BARD

FINAL REPORT

PROJECT No. US-539-82

Detection and Control of *Campylobacter jejuni* in Poultry and Red Meats

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in Poultry and Red Meats"

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ABSTRACT

Several areas were researched pertaining to "Detection and Control of Campylobacter jejuni in Poultry and Red Meats." Included in these studies were (1) understanding how free-radicals and oxygen scavengers influenced oxygen toxicity and aerotolerance of the bacterium; (2) the effects of ascorbic, isoascorbic and dehydroascorbic acids on growth and survival of Campylobacter; (3) cadmium chloride disks for identifying the organism; (4) the influences of gaseous environments on the organism; (5) the growth or killing of the organism in response to ferrous sulfate and cadmium chloride; and (6) optimizing enumeration of the organism from foods and the reduction of the organism by lactic and acetic acids.

In the area of immunological detection of the organism in foods, studies were divided as follows: (1) the production of monoclonal antibodies through the creation of hybridoma cultures and (2) the application of these antibodies from other bacteria as well as to detect Campylobacter jejuni in poultry products. Several hybridoma lines secreting monoclonals were created which fit the criteria of recognizing the organism, while not recognizing non-Campylobacter bacteria. Subsequently, these antibodies were used in creating an enzyme linked immunosorbent assay (ELISA) for speeding the time of detection from about three (3) days to about 24 hours.

The final area of study considered intervening in the colonization of Campylobacter jejuni in the chicken gastrointestinal (GI) tract. Fundamental information about colonization factors such as the variability of the bacterial strains, the oral challenge dose, the chicken age, and competitive exclusion microflora were studied. Outer membrane protein profiles were analyzed by polyacrylamide gel electrophoresis to observe quantitative and/or qualitative shifts in proteins which were correlated to colonization factors. Monoclonal antibodies, produced above, were screened to provide reagents discriminating between strains of Campylobacter jejuni which did, and did not colonize the chicken GI tract. These studies should provide information toward the goal of intervening in colonization of this important human enteropathogen.

III. REPORT

A. INTRODUCTION

Campylobacter jejuni is a major human enteropathogen currently acknowledged to cause gastroenteritis in at least as many cases as do Salmonella spp. The epidemiologic data indicates that chicken products are the direct or indirect vehicle in a vast majority of these human diseases in the developed world.

Detection methodology of all enteropathogens is a function of optimizing growth requirements of the target organism while limiting interference by competing microflora. Application of hybridoma technology for the purposes of detection was applied to C. jejuni. Means for inactivating the organism on foodstuffs required an understanding of the physiologic response by C. jejuni to a variety of conditions and antimicrobial additives. As the organism is exquisitely sensitive to oxidation-reduction potentials, assessment of oxygen toxicity and compounds influencing redox potentials were assessed. Finally, after we determined that serotypes of the organism were associated both with poultry and human disease, we proceeded in assessing selected factors involved in chicken GI tract colonization. The goal of this final approach was to intervene in the colonization of C. jejuni in the chicken; with the concomitant diminished presence of the organism on the food product, there will be a subsequent enhancement of human health.

B. OBJECTIVES AND SIGNIFICANCE OF THE PRESENT RESEARCH

1) Characterization and reduction of C. jejuni presence in foods: We wished to determine conclusively the association of poultry contamination with C. jejuni and human disease. Subsequently, we showed that serogroups #2, #11, and #12 are frequently isolated from humans in Israel and are among those also found associated with chicken carcasses. These data, along with other substantive epidemiological data implicating chicken products as the prominent source for human infection, indicate that to reduce human campylobacteriosis, poultry must be targeted for reduction of C. jejuni. To attain this goal, we used lactic and acetic acids on the poultry carcass to reduce the presence of the organism by slightly less than 1 log 10 colony forming units per carcass when compared to the numbers on the untreated carcass. We demonstrated that C. jejuni survived better in a 100% nitrogen atmosphere when compared with its survival under vacuum. With these approaches applied to chicken processing, a concomittant reduction in human exposure to this causative agent could be realized.

2) Physiological studies on C. jejuni: Through understanding the physiology of the organism we hoped to gain incite to alternative approaches in poultry processing. Ultimately, we wished to reduce the presence of the organism, as well as to optimize detection capability. To these ends we studied (1) the effect of free-radical and oxygen scavengers on photochemically generated oxygen toxicity and on the aerotolerance of C. jejuni, (2) the effect of ascorbic, isoascorbic and dehydroascorbic acids on the growth and survival of C. jejuni, and (3) the response of C. jejuni to combinations of ferrous sulfate and cadmium chloride.

The significance of our findings lie in future applications of selected antimicrobial compounds which we demonstrated to cause substantial reductions in numbers of the organism.

