhella et al. 1991). For example, a decade of applied research toward development of a prototype delivery device for oral raccoon rabies vaccination (Rupprecht et al. 1987) in the Eastern United States, resulted in a fishmeal–polymer bait that was readily consumed by a majority of raccoons under laboratory and field conditions (Rupprecht et al. 1992a). Yet variations in bait density (10–100/ha), distribution season, habitat type (barrier island to forested uplands), or method (hand delivery *v.* aerial), targeting ecotones suggestive of high raccoon activity, were unable to exclude consumption by other mammals (Hanlon et al. 1989 and 1993, Hable et al. 1992, Rupprecht et al. 1992a). Viewed as

Table 2. Biomarker[1] detection in nontarget species from fishmeal–polymer bait consumption: Virginia, Pennsylvania, and New Jersey (1990–93)

Species	No. positive/total	Percent
Opossum *(Didelphis virginianus)*	64/95	67
Striped skunk *(Mephitis mephitis)*	13/32	41
Domestic cat *(Felis domesticus)*	6/20	30
Red fox *(Vulpes vulpes)*	2/6	33
River otter *(Lutra canadensis)*	1/5	20
Porcupine *(Erythizon dorsatum)*	3/36	8
Black bear *(Ursus americanus)*	2/198	1
Norway rat *(Rattus norvegicus)*	1/8	13
House mouse *(Mus musculus)*	2/15	13
Rice rat *(Oryzomys palustris)*	2/7	29

[1] Tetracycline analysis from mandibular bone as described by Hanlon et al. (1989).

a composite (table 2), utilization in excess of 100,000 V–RG vaccine-laden baits specifically for raccoons nevertheless demonstrated contact by a variety of other carnivores and a few rodent species (albeit extremely small numbers). Overall biomarker data indicated bait consumption by a limited variety and number of nontarget species with no evidence of consumption by certain others (such as white-tailed deer during hunting season). These results could not have been predicted a priori, without placebo baiting and nontarget species surveillance. Observed nontarget species outcomes from vaccine exposure in the field have ranged from no apparent effect to immunization. However, what does the bait contact rate of a nontarget, competitor species (e.g., opossums) imply, especially if it approximates or exceeds that of the target species [raccoons]? From a disease control perspective, in which no untoward effects have been demonstrated in the nontarget species at issue, the answer may range from simple nuisance to a resultant economic infeasibility, depending upon the degree of interference and the number of vaccine-laden baits not available to the intended species. Clearly, what looks like a trifling matter—say, an overabundance of opossums vaccinated against a given infectious disease—may not be trivial in regard to immunocontraception, in light of species-specific vectors, antigens, baits, etc. This nontarget species contact problem may figure prominently if the species in question is a keystone species.

Long-term results of applying free-choice oral immunocontraception to free-ranging carnivores (and the associated nontarget milieu) are impossible to predict at present with any reasonable degree of certainty, as regards either safety or efficacy. For example, the efficacy of oral rabies vaccination among a target population may be assessed by (1) confinement studies with baits followed by challenges, (2) capture of free-ranging animals from a vaccinated area for subsequent laboratory challenge (Rupprecht et al. 1993), (3) measurement of seroconversion among free-ranging animals in an area, or simply (4) surveillance for naturally occurring disease. However, the minimal acceptable levels of these assessment techniques that would predict successful disease control or elimination are not known a priori, nor from present data, nor for a variety of complex ecological settings.

A proportion of the population may not consume baits due to a variety of factors. Some heritable behavioral traits, such as temerity in consumption or total avoidance of novel items, like artificial baits, may play a role in the inability to reach a segment of the targeted population. It follows that a particular cohort with a behavioral trait of bait avoidance may gain a competitive advantage. The result would be increasing difficulty in reaching this remaining, actively prepetuating segment of the population via baits. This scenario may be particularly troublesome given the high reproductive capacity of some species. Additionally, because no vaccine is completely efficacious in all individuals, it may be possible to selectively favor nonresponders (the perceived "mangey" or "wormy" individuals), due to major histocompatibility restriction, inherited immunodeficiencies, or immunocompromising infectious agents (Nossal 1989). If these latter theoretical demes gain even a minor reproductive advantage within a population, they may eventually initiate or exacerbate disease and related conditions. Amplification of this particular component of a carnivore population may severely restrict genetic diversity, as in present day cheetah (i.e., *Acinonyx)* (Cohn

1986), and subsequently compromise the overall health and viability of the community at large.

To be ultimately successful, an immunocontraceptive control program will intuitively require reaching a majority of individuals; how can the ideal mix of gene frequencies be ensured in light of this seeming conundrum? At first consideration, a readily transmissible, "safe" recombinant agent (while a bane to regulatory authorities) might appear to overcome the limitation of inequitable bait uptake and biological response. Exposure to a readily transmissible vector could approach unity, successfully reaching all members of a particular carnivore population. But, as evidenced by the global emergence and entrenchment of canine parvovirus within domestic and wild canid populations (Parrish 1994), this strategy may have significant uncontrollable and potentially detrimental effects. Even if a so-called species-specific antigen were discovered, geographic containment of such a highly contagious agent could not be assured. How would programs aimed, for example, at red foxes in the New World prevent exchange to red foxes in the Old World, involvement of related subspecies, or spillover to kin in the same genus, given the frequency of transoceanic travel and exotic and endemic species translocations (Rupprecht et al. 1996)? Similar questions could be raised for other taxa—canid, mustelid, viverrid, etc.

In conclusion, incipient investigations toward immunocontraceptive population management are quite intriguing. Their development for free-ranging carnivores appears well motivated and potentially desirable, at first glance, for numerous applications, given the limitations of available alternatives to reconcile the human–predator interface problem. Nonetheless, it will be crucial to proceed from the outset in as prudent a manner as possible, given the above-voiced concerns. It will be necessary to address, in comprehensive fashion, the potential for untoward events, and objectively divest real from perceived risks, much akin to the scientific scrutiny directed toward recombinant biologics more than a decade ago. In concordance with the recommendations of the World Health Organization (1993) in their Consultation on Reproductive Control of Carnivores, future directions of immunocontraceptive research should include continued efforts to develop species-specific bait delivery techniques, and species-specific contraceptive effects, either through antigen or vector. Given these goals, future research would logically involve international, multidisciplinary, collaborative efforts, strongly based upon objective, testable hypotheses. Until then, free-choice broadcasting of nonrestrictive contraceptive biologics may be unconscionable due to, as yet, unpredictable, undesirable, and potentially far-reaching repercussions, not only in the target species, but also in critical nontargets that share this increasingly burdened and now readily traversed globe.

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Immunocontraceptive Vaccines for Control of Fertility in the European Red Fox *(Vulpes vulpes)*

Mark P. Bradley

Abstract: This paper describes the strategies being employed in the development of an immunocontraceptive vaccine using sperm antigens, to control fox populations in Australia. It is proposed that such a vaccine will be delivered orally in a bait, thereby ultimately stimulating a mucosal immune response within the female reproductive tract. The eventual success in producing such a vaccine requires the identification of gamete antigens that cause immunological infertility, a detailed understanding of the reproductive immunology of foxes, and the selection of the most effective form of antigen delivery system.

Keywords: Sperm antigens, immunocontraception, mucosal immunity

Introduction

Immunocontraception potentially offers the most effective method for the management and long-term population control of vertebrate pest species. The idea of using fertility control for such purposes is not new. In the early 1960's, a number of investigators examined the use of chemical sterilants to limit the reproductive capacity of animal populations (Linhart 1964). None of these methods proved effective, probably because these chemicals lead to castration of the target species. Castration removes the source of the key sex hormones, and this effect has the potential to interfere with the normal social structure of a target population, an undesirable outcome that could lead to a breakdown in established social hierarchies and may result in compensatory breeding.

Immunocontraception for Feral Species

In 1992, the Cooperative Research Centre for the Biological Control of Vertebrate Pest Populations was established in Australia to explore alternative methods of fertility control based on the use of gamete antigens as immunogens. One of the species being targeted in this research is the European red fox *(Vulpes),* which is a major vertebrate pest in Australian responsible for the loss of many native species through predation.

Our approach to immunocontraceptive control of the fox involves developing a bait-delivered oral vaccine. In this paper, I will discuss the experimental approaches used in the development of such a vaccine for foxes. Specifically, the focus will be on the identification of sperm antigens as vaccine candidates, the immunological questions that need to be addressed, and the development of appropriate delivery systems.

Although these considerations are directed toward an application for the fox, many of the concepts are relevant for immunocontraception in other species. The wider concerns relating to species specificity, and the use of recombinant vaccines, will be covered in the paper by Tyndale–Biscoe in this proceedings.

Components of an Immunocontraceptive Vaccine for Feral Species

A successful contraceptive vaccine should (1) block fertilization or early embryonic development; (2) affect both sexes; (3) be species specific; (4) provoke a prolonged and sustained immune response; and (5) not interfere with the normal social function of the animal. In particular, an effective mechanism for transmitting the vaccine throughout the target population must be found. This mechanism must be cost effective to manufacture and administer and must not impose hazards to the environment. These caveats make the development of an immunocontraceptive vaccine for wild animals highly challenging.

Reproductive Studies

Sperm Antigens as Targets for Immunocontraception

Vaccines developed toward sperm antigens would probably be capable of inducing infertility in both males and females. Potentially, this characteristic has the advantages not only of rendering sperm within the male genital tract incapable of fertilization before entry into the female but also of inactivating sperm within

the female genital tract. The direct immunization of males and females with extracts of sperm or testis results in a significant inhibition of fertility (Menge and Naz 1988).

We have tested the antifertility effect of antibodies to sperm in a group of six female foxes (Bradley 1994). Necropsy results revealed that there were 21 ovulations in this group of foxes. In all females examined, no live fetuses were found; 13 of 21 oocytes had been fertilized and embryos implanted, but all failed as determined by the presence of embryonic resorption scars. Examination of uterine flushes failed to find any unfertilized or preimplantation embryos. It was concluded that immunization with sperm results in an immunological block to fertility and that sperm immunization has two effects, one at the embryonic level and the other during fertilization.

The results of this experiment are consistent with those previously reported on the effect of experimentally induced sperm antibodies on fertility (O'Rand 1977, Koyama et al. 1984). Many of these experiments found that anti-sperm antibodies appeared to exert their effect on fertility not at the level of fertilization but rather at later stages ranging from early blastocyst development to implantation (Menge and Naz 1988), suggesting that some antigens are shared between the sperm and the embryo. Immunization against such complex mixtures of antigens is not really a practical approach for vaccine development. Instead, the individual antigenic components capable of causing immunological contraception need to be identified. Indeed, a number of specific sperm proteins can impair fertility when used to immunize animals (Naz 1987, LeVan and Goldberg 1990).

The most common approach to sperm antigen identification and selection is the use of monoclonal antibodies to sperm protein of the species under study (Anderson et al. 1987). One of the best examples is the PH-20 protein originally identified with monoclonal antibodies to guinea pig sperm. Fertility trials have shown that male and female guinea pigs immunized with PH-20 become infertile (Primakoff et al. 1988). More recently, the PH-20 genes from a number of other species have been cloned, leading to the possibility that this antigen may have applicability as a vaccine target in other species.

Another sperm antigen of interest is SP-10. This has been designated as a "primary vaccine candidate" by the World Health Organization Task Force on Contraceptive Vaccines (Herr et al. 1990a and b). Antibodies to SP-10 inhibit the penetration of hamster eggs by human sperm, and trials in baboons currently in progress will assess the applicability of this antigen as an immunocontraceptive for humans. Recently, a homologue of SP-10 (called MSA-63) has been identified in the mouse and cloned (Liu et al. 1990). Furthermore, antibodies to MSA-63 have been shown to have a strong inhibitory effect on the in vitro fertilization of mouse ova, providing good support that this class of antigens is worthy of study as potential targets for immunocontraception.

Fox Sperm Antigens Currently Being Assessed for Use in an Immunocontraceptive Vaccine

A number of monoclonal antibodies have been developed to fox sperm antigens and used to clone the cognate genes from a fox testis cDNA library. One of these candidate antigens, FSA-1r, has been through fertility trial testing and found to have no effect on fertility. Other antigens are currently in the fertility trial phase of assessment.

Fox Acr.1 (Acrosomal Protein 1)

The FSA-Acr.1 protein is located within the acrosomal matrix of fox sperm, and it is first detected during spermatogenesis on the developing acrosome of round and elongating spermatids (Beaton et al. 1995). A monoclonal antibody to FSA-Acr.1 (FSA-10) was used to screen a fox testis cDNA library, and a cDNA clone was isolated. Database searches with the deduced amino acid sequence of FSA-Acr.1 revealed that the clone has high homology to both human and baboon sperm protein SP-10 and the mouse sperm protein, MSA-63. The region of highest homology is within the carboxyl terminus. Within the central portion of the open reading frame, the fox sequence

contains amino acid motifs that are absent from both the human and baboon SP-10 and mouse MSA-63 sequences. We have expressed the FSA-Acr.1 protein in vitro, and this protein is being assessed in fertility trials to determine whether or not antibodies to FSA-Acr.1 can impair fertility.

Fox LDH-C_4

Lactate dehydrogenase C_4 (LDH-C_4) is an intracellular sperm-specific enzyme a portion of which is located on the sperm flagella plasma membrane. A number of studies have previously demonstrated that, when purified LDH-C_4 is used to immunize either mice, rabbits, or baboons, fertility was reduced by 60 to 80 percent (LeVan and Goldberg 1990). Epitope mapping studies of mouse and human LDH-C_4 have identified an antigenic peptide within the N-terminal region of the open reading frame, from amino acids 5–19 (Millan et al. 1987). This sequence also has the greatest variation in sequence between different species. We have recently cloned a fox LDH-C_4 cDNA and have derived sequence information from the 5' region of the open reading frame. This research has enabled us to synthesize a peptide to this region that was subsequently conjugated to the tetanus toxoid protein as an immunogenic carrier protein. This peptide-protein conjugate has been used to immunize female foxes by the intra-Peyers' patch route, and the immune responses and the effects of this immunization on fox fertility are currently being measured.

Fox PH-20

Cloning of the guinea pig PH-20 revealed that it has homology at the protein level with bee venom hylauronidase (Gmachl and Kreil 1993). Hylauronidase enzymatic activity is present within the head of mammalian sperm, and it has been shown that PH-20 has hylauronidase enzymatic activity (Gmachl et al. 1993). We have isolated a cDNA from a fox testis cDNA library encoding PH-20, and partial sequence analysis indicates close homology to PH-20 antigens cloned from other species. The antifertility effects in foxes of PH-20 immunization will be assessed with the whole protein and with selected peptide sequences.

Production of Recombinant Antigens

The in vivo testing of candidate antigens requires the large-scale production of recombinant proteins. A number of commercially available protein expression systems exist for this purpose; however, the selection of the system for a particular purpose eventually depends on the properties of the antigen under study. For example, is the protein highly glycosylated, and how important is this for antigenicity? Or, is the protein composed of multiple subunits? These considerations all have bearing on the success and selection of any one expression system. We have extensively used the maltose binding protein (MBP) expression system for the production of recombinant sperm proteins. This system produces a fusion of MBP to the protein of interest. Following expression, the fusion product is purified by affinity chromatography to yield a hybrid protein. The MBP can be cleaved and purified from the target protein, but this has some drawbacks in that small amounts of MBP still contaminate the antigen preparations, and loss of antigen occurs at each purification step. Unfortunately, the selection of expression systems for production of recombinant proteins is still something of a trial-and-error procedure, and several systems may have to be evaluated before selection of one that suits the purpose of the antigen under study.

In Vivo Fertility Testing of Recombinant Sperm Antigens

Ideally, during the selection of monoclonal antibodies that identify candidate sperm vaccine antigens, part of the testing procedure should include the use of functional assay to determine the effect of these antibodies on either sperm–egg binding or fertilization in vitro. While such assays are readily available for some species, researchers are limited in the fox in the routine use of such assays by both the biology of foxes and the paucity of an in vitro fertilization assay system. Because the fox is a seasonal breeder, the availability of gametes for such studies is restricted to 2 months every year, a fact that severely restricts the opportunity for assays. There is one reported study of attempts to establish an in vitro fertilization system for foxes. But, unfortunately, this procedure is still in the

early experimental stages, and its routine application is not feasible at present (Farstad et al. 1993).

An alternative is to test the antifertility effects of candidate antigen(s) in vivo in female foxes. This approach is both time consuming and expensive, but it is highly informative. For example, we have routinely performed necropsies on the immunized foxes at about 40 days after mating to evaluate the biological implications of the immunization regime(s) on fox reproduction. At the same time, samples of reproductive-tract fluids are collected for assays of the immunoglobulin levels within each section of the tract. This approach provides information on both the immunology of the treatment and the in vivo effects on fertility.

Do We Use Proteins or Peptides as Vaccine Antigens?

Ultimately, the choice will have to be made as to whether a recombinant vaccine for immunocontraception is developed which contains the full target protein or an antigenic peptide. Decisions will have to be based on the considerations of the species specificity of the antigen and the ability of any selected peptide epitope to produce a sufficient immune response to block fertility. The success of ongoing research on the selection, design, and construction of peptide antigens will be vital for the future development of peptide-based vaccines.

Immune Responses to Gamete Antigens

Mucosal Immune Response in the Female Reproductive Tract

The use of a bait-delivered oral immunocontraceptive for foxes requires a detailed understanding of the processes involved in the induction, modulation, and duration of genital tract mucosal immunity. The immune responses within the female genital tract are similar to that observed at other mucosal sites. Vaginal and cervical secretions contain high levels of immunoglobulin A (IgA) that appear to be locally synthesized. It has previously been shown that the reproductive mucosal site is linked to the common mucosal system and that IgA plasma cells stimulated at a distant site, such as the gastrointestinal tract, can rapidly migrate to the female reproductive tract (McDermott et al. 1980, Parr and Parr 1989 and 1990).

Using a model recombinant-derived antigen, maltose binding protein (MBP), we have investigated different immunization regimes to determine which route induces reproductive-tract mucosal immune responses within female foxes. Direct administration of antigen into the Peyers' patches (IPP) has been used because this route effectively mimics the oral presentation of an antigen to the gut associated lymphoid tissue (Dunkley and Husband 1990). Peyers' patch immunization induces a mucosal immune response within the female reproductive tract, but in the absence of a booster immunization, the antibody responses are transitory, particularly for IgA (fig. 1). This fact indicates that maintenance of vaginal IgA antibodies may require the antigen to persist.

To examine this, an experiment was conducted to test the effect of secondary immunizations with MBP on the maintenance of vaginal antibody levels. Three female foxes were immunized IPP and then boosted intradermally (ID) 1 and 2 months later. The results show that the levels of both serum and vaginal antibodies were maintained at higher levels and for longer compared with foxes receiving a single Peyers' patch immunization (fig. 2). In light of this information, female foxes in fertility trials are now routinely immunized IPP followed by a secondary immunization ID, thus ensuring that high antibodies levels are present within the reproductive tract throughout the period of the fertility trial.

Immune Responses and the Endocrine System

A number of studies have demonstrated that the sex hormones estrogen and progesterone can influence immunoglobulin G (IgG) and IgA antibody production within the female reproductive tract (McDermott et al. 1980). Any immunocontraceptive vaccine strategy will have to address the effect that these localized

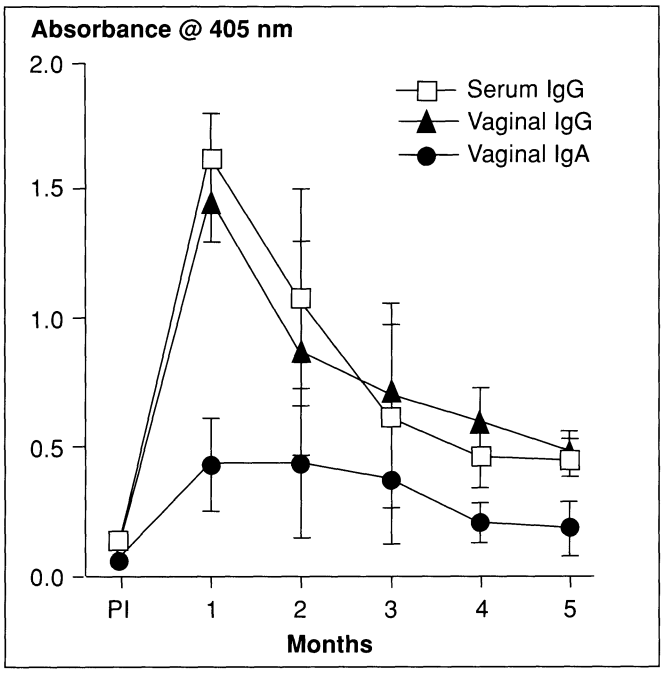

Figure 1. Serum and vaginal fluid antibody responses (Mean ± SD; $N = 3$) in female foxes as determined by enzyme-linked immunosorbent assay (ELISA). Each fox was immunized once with MBP into four Peyer's patches.

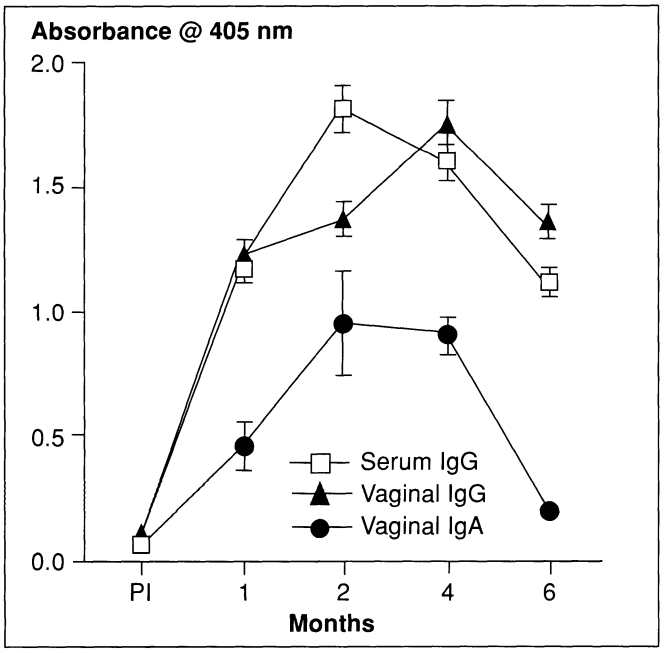

Figure 2. Serum and vaginal fluid antibody responses (Mean ± SD; $N = 3$) in female foxes as determined by ELISA after immunization with MBP into four Peyer's patches, followed by intradermal booster immunizations 1 and 2 months later.

changes in the immune status of females may have on immunocontraception. Effective immunocontraception will depend on the maintenance of high levels of antisperm antibodies within the oviducts, uterus, and vagina during mating. If changes in the localized antibody concentrations are substantial during this critical period, then contraception may be compromised (Wira and Sandoe 1987). Studies are under way in the fox to determine if localized changes are seen in the reproductive tract IgG and IgA during estrus.

Long-Term Maintenance of an Immune Response

A problem that may need to be addressed in the administration of an immunocontraceptive vaccine to an outbred fox population is the variability of the immune response between individuals. Effective application of a vaccine for fertility control requires that a high level of immunity be achieved among individuals exposed to the vaccine. It may, therefore, be necessary to include multiple antigenic determinants within a vaccine to stimulate a broad range of immune responses. In addition, the antigen(s) may need to be presented in conjunction with other highly immunogenic carrier proteins to maintain a contraceptive level of immunity.

