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The History of *Toxoplasma gondii*—The First 100 Years

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ABSTRACT. In this paper the history of *Toxoplasma gondii* and toxoplasmosis is reviewed. This protozoan parasite was first discovered in 1908 and named a year later. Its medical importance remained unknown until 1939 when *T. gondii* was identified in tissues of a congenitally infected infant, and veterinary importance became known when it was found to cause abortion storms in sheep in 1957. The discovery of a *T. gondii* specific antibody test, Sabin–Feldman dye test in 1948 led to the recognition that *T. gondii* is a common parasite of warm-blooded hosts with a worldwide distribution. Its life cycle was not discovered until 1970 when it was found that felids are its definitive host and an environmentally resistant stage (oocyst) is excreted in feces of infected cats. The recent discovery of its common infection in certain marine wildlife (sea otters) indicates contamination of our seas with *T. gondii* oocysts washed from land. Hygeine remains the best preventive measure because currently there is no vaccine to prevent toxoplasmosis in humans.

Key Words. Biology, bradyzoite, diagnosis, life cycle, oocysts, prevention, symptoms, tachyzoite, treatment.

TOXOPLASMA gondii is one of the most well-studied parasites because of its medical and veterinary importance, and its suitability as a model for cell biology and molecular studies with a unicellular organism. There are many thousands of references to this parasite in the literature and it is not possible to give equal treatment to all authors and discoveries (Dubey 2007). In the present presentation the objective is, rather, to provide a history of the milestones in our acquisition of knowledge of the biology of this parasite. Historic landmarks are summarized in Table 1.

THE ETIOLOGICAL AGENT

Nicolle and Manceaux (1908) found a protozoan in tissues of a hamster-like rodent, the gundi, *Ctenodactylus gundi*, which was being used for leishmaniasis research in the laboratory of Charles Nicolle at the Pasteur Institute in Tunis. Nicolle initially believed the parasite to be a piroplasm (see Ajioka and Soldati 2007), then *Leishmania*, but soon realized that he had discovered a new organism and named it *T. gondii* based on the morphology (mod. L. *toxo* = arc or bow, *plasma* = life) and the host (Nicolle and Manceaux 1909). Thus, its complete designation is *T. gondii* (Nicolle and Manceaux 1908) Nicolle and Manceaux 1909. In retrospect the correct name for the parasite should have been *T. gundii*; Nicolle and Manceaux (1908) had incorrectly identified the host as *Ctenodactylus gondi* (Dubey 2007). Splendore (1908) discovered the same parasite in a rabbit in Brazil, also erroneously identifying it as *Leishmania*, but he did not name it.

For the next 30 yr, *T. gondii*-like organisms were found in several other hosts, especially avian species (Dubey 2002a) but viable *T. gondii* was first isolated by Sabin and Olitsky (1937) and proven to be identical with the human isolate of *T. gondii* (Table 1) using cross protection.

Protection to *T. gondii* turned out to be complex involving innate and specific immunity. In the 1940s humoral antibodies were found to kill extracellular but not intracellular tachyzoites (Sabin and Feldman 1948; Sabin and Olitsky 1937). In the next 50 yr protective immunity was found to be mediated largely by immune lymphoid cells (Frenkel 1967; Gazzinelli et al. 1991; Suzuki et al. 1988).

Although *T. gondii* has a worldwide distribution and perhaps the widest host range of any parasite, there is only one species, *gondii* in the genus *Toxoplasma*. Why some hosts develop clinical toxoplasmosis whereas most remain asymptomatic is largely unknown. During the 1980s and 1990s methods were developed to recognize genetic differences among *T. gondii* isolates from humans and animals (Dardé, Bouteille, and Pestre-Alexandre 1987; Howe and Sibley 1995; Pfefferkorn and Pfefferkorn 1980; Sibley et al. 1992; Tibayrene et al. 1991). Mapping of *T. gondii* genes was achieved recently (Khan et al. 2005), and undoubtedly will help in search for better antigens for diagnosis and protection, and mechanism of disease. Until recently, *T. gondii* was considered clonal with very little genetic variability (Howe and Sibley 1995). Lehmann et al. (2006) made the first in-depth study of genetic variability among more than 275 *T. gondii* isolates obtained worldwide from one host (free-range chicken) and in one laboratory (Dubey et al. 2002) and found geographic differences, with some isolates being confined to Brazil whereas others were distributed worldwide. Phenotypically, *T. gondii* isolates from asymptomatic chickens from Brazil were mouse virulent (Dubey et al. 2002). This point is of interest because in my opinion there is no non-pathogenic strain of *T. gondii* and virulence in mice may have no clinical relevance with respect to disease in humans and livestock.