3) Hybridoma technology applied to C. jejuni: We wished to produce monoclonal antibodies to serve as immunological reagents in a rapid detection assays of an ELISA test. We were successful in producing a large number of these reagents, and subsequently, we stabelized the most important hybridoma cell lines for future application. We proliferated the cell lines which we judged as most important. These hybridoma lines produced monoclonal antibodies recognizing about 50 Campylobacter jejuni/coli isolates, while not reacting with non-campylobacter isolates. We subsequently researched and developed an ELISA capable of immunologic detection of the organism in 24 hours as opposed to the 3 or 4 days presently required for cultural detection of C. jejuni. The significance of this contribution, after completion of these studies, will be in providing a rapid and accurate probe protecting the public health.

4) Factors influencing chicken cecal colonization by C. jejuni: With conclusive evidence that chicken serves as the most important vehicle in human infection, and with the fact that a variety of current processing approaches have not been successssful in eliminating the presence of the pathogen, we began to study chicken colonization by the organism. We found that the chicken was highly susceptible to cecal colonization equally well throughout the first 72 hours after hatching, that levels as low as 35 cells of C. jejuni were capable of colonizing the chick, that contrary to reported literature, the protective influence of application of a "competitive exclusion" microflora was ineffective in limiting cecal colonization, and that variability in the potentials for colonization resided with individual isolates of C. jejuni. These data represent an area of study not previously undertaken or reported. Information gathered in this research will lead to an understanding of the factors which influence the colonization by the bacterium. Such an understanding will ultimately bring means for intervention in colonization by C. jejuni, an attendant decrease in human exposure to the agent and, consequently, diminished human gastroenteritis.

C. ORGANIZATION OF REPORT

This report will be presented under the following different sections. Because of the diversity of research and different stages of completeness, it would be confusing to attempt to present it as a "single paper."

D. Incidence of Campylobacter jejuni and Campylobacter coli serogroups in a chicken processing factory. B. J. Juven and M. Rogol. 1986. J. Food Prot. 49:290-292.

E. Cadmium chloride disks as a simple diagnostic tool for the identification of Campylobacter jejuni and Campylobacter coli. M. Rogol, B. J. Juven and N. J. Stern. 1987. Isr. J. Vet. Med. 43:42-45.

F. Enumeration and reduction of Campylobacter jejuni in poultry and red meats. N. J. Stern, P. J. Rothenberg, and J. M. Stone. 1985. J. Food Prot. 48:606-610.

G. Effect of free-radical and oxygen scavengers on photochemically generated oxygen toxicity and on the aerotolerance of Campylobacter jejuni. B. J. Juven and I. Rosenthal. 1985. J. Appl. Bacteriol. 59:413-419.

H. Effect of ascorbic, isoascorbic and dehydroascorbic acids on the growth and survival of Campylobacter jejuni. B. J. Juven and J. Kanner. 1986. J. Appl. Bacteriol. 61:339-345.

I. Survival of Campylobacter jejuni in selected gaseous environments. N. J. Stern, M. D. Greenberg, and D. M. Kinsman. 1986. J. Food Sci. 51:652-654.

J. Response of Campylobacter jejuni to combinations of ferrous sulfate and cadmium chloride. N. J. Stern, S. U. Kazmi, B. S. Roberson, K. Ono, and B. J. Juven. J. Appl. Bacteriol. Manuscript submitted.

K. Cecal colonization of Campylobacter jejuni in the chick. N. J. Stern, J. S. Bailey, L. C. Blankenship, and N. A. Cox. Manuscript in preparation.

L. Production of hybridoma cultures secreting monoclonal antibodies which recognize Campylobacter jejuni. N. J. Stern and A. Jackson. Unpublished.

M. Applications of monoclonal antibodies in Enzyme Linked Immunosorbent Assays (ELISA) for detection and discrimination of Campylobacter jejuni. N. J. Stern and A. Jackson.

N. Effect of ascorbic acids on the survival of Campylobacter jejuni in poultry meat. B. J. Juven and J. Kanner.

IV. DESCRIPTION OF COOPERATION

The research proposal was developed by both the principal and cooperating investigators. The investigators exchanged frequent letters and phone calls, and both have read all manuscripts submitted for publication. They have shared materials when appropriate, as for example the monoclonal antibodies developed and produced against Campylobacter jejuni in Athens, Georgia were confirmed as effective for the detection of the organism at the Volcani Center, Bet-Dagan, Israel.