Antigen Delivery Systems for a Fox Immunocontraceptive Vaccine

At present, three different vaccine delivery systems are being assessed to determine which will be the most effective for inclusion into the bait: (1) recombinant derived gamete antigen(s) encapsulated within microspheres, (2) a recombinant vaccinia virus capable of expressing foreign antigen(s) in the infected host, and (3) selected recombinant bacterial vectors such as the attenuated aroA strains of *Salmonella typhimurium.*

Microencapsulated Antigens

The effective oral presentation of antigens to the lower gastrointestinal tract is hampered by the degradation of protein within the stomach. A convenient way to overcome this is to use biodegradable microspheres that contain the entrapped antigen. These could be ultimately packaged within a bait, providing an effective oral delivery system whereby the vaccine antigen is delivered directly to the gut. The microspheres are taken up by the mucosae with the subsequent induction of a mucosal immune response (McGhee et al. 1992). In recent years, a number of investigators have reported the successful application of this technique for the delivery of antigens and the subsequent generation of mucosal immunity to the encapsulated antigens (Mestecky and Eldridge 1991).

We have recently completed a study to evaluate the efficacy of microspheres containing a recombinant sperm antigen to stimulate a mucosal immune response in rats (Muir et al. 1994). Microspheres were synthesized using the poly-DL-lactide-co-glycolide copolymer incorporating a recombinant source of the fox sperm protein FSA-1r (Bradley 1994). The oral administration of FSA-1r-loaded microspheres to rats resulted in a significant production of cells within the jejunum that were secreting IgA antibodies specific for the FSA-1r antigen. The level of stimulation was comparable to that obtained by either direct immunization of the Peyers' patches with microspheres containing antigen or unencapsulated antigen. These preliminary results indicate that further experiments would be worth pursuing to assess the utility of this approach for antigen delivery to foxes.

Viral Vectors

The use of recombinant vaccinia viral vectors containing the genes encoding selected sperm antigens may offer an excellent delivery system for an immunocontraceptive vaccine. For example, effective vaccination of foxes with a recombinant vaccinia expressing the rabies glycoprotein gene has proved enormously effective for immunizing foxes against rabies (Brochier et al. 1990). Building on these experiences, we are developing vaccinia vectors for application in immunocontraception. Such a system would allow further studies on the enhancement of the mucosal immunity, possibly by constructing vectors that coexpress IgA-specific stimulating cytokines (Ramsay et al. 1994).

A preliminary assessment of the immunological responses in foxes to the oral administration of a recombinant vaccinia viral vector expressing the hemagglutinin antigen (HA) has begun, and the results of these experiments will provide a basis for further experiments designed to test the utility of using a recombinant poxviruses for the delivery of immunocontraceptive antigens.

Bacterial Vectors

Recombinant bacterial vectors are an alternative delivery vehicle for a variety of vaccine antigens (Schodel 1992). The use of attenuated strains of *Salmonella typhimurium* as a live vector would be particularly applicable for stimulating reproductive tract mucosal immunity because *Salmonella* sp. colonize the intestinal tract and proliferate in the gut associated lymphoid tissue (Curtiss et al. 1989).

The use of selected mutant strains of *Salmonella* sp. has the advantage that they can be made avirulent without decreasing immunogenicity and are not infective outside the host. These considerations are important for any recombinant vaccine being considered for environmental release. However, a potential drawback is that immunity toward the carrier organism could develop, making subsequent exposure to the vaccine ineffective.

We have conducted an experiment to assess whether the attenuated *Salmonella typhimurium* aroA mutant strain is capable of stimulating mucosal immune responses in foxes after oral administration. Such information is a prerequisite to any future use of this organism as a vaccine vector. We have found that foxes given oral doses of *Salmonella typhimurium* readily produce serum immunoglobulin M (IgM) and IgG, and vaginal IgG and IgA antibodies to *Salmonella typhimurium* lipopolysaccharide over a 6-week period. The results indicate that foxes can respond immunologically to a single oral dose of *Salmonella*

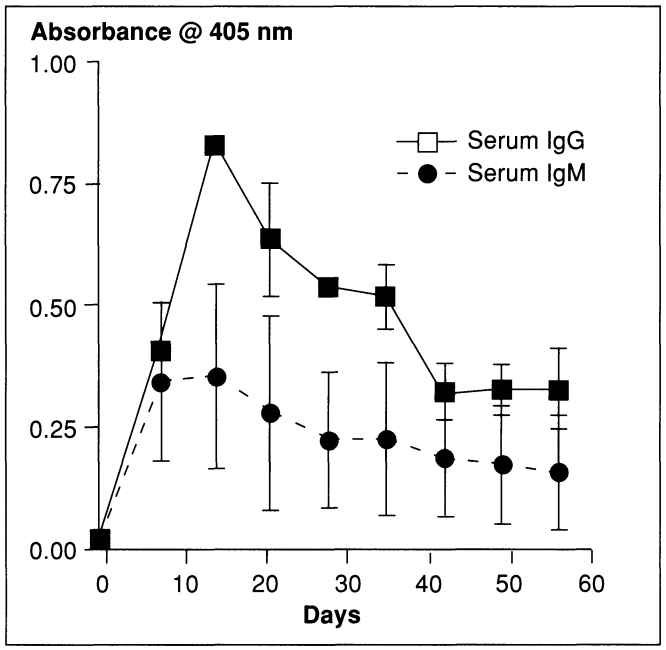

Figure 3. Serum IgG and IgM antibody responses (Mean ± SD; $N = 2$) in female foxes to lipopolysaccharide antigen after one oral dose of 5×10^9 *Salmonella typhimurium* (aroA mutant strain SL3261).

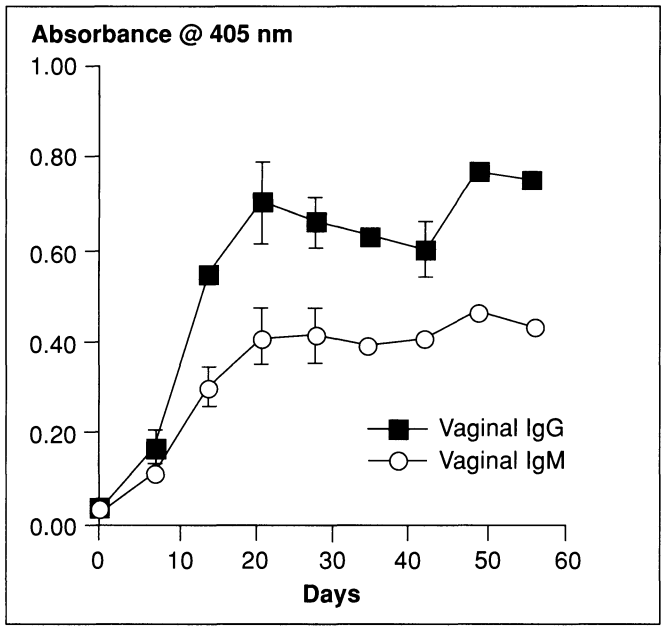

Figure 4. Vaginal IgG and IgA antibody responses (Mean ± SD; $N = 3$) in female foxes to lipopolysaccharide antigen after one oral dose of 5×10^9 *Salmonella typhimurium* (aroA mutant strain SL3261).

typhimurium that is sufficient to produce a high and sustained level of immunity within the female reproductive tract, albeit to a highly immunogenic antigen (figs. 3 and 4). Experiments are now in progress to construct a recombinant *Salmonella typhimurium* capable of expressing selected sperm antigens. Such recombinants will be screened for their ability to induce specific reproductive tract immune responses to the foreign antigen.

Concluding Remarks

Fertility control holds exciting prospects for the future management of wildlife populations. Internationally, a growing number of scientists and wildlife managers regard this approach as the only acceptable future method of managing wildlife populations. However, the obstacles that will be encountered in the development and implementation of such a technology are substantial. If the effort is successful, the rewards will be substantial, too. Each species will yield its own set of unique challenges.

In this overview, I have summarized key aspects relating to the reproductive and immunological studies that are required in the process of developing an immunocontraceptive vaccine for a vertebrate pest species. I have attempted to address, albeit in a rather brief way, the major considerations that need to be taken in to account when contemplating the development of a fertility control vaccine for wildlife. I have not considered the wider ecological implications of fertility control being imposed on a wildlife population. Such studies pose a whole new set of questions and challenges, and any project concerned with fertility control of wildlife will require a large, integral ecology research program to match the other facets of the work. Eventually, it will be the ecological studies that will assess both the impact and long-term consequences of fertility control on a particular wildlife population.

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Potential Use of Contraception for Managing Wildlife Pests in Australia

By Mary Bomford and Peter O'Brien

Abstract: There is an increasing level of interest in contraception to manage wildlife pests in Australia, due mainly to concerns over high recurrent costs, animal welfare, and the failure of current control techniques to prevent damage in some instances. We have developed criteria that need to be met for contraception to be successful for pest control:

- Technology exists to reduce fertility.
- An effective delivery mechanism to treat wild animals exists.
- The end result of reduced animal damage is achieved.
- Effects are humane and nontoxic.
- Product is target specific, cost effective, and environmentally acceptable.

We assessed all available and proposed contraceptive techniques against these criteria to see if any were suitable or promising for use on Australian pests. The present role of contraception in Australia is extremely limited. The main barrier for widespread and abundant pests is the lack of suitable delivery techniques that are cost effective. The probable impact of contraception on wild populations is also poorly understood. High rates of infertility may be necessary to control pest populations and the damage they cause. Even if the fertility of wild pest populations can be reduced, there is no guarantee that this will be as effective as lethal techniques for reducing pest numbers. The longer term potential of contraception in managing wildlife damage will depend on the outcome of future research and development, particularly in the fields of contraceptive delivery and the effects of fertility control on population dynamics.

Introduction

The Australian government is interested in contraception to manage wildlife pests because of concerns over high recurrent costs of lethal controls, and their failure to prevent damage in some instances (Senate Select Committee on Animal Welfare 1991, Wilson et al. 1992). Also, many people are concerned about animal welfare issues associated with lethal techniques used to control vertebrate pests in Australia, particularly the shooting of kangaroos and feral horses. Wildlife contraception is often perceived as a more humane alternative. As a constructive response to this concern, we evaluated the scientific literature on the use of fertility control for wildlife management to assess the potential value of fertility control for wildlife management in Australia (Bomford 1990 and 1990 unpubl., Bomford and O'Brien 1990 unpubl. and 1992). This paper, which summarizes and updates the findings of these studies,

- describes the impacts of pest animals,
- identifies the objectives of wildlife contraception,
- identifies criteria for its successful use,
- evaluates its potential application in Australia, and
- identifies promising research directions.

Impacts of Pest Animals

Australia's main introduced vertebrates that have established wild pest populations are European rabbits *(Oryctolagus cuniculus),* European red foxes *(Vulpes vulpes),* horses *(Equus caballus),* cats *(Felis catus),* dogs and dingoes *(Canis familiaris),* goats *(Capra hircus),* pigs *(Sus scrofa),* buffalo *(Bubalus bubalis),* donkeys *(Equus asinus,)* house mice *(Mus domesticus),* and European starlings *(Sturnus vulgaris).* All these species are widespread and abundant, and many are perceived to cause losses to conservation values and agricultural production over much of their range, which makes their control expensive (Wilson et al. 1992).

Rabbits, Australia's most significant vertebrate pest, have been estimated to cost $50 million (U.S.) a year in lost agricultural production (Flavel 1988). This figure does not include the damage rabbits inflict by competing with our native animals and destroying their habitat, preventing tree regeneration, and contributing to soil erosion (Williams et al. 1995).

Foxes are major predators of wildlife (Kinnear et al. 1988, Saunders et al. 1995). Their distribution corresponds to areas where there have been many extinctions of small and medium-sized native mammals and where many more species are endangered (Wilson et al. 1992). Foxes also prey on lambs (Saunders et al. 1995), and there is a small risk that

foxes could become a rabies vector should this disease be introduced to Australia (Forman 1993).

Feral horses are believed to compete with native species and livestock for pasture and water and cause soil erosion (Dobbie et al. 1993). There are estimated to be more than 300,000 feral horses in Australia, about four times the number in the United States (McKnight 1976, Clemente et al. 1990). They often inhabit remote regions, where they build up to high numbers during good years, and many starve during drought (Wilson et al. 1992).

Some native species are also a problem. For example, native parrots damage cereal and fruit crops (Bomford 1992). The large red and grey kangaroos *(Macropus rufus, M. giganteus,* and *M. fuliginosus)* have increased in range and abundance since European settlement due to the provision of livestock watering sites and extension of grasslands (Robertson et al. 1987). They compete with livestock for pasture and also reach extremely high densities in some national parks, sometimes threatening the survival of native plant communities in these reserves (Caughley 1987, Shepherd and Caughley 1987).

Wildlife managers currently control pests by poisoning, shooting, and habitat manipulation, with trapping, biological control, and exclusion being used to a lesser extent (Wilson et al. 1992). These are currently the only cost-effective means known for wildlife damage control.

Objectives of Fertility Control

The objective of fertility control for wildlife management may be one or more of the following:

- Reduce control costs,
- Achieve more humane control,
- Minimize impact on nontarget species,
- Reduce population growth, and/or
- Reduce animal damage.

When native species are a pest, the control technique used to reduce damage must not put the survival of the species at risk.

Criteria for Successful Use

We believe the following set of seven criteria need to be met for successful wildlife contraception. We examine currently available and proposed fertility control techniques to see how well they meet these criteria.

Criterion 1: Available Drug or Technique To Reduce Fertility

Many chemicals and techniques are known to cause infertility in captive animals (Kirkpatrick and Turner 1985, Marsh 1988, Kirkpatrick et al. 1990 and 1992, Bomford 1990). Much of this knowledge has been acquired from the huge investment in human contraceptive research. The use of contraception for wildlife management is not restricted by a lack of suitable techniques or drugs. So the availability of suitable agents for causing infertility in wildlife is unlikely to be a barrier for pest management.

Criterion 2: Effective Delivery Mechanism To Treat Wild Animals

The lack of practical techniques to deliver drugs to wild populations is a major obstacle to using contraception for controlling wildlife pests. Many tests on captive animals have relied on drugs delivered by surgical implantation, injections, biobullets, or by frequent oral dosing (Noden et al. 1974, Marsh 1988, Plotka and Seal 1989, Plotka et al. 1992). Such delivery techniques are either technically impossible or prohibitively expensive for reducing the damage caused by widespread and abundant wildlife, such as the estimated 200 million to 300 million wild rabbits that cause damage over much of Australia's rangelands (Flavel 1988, Wilson et al. 1992, O'Neill 1994, Williams et al. 1995). No remotely deliverable contraceptive agents cause infertility for more than 1 year, so delivery has to be repeated at least on an annual basis. Many orally active synthetic drugs require frequent ingestion, or delivery has to be precisely timed in relation to the breeding cycle, which may vary with environmental conditions. The suitable period may be as short as 2 weeks for some birds (Lacombe et al. 1986). It is extremely doubtful that these limita-

tions to chemical fertility control could be overcome for effective pest management in Australia.

Development of a single-dose, long-acting or permanent contraceptive would reduce the difficulty and cost of delivery using the techniques described above (Marsh 1988, Berman and Dobbie 1990 unpubl.). The use of a live disseminating-recombinant virus for delivery, which is species specific to the target pest, could further reduce the technical difficulty and expense for some pests (Tyndale–Biscoe 1991 and 1994). But this technology is still under development and even if it is successful, it is unlikely to be available for another decade.

Criterion 3: End Result Is Reduced Animal Damage.

The focus of research on wildlife contraception has been on reducing fertility of pest animals. Doing that is not enough. The goal must be to reduce pest numbers and so reduce damage caused by the pest (Braysher 1993). We found no field studies that demonstrated such effects. Without field studies to examine, we turned to population theory to see what could be expected.

Australia has a highly variable rainfall. Many pest animals build up to high numbers in good seasons when food is abundant and then have their most severe impacts during droughts (Morton 1990, Dobbie et al. 1993, Williams et al. 1995). At these times, they compete with stock and native species for food and water, prey on native species in refuge habitats, and overgraze the land, causing erosion and killing tree seedlings. Many pests, such as feral horses, kangaroos, and rabbits, naturally stop breeding during droughts (Shepherd 1987, Wilson et al. 1992, Williams et al. 1995), so fertility control is not a useful population control tool at such times.

The theoretical effects of killing or sterilizing animals were compared to assess the potential value of contraception as an alternative to lethal controls (Bomford 1990, Bomford and O'Brien 1990 unpubl.). Expanding populations which were unlimited by resources were examined first (fig. 1). In such populations, it is usual for most healthy adults to breed, for juvenile survival to be high, and for the population to have exponential growth.

If half the adult population is killed (fig. 1A), exponential growth resumes, and the population soon recovers to its original density. If half the adult population is sterilized (fig. 1B), using a technique that causes loss of fertility without altering behavior,

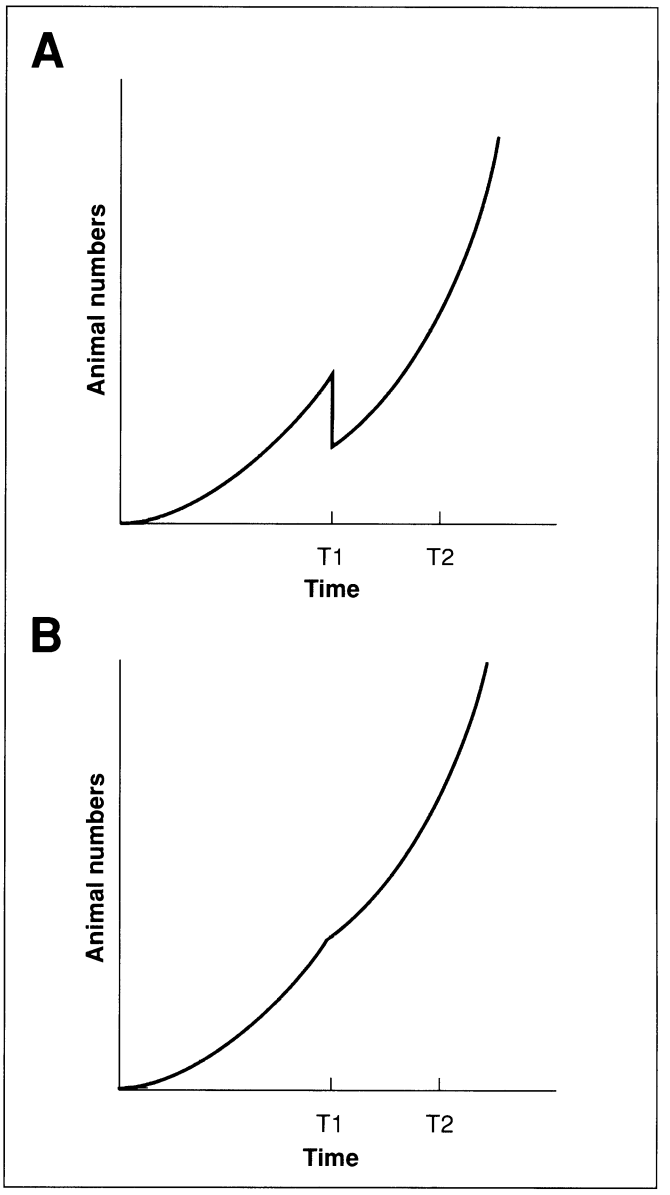

Figure 1. Exponential density-independent population growth. (A) Half population killed at time T1. (B) Half population sterilized at time T1. Killing is more effective for reducing population size.

population growth continues but at a slower rate than would have occurred in the absence of sterilization. Hence, for growing populations, killing or culling is more effective for reducing population growth rates than sterilizing an equivalent number of individuals. This conclusion was also reached by Garrott (1991) through mathematical modeling of the response of feral horse herds to changes in survival or fecundity.

If repeat treatments are used to kill or sterilize new animals over time, as opposed to the single treatment illustrated in figure 1, or if a higher proportion of animals is treated, population growth rates will flatten for both killing and sterilizing treatments, especially at low densities. But the same principle applies, and killing acts to double advantage: not only are dead animals removed from the population, they also do not breed. So by simple arithmetic, it is clear that killing will reduce the population more than contraception if the same number of animals are treated.

We concluded from this that sterilization is likely to be most effective to slow the rate of recovery of a population after some other factor, such as poisoning, shooting, drought, or disease, has reduced numbers to low levels. Hone (1992) also reached this conclusion from his mathematical modeling of population responses to contraception. Killing equal numbers of animals will be more effective than contraception for growing populations, irrespective of the proportion of the population treated.

Stable populations with density-dependent regulation at environmental carrying capacity, limited by available resources, such as space, food, or nest sites, were also examined (fig. 2). In such populations, dominance or territorial behavior often prevents some healthy adults from breeding or causes them to breed in suboptimal habitat or under social conditions where success is low. Juvenile survival is usually poor.

If half the adult population is killed (fig. 2A), logistic growth occurs and the population recovers rapidly. If half the adult population is sterilized (fig. 2B), several different responses in the population are possible, depending on the nature of the density-

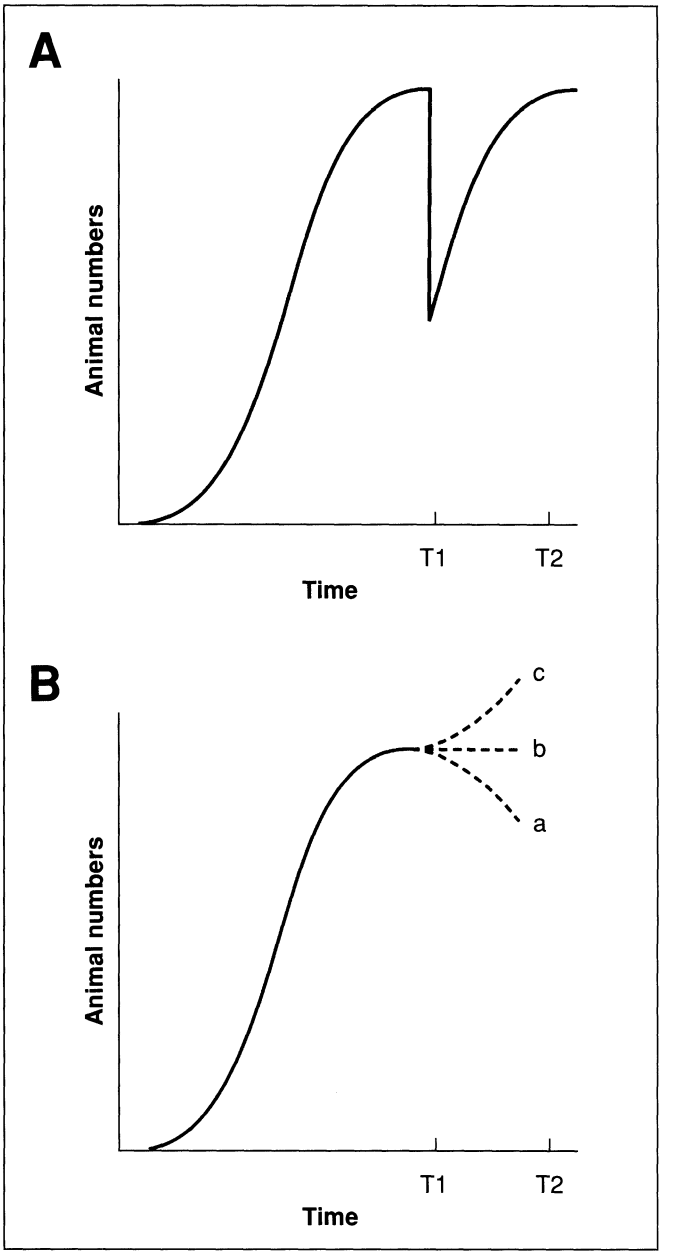

Figure 2. Logistic density-dependent population growth. (A) Half population killed at time T1. (B) Half population sterilized at time T1. In the short term, killing is more effective for reducing population size. In the longer term, the relative advantages of killing or sterilizing depend on the population response to the contraceptive treatment (B—lines a, b, and c), particularly in relation to the duration of sterilization, behavioral changes in treated animals, and compensatory changes in reproductive success of untreated animals and in survival.

dependent regulation and the response of the population to the treatment. A decline in population density (fig. 2B.a) is the response most people expect. Such declines may well occur in certain circumstances, but in some instances contraception may not cause a decline (fig. 2B.b), or it may even destabilize social behavior and lead to a population increase (fig. 2B.c).