PARASITE MORPHOLOGY AND LIFE CYCLE

Tachyzoites. The tachyzoite (Frenkel 1973) is lunate and is the stage that Nicolle and Manceaux (1909) found in the gundi. This stage has also been called a trophozoite, the proliferative form, the feeding form, and the endozoite. It divides into two by a specialized process called endodyogeny (Goldman, Carver, and Sulzer 1958).

Bradyzoite and tissue cysts. The term ''bradyzoite'' (Gr. brady = slow) was proposed by Frenkel (1973) to describe the stage encysted in tissues. Bradyzoites are also called cystozoites. Dubey and Beattie (1988) proposed that cysts should be called tissue cysts to avoid confusion with oocysts and pseudocysts. Jacobs, Remington, and Melton (1960a) first provided a biological characterization of cysts when they found that the cyst wall was destroyed by pepsin or trypsin, but the cystic organisms were resistant to digestion by gastric juices (pepsin–HCl), whereas tachyzoites were destroyed immediately. Thus, tissue cysts were shown to be important in the life cycle of *T. gondii* because carnivorous hosts can become infected by ingesting infected meat.

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Table **1.** Summary of landmarks in the history of *Toxoplasma gondii*

Finding	Reference
Etiologic agent	
Protozoa found in the rodent, *Ctenodactylus gundi* in Tunisia	Nicolle and Manceaux (1908)
Protozoa found in a rabbit in Brazil	Splendore (1908)
Name *Toxoplasma gondii* proposed (taxon = bow, plasma = image)	Nicolle and Manceaux (1909)
First viable *T. gondii* isolate obtained from an animal	Sabin and Olitsky (1937)
First isolate of *T. gondii* from human	Wolf et al. (1939)
Human and animal *T. gondii* proven identical	Sabin (1941)
Pathogenesis of toxoplasmosis, including hydrocephalus	Frenkel and Friedlander (1951), Frenkel (1953,1956)
Parasite morphology and life cycle	
Tachyzoite (trophozoite, feeding form, proliferative form, endodyozoite)	
Term tachyzoite proposed (tachy-fast, zoite- life)	Frenkel (1973)
Endodyogeny described	Goldman et al. (1958)
Ultrastructure described	Gustafson, Agar, and Cramer (1954), Sheffield and Melton (1968)
Tissue cyst, bradyzoite, cystozoite	
Cyst recognized	Levaditi, Schoen, and Sanchis Bayarri (1928)
Cyst described cytologically	Frenkel and Friedlander (1951), Frenkel (1956)
Ultrastructure described	Wanko et al. (1962), Ferguson and Hutchison (1987)
Term bradyzoite proposed (bradys = slow, zoon = animal)	Frenkel (1973)
Term tissue cyst proposed	Dubey and Beattie (1988)
Bradyzoite resistance to digestive enzymes recognized	Jacobs et al. (1960a, b)
Development of tissue cysts and bradyzoites described	Dubey and Frenkel (1976)
Complete biology of bradyzoites and tissue cysts reviewed	Dubey et al. (1998)
Feline entroepithelial stages	
Coccidian phases described	Frenkel, Dubey, and Miller (1970), Hutchison et al. (1970), Dubey and Frenkel (1972), Sheffield and Melton (1970)
Oocyst morphology described	Dubey et al. (1970b)
Five asexual *T. gondii* types (A–E) described	Dubey and Frenkel (1972)
Ultrastructure of coccidian stages described	Sheffield (1970), Piekarski, Pelster, and Witte (1971), Ferguson et al. (1974, 1975, 1979a,b), Christie, Pappas, and Dubey (1978), Speer, Clark, and Dubey (1998), Speer and Dubey (2005)
Transmission	
Congenital	