Drs. Stern and Juven met initially in 1983 at an Institute of Food Technology Annual Meeting in New Orleans, to discuss and develop the current BARD proposal. Subsequently, the cooperators met several times to further define the nature of their independent and cooperative areas of study. In 1984, Dr. Juven visited Dr. Stern's laboratory in Beltsville, Maryland to review progress in their respective areas of research, and to refine their studies. In 1985, Dr. Juven came to Dr. Stern's laboratory to work on the second annual BARD report for this project, to finish a collaborative manuscript with Dr. Stern, to become familiar with the ELISA methodology and to present a seminar on "The effect of free-radical and oxygen scavengers on photochemically induced oxygen toxicity and on photochemically induced oxygen toxicity and on the aerotolerance of C. jejuni." In 1986, Dr. Juven came to Dr. Stern's laboratory at the Russell Agricultural Research Center in Athens, Georgia to further review research progress, to refine their current studies, to write the third annual BARD report, and to present a seminar on "The mechanism of toxicity of ascorbic acid to Campylobacter jejuni." In May, 1987, Dr. Stern came to Dr. Juven's laboratory in Bet-Dagan, Israel, to provide the immunological reagents prepared in Dr. Stern's laboratory and to demonstrate the immunoassay for detection of the organism on chicken carcasses, to review the progress made throughout the life of the current BARD grant, to prepare this final report, to present a seminar on "Colonization and translocation of Campylobacter jejuni in the ceca and gallbladders of chicks," and to plan for future research cooperative ventures.

V. BENEFIT TO AGRICULTURE

If it is not the major human enteropathogen, Campylobacter jejuni is certainly one of the most prominent of causative agents in cases of human gastroenteritis. Poultry products are recurringly implicated as the most important vehicle in transmission to humans. National pressures brought upon the poultry industry in the United States to eliminate or diminish bacterial pathogens associated with chicken have begun to have economic consequences for the industry. International trade, in some instances, has also forbidden the import of food products contaminated with human enteropathogens. With these consequences and the growing awareness of the role of C. jejuni as a human pathogen, the benefit of our research has increased with time.

Through our research demonstrating the relation of C. jejuni presence on chicken carcasses and human disease, we have helped direct appropriate concern toward poultry food products. With this awareness, we could then

focus on intervening in the presence of the pathogen on or in the chicken. Our work has provided state of the art methods for detecting and enumerating the bacterium in chicken carcasses, and this will serve to better monitor our food supplies and provide benefit for the public health. Economic benefits for the industry are subsequently accrued as consumer confidence is increased with a perception of enhanced safety. We have also provided basic knowledge in the area of Campylobacter spp. physiology. This information is scientifically of interest, but also has the potential for applied anti-campylobacter influences. Again, such applications would decrease public health risks and enhance the image of chicken as a safe product. Finally, our long-term, high-risk research on factors involved in the colonization of campylobacters in the chicken will provide an answer for totally removing this enteropathogen in its association with the chicken.

It is difficult to assess the real costs of disease attributed to consumption of poultry contaminated with C. jejuni. Certain estimates made on this subject suggest that many billions of dollars are lost to the American economy each year because of foodborne illness. Apart from this very important economic factor, the costs of human suffering and death associated with campylobacteriosis is an eye-opening reality. We believe that our research contributes to alleviating this ongoing human concern.

VI. LIST OF PUBLICATIONS

- B. J. Juven and M. Rogol. 1986. Incidence of Campylobacter jejuni and Campylobacter coli serogroups in a chicken processing factory. J. Food Prot. 49:290-292.
- M. Rogol, B. J. Juven and N. J. Stern. 1987. Cadmium chloride disks as a simple diagnostic tool for the identification of Campylobacter jejuni and Campylobacter coli. Isr. J. Vet. Med. 43:42-45.
- N. J. Stern, P. J. Rothenberg, and J. M. Stone. 1985. Enumeration and reduction of Campylobacter jejuni in poultry and red meats. J. Food Prot. 48:606-610.
- B. J. Juven and I. Rosenthal. 1985. Effect of free-radical and oxygen scavengers on photochemically generated oxygen toxicity and on the aerotolerance of Campylobacter jejuni. J. Appl. Bacteriol. 59:413-419.
- B. J. Juven and J. Kanner. 1986. Effect of ascorbic, isoascorbic and dehydroascorbic acids on the growth and survival of Campylobacter jejuni. J. Appl. Bacteriol. 61:339-345.
- N. J. Stern, S. U. Kazmi, B. S. Roberson, K. Ono, and B. J. Juven. Manuscript submitted. Response of Campylobacter jejuni to combinations of ferrous sulfate and cadmium chloride. J. Appl. Bacteriol.
- N. J. Stern, J. S. Bailey, L. C. Blankenship, N. A. Cox and F. McHan. 1987. Colonization and translocation of Campylobacter jejuni in the ceca and gallbladders of chicks. 87th Annual Meeting of the American Society for Microbiology, Abstract P 8.
- N. J. Stern, M. D. Greenberg, and D. M. Kinsman. 1986. Survival of Campylobacter jejuni in selected gaseous environments. J. Food Sci. 51:652-654.