Compensatory responses can prevent population declines, even if the contraceptive treatment does not interfere with sexual or social behavior. Compensatory responses may include increased survival, increased birth rates in untreated fertile individuals, increased immigration, and reduced dispersal. For example, many populations have high juvenile mortality. Sterilization may simply prevent the birth of young that would otherwise die or disperse without breeding. Even quite high reductions in fertility will not reduce population density if birth rates are still sufficiently high to allow normal numbers of young animals to join the adult population.

The extent of compensation determines whether fertility control will work and how well it will work. Unfortunately, we know little about the extent to which compensatory factors operate in pest populations following contraceptive treatment. We found many of the published models on the effects of contraception on population dynamics took inadequate account of such compensatory factors (Sturtevant 1970, Knipling and McGuire 1972, Spurr 1981, Bomford 1990, Bomford and O'Brien 1990 unpubl.).

Compensatory responses, such as increased breeding, survival, or immigration, can also be expected following population control by killing and can lead to rapid recovery of culled populations. Annual rates of increase in culled populations have been estimated at 20 percent for feral horses (Eberhardt et al. 1982), 23 percent for feral donkeys (Choquenot 1990), and 75 percent for feral goats (R. Henzell, pers. comm.). We could find no research that compared the extent of compensatory responses following killing or contraception in wildlife populations. Stenseth (1981), however, modeled pest control processes, including parameters for natality, mortality, dispersal, and immigration rates, all of which allowed for the effects of compensation. He found the higher the age-specific mortality rate (population turnover rate) of an uncontrolled population, the more likely it is that reduction in reproduction will be the optimal pest control strategy (as opposed to increased mortality or decreased immigration). If the equilibrium density of the population is low, the optimal pest control strategy will most often be to increase mortality rates as much as possible, especially if the mortality rate is naturally low. If, however, a pest species is long lived, and a contraceptive that lasts several years following a single treatment is used, the proportion of sterile individuals in the population may increase with successive treatments. In such circumstances, sterilizing animals may be more effective for reducing population growth rates than killing equal numbers.

When drugs used to sterilize animals cause a change in social behavior and territorial behavior or dominance is lost, a population could increase. This has been demonstrated in a model published by Caughley et al. (1992) showing that random contraception of a proportion of the females in a population could lead to increased production of young if the contraceptive treatment overrode suppression of breeding exerted by dominant females over subordinate females within social groups. The occurrence of this response would depend on social group and litter sizes, and in most circumstances the model of Caughley et al. (1992) indicated that contraception would reduce breeding.

A field study conducted on sheep on Soay Island showed that if contraception alters social behavior it may be counterproductive in terms of damage control (Jewel 1986). Male lambs were castrated in feral sheep flocks which had density-dependent regulation of numbers through food supply. After 4 years, 61 percent of castrated males had survived, in contrast to only 6 percent of untreated males. Sterilized males also spent more than twice as much time feeding as fertile males. Hence, in this study, sterilizing part of the population increased survival and may have increased food consumption. This important finding illustrates the need for contraceptive approaches that do not cause undesirable changes to endocrine function and behavior.

If damage mitigation rather than lower reproductive success is the objective, fertility control may not be an advantage. It may even be counterproductive, if it allows large numbers of nonbreeding individuals to remain in a population. So we concluded that scientists need to greatly improve understanding of the factors regulating populations of pest species and how these are affected by fertility control. Without precise information on these relationships, scientists cannot predict whether contraception will be an effective tool for controlling wildlife damage. More sophisticated models, based on good field data, are needed. In particular, investigators need a better understanding of the proportion of animals in pest populations that need to be rendered infertile to bring densities down to levels where damage is controlled.

Criterion 4: Humane and Nontoxic Effects

Fertility control drugs can affect animal health. Some have unpleasant side effects, and some are toxic or carcinogenic (Lofts et al. 1968, Cummins and Wodzicki 1980, Johnson and Tait 1983). But in general, this is an area where fertility control performs well relative to lethal control techniques.

Criterion 5: Target Specificity

Unfortunately, few fertility-control drugs are species specific, so nontarget wildlife, domestic species, or people could be affected. This is, of course, also true for many lethal control techniques (McIlroy 1986). The doses of chemosterilants necessary to cause infertility in target pests may be toxic or lethal for other species (Ericsson 1982, Johnson and Tait 1983, Saini and Parshad 1988). Immunological fertility-control agents spread by genetically engineered organisms could be made target specific for some species. But this may be a problem for feral pests with domestic counterparts, or those closely related to protected native species. There is also a risk that modified viruses could mutate to infect species other than their original hosts (Tiedje et al. 1989). But mutation would not cause the new hosts to become infertile if the virus were engineered to affect genes or proteins present in the target species only.

Criterion 6: Environmental Acceptability

In contrast to many vertebrate poisons, most fertility-control drugs do not leave residues that are harmful to the environment, though some chemosterilants could be unsuitable for use in food crops (Marsh and Howard 1973).

Criterion 7: Cost Effectiveness

Pest-control benefits must exceed costs. Preferably, the technique chosen and level of application should maximize the benefit–cost ratio. In calculating the relative costs and benefits of alternative techniques, assessments of the value of moral and animal welfare issues need to be considered. Some benefits may be difficult to quantify, such as the benefits of protecting endangered native species. Cost effective damage control occurs when the cost of pest control is more than met by savings in protecting all values society wants (Braysher 1993).

Cost is a major obstacle in the employment of fertility control as a wildlife management technique using current technology. Although the technology for fertility control of individuals does exist, contraceptive chemicals and their delivery can be prohibitively expensive for widespread and abundant pests (Matschke 1980, Berman and Dobbie 1990 unpubl.). Most of the more expensive techniques for fertility control, such as those requiring surgery, implants, or frequent or continuous dosing over extended periods, are likely to be cost effective for only small numbers of valuable animals, such as those in exhibition parks or small private collections. In contrast, lethal control techniques are often cost effective for pests such as rabbits, feral horses, and foxes (Dobbie et al. 1993, Bomford and O'Brien 1995, Williams et al. 1995).

Application to Australian Pests

Control on a National Scale

For widespread and abundant pests, such as rabbits, rodents, foxes, and feral cats, horses, and pigs, no currently available contraceptive technique can provide cost-effective damage control. Only research

into contraceptives disseminated passively by live organisms has promise for wide-scale control of such pests in the future. Research is currently being conducted in Australia on viral-vectored immunocontraception for the control of rabbits and is planned for wild house mice. Viral-vectored immunocontraception has the potential to bring great benefits to wildlife pest management in Australia and its development is a current research priority. There are, however, many technical hurdles to be overcome, and it is too early to predict whether the research will be successful. In addition, there are social considerations that may impede the development and use of viral-vectored immunocontraceptives. For example, there is a risk that a live immunocontraceptive virus for rabbits could be accidentally transported to other countries where lagomorphs are not pests. It is also probable that some sections of the community would oppose the release of an immunocontraceptive virus due to perceptions of risk to nontarget species.

Control on a Local Scale

Contraception could also be used in Australia for localized control of relatively small numbers of pest animals. Contraceptives delivered through baits, implants, or injections might be used to reduce the damage caused by small numbers of pest animals such as kangaroos, feral horses, or foxes in a localized area. An example might be to use contraceptives to reduce pest numbers to protect endangered flora or fauna in a reserve. The technology is certainly achievable. Delivery would be a major expense, but in an intensively managed area, where shooting or other lethal controls are unacceptable for public-safety or public-relations reasons, or due to the risks to nontarget species, the high cost of delivery using baits or remotely delivered injections might be acceptable. Contraceptives are most likely to be suitable for species with short breeding seasons, where drug delivery is necessary for only a few weeks each year. For species with longer breeding seasons, a contraceptive would need to be developed for which a single dose lasts for at least 3 years to reduce delivery costs.

Research is currently being conducted to develop an immunocontraceptive for fox control. Despite an extensive search, no suitable live vector has been found for its delivery. But if a fox immunocontraceptive is successfully developed, it may be possible to use a bait delivery system for fox control in localized areas.

Conclusion

Currently available contraceptive techniques cannot be used to control Australia's widespread and abundant pest animal species. There are two main problems for using contraception for wide-scale control of any of our major pests. First, there are no suitable techniques for cost-effective delivery, which will be prohibitively expensive for broad use unless passive delivery via a live agent becomes available. Second, researchers lack knowledge about the factors regulating pest populations and the potential effect of fertility control on pest population dynamics. Field experiments are needed to determine if immunocontraceptives can reduce pest populations to the extent needed to control damage. Australian research is focused in these priority areas, but there are many technical hurdles, and success, if it comes, will not be for some years.

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Research To Develop Contraceptive Control of Brushtail Possums in New Zealand

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Abstract: Common brushtail possums are serious pests in New Zealand, where they threaten the survival of native plants and animals and spread bovine tuberculosis. A National Science Strategy Committee established in 1991 to coordinate possum research gave high priority to research aimed at biological control of possums, particularly contraceptive control. Surveys are identifying pathogens and potential vectors, and research has begun on immunology, gene transcription, potential contraceptive targets, and sociobiology. As there are more than 60 million possums in New Zealand, contraceptive vaccine delivery systems need to be cost effective, and they must be publicly acceptable. A vaccine could be included in a bait, but long-term cost-effective control will probably require a biological vector. Eventually the best control strategy will probably combine traditional control and immunocontraception.

Keywords: brushtail possums, immunocontraception, *Trichosurus vulpecula,* vectors

The Possum Problem

The common brushtail possum *(Trichosurus vulpecula),* a native Australian marsupial, was introduced into New Zealand in the last century to start a fur industry. Possums are folivorous and largely arboreal, and they average about 3 kg in weight. They are relatively widespread in Australia, although not often in high number. Like all marsupials, they are legally protected in Australia (How 1983). In contrast, possum population densities are 5 to 20 times higher (up to 25/ha [Green 1984]) in New Zealand, where the possum is acknowledged as the most serious vertebrate pest (Parliamentary Commissioner for the Environment 1994).

Possums established themselves so successfully in New Zealand partly because the forests evolved in the absence of any mammalian browser. Lacking specific chemical defenses against such an attack, many native trees and shrubs are highly palatable to introduced herbivores. There are also no significant possum predators in New Zealand. The large possum population is degrading the composition and structure of some forest types (Payton 1987, Stewart and Rose 1988, Allen et al. 1989). Populations of native animals are also affected because possums compete with them for fruit and nectar supplies (Cowan 1991). Predation, disturbance, and competition are reducing the survival and reproductive success of endemic and highly endangered species such as the kokako *(Callaeas cinerea)* and the short-tailed bat *(Mystacina tuberculata)* (Leathwick et al. 1983, Brown et al. 1993).

In addition to such impacts on conservation values, possums are the major wildlife host of bovine tuberculosis (Tb) in New Zealand. Possums maintain this disease and spread it to cattle and deer herds (Livingstone 1986, 1991), threatening the country's livestock exports. In the 1993–94 financial year, NZ$17 million was spent on Tb eradication programs in cattle and deer herds and NZ$18 million was spent on possum control in Tb endemic areas (Parliamentary Commissioner for the Environment 1994). Possum control is chiefly dependent on the use of Compound 1080 (sodium monofluoroacetate) baits, but public opposition to the widespread use of this poison is increasing with particular concern about possible environmental contamination.

In October 1991, the New Zealand Government recognized the seriousness of the possum threat to the economy and environment by establishing a National Science Strategy Committee (NSSC) to coordinate possum research. The NSSC has given a high priority to research on the biological control of possums, with contraceptive control as a major focus (Atkinson and Wright 1993). Biological control options for possums were discussed at a workshop hosted by the NSSC for New Zealand and Australian scientists in October 1992. There was general consensus that too little was known about some fundamental aspects of possum biology to embark on a focused project like that for rabbit and fox control in Australia (see Tyndale–Biscoe, this volume). It was agreed that basic research was required in seven priority areas

(table 1). This chapter describes some of the research that has been initiated and the philosophy underlying the research priorities.

Table 1. Priority research areas (not in order) for the biological control of common brushtail possums in New Zealand

1	*Screening for pathogens and potential vectors*	The National Science Strategy Committee is open to the possibility of identifying organisms that could be used for classical biological control as well as organisms that could be used to vector contraceptive vaccines.
2	*Immunology*	These are both vital for the development of contraceptive vaccines.
3	*Gene transcription*	
4	*Control of reproduction through the central endocrine system*	Studies in these fields aim to identify potential contraceptive vaccine targets.
5	*Biology of sperm, eggs, and the reproductive tract*	
6	*Control of lactation*	
7	*Sociobiology*	This is an important area of research in regard to the transmission of vectors and the behavioral consequences of contraceptive vaccines.

Research Priorities

Pathogens and Potential Vectors

Possum parasites and pathogens are being comprehensively surveyed in New Zealand and Australia to identify organisms for use as classical biological control agents or as vectors of contraceptive vaccines (Heath et al. 1994). Because New Zealand's possum population was founded from only a few hundred animals and some intermediate hosts are not present in New Zealand, possums in this country probably carry fewer types of parasites and pathogens than those in Australia (Cowan 1990). Also, because New Zealand's possum population has been isolated for more than 100 years, it may be more susceptible than the Australian population to the broader range of parasites and pathogens present in Australia.

In New Zealand, approximately 200 possums were trapped in 1993 and 1994 in each of eight locations close to the original possum release sites. These animals have been examined for the presence of ectoparasites and endoparasites, and samples from the heart, lung, liver, spleen, and gut have been screened for bacteria and viruses. Comparative studies will begin in Australia shortly.

Immunology

The New Zealand Pastoral and Agricultural Research Institute (AgResearch) is working on the immune response of possums. This research is primarily focused on the kinetics of antibody production in the possum against particulate and soluble proteins, and on determining optimal systems for stimulating high antibody responses at both the parenteral and secretory level. Early results indicate that cell-mediated responses are generally poor in the possum, which relies more on antibody responses. Responses to particulate antigens (e.g., whole sperm) are considerably stronger than responses to small soluble antigens (B. Buddle, pers. comm.). The cytokines that regulate immune responses in possums are being identified.

Gene Transcription

Although gene transcription is one of the research priorities, there appears to be little current research. AgResearch has started investigating how foreign DNA is expressed in nematodes and considering how nematodes might be used as vectors of contraceptive vaccines. A number of groups are preparing c-DNA libraries, including those for the pituitary (AgResearch, Wellington), the mammary gland (University of Otago, Dunedin), and the testis and female reproductive tract (Marsupial Cooperative Research Centre, Macquarie University, and La Trobe University, Australia).

Potential Contraceptive Targets

Central Endocrine System—Gonadotropin-releasing hormone (GnRH) is central to the regulation of reproduction and has therefore been suggested as one possible target of a contraceptive vaccine. Vaccines against GnRH are easily produced. Because the

hormone is present in the body in tiny amounts, antibodies produced by GnRH vaccines can readily remove the hormone from circulation.

A major disadvantage is that the structure of this hormone is identical in all mammals and birds, so GnRH vaccines would not be possum specific. It is possible, however, that species-specific regions may be present on GnRH receptors or that gene regulators may have species-specific regions. AgResearch in Wellington is investigating the regulation of GnRH gene expression.

Another disadvantage of GnRH vaccines is that they effectively neuter animals so that social behaviors that are dependent on sex steroids are likely to change in vaccinated animals. At Landcare Research, Christchurch, investigators are testing the behavioral effects on possums of a commercial GnRH vaccine registered for use in cattle in Australia (Hoskinson et al. 1990). Captive groups of one male and two female possums are observed regularly after the dominant female has been vaccinated with the GnRH vaccine. This work is still at an early stage, but the vaccine does not seem to affect the social status of the dominant possums.

Biology of Sperm, Eggs and the Reproductive Tract—At Landcare Research, we are focusing on sperm, eggs, and the fertilization process. We are investigating the immunological responses of possums to sperm vaccines and collaborating with Macquarie University, Australia, to characterize specific antigens of possum sperm and zona pellucida. Targeting gametes has advantages because many antigens are potentially possum specific, and an immunological attack on sperm or eggs should not affect behavior. Sperm vaccines are attractive. If sperm are attacked in the female tract before fertilization, this will disrupt fertility in females as well as in males. Repeated insemination of infertile females may also act as a booster vaccination and result in a long-lived immunity.

Vaccines have been prepared from whole sperm using Freund's Complete and Freund's Incomplete adjuvants. We are studying the effect of the vaccine on fertility and the relationship between fertility and antibody titre. The Macquarie group is also working on in vitro fertilization (IVF) systems for possums using Tammar wallabies *(Macropus eugenii)* as a marsupial model (Mate and Rodger 1993). An IVF system will greatly facilitate the characterization of gamete antigens and ultimately be essential for screening potential vaccine antigens. Because possums are marsupials, we hope that the gamete antigens are different from those of eutherian mammals. However, there appears to be some homology between possum and eutherian zona pellucida proteins (J. Rodger, pers. comm.); detailed characterizations are yet to be done on the sperm surface proteins of the possum.

Secretions of the female reproductive tract are important for the transport of sperm, eggs, and early embryos, as well as being vital for growth and survival of the conceptus. The Macquarie University group plans to characterize some of the secretory proteins as an immunological attack directed at some of these may result in infertility. Workers at AgResearch in Dunedin are identifying functional aspects of the female reproductive tract that may be susceptible to disruption by estrogenic plant compounds. This group is considering the possibility of being able to genetically modify food plants favored by the possum so that they deliver contraceptive compounds (B. McLeod, pers. comm.). Initial work concentrates on normal function of the reproductive tract, looking at the cyclical changes in the ultrastructure of the tract, secretory organelles, and mucoid secretions.

Control of Lactation—Disruption of lactation is not true contraception, but is an option with possums, which, like all marsupials, invest in lactation rather than pregnancy. Pregnancy in possums lasts only 17 days; lactation lasts up to 230 days (Pilton and Sharman 1962). An immunological attack could suppress milk production or change the composition of the milk so that it does not provide the appropriate nutrients. In possums and some other marsupials, cessation of lactation is a natural method of population regulation: when conditions in the wild are unfavorable, lactation ceases and pouch young die. In some years, up to 50 percent of possum pouch young may die in this way. The pouch young has limited sensory

development until it is nearly 3 months old (Lyne and Verhagen 1957, Hughes and Hall 1984), so targeting lactation in the early stages of development is considered relatively humane.

The composition of the milk undergoes marked changes during the long suckling period in marsupials (Tyndale–Biscoe and Renfree 1987). At Landcare Research, we are investigating changes in the elemental composition of possum milk during the course of lactation. Sodium, potassium, iron, and copper all show marked changes. We are especially interested in milk calcium levels. Brushtail possums develop metastatic calcinosis when fed a diet high in calcium, and calcium metabolism is readily disrupted in possums by cholecalciferol (Eason 1991). Also in progress is work to model the effects of lactation disruption on pouch young using the prolactin inhibitor bromocriptine. The aim of this project is to quantify the relationship between milk production and growth and survival of the pouch young.

Otago University is characterizing possum milk proteins. Researchers at the university have identified some unusual proteins associated with certain stages of lactation that are analogous to late lactation protein in Tammar wallabies *(Macropus eugenii)* (Nicholas et al. 1987), but the significance of these to the developing pouch young has not yet been established (M. Grigor, pers. comm.).

Sociobiology

Behavioral changes caused by contraceptive vaccines could compromise their efficacy. Possum social organization is based around dominance hierarchies (Winter 1976, Biggins and Overstreet 1978). If sterilization affects social behavior, changes in social structure could allow subordinate individuals to increase their breeding success. Landcare Research has been studying whether sterilization will affect social status. Females are more aggressive than males (Winter 1976, Oldham 1986), and our trials have shown that in captivity females retain their dominance status even after ovariectomy. The status of males also appears unchanged by castration (P. McAllum, unpubl. data). We plan further work to untangle the behavioral effects of sex steroids from learned behaviors and to ascertain that the results of pen trials are applicable to free-living populations.

Future Directions

Vectoring a Vaccine

As there are more than 60 million possums in New Zealand (Cowan 1991), vaccine delivery must be cost effective. A vaccine included in a bait is the likely research target for the medium term (6 or 7 years). However, long-term, cost-effective control will probably need a self-sustaining biological vector such as a genetically modified virus (as proposed for rabbit and fox control in Australia; Tyndale–Biscoe, this volume), bacterium, or nematode. At this early stage, the New Zealand research program is keeping all options open.

Any vector would need to meet rigid specifications to allay the concerns of the public and New Zealand's trading partners. It would need to be humane and unable to survive away from possums, so that it could not cross to Australia, where possums are protected. The vector must infect only possums, and its mode of action must also be possum specific. The last two criteria act as a double safeguard.

Expectations

A biologically vectored vaccine is unlikely to eradicate possums because its success would be inherently dependent on the density of the possum population. When a population is dense, the level of possum to possum contact is high, and a vectored vaccine would have a significant impact. As a population declines and possums became more sparsely distributed, transmission of the vectored vaccine would be reduced. The population would equilibrate at a new lower level but not decline to extinction. This density-dependent effect could largely be circumvented by the use of a sexually transmitted vector. Possums actively seek partners in the breeding season, so a venereal infection is likely to persist even at low population levels.

A venereal vector would have other advantages. Any contraceptive vaccine would probably rely on the mucosal immune system to cause infertility, so sexual transmission of the vector delivers the vaccine to exactly where it can be most effective. The use of a sexually transmitted organism would also allay the concern that the vector might cross to Australia and infect protected possums there.

Computer modeling is currently defining the ecological and epidemiological criteria that need to be met to minimize the impact of possums. Modeling suggests that the possum population must be reduced to about 40 percent of its present level for 8–10 years to eradicate bovine tuberculosis (Barlow 1991). Such a reduction is theoretically achievable with a vectored contraceptive vaccine. Target reductions in possum numbers to protect conservation values are more difficult to quantify because of the diversity of the conservation values society wants to protect. This is an area of active research. The likely timetable for research and development of effective biotechnological control strategies for possums is in the order of 10 to 20 years, so New Zealand will have to depend on traditional pest control technologies for some time yet. Eventually the best control strategy will probably combine traditional control and immunocontraception (Barlow 1994).

In the coming years, new technologies will offer new possibilities for the control of vertebrate pests. The future promises some exciting research. We believe that it is only through the use of biotechnologies such as vectored immunocontraception that control of this pest can become truly sustainable.

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Immunosterilization for Wild Rabbits: The Options

C. H. Tyndale–Biscoe

Abstract: Control of wildlife pest populations by sterilization could be more effective than conventional mortality agents, provided that two conditions are met: (1) the endocrine function of affected animals is not compromised, so as to exploit the natural suppression of reproduction of subordinate members of the population that occurs in many species; and (2) the incidence of sterility is sufficient to lower population recruitment and growth. Both conditions could, theoretically, be met by use of an infectious recombinant virus, expressing genes for specific reproductive antigens. Using the rabbit, I describe the research required to test the concept and discuss the legal and ethical consequences that may arise from a positive outcome to the research.

Keywords: Gamete antigens, population ecology, recombinant virus, risk assessment, social hierarchies

Introduction

The idea that fertility control has a potential for the management of wild species has been recognized in recent years, as shown by a 1990 meeting in Melbourne, Australia (Tyndale–Biscoe 1991) and a 1993 meeting in Denver, CO. While considerable support for this concept exists on grounds of humane control of wildlife, several important and as yet unresolved matters remain regarding its safety. The biological and ethical issues largely resolve into whether we are concerned with controlling populations of desirable species at appropriate levels with the option for reversing the effects in future years, or whether we are concerned to control populations of undesirable species of wildlife at very reduced levels indefinitely and at minimum cost. In North America, the first concern is the overriding one; in Australia and New Zealand, the second is.

The European wild rabbit, *Oryctolagus cuniculus,* is an excellent example with which to explore some of the biological and ethical questions surrounding the use of sterility as a means of management of wildlife. In some countries, it is a highly regarded wildlife species while in others it is the most serious and intractable of all pests (Thompson and King 1994). In addition, there are many other species of lagomorph around the world that are well regarded, and some are endangered (Chapman and Flux 1993). Clearly, methods developed for the control of the common rabbit must not affect these other relatives.

The rabbit is indigenous to southern Europe, where it is regarded as a desirable element of the fauna. In Spain, it is the main prey of eight raptors, two snakes, and six species of mammal, including the endangered lynx, and it is also a prized game animal. It was domesticated by French monks and taken by the Normans to Britain (Rogers et al. 1994). Domesticated varieties of the rabbit are raised throughout the world for meat, skin, and fur. It is also an important laboratory species, and there is a strong culture of breeding distinct varieties by rabbit fanciers. This range of interests in the rabbit involves a large and worldwide trade and distribution of rabbits and rabbit products. The nature of this interchange was dramatically demonstrated by the newly recognized rabbit calicivirus, which causes a rapidly fatal disease in rabbits (viral/rabbit hemorrhagic disease, VHD/RHD), after its discovery in China in 1984 (Liu et al. 1984) and its spread to southern Europe in 1988 and to Mexico in 1990.

In Britain, the long-term impact of the rabbit on vegetation was not appreciated until the demise of rabbits after the myxomatosis epizootic of the 1950's (Thompson 1994). Since the rabbit's subsequent recovery, the value of the damage rabbits now cause has been estimated at $180 million each year.
In the nineteenth century, the rabbit was released on many islands as a source of emergency food for castaways (Flux 1994), and it was also introduced by British colonists to Australia and New Zealand for a variety of reasons, including sentimental ones of enjoying the presence of a familiar animal in an alien country. In many cases, these introductions had severely deleterious consequences to the vegetation and indigenous fauna. In recent years, some of the islands under Australian and New Zealand control have been cleared of rabbits at very great expense (Burbidge 1989, Towns et al. 1990), and the vegetation is recovering. However, this is not a practical strategy for larger areas or for continental Australia.

The Rabbit Problem in Australia

Within 20 years of the introduction of rabbits to Australia, it was evident that they were a serious problem. Their explosive spread across the continent was complete by the early years of this century and resulted in gross overgrazing of the native grasslands, permanent degradation of the semiarid region, and widespread soil erosion. The long-term damage that the rabbit is doing in this large region of Australia is serious because it is preventing the regeneration of the long-lived plant species. When the old plants die, therefore, the whole ecosystem is irrevocably changed. The rabbit has been a major factor in the extinction of the small to medium-sized marsupials that were indigenous to this region and the value of losses to the pastoral industry each year has been estimated to be $500 million. The rabbit is acknowledged to be the most serious of all animal pest species in Australia and its control the most urgent.

In 1888, the Intercolonial Rabbit Commission was set up ". . . to rid the country of this menace." In the century since, many schemes to control the rabbit have been proposed, but only one has come near to accomplishing it. At the end of 1950, the myxoma virus was released. It spread rapidly across the southern half of Australia, causing massive mortality among rabbit populations. This was the single most effective control of a pest mammal ever achieved, and the effects of it are still apparent in most regions of Australia (Parer et al. 1985). Within a few years of the release, attenuated strains of the virus had evolved, and resistance to the virus had developed in the rabbit populations (Marshall and Fenner 1958, Fenner and Ratcliffe 1965). In order to counter this apparent decline in the effectiveness of the virus, the highly virulent Lausanne strain was imported from Europe and for 20 years was regularly released by land managers and owners for rabbit control. It probably caused high mortality at the site and time of each introduction but did not persist or spread very far. Recent evidence (P. J. Kerr, pers. comm.) suggests that the Lausanne strain has not displaced the preexisting strains, and its value for rabbit control is unclear.

The efficacy of myxoma virus for broad-scale rabbit control is critically dependent on insect vectors, and in Australia the initial epizootic was effected by two species of mosquito. In Europe, however, the spread of the virus was largely due to the rabbit flea, *Spilopsylla cuniculi.* In 1960, this species was introduced to Australia and has become an important additional vector in the higher rainfall regions of the continent. A third vector, the Spanish flea *(Xenopsylla cunicularis),* which can survive in more arid environments, was released in South Australia in 1992. Hopefully, the Spanish flea will be an effective vector of the myxoma virus in the arid regions of the country, where rabbits are abundant in years of high rainfall.

Rabbit calicivirus was also being assessed in 1995 for possible release in Australia as another mortality agent. Unfortunately, it escaped from quarantine and is now widely dispersed. In addition to these measures, substantial resources have been directed by the Australian Government, through the Cooperative Research Centre for Biological Control of Vertebrate Pest Populations (1993, 1994), to investigate the potential for fertility control. In this approach, the myxoma virus would be used as a vector to introduce an immunocontraceptive to populations of wild rabbits.

Fertility Control of Rabbits

All previous attempts to control the rabbit have depended on developing methods to enhance mortality, such as disease, natural predators, commercial trapping, and shooting or poisoning with strychnine, arsenic, phosphorus, or sodium fluoroacetate (Compound 1080). Most of these methods are now illegal because of the pain they inflict on the animals in the process of killing them. In a recent study to compare the efficacy of these different methods, Williams and Moore (1995) found that the destruction of rabbit warrens was far more effective and long lasting than poisoning. When the warrens were left intact after fumigation or poisoning, rabbit populations recovered very rapidly because the warrens could be reoccupied and breeding could recommence. Similarly, after the

initial highly successful reduction of rabbits in 1951–54 with the myxoma virus, rabbit populations recovered in some areas because warrens were left intact, reproduction of the survivors was not curtailed, and resistance to the virus thus evolved rapidly (Marshall and Fenner 1958). Clearly, the most important factor in rabbit control is the rate of recovery after a treatment has been applied: if that could be curtailed, as with warren ripping, the effect of all methods would be enhanced.

Fertility control is sometimes regarded as mortality applied at an earlier stage of the life cycle, but for many species it could be much more than this. Many studies on wild mammals have shown that reproductive success is closely linked to high rank in the social hierarchy of the population. Lower ranking animals either do not breed or fail to rear their young to independence (Wasser and Barash 1983, Abbot 1988). Failure to breed has been shown in wild foxes in Britain to be effected by the dominant members of the group (McDonald 1987). In the wild rabbit, there is no evidence that dominant females suppress breeding by subordinates, but the survival of the kittens of dominant females is significantly greater than for those of subordinate females (Mykytowycz 1959, Mykytowycz and Fullager 1973, Cowan 1987). Thus, sterilization of dominant members could affect fecundity of the population disproportionately, provided that the sterilized individuals remain sexually active and retain or improve their status in the social hierarchy of the population, and that a sufficiently high proportion of the population is sterilized (Caughley et al. 1992, Barlow 1994). While these conditions have long been recognized (Knipling 1959, Davies 1961), the problem has been how to achieve them for control of a wildlife species.

For many species with strong social structures, it is therefore important that a sterilizing agent not compromise the hormonal function of the gonads of the target animal. Because rank order is related to levels of sex hormones, a castrated animal is rapidly replaced in the social hierarchy and exerts little or no influence on the reproductive potential of other members of the population. Methods of fertility control that rely on exposing the target animal to steroids of one sort or another usually affect endocrine functions and consequently the sexual and social behavior of the animal (Bomford 1990 and this volume). Likewise, the use of agents that immunize the animal against gonadotropin hormone-releasing hormone (GnRH) or block receptors for GnRH in the pituitary seriously affect the steroidal functions of the gonad as well as its gametogenic function. While these methods have wide application in livestock management, and could be useful for the control of breeding in local populations of wildlife or for those species in which breeding suppression does not occur, they are of little potential use for pest species where social and sexual behavior must not be compromised. Agents that affect gametes, fertilization, or implantation must be sought and then presented to the target animal in such a way as to induce a strong and persistent immune response that prevents or compromises pregnancy.

A second requirement for a widespread pest species like the rabbit is a means of delivering the agent to a large proportion of the population. The concept that we are investigating for the rabbit is to clone the genes encoding proteins that are critically involved in fertilization or implantation and insert them into the myxoma virus. Rabbits infected with the recombinant myxoma virus would simultaneously raise antibodies to the virus and to the reproductive antigen, and fertilization or implantation would be prevented. Because this immunization would not affect the hormonal status of the rabbit, it would not affect its sexual activity or social status in the population. For the wild rabbit, five key questions derive from this concept:

1. What proportion of females in a wild population must be sterile in order to reduce significantly the rate of growth of the population?

2. Can gamete-specific proteins be presented to the animal in such a way as to provoke an effective and long-lasting immune response that interferes with fertilization or fetal development?

3. Can recombinant myxoma viruses that express the genes encoding the gamete proteins be constructed in such a way that they can act as vectors to

immunosterilize the proportion of the wild population identified in the first question?

4. How and when will selection forces diminish the effect of the recombinant virus?

5. Can this be achieved in a way that does not put at risk other species in Australia or rabbits in other countries?

Effects of Sterility on Rabbit Population Dynamics

In Western Australia and in New South Wales, two large experiments on wild rabbits were begun in 1993 to test the effect of sterilizing various proportions of the female population on rate of increase and survival of young. In each experiment, 12 free-range populations, each initially of 50 to 100 adult rabbits, were isolated by combinations of fences and buffer strips, and each was allocated randomly to 1 of 4 treatments. On each site, all the rabbits were caught and marked, and 80 percent of the adult females were subjected to surgery, either laparotomy or ligation of the oviducts. The proportions sterilized on three sites each were 0 percent, 40 percent, 60 percent, or 80 percent. In addition, the impact on the European flea, *Spilopsyllus cuniculi,* and the incidence of infection with myxoma virus are being investigated. This flea is being studied because its life cycle is intimately tied to the reproductive cycle of the rabbit, particularly females in late pregnancy and newborn kittens. With a high proportion of the females sterile, will the flea population be able to survive and transmit myxoma virus?

These experiments have been run for 3 years to determine how the productivity of the populations is affected by the different levels of sterilization. Preliminary results from the first year suggest that, while the number of kittens was reduced on sites where females were sterilized, survival of these kittens was higher than on the control sites, so that, by the start of the next breeding season, the net production between sites was not different. However, survival of sterilized females appears to have been higher than for intact females and males, and sterilized females entered the next breeding season heavier than intact females (Williams and Twigg 1996). In the second and third years, the treatments were repeated on new recruits to the populations. In both experiments, climatic vagaries during the 2 years affected reproduction and survival. In general, however, the effect of sterilization followed the pattern of the first year. The most notable result was that the higher levels of sterility reduced the annual cohort of recruits; populations with 80-percent sterility tended to have a flat trajectory over time, unlike the fluctuating pattern in the experimental controls (L. Twigg and C. K. Williams, pers. comm). Knowledge of the longer term effects of sterility must await detailed analyses of these experiments and their application to mathematical models for extrapolation. This information will be crucially important in deciding the requirements for an effective recombinant virus.

Gamete Antigens for a Rabbit Immunocontraceptive

Effort is presently concentrated on interfering with fertilization by identifying those proteins present on the surface of the sperm and the ovum, which are involved in the processes leading to fusion of the male and female nuclei. These are

(1) locomotion of the sperm, which brings it into the vicinity of the of the ovum;

(2) the first contact between the sperm head and the zona pellucida, which induces the acrosome reaction of the sperm;

(3) release from the acrosome of enzymes that break down the zona and allow the sperm to pass through and lie against the plasma membrane of the ovum; and

(4) proteins on the equator of the sperm head that are thought to be critically important in causing the fusion of the sperm and egg plasma membranes, so that the sperm nucleus can enter the egg and fuse with its nucleus.

The approach being used to identify and isolate selected antigens is to develop polyclonal and monoclonal antibodies to gamete antigens and, if possible, assess the effect of these antibodies on sperm–egg binding, on in vitro fertilization, and on fertility in intact animals.

To date, 35 monoclonal antibodies have been prepared against rabbit sperm, and some of them have been shown to block sperm–egg binding and prevent fertilization in vitro. A cDNA library has been prepared from rabbit testis, and the gene encoding for one of these proteins (Pop1) has been sequenced and appears to be a novel testis-specific protein (M. Holland, pers. comm.). In addition, DNA probes, derived from sperm antigen genes of other species, are being used to isolate the rabbit homologues from the rabbit testis cDNA library. The first of these to be characterized are the homologues of the genes for the guinea-pig sperm antigens PH20 (Holland et al. 1997) and PH30 (Hardy and Holland 1996).

For effective immunocontraception, the antigen must provoke a strong and sustained immune response that will interfere with the functions of the gametes. This process involves the appropriate presentation of the antigen to the immune system to induce strong memory and the development of high titres of appropriate antibodies at the time that fertilization is most likely to occur. Gamete antigens are self proteins and therefore may not induce a strong immune response alone. However, spermatozoa, which are normally not presented to the immune system of the male because of the blood–testis barrier, may provoke an immune response when presented to the systemic circulation of males.

Effective application of a vaccine for fertility control requires that a high level of immunity be achieved amongst individuals exposed to the vaccine. In outbred populations of wild mammals, heterogeneity of the immune response between individuals may make it hard to reach or sustain that level. It may therefore be necessary to include several antigenic determinants together, so as to stimulate a broad range of immune responses within the population. In addition, the antigen(s) may have to be presented in conjunction with other highly immunogenic carrier proteins in order to induce a strong and lasting immunity. This could include species-specific cytokines, such as interleukin–6, which has recently been shown to enhance the immune response in vivo in mice (Ramsay et al. 1994).

Molecular Virology and Antigen Delivery

For immunosterilization to be effective in a wild population, the gamete antigens must reach a large proportion of the exposed population. Delivery systems can utilize

(1) direct presentation of the antigen (in baits or by projectiles, which is costly but safe),

(2) oral administration of nondisseminating recombinant micro-organisms (which carry the genes encoding the gamete antigens and immunogenic carrier proteins), or

(3) a recombinant micro-organism that spreads through the target population by sexual transmission, contagion, or arthropod vector.

The delivery of immunosterilizing agents by bait has considerable value in circumstances where the target population is restricted in distribution or in time. However, the control of rabbits in Australia calls for a more cost-effective means of delivery that would spread the agent through the population independently. This of course, brings with it a much higher degree of risk—but not so high that the concept should not even be investigated or contemplated.

Over the past decade, recombinant viruses, carrying gene sequences derived from other organisms, have been constructed to function as living vaccines. The best example is the vaccinia virus recombinant expressing a portion of the rabies virus genome, which has been used very successfully in Europe to immunize populations of wild foxes (Brochier et al. 1990). The success of this project is due to the ability of the recombinant virus to replicate in the oral cavity of the infected fox and because the rabies glycoprotein expressed by the inserted gene is highly immunogenic.

The myxoma virus has been circulating in the rabbit populations of Australia for more than 40 years, and no evidence has been found to indicate that it infects species other than rabbits. Myxoma virus is a large DNA leporipoxvirus, related to vaccinia virus, so technologies already developed for preparing recombinant poxviruses can be adapted for the construction of recombinant myxoma viruses. During the past 9

years, the structure of the virus genome has been investigated (Russell and Robbins 1989), and a number of open reading frames in the central region of the genome have been identified (Jackson and Bults 1990 and 1992a, R. J. Jackson et al. 1996), including insertion sites homologous to those used in the vaccinia virus work. The aim here was to preserve the viability and infectivity of the native myxoma virus by inserting foreign DNA at intergenic sites, and not to compromise the virus by inserting foreign DNA intragenically.

Plasmid transfer vectors, based on the myxoma virus, have been constructed. These vectors contain a multiple cloning site adjacent to vaccinia virus promoter elements inserted in an intergenic region between the myxoma virus *tk* gene and open reading frame MV8a (Jackson and Bults 1992b). These vectors were used to generate recombinant myxoma viruses in cell culture and demonstrated that the myxoma virus can carry additional DNA and express the product in a culture system (Jackson and Bults 1992b), and without associated attenuation (R. J. Jackson et al. 1996).

Other recombinant myxoma viruses have been constructed using an attenuated strain, which express the hemagglutinin antigen of influenza virus (Kerr and Jackson 1995). These recombinants express cell-membrane-bound hemagglutinin, which can be detected by immunofluorescence. In live rabbits, the recombinants provoke strong antibody titres to the myxoma virus and very strong antibody titres to the hemagglutinin antigen. This demonstration opens the way for the insertion of other genes, such as those encoding for reproductive antigens.

Competition Between Recombinant and Native Strains of Virus

Concurrently with the reproductive and viral programs, it is important to determine the conditions under which dissemination of the recombinant virus, expressing sterilizing genes, may be able to outcompete existing field strains. To assess such potential competition, the pathogenesis and epidemiology of the recombinant virus must be compared to that of purified strains of the virus and, in the case of myxoma virus, the various field strains that have evolved over the past 40 years in Australia. Using the techniques of restriction length fragment polymorphism and polymerase chain reaction, P. J. Kerr (pers. comm.) has been able to identify field strains of myxoma virus by criteria that are independent of the virulence or pathogenesis of the strain. This work is not only providing a far greater appreciation of the regional differences in the virus and its host but will enable the selection of strains with which to prepare recombinants much more effectively targeted to rabbit populations.

The other question here is the fitness of the sterilizing recombinant virus to survive in the host population. In preliminary modelling for a recombinant myxoma virus expressing a sterilizing gene, being undertaken by R. Pech and G. Hood (pers. comm.), persistence of strains depends on the rate at which new susceptible rabbits enter the population. Even virulent strains can persist in the population if the birth rate is high; at lower birth rates, however, only avirulent strains with a long period of infectivity survive. The persistence of sterilizing strains may be even more constrained because there will be fewer opportunities for transmission to new, susceptible rabbits. In a different model, in which the sterilizing virus is assumed to be sexually transmitted and to persist in the infected host, Barlow (1994) has estimated that the recombinant virus will be at a selective advantage over the native strain because the more frequent return to estrus by sterilized females will provide more opportunities for transmission. In this situation, he concludes that the recombinant virus could persist in a population that stabilizes at a substantially lower level.

Legal and Ethical Issues of Viral-Vectored Immunosterilization

The development of immunosterilizing vaccines that can be delivered to wild animals raises a number of important issues about the international consequences of the impact of an agent designed for a species that is a pest in one country but a desirable or even endangered species in another. One view is that the outcome of the concept is so uncertain and the risks are so great that approval for release will never be

given; therefore, the research should not proceed at all. Implicit in this view is the inference that these other issues will always outweigh the problem of the rabbit in Australia, a problem of great magnitude. An opposing view, which we favor, is that the research should proceed incrementally with public discussion and proper scrutiny at each step, so that, if the concept is shown to be valid, its potential use can be assessed properly against the risks. This view recognizes that understanding of the control processes in gene expression is advancing so fast that difficulties that now seem insuperable may not be so in a few years. However, if we wait until then before embarking on the basic research required to develop the concept, we will have delayed the time when it can be used. Delay may lead to further deterioration of threatened ecosystems, which is an issue of great concern in Australia. The debate is just beginning, and it is too early to establish rigid directives on this matter. Rather, it is important to establish first, whether it is possible to control populations by immunosterilization and second, if it is, to explore the range of options for delivery of the immunogen. Options range from the direct delivery of a nontransmissible immunogen, which is costly but has a low risk, to delivery by a disseminating microorganism in which the unit cost is very low but the risk is higher.

The risks relate to (1) the effect on species other than the prime target in the country with the pest problem, which are primarily national risks, and (2) the risks to the target species and related species in another part of the world, where they are valued highly. The latter risks are mainly international.

National Aspects—The important questions relate to species-specificity of the reproductive antigen complex, specificity of the virus to be used as the vector, and specificity of the means by which it will be transmitted.

Gamete antigens that have been characterized in recent years show considerable homology between species at the genomic level, and researchers need to know whether antigenic epitopes can be identified that affect fertilization only in the target species. It is likely that such epitopes, if they exist, may not provoke a strong immune response by themselves but may do so when coupled with some other protein or when expressed in conjunction with cytokines. If the cytokines themselves are specific to the target species, both specificity and immunogenicity of the antigen would be enhanced. Alternatively, if species-specific gamete antigens cannot be identified, then other antigens involved in reproduction may need to be considered. In the case of the rabbit, the protein uteroglobin, which is associated with implantation (Beier 1982), is specific to the rabbit and the gene encoding it has been cloned (Bailly et al. 1983). However, the evidence that it is essential for implantation is not strong, and the case against gamete antigens would have to be very strong before attempting to exploit uteroglobin for immunosterilization.

In the choice of vectors for the delivery of sterilizing antigens, the important aspect is the degree to which the viral vector is specific to the target species and is incapable of replicating and provoking an immune response in nontarget species. For myxoma virus in Australia, the case is strong that it is specific to the rabbit. In the past 40 years, as mentioned earlier, no evidence has been produced to indicate that the virus affects any species other than the rabbit, and humans exposed in an outbreak of myxomatosis did not seroconvert (E. W. Jackson et al. 1996). However, no critical tests have been done to determine that it does not undergo minimal replication in nontarget species, and no nontarget species has yet been screened for seroconversion. That can now be done for myxoma virus, using an enzyme-linked immunosorbent assay (ELISA) developed in our Center (Kerr 1997).

The third level of specificity is the way in which the virus is conveyed from one member of the target species to another. Contagious or insect-borne transmission involves a risk of cross-species infection, unless the insect vector is specific to the target species. Neither of the two species of rabbit flea introduced to Australia feeds on species of vertebrate other than the rabbit, and *Spilopsyllus cuniculi* can complete its own life cycle only on rabbits. However, the myxoma virus can be transmitted by other biting insects, a fact that reduces its specificity. Transmis-

sion of a viral vector by sexual contact or placental transfer would confer the greatest degree of specificity. In polyestrous species, sexual transmission would also confer a selective advantage on the recombinant over the native virus because, as mentioned above, females infected with the recombinant would undergo estrus more often and hence provide more opportunities for virus transmission (Barlow 1994). In this regard, herpesviruses that are sexually transmitted, show persistent infection, and are species specific may be particularly suitable candidates as viral vectors (Shellam 1994).

International Aspects—Another major concern about immunosterilization is international, centering on the risk posed by immunosterilization to a target species in countries where it is indigenous and well regarded. The concern is the risk of accidental or malicious export of the agent from a country where it is used for pest control to another country. For the rabbit, these concerns relate not only to the impact that the recombinant virus might have on *Oryctolagus cuniculus* but on other leporids that are susceptible to infection with leporipoxviruses. In North America, there are 17 species of *Sylvilagus* that could be infected, some of which are considered to be endangered species (Chapman and Flux 1993). In addition, there are other rare leporids in Mexico, Indonesia, and Japan that might be susceptible. The International Union for Conservation of Nature/Status Survey and Conservation Lagomorph Specialist Group has expressed strong reservation about the use of genetically engineered myxoma virus because of the potential risk to these species. What steps would be required to address this strongly expressed concern? A risk assessment needs to be undertaken that would place probability values on (1) transfer from the user country, (2) establishment in a second country, and (3) means to contain its spread, should an outbreak occur. These are similar to the concerns of international agencies that currently deal with other infectious organisms.

Probability of Transfer From the User Country—This probability would only include accidental or illegal transfer because presumably the power of legislation and its implementation with regard to the export of micro-organisms would be exercised under current international obligations. If the user country were Australia and a recombinant myxoma virus were being considered, risk assessment should include the past history of the virus in the country, the conditions required for initial establishment of the virus, and the evidence, if any, of the occurrence of genetically identifiable Australian strains of the virus anywhere else. The present evidence is that Australia is the only country that used the Standard Laboratory Strain (SLS) of the myxoma virus, and all field strains in Australia appear to be derived from it (P. J. Kerr, pers. comm.). There is no evidence that any strain of myxoma virus from Australia has been deliberately or accidentally spread to any other country. With techniques now developed, it would be possible to determine whether SLS-derived strains of the virus occur elsewhere.

It is perhaps significant that several attempts to release the virus in Australia before 1950 failed. The successful outbreak was due to the coincidence of widespread rains and large populations of two species of mosquito (Fenner and Ratcliffe 1965, Fenner and Ross 1994). In the early 1950's, attempts were also made to introduce the SLS myxoma strain to New Zealand, but they failed. At the time, investigators concluded that this happened because the appropriate insect vectors were not available to transmit the virus (Gibb and Williams 1994). More recently, there were moves to introduce the rabbit flea to New Zealand so as to provide a suitable vector, but the New Zealand Government decided in 1993 to forbid the release of the flea and the virus. There are several potential insect vectors of myxoma virus in New Zealand and many people there who would like to see the virus used. Despite this, legislative controls have been a sufficient barrier to its entry for 44 years.

Probability That the Agent Could Become Established in a Country Where the Target Species or Related Species Are Endemic—Whether leporipoxviruses are already present in the nontarget populations would influence whether the new virus could become established. Where related viruses or the same virus is already present, the probability of the recombinant strain becoming established may be low because of the so-called founder effect and also

because genetic manipulation may render the recombinant less competitive. In Australia, despite massive and repeated release of the Lausanne strain since 1957, particularly in Victoria (Fenner and Ross 1994), all field isolates so far examined are genetically derived from SLS (P. J. Kerr, pers. comm.). This finding suggests that the Lausanne strain has been unable to establish in the face of preexisting strains. In an analogous way, Japanese B encephalitis flavivirus has never become established in Australia, where Murray Valley encephalitis virus occurs, despite annual introductions in migratory birds from Japan (Davey et al. 1982).

In Europe, where the Lausanne strain of myxoma virus was released, it underwent a parallel but wholly independent evolution of attenuation (Fenner and Ross 1994). If the founder effect applies to virus strains, Australian SLS-derived strains would be unable to establish in Europe. This idea could be tested on Macquarie Island, where Lausanne was the only strain released, by introducing genetically distinct SLS strains.

The same principle applies to establishment of an immunosterilizing virus in Australian wild rabbit populations. Competition with field strains probably will pose a severe barrier to introduction and transmission, and special strategies for seeding and timing will probably need to be adopted. Such conditions would suggest a very low probability of transmission by limited events, such as accidental or malicious release in another country. Nevertheless, if research enables a highly competitive immunosterilizing strain to be produced and/or foreign populations or species are shown to be at risk, careful consideration must be given to the strains used and the safeguards to be built into the genetic manipulations and to whether these safeguards would provide adequate protection.

In America, various strains of myxoma virus are endemic in species of *Sylvilagus* in the Western United States and several countries in Central and South America, but the incidence of seropositivity in wild populations has been determined in a limited way for *S. bachmani* in California only (Regnery and Miller 1972). This strain was shown to be capable of infecting four other species of *Sylvilagus,* but it could not be transmitted by mosquito from any of the primary hosts because the amount of virus was insufficient for effective mosquito transfer (Regnery and Marshall 1971). A more extensive assessment of this would be required to develop probabilities of a recombinant strain becoming established. Laboratory testing of susceptibility to infection and transmission would determine whether the risk is real for these other species and so contribute to assessment of their magnitude. In other parts of the world, where leporipoxviruses do not occur, the susceptibility of rare leporids might have to be determined.

Develop Means To Contain Its Spread, Should an Outbreak Occur.—From the foregoing, the probability of an outbreak spreading in a population of rabbits that are already exposed to leporipoxvirus is low. However, in a naive population the myxoma virus can spread rapidly, as occurred in Europe in 1952 (Fenner and Ross 1994). If other leporids are shown to be susceptible to the virus and there was deemed to be a risk of transfer of the immunosterilizing virus, contingency plans would have to be considered. In Australia, contingency plans have been developed for containing an outbreak of major diseases of domestic stock, such as foot-and-mouth disease virus in feral pigs (Pech and McIlroy 1990), and similar models could be developed for myxoma virus. The important factor is to recognize the outbreak at an early stage and to reduce the proportion of susceptible animals rapidly, either by destroying them or by immunizing them against the pathogen. In the case of myxoma virus, there are now several isolates of the virus that are attenuated but immunogenic. P. J. Kerr (pers. comm) is currently investigating the genetic basis of pathogenesis and virulence using these isolates. These studies may provide strains of the virus that could be used to deliver broad-scale immunization to contain an unwanted outbreak. In California and in France, highly attenuated strains of myxoma virus have been developed as vaccines to protect domestic and wild *O. cuniculus,* but attempts to produce an inactivated myxoma virus vaccine have been unsuccessful (Fenner and Ross 1994).

Conclusion

Effective control of pest mammals is immensely difficult. While current methods using specific disease organisms or poisons may have some benefit, it is widely acknowledged that none has long-term promise. So far as poisons are concerned, there is doubt even of their medium-term efficacy. A new approach to pest animal control is urgent; the concept of a viral-vectored immunosterilant is such an approach. Because it is novel, the outcome is uncertain, and the risks are considerable. However, if the risks can be reduced to an acceptable level and this methodology is effective in controlling rabbits, the benefits will be very great. Furthermore, because the concept is generic, if it is effective in this species, it has the potential to be applied to other species as well. Three species are already being investigated—the European red fox, *Vulpes vulpes* (Bradley, this volume), and the brushtail possum, *Trichosurus vulpecula* (Jolly, this volume), in New Zealand; and the wild house mouse, *Mus domesticus* (Shellam 1994), in Australia.

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The Development of Contraceptive Methods for Captive Wildlife

Cheryl S. Asa

Abstract: Contraception has become critical in managing zoo populations, both to limit production of surplus animals and to promote genetic health. One role of the Contraception Advisory Group, formed in 1989, is to coordinate research to develop new contraceptive methods. Because melengestrol acetate (MGA) implants, which have been used by zoos for almost 20 years, recently have been associated with uterine pathology in felids, several new contraceptive techniques are being evaluated. These include other steroid hormone formulations, such as the birth control pill Depo-Provera,® the Norplant® implant, and MGA added to feed; bisdiamine, an oral male contraceptive; zona pellucida (ZP) vaccine; and vas plugs. Bisdiamine reversibly blocked spermatogenesis while sparing testosterone in its first test in gray wolves. ZP vaccine has been effective in preventing births in a variety of species of hoofstock, primates, and carnivores; however, long-term deleterious effects on the ovaries have been found in some controlled trials. Injectable vas plugs that conform to the shape of the vas make it possible to successfully treat a wide variety of species; reversal trials are currently underway. As research efforts continue, we hope to expand our collaborations with scientists working on contraceptive development for humans, companion animals and wildlife, to better make use of the limited resources available for these investigations.

Keywords: contraception, captive animals, zoo, vas plugs, bisdiamine, indenopyridine, zona pellucida vaccine, GnRH agonist, LHRH vaccine

Introduction

The modern zoo now faces the consequences of becoming too successful at breeding and maintaining the animals in its care. Advances in husbandry, nutrition, and medicine have resulted in more births and longer lives for most captive species. The major benefit of this success is that these captive populations can be self-perpetuating and not dependent on importation of animals from the wild. However, due to limited space and resources, zoos cannot allow uncontrolled reproduction.

In addition, zoos give high priority to proper genetic management, that is, reduction of inbreeding and balanced genetic representation of the founders of captive populations. Both genetic management and limiting production of surplus animals are being accomplished through contraception.

Although physical separation of males and females can prevent unwanted conceptions, most zoos consider this an undesirable measure. Not only can such an arrangement be stressful for species in which males and females typically associate, lone animals present an unnatural view of that species to the visiting public. A primary mission of zoos is education, a mission that is better served by maintaining animals in social groupings which are as representative as possible of the organization that would occur in the wild.

In recognition of the importance of contraception in responsible animal management, the American Association of Zoos and Aquariums approved formation of the Contraception Advisory Group in 1989. Composed of zoo curators, veterinarians, and reproductive physiologists, the group surveys the use of contraception in zoos for inclusion in a computerized data base, advises zoo personnel, and initiates and coordinates research into alternative methods.

The International Wildlife Contraception Database, housed at the St. Louis Zoo, was developed and is maintained by Ingrid Porton and Betsy Hornbeck of the Contraception Advisory Group. Begun in 1990 and updated from yearly surveys, it currently contains more than 2,000 complete records on 184 species and serves as a resource for zoo animal managers and for researchers.

Contraception of captive wildlife in zoos began in the mid-1970's. After preliminary tests with lions and tigers (Seal et al. 1976, Seal and Plotka 1978) comparing medroxyprogesterone acetate (Provera,® The Upjohn Co., Kalamazoo, MI, USA) and melengestrol acetate (MGA, also Upjohn), MGA in silicone implant form was selected as the more suitable. Of the more than 80 percent of North American zoos that reported using contraception (Porton et al. 1990), most use the MGA implant.

However, concern has arisen about possible pathology in progestin-treated carnivores (Kollias 1988, Kollias et al. 1984) because deleterious effects have been associated with progestin use in domestic dogs and cats (reviewed in Asa and Porton 1991, Asa et al. 1996). Indeed, histological examination of uteri from MGA-treated felids has revealed significantly more pathology than those from untreated felids (Munson and Mason 1991).

No comparable effects are expected in primates, based on the extensive studies that have been conducted in laboratory primates, as well as decades of progestin administration to human females. In general, birth control methods that have been approved for human use should be safe and effective for nonhuman primates. Birth control pills (combination progestin and estrogen, various formulations commercially available), Depo-Provera, Norplant (levonorgestrel implant system, Wyeth–Ayerst, Philadelphia, PA, USA), and intrauterine devices have been used in primates, especially in the great apes (Porton et al. 1990). However, few data exist on progestin effects in ungulates and other mammals.

A number of research trials are under way to evaluate new approaches with the hope of providing a broader selection of contraceptives, especially alternatives to the progestin-based formulations for carnivores. These include vas plugs, the antispermatogenic compounds bisdiamine and indenopyridine, and short-term Depo-Provera for seasonal breeders. Additional work is focusing on specific aspects of MGA treatment. For a discussion of the progress of zona pellucida trials in zoos, see Kirkpatrick et al., this volume.

Melengestrol Acetate

As mentioned, MGA is the most frequently used contraceptive for captive mammals. MGA is incorporated into silicone rods by E. D. Plotka (Marshfield Medical Research Foundation, Marshfield, WI) and distributed to zoos throughout the world. When inserted subcutaneously, the implants provide effective contraception for at least 2 years (Porton et al. 1990). The only side effect noted in primates has been weight gain (Portugal and Asa 1995), which is also common in human females treated with progestins.

Because of concern about possible effects on social behavior, a study was conducted with a troop of hamadryas baboons *(Papio hamadryas)* at the St. Louis Zoo (Portugal and Asa 1995). Administration of MGA was not associated with social disruption. Treated females were involved in fewer affiliative interactions, but there was no increase in aggression compared to control animals.

Although MGA implants have been effective in a wide range of monkeys and apes, some New World primate species have proven resistant. Because they have naturally high levels of endogenous steroids, these species may require larger exogenous doses to achieve contraception. Research is being conducted to test this hypothesis (E. Plotka, pers. comm.).

Recognizing that individual capture and immobilization for implant insertion may be inadvisable in some situations, Bronx Zoo veterinarians (B. Raphael, pers. comm.) have tested adding MGA to feed for herds of antelope and deer. Although this procedure is generally successful, drawbacks include contraceptive failure in some subordinate animals that apparently did not ingest a sufficient dose and alteration of the antler cycle in male barasinga *(Cervus duvauceli)*.

Pathological effects of MGA and related progestins are being investigated by Linda Munson (University of Tennessee, Knoxville). Early work concentrated on reproductive tracts of felids (Munson and Mason 1991), and research is now extending to other carnivores and primates. In general, progestins have been found to stimulate growth of the uterine lining of felids and canids, resulting in hyperplasia, pyometra, and neoplasia (reviewed in Asa and Porton 1991). Effects of dosage, length of treatment, and age during treatment are currently being studied.

Depo-Provera

The belief that side effects can be minimized by a shorter treatment period has created interest in the progestin medroxyprogesterone acetate in its injectable, slow-release formulation. In particular, seasonal breeders that are fertile for only part of the year might benefit from progestin exposure for only that period, as opposed to the continual, long-term exposure imposed by an implant. An injection every 2–3 months is thought to be preferable to the repeated surgical procedures required for implant insertion and removal.

Dose/response trials and evaluation of weight gain have been conducted with ruffed lemurs *(Varecia variegata)* and black lemurs *(L. macaco)* at the St. Louis Zoo, in collaboration with the Henson–Robinson and Metro Toronto zoos. Using vaginal cytology to monitor suppression of cycles, researchers found the minimum effective dose to be 5 mg per kilogram of body weight. Pelage darkening in treated black lemur females was an unexpected side effect, probably related to the ability of this progestin to bind androgen receptors (Labrie et al. 1987). (Black lemurs are sexually dimorphic in color, with males having a black and females a brown coat).

Depo-Provera also is being used in hippopotamuses *(Choeropsis liberiensis),* giraffes *(Giraffa camelopardus),* sea lions *(Zalophus californianus),* and gray seals *(Halichoerus grypus),* although not part of controlled research projects. There is concern about sea lions and seals, in particular, because they are closely related to the carnivores and because they have extensive fat stores that may absorb and hold steroids, which are lipophilic. Fat stores may also present a problem with hippos.

Norplant

Although no controlled studies have been published, several zoos are using these implants, which contain levonorgestrel, a synthetic progestin related to both MGA and Provera. Its advantage over MGA is the much thinner capsules, which can be inserted with a trocar. Compared to the incision and sutures needed for the MGA implants, the small puncture site attracts less grooming and reduces the chance of loss.

Birth Control Pills

As with Norplant, some zoos opt to use oral birth control pills, another product developed for the human market. The willingness of many apes to take pills placed in food treats dispenses with the need for immobilization and insertion of an implant. The vast majority of pills contain estrogen in combination with a much lower dose of progestin than is present in the progestin-only forms discussed above. Although this combination does not significantly alter the associated side effects for primates, it is not appropriate for carnivores. Adding estrogen to progestin in formulations given to dogs exacerbates uterine pathology (Teunissen 1952).

Megestrol Acetate

Another synthetic progestin—available as Megace® (Mead Johnson Laboratories, Evansville, IN, USA) or Ovaban® (Schering–Plough, Union, NJ, USA)—is sometimes used as a contraceptive in domestic dogs and cats. However, because of the attendant risk of hyperplasia and pyometra, this synthetic it is not recommended for more than two consecutive treatments.

Lupron Depot®

An agonist of GnRH (gonadotropin-releasing hormone also called LHRH, luteinizing-hormone releasing hormone), Lupron Depot (TAP Pharmaceuticals, North Chicago, IL, USA) has been used in males of several species in attempts to block spermatogenesis. Although theoretically tenable, this approach has been unsuccessful due to incomplete suppression of sperm production. Sperm numbers must be reduced below the level required for fertilization for this to be an effective contraceptive. Because the GnRH agonists and antagonists block production of testosterone, they

also can find application in suppression of aggression in some individuals.

Lupron Depot also has been given to females to suppress cyclicity. However, because agonists first stimulate the endocrine cascade that results in ovulation, administration to induced ovulators, such as the felids, may stimulate ovulation and pseudopregnancy before suppressing cycles by negative feedback.

Although analogues show promise in providing the equivalent of reversible chemical castrations, their current cost prohibits for widespread use. Long-term delivery also is a problem. Because these compounds are not orally active and do not follow the same diffusion dynamics as steroids, traditional delivery methods such as silicone implants have not been effective. Both a silicone elastomer matrix and a reservoir system are being tested (Vickery et al. 1989).

LHRH Vaccine

Immunization against LHRH can provide contraception for both males and females (Fraser 1986). LHRH initiates the cascade of hormonal events that results in testosterone and sperm production in males and in production of estrogen and progesterone and ovulation in females. Because this vaccination would accomplish the equivalent of a reversible chemical castration, it can be especially appropriate for males to suppress testosterone and thus aggression and for female carnivores to completely suppress secretion of progesterone. Vaxstrate, an LHRH vaccine that is commercially available in Australia (Arthur Webster Pty. Ltd., New South Wales, Australia) for domestic cows (Hoskinson et al. 1990), has also been used with success in some exotic species at the Western Plains and Perth zoos (D. Blyde and S. Haigh, pers. comm.). In this country, both The Population Council in New York and Colorado State University are developing LHRH vaccines.

Bisdiamine

All the steroid hormone preparations in use in zoos target the female. However, if prevention of reproduction is desired in polygynous social groups, it is more efficient to treat the males than the females. The bisdiamine WIN 18,446 (Sterling Winthrop, Rensselaer, NY, USA) was tested in the early 1960's as a birth control pill for men. It works by selectively interfering with spermatogenesis but not testosterone production. Although initial trials demonstrated the drug to be effective, safe, and reversible, it was soon discovered to interact with an enzyme that detoxifies alcohol. Thus, men taking bisdiamine who then drank alcohol became ill, making the compound unsuitable for general marketing. Because we assume that we can prevent alcohol consumption in captive animals, bisdiamine may be a feasible contraceptive alternative for this application.

The first test of bisdiamine in a wildlife species was conducted with a captive colony of gray wolves (Asa et al. 1995). Daily administration in ground meat at a dose of 200 mg/kg suppressed spermatogenesis without affecting mating behavior. In the subsequent breeding season, semen samples of the previously treated males were comparable to those of controls, confirming reversibility.

Indenopyridine

This drug (Research Triangle Institute, Research Triangle Park, NC, USA) is similar to bisdiamine in that it is orally active and blocks sperm production without interrupting testosterone secretion. To date, it has been tested only in rodents (Fail et al. 1991, Gurtler and Donatsch 1979, Hodel and Suter 1978), so the extent of its efficacy and safety has not been adequately determined. A dose response trial with domestic cats, as a model for exotic felids, is currently being conducted (Fail et al., unpubl.)

Vas Plugs

L.J.D. Zaneveld and I investigated vas plugs. Silicone injected into the vas deferens, the tube carrying sperm from the testis, hardens to form a barrier that prevents the passage of sperm. Plugs were placed in 59 mammals, including marsupials, felids, primates, and ungulates, at 17 different zoos. Early use of the preformed plug (Zaneveld et al. 1988), which is also in clinical trials with humans, proved inadequate for the range of vas sizes encountered in zoo animals. The injectable plugs were successful in blocking passage of sperm, but fertility was not restored after removal.

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Contraception in Wildlife Management: Reality or Illusion?

David C. Guynn, Jr.

Abstract: Nuisance wildlife in areas where hunting is not an accepted practice and declining public support of lethal control measures have prompted research on contraceptives as a way to manage population levels. However, complex legal, biological, economic, and ethical issues should be addressed before such techniques are tested even on small, isolated populations. Regulatory authority by State and Federal agencies must define protocols for using contraceptive materials in wild populations. Registration of wildlife contraceptives either as pesticides or vaccines will likely be necessary. Health-related issues include harmful effects on target species, nontarget species and humans who may consume carcasses. Models for evaluating population impacts and genetics are needed. Cost effectiveness itself and who will pay these costs must both be considered. Disruption of behavioral mechanisms and resulting population impacts raise ethical considerations. Contraception may have application with limited, isolated or confined populations, but its eventual use on free-ranging wildlife populations is questionable.

Keywords: Wildlife contraception, State and Federal regulations, impacts on animal behavior

Nuisance wildlife, particularly high densities of white-tailed deer *(Odocoileus virginianus),* have become a problem in many areas of the United States (Warren 1991). Significant economic losses can result from damage to crops and landscape plantings and from deer–vehicle collisions. Regulated hunting can be an effective means of controlling deer populations (Behrend et al. 1970). However, problems in areas where hunting is not an accepted practice (e.g., national parks and suburban areas) and declining public support of lethal control measures have prompted research on contraception as a means of managing population levels. Recent studies on immunocontraception of free-ranging feral horses *(Equus caballus)* (Goodloe 1991, Kirkpatrick and Turner 1991) and deer (Turner et al. 1992, Warren and White 1995) indicate that an effective vaccine and oral delivery system could be developed. However, complex legal, biological, economic, and ethical issues should be addressed before such techniques are applied even on small, isolated populations. This chapter will attempt to identify some of the key points of these issues with focus on management of white-tailed deer.

Legal Issues

Although wildlife contraception is a potential management tool, contraception research is being conducted outside of the State and Federal agencies having primary responsibility for management of wildlife populations. Except for migratory species and species afforded protection under the Endangered Species Act, the State wildlife and fisheries agencies are empowered to manage wildlife populations. Each State has a unique set of statues and regulations defining legal utilization and protection of wildlife to include status as a hunted or nonhunted species, season lengths, bag limits, baiting and feeding, sale of animal parts, appropriate nuisance control methods, and use in scientific research. In some States, other legislative agencies dealing with domestic animals and veterinary practice may regulate use of wildlife contraceptives. The situation is further complicated by land ownership patterns. A recent report by the Southeast Deer Study Group (1993) indicated that 90 percent of the white-tailed deer habitat in the 16 member States is in private ownership. Thus at the State level, there is concern whether current regulations and authorities adequately define control over determining when, where, and how contraceptives may be used with wildlife populations. Most States would probably need new legislation to clarify issues pertaining to permitting, reporting, training and qualification of personnel, and protocols for administering contraceptives to specific wildlife species.

Uncertainty also exists concerning regulation of wildlife contraceptives by Federal agencies. The Subcommittee on Wildlife Contraception of the International Association of Fish and Wildlife Agencies reviewed regulatory authority over these drugs (Southeastern Cooperative Wildlife Disease Study Group 1993). The subcommittee reported that no registration

of a wildlife contraceptive vaccine either as a pesticide (U.S. Environmental Protection Agency) or a vaccine (U.S. Department of Agriculture or U.S. Food and Drug Administration [FDA]) has been applied for or approved. Mallory (1993 unpubl.) stated that wildlife contraceptive vaccines are regulated by the Center for Veterinary Medicine at FDA. The Food, Drug and Cosmetic Act of 1938 (FDCA) requires FDA approval before marketing any drug not generally recognized as safe. A new animal drug is presumed unsafe with respect to any particular use or intended use unless an application pertaining to such use or intended use is approved by FDA. In general, approval of a new animal drug application by the FDA is a lengthy and expensive process.

Biological Issues

Health-related issues concerning use of wildlife contraceptives include effects on target and nontarget species and effects on humans who consume carcasses or have other contact with contraceptive materials. Nettles (1993 unpubl.) identified the following concerns about use of contraceptives in white-tailed deer; however, many of these concerns would apply to other species as well:

1. Will contraceptives cause females to experience an abnormal number of estrous cycles, expending stored energy and increasing predation on deer?

2. Will males expend themselves by repeatedly breeding sterile females that are constantly recycling?

3. What effects will contraceptives have on pregnant animals concerning abortion, fetal resorption, uterine infection, birthing difficulties, and lactation failure?

4. What effects will contraceptives have on prepubertal animals concerning permanent sterility and growth defects?

5. What effects will contraceptives have on sex characteristics such as antler cycles?

6. An antisperm membrane vaccine for deer is under study (White et al. 1993). Will vaccinated does exposed to deer sperm experience anaphylactic shock? Will orchitis, epididymitis, or anaphylaxis occur in males inadvertently injected with antisperm vaccine?

7. Will remote injection or implantation of contraceptives cause traumatic injury problems or infection?

McShea et al. (1994) report that immunocontraception of does has dramatic effects on mating season and activity budgets of white-tailed deer. In that study, 30 does were captured from a wild population and porcine zona pellucida was remotely administered by darts to 20 does during October 1992. The 30 does were exposed to 5 bucks from November 1992 through March 1993. Although control does mated in December, contracepted does exhibited estrus behavior through February. Whereas locomotion constituted 18 percent of the activity budget of control does, it constituted 32 percent of the activity budget of contracepted does and 39 percent of the activity budget of males.

Nettles (1993 unpubl.) reports that although wildlife contraceptives currently being evaluated for deer are delivered by injection or implant, the final goal is to have an oral vaccine. Such an oral vaccine would probably be genetically engineered and would use a live virus or bacteria as a carrier. But there are several potential hazards associated with this approach:

1. The carrier virus or bacteria could be pathogenic to the target or nontarget animals. This concern would include safety of vaccinated animals for human consumption.

2. The carrier organism could be highly transmissible from the initial vaccinate to secondary nonspecific animals. This situation could result in a reproductive disease that—once introduced—might be impossible to remove from a wild population.

3. In the carrier organism, a genetic reassortment or mutational change might occur that would increase virulence and/or transmissibility.

Other concerns have been expressed concerning impacts of contraceptives at the population level (Nettles 1993 unpubl.). The efficiency of immunocontraceptives is dependent upon an effective immune response in the target animal. When contraceptive vaccines are administered, the animals with the best immune systems will be the most susceptible to

Table 1. Reported harvest of white-tailed deer in Jasper County, SC (1974–94)

Year	Club areas reporting	Antlerless tags issued	Bucks harvested	Does harvested	Total harvested	Harvest rate (deer/mi^2)
1974	39	—	2,120	687	2,807	5.8
1980	67	—	1,669	915	2,584	5.3
1985	62	1,706	1,853	1,016	2,869	5.9
1988	82	2,468	2,228	1,800	4,028	8.3
1990	19	3,824	2,381	2,536	4,917	10.2
1993	79	4,029	2,239	2,837	5,096	10.5

sterilization while those with the poorest immune systems will be the most refractory. Thus, the deployment of contraceptive vaccines could shift the gene pool in favor of immunodeficient animals with resultant increased susceptibility to pathogenic organisms. Another concern is the capability of a contraceptive-treated population to respond to a natural disaster that would not be selective in regard to sterile *v.* fertile animals. Thus, a contraceptive-controlled population could theoretically be pushed to the brink of extinction directly or through creation of a genetic bottleneck.

Potential impacts are not limited to the target species. The reproduction of nontarget species that consume oral contraceptives placed for target species or that consume carcasses of target species through predation or scavenging could be affected. Populations of predators or scavengers that use the target species as a food source could be reduced. There is also concern over the safety for humans who use contraceptive-treated animals for food, particularly with implanted materials, or for people who have particular sensitivity to drugs, such as pregnant women with potential impacts on a developing human fetus.

Economic Issues

Although the effectiveness of experimental treatments with contraceptives of wild populations of feral horses looks promising (Kirkpatrick 1993, Kirkpatrick et al. 1990), two important questions must be examined before application to wild populations of ungulates is considered: (1) What proportion of the populations must be treated, and (2) How much will it cost? The management of white-tailed deer populations in Jasper County, SC, will be used to illustrate the relevance of these questions.

Jasper County is located within the Coastal Plain of South Carolina. Land use is predominantly agriculture and forestry. Deer densities are estimated to be as high as 1 deer/5 acres in some areas (Lewis Rogers, pers comm.), and deer-caused damage in this area has reportedly caused repeated crop failures.

Expenditures for recreational hunting contribute significantly to the local economy. The annual economic impact of hunting on private land in Jasper County during 1990–91 was estimated at $9 million (Richardson et al. 1992). The deer-hunting season in this area extends from August 15 to January 1 with no limit on antlered bucks. Antlerless deer may be taken by permit from October 1 to January 1. Harvest trends from 1974 through 1993 reflect efforts by the South Carolina Department of Natural Resources to curb increases in deer density (table 1). During this period, total reported harvest nearly doubled while doe harvest increased fourfold.

Several studies suggest that 35–40 percent of adult does must be removed annually to stabilize a deer population at levels substantially below (60–70 percent of) carrying capacity (McCullough 1979, Downing and Guynn 1983, Guynn 1985). Thus, it can be assumed that 35–40 percent of the adult does would have to be treated annually with contraceptives to achieve this same level of population regulation.

About 25 percent of the total doe harvest in Jasper County is fawns; thus, of the 2,837 does reported harvested in 1993 (table 1), about 2,128 were adults. The current level of harvest does not appear to be constraining populations within acceptable levels; obviously, treating less than 2,100 adult does with contraceptives every year would not alleviate crop depredation problems. Administering contraceptives with darts to this number of animals would be impractical. An oral delivery system would be needed.

The costs of administering a contraception program plus the forgone economic losses from reducing or eliminating a major recreational hunting opportunity would be substantial. Who would pay the costs—Federal agencies, the South Carolina Department of Natural Resources, the county, landowners, or the members of the local community? It is doubtful that any or all of these groups collectively would be able to pay for such a program. The overall impact of attempting wildlife contraception as an alternative to sport hunting for managing deer populations in Jasper County could easily exceed $10 million annually.

Ethical Issues

Species such as the white-tailed deer have evolved with complex behavioral mechanisms that keep populations and their individual members fit and competitive. The disruption of these mechanisms and the resulting population impacts imposed by sport hunting, contraception, or any other management practice should concern everyone. Preservation of the natural processes that define free-ranging populations of wildlife should concern everyone as well as the welfare and death of individuals. A large part of this dilemma can be attributed to the way in which people view the natural world.

In a video for the American Forest Council (1991), Gustare Repie discussed the forest archetype of American culture. An archetype is simply the way people think about any one certain idea or object in a given culture. As an illustration, he described the failure of marketing French cheese products in the United States. In France, cheese is displayed in the open without refrigeration. Customers can smell, feel, and taste the cheese, buying whatever amount they desire. Cheese is a living thing to the French. In contrast, Americans are accustomed to seeing cheese highly processed, wrapped in cellophane, and refrigerated. Cheese is dead. Marketing cheese in American stores in the typical French manner was offensive to Americans and sales of the product were a dismal failure.

Repie's forest archetype assumes three perceptions: the natural forest, the managed forest, and the jungle. The natural forest perception resembles the fantasy of Disneyland—there is no death, predation is bad, there are no humans, and the hand or influence of humans is unseen. Humans constitute a visible part of the managed forest with destruction, cutting of trees, and exploitation being the norm. Connotations include killing of the bison and removal of the Native Americans from their homelands, for example. In the jungle is the true natural forest—every living thing is subject to death, competition for basic resources is universal, and humanity is at best an abstract concept. Few Americans appreciate this perception, especially if humans are viewed as part of the jungle rather than separate from the jungle.

If they deliberately tamper with natural interactions, scientist-managers must be careful to consider all the impacts that any management approach may have on individual species of wildlife and the ecosystems in which they live. Consideration must be given to species populations and their function as well as the humane treatment of individuals. Those who utilize contraception methods must convey the limitations of contraception as well the positive attributes so that society does not view this tool as a cure-all for wildlife management problems.

Conclusion

Is wildlife contraception a reality or an illusion? I conclude that it's both. The technology exists to make wildlife contraception a reality for controlling populations of large ungulates on small confined areas such as zoological settings and islands. The potential in

these situations to prevent environmental damage and provide esthetic benefits is great. As a generalized tool for managing species with high reproductive rates, such as the white-tailed deer, in unconfined free-ranging populations, contraception is currently an illusion. Even if the technology were currently in place, the legal, biological, economic, and ethical issues that must be considered will likely require decades for resolution.

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Human Dimensions of Contraception in Wildlife Management

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Introduction

Wildlife damage management was so much simpler in the good old days. If deer *(Odocoileus viginianus),* beaver *(Castor canadensis),* or other animals were a problem in a particular situation, people simply had them shot, trapped, or poisoned. Not many years ago, most people would go along with this approach, and those who didn't like it were marginalized as the "radical fringe." Not so today. Greater and more diverse segments of the public want a say in what professionals decide to do with *their* wildlife. The public wants to participate in setting objectives for management and in approving the methods for accomplishing those objectives. Kania and Conover (1991) emphasized that wildlife agencies should respond to these societal changes rather than resist them, thereby enhancing the value of the wildlife resource for *all* people. Changes in sociopolitical values have resulted in more stakeholder groups who want to be included in wildlife management decisions today than at any other time since the advent of applied wildlife management in North America (Curtis and Richmond 1992).

Although public attitudes and beliefs regarding wildlife have always been dynamic, public interest in wildlife and desire for input into management of wildlife have increased since the early 1970's. In response to this phenomenon, an area of social science inquiry and application to management has developed within the wildlife management profession—the human dimensions of wildlife management. Basically, human dimensions efforts focus on identifying what people think and do regarding wildlife, understanding why, and applying that understanding to the wildlife management decisionmaking process.

Some wildlife management professionals operating in the human dimensions arena have advocated the notion that we are now working within a new paradigm for management, one that strives to integrate the biological and human dimensions of wildlife management for improved decisionmaking and objective accomplishment (Decker et al. 1992). This represents a philosophical and pragmatic shift from an approach where biological science was the primary source of information for decisionmaking and the pervasive public sentiment of the time was in line with management professionals' values (Decker et al. 1991). However as a diversity of stakeholders emerged, wildlife managers were confronted with conflicting points of view. Under the new paradigm, social and biological information, as well as management experience, are part of the information base used in decisionmaking (Decker et al. 1992). This contemporary paradigm for wildlife management recognizes that decisionmaking occurs in an environment having sociocultural, economic, physical, legal, and administrative aspects, as well as biological components (Decker et al. 1992, Slate et al., 1992).

The new paradigm also includes consideration of the human dimensions when determining goals and objectives for management and in measuring outcomes of specific actions (Knuth and Nielsen 1989, Decker et al. 1992). In contemporary wildlife management, we recognize that many people representing a variety of views are legitimate stakeholders in management. Some of these people have no particular "use" for wildlife (i.e., food, recreation, or other utility). They may simply value wildlife for esthetic attributes or other nonconsumptive values. Thus, several different human values, beliefs, and attitudes (Kellert 1980) are playing an increasing role in establishment of wildlife management goals and objectives. Such human attributes are also playing greater roles in determining the social acceptability of management decisions and actions, including selecting and applying population control methods. In fact, Schmidt (1992) argues that natural resource management decisions, previously thought to be defined by science and economics, are driven by human values.

Knowledge concerning various stakeholders' reactions to conventional management approaches in nontraditional situations (i.e., wildlife management in

urban and suburban environments) is imperfect. Recent, accumulating experiences indicate that these nontraditional wildlife management settings call for innovative approaches, including the development and application of new technologies. However, new technologies need to be developed and applied on a limited trial basis, with an eye toward anticipating and evaluating social acceptability. As new technologies are being considered, one needs to ask whether the innovation hoped for is consistent with the beliefs and values of affected stakeholders. Although minority opinions can be problematic to management programs, these viewpoints can provide important balance to a decisionmaking or planning process.

The purpose of this paper is to begin discussion of the human dimensions of contraception in wildlife management that developers of this emerging technology should consider as biological research proceeds. We draw limited inference from literature about human values toward wildlife, and human use and management of wildlife, to the use of contraception in management. We also identify issues that managers and other decisionmakers who formulate wildlife policy should consider as they contemplate applying contraception as a wildlife management tool. Because no studies have focused on identification and explanation of people's beliefs and attitudes about this new technology, we caution that this discussion is exploratory, not definitive. We also identify additional research needs in the area of human dimensions that could provide valuable insight concerning wildlife contraception issues.

Wildlife Management Stakeholders

Researchers who are developing wildlife contraception technologies need to understand the views that stakeholder groups hold concerning the application of new contraception methods and why differences exist. Beliefs and values that underlie various perspectives and the acceptability of wildlife contraception should be considered during research and development, before too much time and money are invested in approaches that may later prove to be morally or ethically unacceptable.

For example, Turner et al. (1992) noted that female white-tailed deer treated with a porcine zona pellucida (PZP) vaccine continued to cycle after not becoming pregnant. It is possible that PZP treatment could affect long-term patterns of deer behavior and social organization. Deer that expend extra energy for breeding activities may not survive a harsh winter.

Moen (1976) noted that seasonal physiological changes occur, and deer conserve energy in winter by reducing their general level of activity. Consequently, deer should remain as undisturbed as possible during winter, and increasing the length of the breeding season will likely have serious impacts on seasonal changes in deer physiology.

These changes in deer reproductive biology raise serious ethical and management questions, and they may influence stakeholders' perceptions of this contraceptive technique. Stakeholders must understand the full range of effects that different contraceptive methods may have on deer populations before making decisions to accept or reject their use. Also, sensitivity to key stakeholders' values and beliefs during the development stages, prior to widespread field applications, are extremely important. The wildlife profession may spend millions of dollars developing and registering new contraceptive technologies yet still face public controversy if the interests and concerns of all stakeholder groups are not carefully considered and addressed in advance of implementation.

Who are key stakeholders in the wildlife contraception arena, and what can managers conjecture about stakeholders' opinions on contraceptive applications? Identification of stakeholders is an essential human dimensions component when considering various management options. Key stakeholders would be similar in population management situations whether wildlife contraception or other direct management methods (i.e., shooting, trapping, etc.) are being considered or used. The claims made by stakeholders may seem different, but the fundamental values that lead to their expressed views likely will be consis-

tent with those observed in past studies unless contraception technology taps into different values and beliefs. Stakeholders include wildlife management professionals, researchers developing the technology, industry representatives hoping to produce and market the technology, potential regulating agencies (e.g., the Department of Health and Human Services' Food and Drug Administration, the Environmental Protection Agency, State health departments, etc.), Federal and State land-management agencies, wildlife damage/nuisance control operators, people experiencing damage or wildlife-related health and safety risks, extension wildlife specialists, people concerned about animal welfare, animal rights advocates, elected public officials, hunters concerned about a competing management tool, environmentalists, taxpayers concerned about costs, media representatives, and religious leaders in communities. Depending upon the site-specific situation, this list of stakeholders could be expanded or condensed.

Anticipating Issues Regarding Contraception Technology

Animal Rights and Animal Welfare Concerns

Schmidt (1990) proposed a distinction between animal rights and animal welfare advocates that has great bearing on how we think different stakeholders will view wildlife contraception. Animal rightists fundamentally believe that animals should be extended rights similar to humans, because to do otherwise would constitute speciesism (Singer 1980). This belief differs from that of animal welfare supporters, who focus primarily on the humane treatment of animals, though these people may not believe that animals and humans have equal rights. Although most of the animal rights confrontations with wildlife management have focused on hunting and trapping, there are few indications that animal rights advocates would find contraceptive use in wildlife management much more acceptable philosophically. We speculate that denying animals the right to procreation, giving them no "say" in the decision, or manipulating individual animals to further human needs, seems to be as great a violation of the animals' rights (thinking of the human analogy) as taking their lives through hunting. Thus, we forecast no significant improvement in relations between wildlife managers and animal rights advocates because of contraception technology.

Animal welfare advocates will likely favor contraceptive technology if pain and stress to wildlife, unnecessary animal deaths, or other concerns about humane treatment of animals are minimized. Some momentary stress or pain will be acceptable if, on balance, contracepting wildlife will reduce mortality of animals by starvation, disease, motor vehicle accidents, selective culling, or other factors. However, opposition may mount if contraceptive materials affect animal breeding biology (e.g., late-born fawns, extending the buck rut into midwinter, etc.) or other behaviors that raise serious welfare concerns.

As something of an aside, we do see a new dynamic occurring relative to animal rights advocates and contraceptive technology issues. Unlike most battles between animal rightists and wildlife management advocates, where hunters bear the brunt of public scrutiny, it is likely that hunters may be spectators in many situations where contraception is being considered for application, especially in residential or park landscapes where hunting is less feasible. Values and beliefs of many suburban property owners (i.e., homeowners, motorists, gardeners, etc.) who desire relief from nuisance wildlife and are concerned about wildlife-related health and safety risks to people (e.g., Lyme disease, rabies, deer–vehicle collisions, etc.) will be opposed by groups who espouse the animal rights philosophy. Animal rights advocates may find themselves battling those people who previously have been part of the silent majority concerning wildlife management issues, rather than focusing on hunters and trappers.

Contraception v. Hunting

As the development of contraceptive methods moves forward, we anticipate wildlife contraception will affect public perceptions of the necessity for hunting. For perhaps 50 years, the wildlife profession has told hunters and the public at large that hunting is the most

cost-effective tool for management of overabundant wildlife populations. That statement has become a standard defense for the justification of regulated hunting. Of course, hunting is the primary method used for managing a few wildlife species (e.g., deer and elk *[Cervus elaphus]);* however, it has not been proven that regulated harvests can effectively control other wildlife populations. So what are the consequences for hunting if an effective wildlife contraception method is developed and society is willing to pay for its application? Will wildlife contraception signal the demise of hunting?

The future of hunting was recently discussed at the North American Hunting Heritage Symposium. Decker et al. (1993) asserted that the future of hunting lies in public understanding and acceptance of the sociocultural values related to hunting, not for its value as a form of recreation or a tool for wildlife population management. Thus, some researchers believe that whether or not contraception technology evolves to become economically feasible, the future of hunting is dependent upon other factors, such as maintaining a rural cultural tradition.

Contraception v. *Other Direct Lethal Methods*

Direct (e.g., shooting, kill-trapping, poisoning, etc.) and indirect (e.g., induced abortion, etc.) methods for lethal management of wildlife populations have been applied or tested experimentally in the past. Public acceptability of these lethal methods depends on the species of wildlife in question and perceived human health and safety risks. Based on a survey of homeowners *(n* = 391) with nuisance wildlife problems (Braband and Clark 1992), most respondents approved of lethal control for rats and mice (Cricetidae, 95 percent), moles (Talpidae, 79 percent), snakes (Serpentes, 74 percent), bats (Chiroptera, 71 percent), pigeons *(Columba livia,* 60 percent) and skunks *(Mephitis* spp., 57 percent). However, most people disapproved of lethal control for deer (70 percent), geese *(Branta canadensis,* 67 percent), woodpeckers (Picidae, 65 percent), and squirrels (Sciuridae, 59 percent). For many respondents, humaneness was equated with nonlethal control, and nearly 90 percent indicated that humane treatment of nuisance animals was important.

It seems certain that nonlethal methods would be preferred over direct lethal methods by animal welfare and other similar stakeholder groups if the nonlethal approaches are equally effective and carry similar costs. It is also likely that lethal methods which have added benefits would be preferred over lethal methods that have no added value (e.g., recreational hunting would be preferred over poisoning). This idea of relative acceptability has not been adequately investigated and deserves future inquiry. Increasing professionals' understanding of public acceptability of various lethal and nonlethal methods would be useful in management decisionmaking and in developing research agendas to meet future wildlife management needs of society. The findings of such a line of inquiry might also be useful for creating educational programs concerning tradeoffs about which approaches to take in various situations.

So far in this discussion, we have treated wildlife contraception as a nonlethal management technique. Yet even this assumption may be questioned by some stakeholders in wildlife management decisions. This is both a value-based (Decker et al. 1991) and biologically based judgment. Prevention of conception may be equated with the "unnatural" death or management of an animal by some segments of society. Even when groups of animals such as deer are trapped and transported to another location, some mortality typically occurs during transport. Defining what are lethal *v.* nonlethal techniques may not be as obvious as initially expected. Such thoughts call for additional public retrospection about the value of "wild" in wildlife populations.

Contraception v. *Nonmanagement*

A segment of society supports the nonmanagement viewpoint. That is, some people believe that humans should simply "leave nature alone" and "learn to live with wildlife." This perspective does not fully recognize the immutable impacts that humans have had, and will continue to have, on the environment for

centuries to come. Certainly, the "leave it alone" perspective is attractive because proponents believe that wildlife populations will take care of themselves; however, the consequences of wildlife extinction or overpopulation may damage both ecosystems and people. Regardless, wildlife contraception would likely be unacceptable to many nonmanagement advocates, as would any other wildlife management tool. Any purposeful intervention by people to manipulate wildlife could be viewed as altering the "naturalness" or "wildness" of animal populations. However, faced with animal overabundance and deterioration of habitat quality, research and management professionals from some areas (e.g., national parks and wildlife refuges, State parks, etc.) that have typically been managed under a natural-systems approach are actively exploring the development and application of contraceptive technologies to control large ungulates (Kirkpatrick et al. 1990, Turner et al. 1992).

Public Beliefs and Values About Wildlife and Wildlife Management Via Contraception

Our literature review uncovered no research concerning public attitudes toward contraception in wildlife. Position papers and other nontechnical writings abound, but we were unable to find a single comprehensive study describing the nature and basis for public attitudes toward either wildlife or human contraception. Similarly, we were unable to find studies of people's attitudes about contraception in companion animals. Thus, we draw entirely on research about human attitudes toward wildlife and various uses of animals when discussing attitudes and beliefs that likely will be pertinent in assessing the degree of public acceptability for contraception in wildlife.

It's important to remember that people's beliefs and attitudes about wildlife are formed, exist, and change in a context of broader attitudes and values concerning several domains of their lives. For example, people's broader world view concerning what constitutes appropriate human interaction with the environment or nature has profound effects on how people view human–wildlife interactions. Wildlife-associated attitudes and values are also related to other major world views, such as religious beliefs, beliefs about safety and security (both physical and financial) of family and community, and beliefs about individual freedom of choice in dealing with problems (i.e., those caused by wildlife).

Studies by the Human Dimensions Research Unit in the Department of Natural Resources at Cornell University have examined the wildlife-related attitudes and values of thousands of people on a variety of subjects during the last 15 years. Based on these studies, a Wildlife Attitudes and Values Scale (Purdy and Decker 1989) was developed and applied in over a dozen studies. Essentially, this work identified the existence of three broad dimensions of public attitudes toward wildlife: wildlife use, wildlife preservation, and wildlife damage/nuisance tolerance.

The wildlife-use category includes a traditional wildlife conservation philosophy that supports use of wildlife for human benefits and management to accomplish such purposes. Attitudes and values associated with hunting, trapping, and similar activities would be reflected in this dimension.

The wildlife-preservation category embodies concerns for individual animals and for their continued existence in nature. Animal rights notions would be on the extreme end of this set of attitudes and values.

The wildlife-problem-tolerance set of attitudes and beliefs is interesting conceptually because they discern that people have a wide range of acceptability of various human–wildlife interactions. Other research and observation lends credence to the existence of thresholds of tolerance for wildlife-caused problems depending upon economic or health and safety risks. For example, some people will incur a high level of economic damage from wildlife before they find the tradeoff tips toward wanting relief. Damage tolerance has been documented for both farmers (Decker and Brown 1982, Decker et al. 1984) and homeowners (Sayre et al. 1992). However, when the perceived risk of health and safety problems associated with wildlife (e.g., rabies, Lyme disease, motor vehicle accidents, etc.) reaches even modest levels, tolerance of wildlife

presenting the risks is reduced markedly (Connelly et al. 1987, Stout et al. 1993). A recent survey of Tompkins County, NY, residents indicated the perceived risk of being involved in a deer–vehicle accident, along with attitudes toward deer and the degree of personal involvement with deer–vehicle collisions, predicted the likelihood that a person would support reducing the local deer population (Stout et al. 1993). Results from this New York experiment suggest that people change their attitudinal orientation if perceived risks of economic loss or health and safety impacts exceed certain thresholds of tolerance (which need further assessment for precise estimation).

To learn more about public attitudes toward a variety of deer-management alternatives in a suburban environment, we conducted a survey of property owners in the greater Rochester, NY, metropolitan area. The paucity of scientifically obtained information documenting people's beliefs about contraception in wildlife management, and lack of management experience in this new arena, encouraged us to explore these issues. The survey instrument included several questions concerning contraceptive management of a locally overabundant deer herd. Public attitudes and values related to the acceptability of contraception as a deer management technique are discussed further below.

Identifying Public Acceptance of Contraception: A Pilot Study

Wildlife managers considering the use of contraception for resolving wildlife problems need knowledge of the specific attitudes held by stakeholders in a given management situation. The greater Rochester area was selected as the site for a pilot study because of a long-standing deer-management controversy surrounding Durand Eastman Park and implementation of a public involvement process for setting deer management objectives (Curtis et al. 1993).

To determine the attitudes of suburban residents toward deer management, a questionnaire was sent to 1,590 residents living in the Rochester area during 1992. Questions were developed with input from New York State Department of Environmental Conservation (DEC) staff. The primary objectives of the survey were to assess public attitudes about deer, perceptions about deer-management methods, and the public acceptability of various management options, including contraception.

Approximately 750 residents completed the questionnaire (a 47-percent response rate). A followup phone survey of people who did not respond to the questionnaire indicated that many people were either not interested in deer-management issues or had difficulty understanding the questions and concepts. The majority of respondents selected either contraceptive methods, managed hunting, or trapping and releasing deer to the wild as their preferred deer-management option.

People who supported contraception were more interested in minimizing the suffering of deer than respondents who did not support contraception. Respondents who thought deer contraception was an extremely acceptable management option were also more likely to be dissatisfied with DEC's deer-management program and tended to agree with the statement that "herd size should be guided by nature alone."

Important considerations of those opposed to contraception included maximizing hunting opportunity and minimizing economic costs to society. In addition, people who were satisfied with DEC's deer-management approach were more likely to view contraception as unacceptable.

The credibility of 21 potential sources of deer-management information was associated with the acceptability of deer contraception as a preferred management option. People who selected contraception as their preferred option tended to rate the Humane Society of Rochester, Save Our Deer, and Helmer Nature Center with greater credibility than respondents who preferred other deer-management methods. Conversely, those who did not select contraception ranked the local hunting club and the Irondequoit Deer Action Committee with greater credibility. However, DEC ranked as the single most believable source for deer information among both supporters and opponents of deer contraception.

It is not surprising that respondents who were interested in maximizing deer-hunting opportunities and reducing economic costs were generally opposed to contraception. Because hunting is the primary method used by DEC to manage deer in New York, respondents who were satisfied with DEC's deer-management program were also more likely to view contraception as an unacceptable alternative. However, it's important to note that about 50 percent of respondents selected either minimizing human health and safety risks or maintaining a healthy deer population as the most important deer-management consideration, regardless of whether respondents supported or opposed contraception.

Research Needs for Human Dimensions of Wildlife Contraception

This pilot investigation of citizens' attitudes toward deer contraception can contribute to a broader understanding of public beliefs about contraception in wildlife. In similar situations, it's important to identify relevant stakeholder groups along with their size, position on the issue, salience of the issue to them, perceived stake in the issue, power in decisionmaking (political influence), knowledge of the issue, and socioeconomic and demographic characteristics. Inquiry must go beyond description because wildlife managers and policymakers need to know why people hold various beliefs and attitudes and if these attitudes are based on accurate perceptions of wildlife ecology and contraceptive techniques. That information will help professionals identify the extent and nature of educational communications need. Also, it's useful to know how rigid or malleable attitudes are. Obviously, educational programs can influence change only if the public's attitudes are flexible.

One limitation of our pilot study is that we painted contraception for deer management with a very broad brush and did not define specific technologies or delivery systems. For example, delivering contraceptive vaccines via oral baits, dart guns, "bio-bullets," or arthropod vectors may have characteristics that tap into different underlying values held by various stakeholder groups. Also, specific technologies (i.e., using genetically recombinant proteins or genetically altered viruses, etc.) for developing vaccines for reproductive inhibition may be unacceptable to some publics. Mammalian reproductive biology is similar across species, and the chance of mutations in genetically altered viruses may pose substantial risk. Consequently, when examining attitudes and beliefs of people toward contraception in wildlife management, it will be extremely important to identify both the specific material and delivery system that will be used and to be certain that stakeholders understand how they work.

Public Involvement Strategies for Making Management Decisions

In addition to human dimensions research, increasingly the wildlife management profession is finding that public-involvement techniques are helpful in reaching community consensus on controversial wildlife management issues (McMullin and Nielsen 1991, McAninch and Parker 1991, Nelson 1992, Curtis et al. 1993, Stout et al. 1993). Conceived carefully and implemented effectively, citizen participation strategies present educational opportunities, improve agency image as being responsive to stakeholder needs, and lead to more acceptable, if not universally embraced, decisions and actions to solve management problems (Stout et al. 1993). Different public-involvement models have been used in Minnesota (McAninch and Parker 1991) and New York (Curtis et al. 1993), and these can be assessed for suitability and adapted to fit other situations. In New York, the work of citizen task forces was greatly enhanced by the availability of systematically collected human-dimensions data gathered from the community at large or from members of specific stakeholder groups. Results from systematic, ongoing evaluations of citizen participation activities can be used to feed into and improve the process as it is being carried out and are invaluable for effectively managing the process (Stout et al. 1992).

Human dimensions studies and citizen participation strategies take time and money, but there is no indication that these costs are any larger than those incurred when such strategies are not included in the development of management policy. The difference is that proactive efforts are more predictable and manageable than the time and cost of reacting to problems after an unacceptable decision is made and the management agency has to resort to the typical "damage control" mode of operation.

A special group of stakeholders should be the focus of immediate inquiry—wildlife management professionals. Whether it's wildlife contraception or any other innovation or deviation from traditional approaches to wildlife management, members of the profession are extremely important stakeholders. These professionals have the credibility to scuttle innovation or to accelerate its adoption. Some members also have loyalties to the conventions of the profession, and basic beliefs and values are fundamentally difficult to alter (Sanborn et al. 1994). We believe that the advent of contraception in wildlife management may signal a significant change in the way wildlife managers do business. If that prediction is on track, then it is clear that resistance to contraceptive technology will emerge. Thus, we believe it is important to understand the attitudes of members of the wildlife management profession on this topic. Publications such as this facilitate discussion, reveal positions, etc.; however, we also need empirical analyses to help the profession grapple with contraception in wildlife management and related issues looming on the horizon.

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Thunder in the Distance: The Emerging Policy Debate Over Wildlife Contraception

R. Bruce Gill and Michael W. Miller

Abstract: Wildlife contraception is only now emerging as a wildlife policy issue. It will emerge into a sociopolitical environment that is already polarized from a clash of ideologies. The wildlife conservation/hunting community strives to preserve the status quo while animal welfare and animal rights activists struggle to change wildlife management philosophy and practice to conform to their respective beliefs. Recent professional and popular literature reveal at least four major areas of conflict: (1) antimangement sentiment, (2) antihunting sentiment, (3) animal rights sentiment, and (4) animal welfare sentiment. Wildlife managers anticipate that the conflict over the use of contraceptives will involve value and belief conflicts between traditional wildlife management and animal rights proponents. We believe instead that the primary conflicts will revolve around pragmatic issues such as when, where, and in which circumstances managers will use the contraceptive tool. In this context, wildlife contraception will be regarded as a "mixed bag." Given the nature and potential polarity of the wildlife contraception issue, wildlife agencies will have to behave proactively by projecting themselves into their future. Currently, wildlife agencies respond to many policy challenges reactively and defensively in an attempt to preserve their past. If a productive compromise can be reached over the issue of if, how, when, and where to use wildlife contraception, the wildlife policy decision process must be visionary, wise, bold, accessible, adaptable, and, most of all, fair.

Keywords: wildlife contraception, antimanagment, antihunting, animal rights, animal welfare, wildlife policy decision process

Introduction

> No policy that does not rest upon philosophical public opinion can be maintained.
> —Abraham Lincoln

History is a thread. It weaves from the past through the present and, inevitably, binds to the future. Earlier in this decade, wildlife policymakers in Colorado experienced an historical precedent event. On November 3, 1992, voters successfully overturned Colorado Wildlife Commission policy and outlawed the practices of hunting black bears in the spring and hunting them with bait and dogs (Loker and Decker 1995). This was the first time in Colorado history where wildlife policy was established by a citizen-referred ballot initiative. That historic event will ineluctably bind the State's past to the future because it marked a monumental failure in the policy decision process and strained State officials' credibility to deal with future controversial wildlife management issues.

In the black bear management controversy, agency officials failed to *see* when they looked. They failed to *listen* when they heard, and they failed to *act* while there was time. They did not see a subtle evolution of public wildlife values. They did not listen to the growing chorus of public discontent. They did not act while the management environment was still tractable. We believe this failure resulted because wildlife policymakers in Colorado were unaware of or insensitive to the social *context* into which the bear hunting issue intruded. This, in turn, allowed the issue to evolve into a polarized *controversy* before policymakers attempted to forge effective *compromises*. Furthermore, we believe the wildlife contraception issue has similar characteristics to follow a parallel evolutionary path unless policymakers assume a proactive posture from the outset.

Context

Wildlife contraception is only now emerging as a practical tool to control growth of wildlife populations (Kirkpatrick and Turner 1991, Garrott et al. 1992). Expectations have been raised which already seem to exceed the likely potential of the technology. Indeed, its emergence is being hailed by some as the "magic bullet" to solve the problem of controlling wildlife populations where hunting is not a viable option (Kirkpatrick and Turner 1991). Nontheless, this genesis promises to be anything but tranquil.

First, wildlife policymakers will be unable to control either the development of animal contraceptive technology or its availability. Pharmaceutical companies currently project two major markets for animal

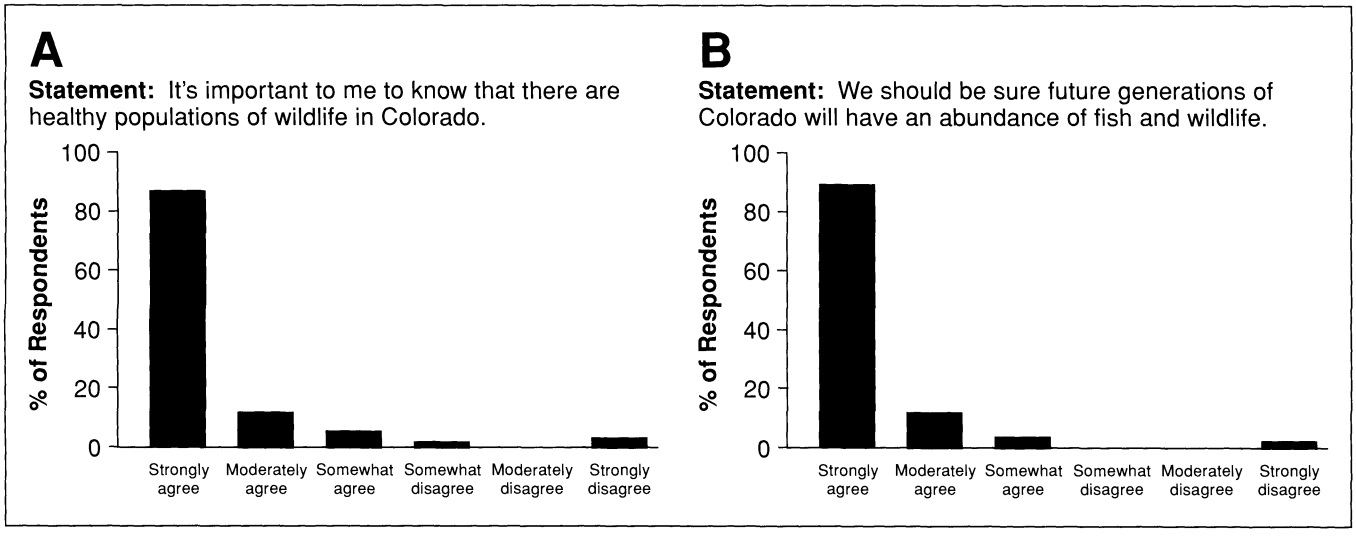

Figure 1. Indexes to the value Coloradans place on their wildlife: (A) Existence value, (B) Preservation value.

contraceptives, animal production and pet neutering. They also project it will be a multimillion to multibillion dollar industry. For example, one estimate suggests that between 5.7 and 12.1 million dogs and cats are euthanized each year in America due to pet overpopulation (Olson et al. 1986). Contraception is regarded both as a more humane and a more economical solution to pet overpopulation than euthanization or surgical sterilization (Maggitti 1993). Consequently, animal contraception will be available as an alternative to lethal wildlife population control irrespective of the desires of wildlife agency policymakers.

Second, environmental values have been metamorphosing throughout the world for several decades. Whereas *laissez faire* attitudes predominated in the last century, twentieth century values have grown increasingly "green" (O'Riordan 1971, Dunlap 1991, Kellert 1993, McAllister and Studlar 1993). Contemporary environmentalism, with its emphasis on environmental protection, now enjoys widespread public support (Sagoff 1990). Wildlife agencies, on the other hand, increasingly find themselves stuck in the backwater of a bygone era of maximum sustainable use. Public support for wildlife policies based upon wildlife uses seems to be waning. As a result, support for agency wildlife management policies has weakened as opposition has intensified.

Contemporary Public Attitudes

Colorado has long been regarded as a political bellwether State because of its geographically and philosophically diverse population. If so, perhaps the situation in Colorado forecasts trends in public wildlife values as well. The Colorado Division of Wildlife has been conducting public opinion and attitude surveys concerning wildlife issues at least since 1986. When we review the context of public attitudes, we see both consensus and conflict. We have consensus that wildlife is highly valued and conflict over how it should be valued. Consider the statement: *"It's important to know that there are healthy populations of wildlife in Colorado."* Virtually everyone concurs (fig. 1A). Similarly, when we ask if wildlife preservation should be a priority wildlife agenda item, affirmation is equally strong (fig. 1B).

Consensus dissolves, however, when we infer purpose from value. Colorado statutes declare it State policy to manage wildlife for *"the use, benefit, and enjoyment of people."* Although most would agree with managing for benefits and enjoyment, public values begin to diverge over the issue of use. Some say wildlife should be managed for consumptive uses, others say it should be managed for nonconsumptive enjoyment, while still others say we should manage

people for the benefit of wildlife. Recent professional and popular wildlife literature reveals at least four major areas of conflict: (1) antimanagement sentiment, (2) antihunting sentiment, (3) animal rights sentiment, and (4) animal welfare sentiment (Goodrich 1979, Decker and Brown 1987, Schmidt 1989, Richards and Krannich 1991).

Antimanagement Sentiment.—Among Coloradans, public sentiment is divided over whether hunting is one of the worthy purposes of wildlife management. Surveys suggest that wildlife professionals and hunting advocates have overrated public sentiment against management. For example, a recent planning survey conducted for the Division of Wildlife by the Human Dimensions in Natural Resources Unit of Colorado State University asked Coloradans to express their agreement or disagreement with the statement: *"It is important for humans to manage populations of wild animals."* More than three-fourths of the respondents agreed that wildlife management is important (fig. 2A).

However, approval of wildlife management is conditioned by perceptions of management intent. When management is directed toward animal benefits,

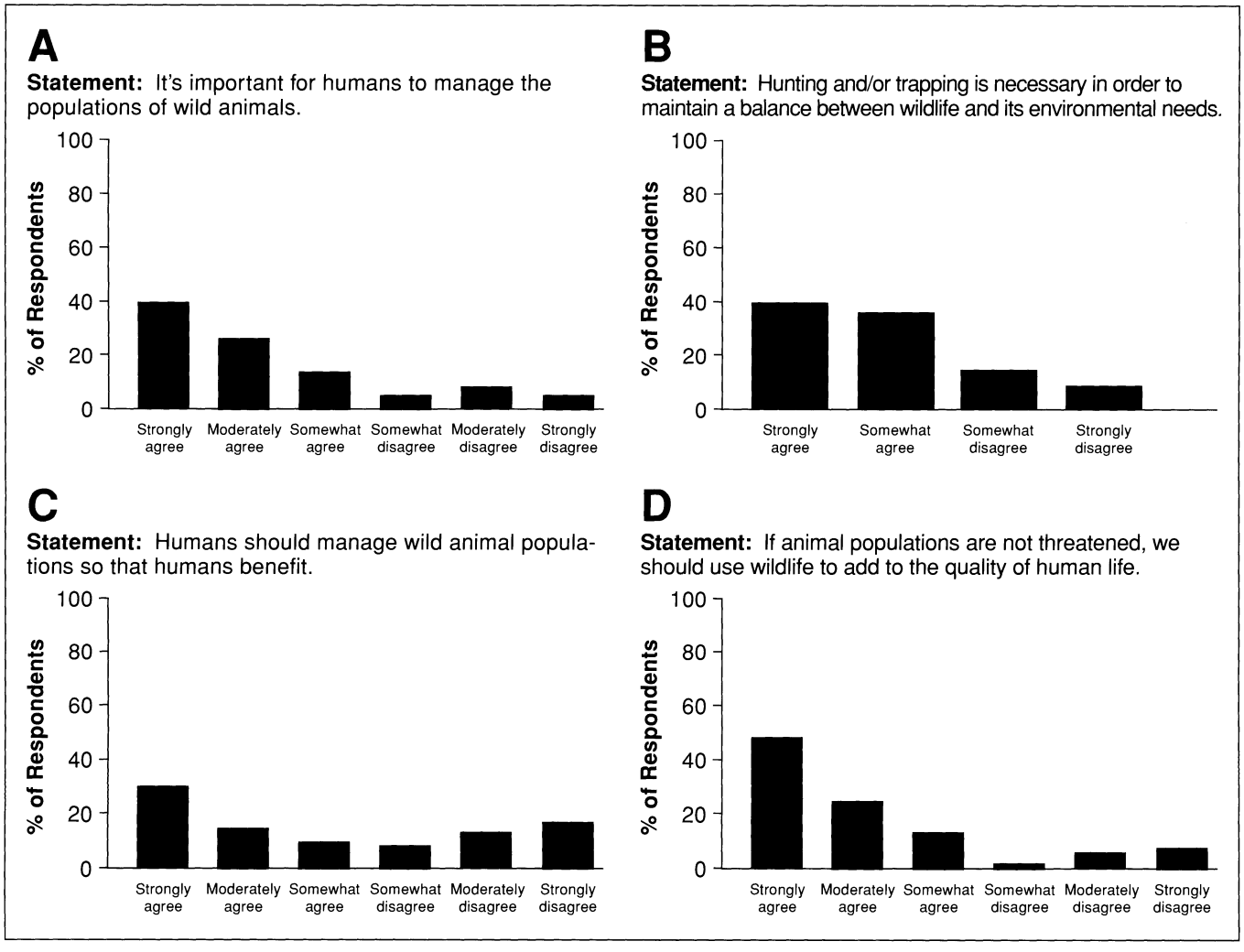

Figure 2. Indexes to antimanagement sentiment among Coloradans: (A) Support for wildlife management, (B) Support for hunting and/or trapping, (C) Support for managing wildlife for human benefits, and (D) Support for human use of wildlife to enhance the quality of life.

approval is strong. In a 1986 survey, nearly 75 percent of the respondents agreed that *"Hunting and/or trapping are necessary in order to maintain a balance between the number of wildlife and its environmental needs"* (fig. 2B). On the other hand, only 50 percent of Coloradans agree that *"Humans should manage wild animal populations so that humans benefit"* (fig. 2C). But as human benefits are clarified and conditioned—as in the statement *"If animal populations are not threatened, we should use wildlife to add to the quality of human life,"*—again, implicit support for wildlife management is high (fig. 2D).

It would seem that antimanagement sentiment per se is an unimportant public wildlife issue. Rather, the issue of management focuses on management outcomes. Management aimed at protecting wildlife populations from detrimental effects of their own excesses and focused on wildlife uses which enhance the quality of our lives is strongly sanctioned. Support declines, however, as the perceived nobility of purpose declines.

Antihunting Sentiment.—In general, the public does not appear to be prescriptively antihunting. When directly asked if wildlife agencies should disallow hunting, time and again the public responds that they should not. Even in the hotly contested black bear management controversy, antihunting sentiment was not a major factor affecting the outcome. For example, when a sample of prospective voters were asked to respond to the statement, *"As I read the following four statements about hunting please tell me which one comes closest to your views: A. Don't allow any hunting; B. Allow hunting only by wildlife professionals to control animal overpopulations; C. Allow hunting by licensed sportsmen; and D. Disallow hunting only when necessary to protect wildlife populations because hunting is a basic right,"* only 7 percent of Colorado's voting population supported the abolition of all hunting. Nearly 80 percent supported legal sport hunting so long as wildlife populations were protected from overharvest (fig. 3A).

Again, however, public support for hunting is conditional. Steve Kellert's earlier survey (Kellert 1980) and our more recent one found strong public support for meat hunting, less for recreational hunting, and little support for trophy hunting (fig. 3B). As was the case for management, the public seems to be saying, "We support hunting if it serves worthwhile social purposes, such as providing food for one's family." But when hunting deviates from the norm of public worthiness, it loses support.

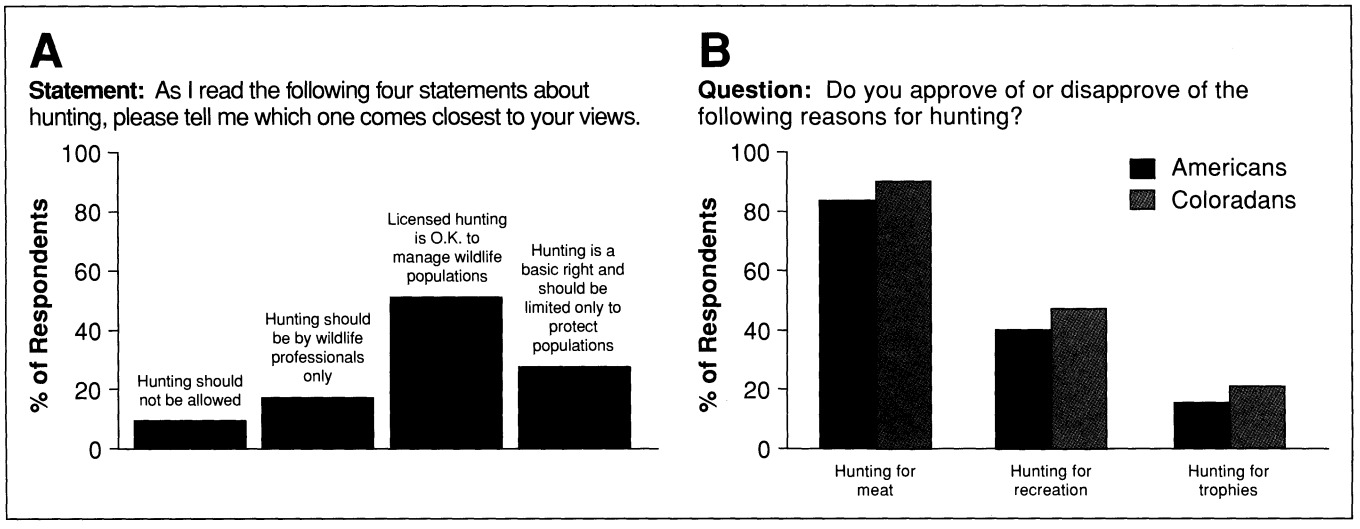

Figure 3. Indexes to antihunting sentiment among Coloradans: (A) Support for hunting, (B) Approval of reasons for hunting.

Table 1. Comparison of animal welfare and animal rights organizations (after Macauley 1987a–c)

Attribute	Animal welfare organizations	Animal rights organizations
Philosophies	Legalistic	Moralistic and legalistic
	Humanistic, benevolent	Libertarian, vegetarian, revisionist
	Reduce cruelty, unnecessary pain and suffering.	Eliminate suffering; elevate moral standing.
Concerns	Companion animals and endandangered species, whales, seals, some experiments	Factory farming and experimental animals
	Public abuses	Private as well as public abuses
	Individual abuses and species preservation	Institutional exploitation
Motivations	Emotional, ecological	Just, ethical
	Sympathy, kindness to animals	Philosophical
Strategies	Moderate	Radical or militant
	Regulationist, incremental	Abolitionist, revolutionary
	Educational, informational, preventative	Political, legal, reconstructive
Organizations	Comparatively large, established, national	Comparatively small, emergent, local or regional
	Well-endowed, hierarchical	Poorly funded, relatively decentralized
	Homogenous, wealthy, professional membership	Heterogenous, less affluent, diversely employed membership

Animal Rights Sentiment.—Wildlife professionals and hunting advocates infer cause and effect between animal rights sentiment and antihunting activism (Goodrich 1979, Richards and Krannich 1991). Despite this opinion, few public attitude surveys have investigated this connection. Much of the rhetoric and reaction to animal rights fail to separate public attitudes about animal rights from sentiments for animal welfare. Macauley (1987a–c, 1988a and b) conducted an intensive study contrasting animal welfare organizations with animal rights organizations. In general, animal welfare organizations oppose *unnecessary* pain and suffering among animals, including wildlife, whereas animal rights groups are generally opposed to human intervention in the lives of animals. Macauley concluded that animal welfare advocates are better organized, better funded, and more politically adept than animal rights groups. Strategies of animal welfare groups to change American values toward animals tend to be moderate, long-term, and educational in contrast to those of animal rights activists, which tend to be radical, immediate, and sensational (table 1). Regan and Francione (1992) characterize the philosophy of animal welfare advocates as "gentle usage" and contrast it with an animal rights philosophy which calls for "nothing less than the **total** liberation of nonhuman animals from human tyranny." We believe that general public values are more attuned to animal welfare than to animal rights philosophy.

We tried to tease these issues apart by examining responses of Coloradans to a variety of questions about animal rights and animal welfare issues. Animal rights sentiment was indexed by the statement: *"Animals should have rights similar to humans."* Astonishingly, perhaps, 60 percent of the respondents agreed, and one-third of these agreed strongly (fig.4A). What does this mean in terms of public attitudes to wildlife uses? In response to the statement, *"The rights of wildlife are more important than human use of wildlife,"* more than half of the respondents agreed, and of these, one-third strongly agreed (fig. 4B).

Nevertheless, when asked to make choices between rights and uses, once again the public discriminates. In response to the statement, *"I object to hunting because it violates the rights of an individual animal to exist,"* nearly two-thirds of the respondents disagreed, and one-third of these disagreed strongly (fig. 4C).

Animal Welfare Sentiment.—It would seem that Coloradans agree with the general notion that animals should have rights, but these rights should protect them from abusive uses, not all uses. Indeed, much of the conflict between animal uses and animal rights seems to center on the issue of animal welfare, and on this issue the public is much less equivocal. For example, the statement, *"I see nothing wrong with using steel-jawed leghold traps to capture wildlife,"* evokes strong opposition from most of the public (fig. 5A). What about perceptions of the humaneness of hunting? Here the public is divided. About one-half agree and one-half disagree with the statement, *"Hunting is cruel and inhumane to animals"* (fig 5B). In effect, the public seems to be saying, "No matter how important the management outcome, the end does not justify the means."

Controversies

So far, Statewide policymakers have treated public attitude responses as though the public was monolithic. This is clearly not the case. Wildlife values of Coloradans tend to cluster into four distinct types. Nearly one-third share the attitude that people can use wildlife to their benefit if wildlife populations are not endangered. Additionally, this sector believes that wildlife has the right to protection from abusive uses. Another cluster of similar size places high emphasis on commodity and recreational values of wildlife. A third cluster, representing about 25 percent of the population, strongly believes wild animals ought to have rights protecting them from human exploitation. A fourth cluster, representing less than 10 percent of the population, supports the use of wildlife for human benefits, such as food, fur, and fiber, but seems to be ambivalent toward recreational uses of wildlife. These

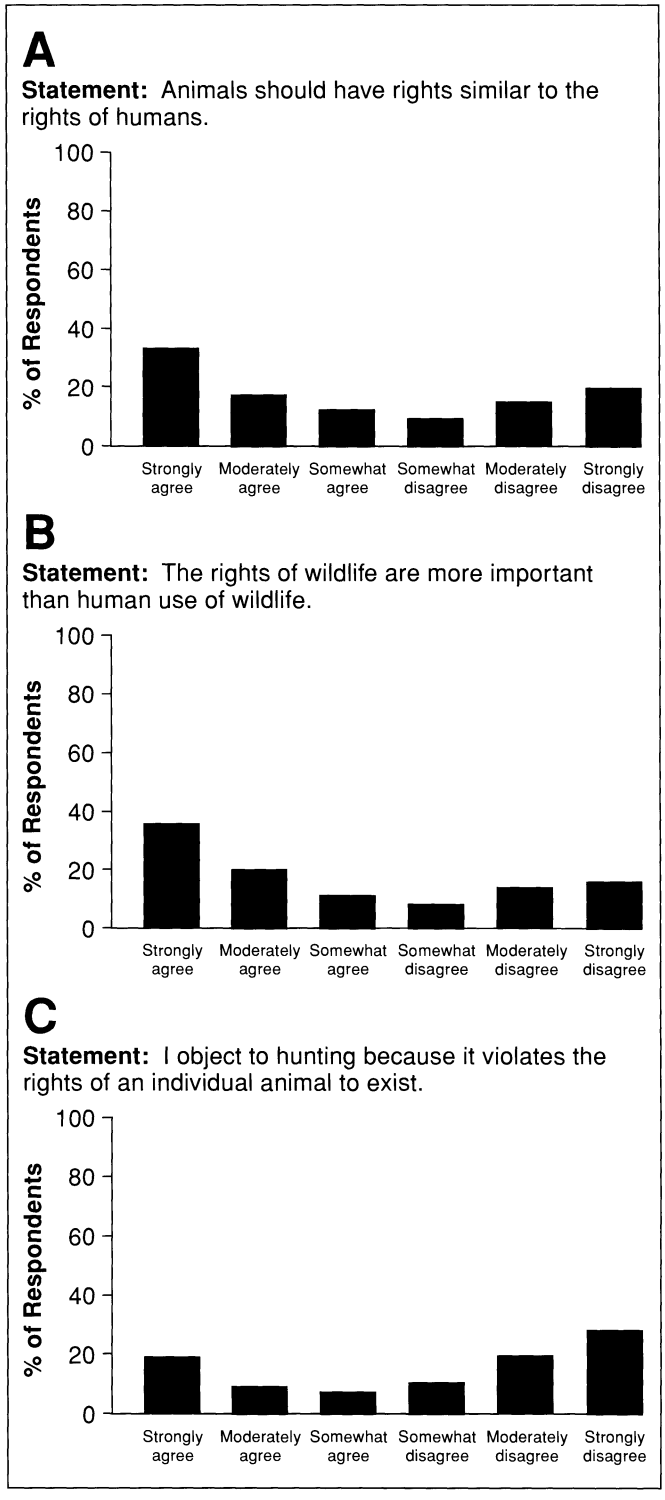

Figure 4. Indexes to animal rights sentiment among Coloradans: (A) Support for the concept that animals have rights similar to those of humans, (B) Support for the concept that animal rights supersede human uses of animals, and (C) Support for the concept that hunting violates the rights of animals.

people strongly oppose the concept that animals have rights (fig. 6A).

Given this fabric of social context, how are these contrasting publics likely to respond to the issue of wildlife contraception? We predict the following controversies will emerge. Those who strongly support hunting and animal uses will see wildlife contraception as a threat to hunting and will oppose its use vigorously. The animal rights community will be divided on the issue of wildlife contraception. Some will see it as a much preferred alternative to hunting because it is nonlethal and will insist it replace hunting as a wildlife population control tool. Others in this cluster will see wildlife contraception as just another interventive tool for humans to dominate animals. Those who moderately support animal rights and uses will support wildlife contraception to manage nuisance wildlife and will judge its utility to other management issues on a case-by-case basis. Those who are moderate toward animal uses, low on support for hunting, but strongly against animal rights will have mixed responses. Some will support wildlife contraception if it is more effective than hunting or trapping to control wildlife populations. However, most will

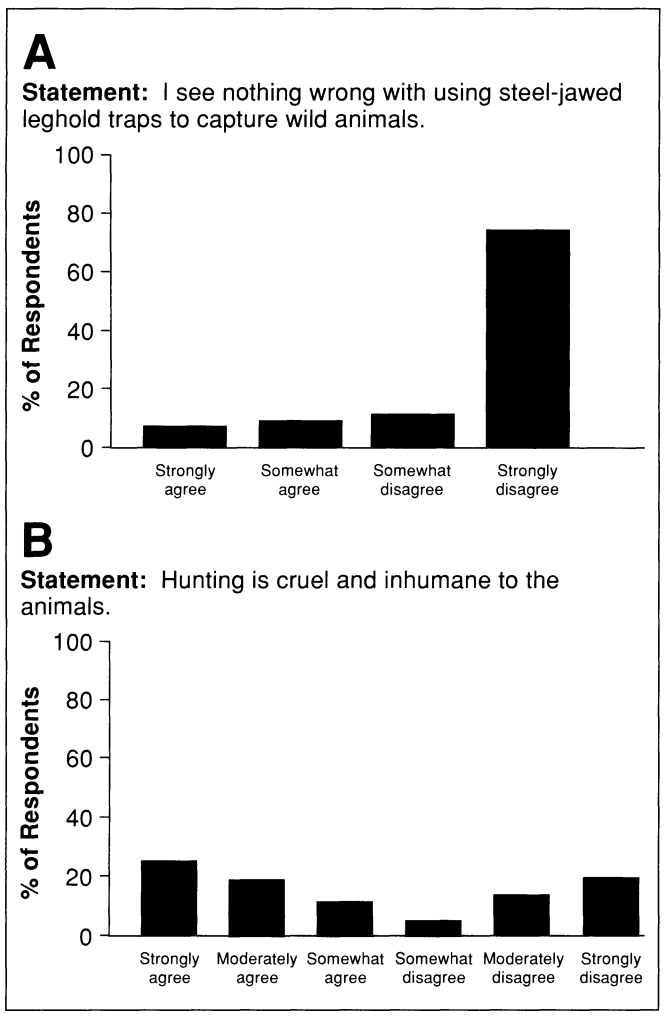

Figure 5. Indexes to animal welfare sentiment among Coloradans: (A) Opposition to the use of the steel-jawed trap, and (B) Support for the concept that hunting is cruel and inhumane to animals.

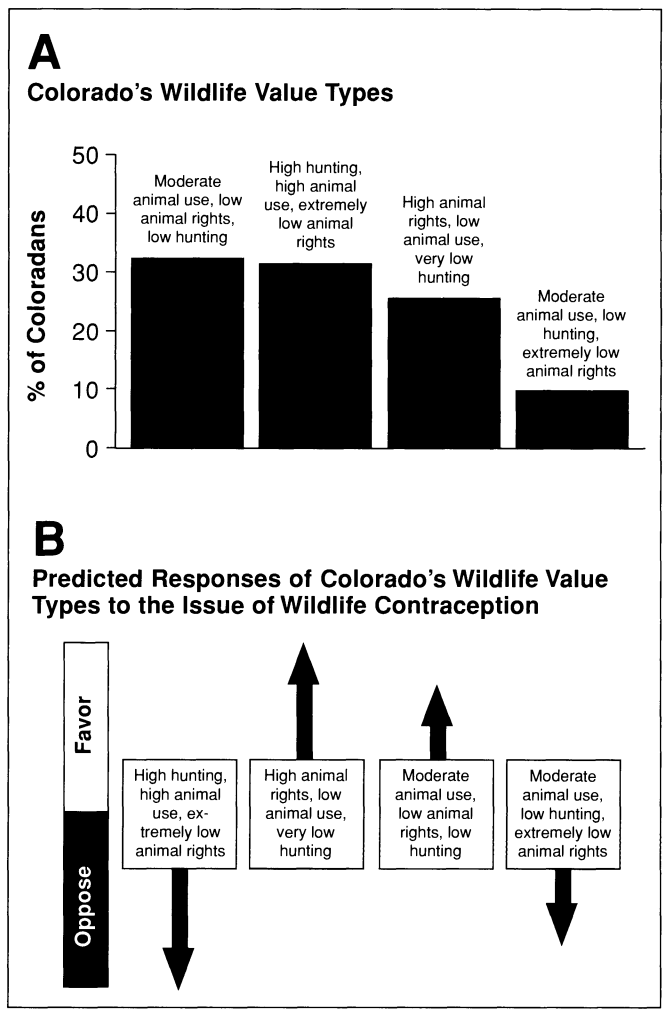

Figure 6. (A) Clusters of wildlife value types in Coloradans, and (B) Predicated responses of Colorado's wildlife value types to the issue of wildlife contraception.

Table 2. Advantages and disadvantages of competing wildlife contraception technologies

Contraceptive technology	Advantages	Disadvantages
Steroidal contraceptives	Readily available	Remains in the food chain
	Orally active	Lengthy Food and Drug Administration approval
	Reversible	Slow biodegradation
Immunocontraceptives	Reversible	Requires multiple treatments
	Inexpensive	Currently not completely efficacious
	Amenable to remote delivery	Must be developed specifically for each species
	Minimal side effects	
	Rapid biodegradation	
Hormonal toxin contraceptives	Requires only a single treatment	Irreversible
	Amenable to remote delivery	Alters reproductive behavior of treated individuals
	Equally efficacious to both sexes	
	Single chemical formulation efficacious across all vertebrate species	
	Rapid degradation	

oppose moralistic-based efforts of animal rights activists to substitute wildlife contraception for all hunting (fig. 6B).

Compromise

Left unmanaged, the wildlife contraception controversy will devolve into confrontational questions of *will we* or *won't we*. The challenge of the wildlife policy decision process will be to focus the debate on circumstantial questions such as *how will we* or *where will we*.

Currently, three distinctly different contraception technologies are being developed and tested for use in free-ranging wildlife populations: contraceptive steroids, immunocontraceptives, and chemosterilants such as hormonal toxins. Each technology has its advantages and disadvantages (table 2). Regardless of which technology is used, modeled responses of simulated populations suggest that applied wildlife contraception will be both prohibitively expensive and logistically daunting unless a single treatment endures for the reproductive lifetime of each treated individual (N. T. Hobbs, pers. comm.). Furthermore, the most efficacious treatments involved a combination of hunting (or culling) to lower population levels and contraception to maintain them at the desired level. In addition, the use of contraception to maintain wildlife populations is more precarious than shooting because much of the reproductive portion of the population has been uncoupled from density-dependent reproductive responses. Based upon what wildlife biologists now know, a prudent answer to the *how will we* question might be to control populations with both hunting and contraception.

Moreover, it seems unlikely that wildlife contraception will replace hunting as the wildlife population control of choice even if that were the most desired option. Hunting provides for an efficacious control on large-animal populations because an army of volunteer hunters not only donates its time but also pays for the opportunity. Consequently, hunting is not only effective, it is also economical. The niche for wildlife contraception most likely will be to control wildlife populations in areas such as nature preserves, wildlife parks, and urban open space, where control by licensed hunters is either impractical, undesirable, or unsafe (Hoffman and Wright 1990, Underwood and Porter 1991, Warren 1991, Curtis and Richmond 1992, Porter 1992).

Table 3. Contrasting characteristics of proactive *v.* reactive agencies

Proactive agencies	Reactive agencies
Driven by vision	Shackled by tradition
Committed to planning	Addicted to action
Planning anticipates the need for action.	Action precipitates a need for crisis planning.
Policy is by design: from the top down.	Policy is by default: from the bottom up.
Macromanagement: focuses on outcomes	Micromanagement: focuses on activities

Deflecting the wildlife contraception debate from confrontation to compromise will require a policy decision process that informs, educates, involves, and responds to the values of all stakeholders. Is the current process up to the challenge? Not without change.

In the first place, the current policy decision process is fundamentally reactive, not proactive. Wildlife agencies, for the most part reflect the philosophy, "if it ain't broke, don't fix it." Consider the contrasts between reactive and proactive organizations. Reactive organizations tend to be shackled by tradition and addicted to action. That action often leads to defensive planning. Policymaking tends to come from the bottom up, and there is a compulsion to micromanage activities and ignore or overlook the larger policy issues. In contrast, proactive agencies are driven by vision and committed to planning which, then, leads to action. Policy is formulated by design and implemented from the top down. Implementation is macromanaged by focusing on outcomes rather than activities (De Greene 1982, Gawthrop 1984, Morgan 1988). Reactive agencies look over their shoulders, fixed in their past. Proactive agencies, in contrast, scan the horizon in search of their future (table 3).

Attitudes of wildlife agency employees reflect a fixation on the past by clustering more closely toward traditional clients than toward the general public (Kennedy 1985, Peyton and Langenau 1985). For example, one of our Colorado surveys contrasted attitudes of bighorn sheep hunters, the general public, and Colorado Division of Wildlife employees. When asked whether they agreed or disagreed with the statement, *"Hunting male bighorn sheep is a form of sport and recreation, and people who want to hunt them should be allowed to do so,"* large majorities of both agency employees and bighorn sheep hunters agreed. In contrast, a substantial majority of the general public disagreed with the statement (fig. 7).

If most wildlife agencies are, indeed, fundamentally reactive, first and foremost they need to change their basic management philosophy from "if it ain't broke, don't fix it" to "if it ain't broke, break it" because management environments change constantly and management responses also must change constantly to keep pace. Wildlife agencies will have to break from their traditional biases to form effective partnerships with all of their publics to develop and evaluate truly *public* wildlife policies (Anderson 1975, Clark and Kellert 1988).

In the case of the pending wildlife contraception controversy, wildlife agencies still have an opportunity to be proactive. None of the developing technologies is yet operational. As a result, the management environment remains relatively unpolarized over the contraception issue. Thus, the future can be influenced and will depend largely on how agencies

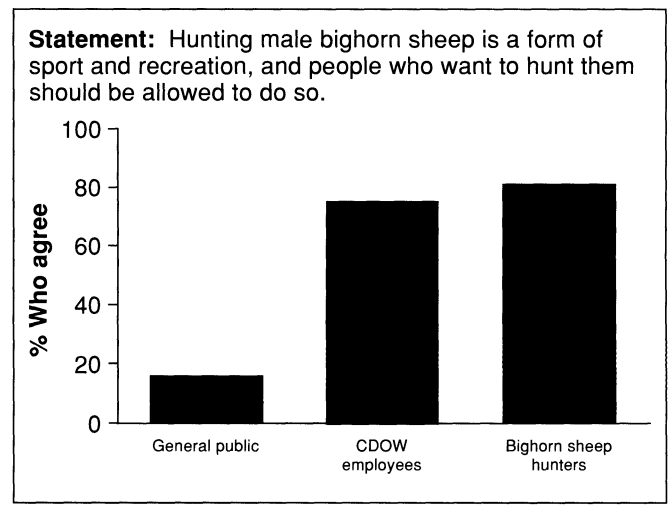

Figure 7. Contrasts between the attitudes of Colorado Division of Wildlife employees, bighorn sheep hunters, and the general public over whether bighorn sheep hunting for sport and recreation ought to be permitted.

respond to contraception as an emerging wildlife management tool. Proactive wildlife agencies dedicated to the overall public interest will respond with a combination of vision, wisdom, courage, accessibility, adaptability, and fairness.

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