Transmission demonstrated in human	Wolf et al. (1939)
Repeated transmission found in house mouse	Beverley (1959)
Congenital transmission found in a large wild-animal species, white tailed deer	Dubey et al. (2008)
Carnivorism, transmission by meat of intermediate hosts	
Suggested carnivorous transmission	Weinman and Chandler (1954)
Transmission by meat found in humans	Desmonts et al. (1965)
Fecal—oral	
Transmission by a resistant fecal form of *T. gondii* demonstrated	Hutchison (1965)
Coccidian phase recognized	Hutchison et al. (1970, 1971), Frenkel et al. (1970), Dubey et al. (1970a,b), Sheffield and Melton (1970), Overdulve (1970)
Definitive and intermediate hosts defined, including shedding of oocysts only by felids	Frenkel et al. (1970), Miller et al. (1972), Jewell et al. (1972)
First oocyst-inhaled/ingested human toxoplasmosis outbreak described	Teutsch et al. (1979)
Genetics and different genetic *T. gondii* strains	
Recombinants and genetic crosses produced	Pfefferkorn and Pfefferkorn (1980)
Isoenzyme differences used to distinguish *T. gondii* strains	Dardé et al. (1987), Tibayrene et al. (1991)
Restriction fragment length polymorphism used to group *T. gondii* strains into 3 Types (I,II,III)	Sibley et al. (1992), Howe and Sibley (1995)
National, continental, intercontinental, and pandemic *T. gondii* strains distinguished	Lehmann et al. (2006)
T. gondii genome anointed	Khan et al. (2005)
Immunity and protection	
T. gondii neutralizing antibody recognized	Sabin and Ruchman (1942)
Antibodies found to kill extracellular but not intracellular *T. gondii*	Sabin and Feldman (1948)
Protection transferred by immune lymphoid cells but not by antibodies	Frenkel (1967)
Interferon γ found to be main cytokine for protection	Suzuki et al. (1988)
Role of CD4+and CD8+cells in protection defined	Gazzinelli et al. (1991)
Toxoplasmosis in humans	
Congenital	
First proven case of congenital toxoplasmosis described	Wolf et al. (1939)
Typical tetrad clinical signs described (hydrocephalus or microcephalus, chorioretinitis, intracerebral calcification)	Sabin (1942)

Table **1.** (Continued).

Finding	Reference
Acquired	
First case in a child	Sabin (1941)
Fatal toxoplasmosis in adults found	Pinkerton and Weinman (1940)
Lymphadenopathy recognized as the most frequent symptom	Siim (1956), Beverley and Beattie (1958)
Susceptibility to toxoplasmosis in AIDS patient recognized	Luft et al. (1983)
Chronic infection	
Cysts found in autopsy slides, indicating chronic asymptomatic infection	Plaut (1946), Kean and Grocott (1947)
Toxoplasmosis in other animals	
Toxoplasmosis found in a domestic animal, dog	Mello (1910)
Immunosuppressive Canine Distemper Virus influenced clinical toxoplasmosis outcome in dogs	Campbell, Martin, and Gordon (1955)
Epidemic toxoplasmosis abortions in sheep recognized	Hartley and Marshall (1957)
Toxoplasmosis in animals reviewed critically	Dubey and Beattie (1988)
Toxoplasmosis found a common infection in a marine mammal species, sea otter	Cole et al. (2000)
Diagnosis	
Novel Sabin–Feldman dye test described	Sabin and Feldman (1948)
Toxoplasma skin test as a survey tool	Frenkel (1948)
Tests developed to detect IgM antibodies in cord blood	Remington et al. (1968), Desmonts et al. (1981)
Simple direct agglutination test developed (DAT, MAT)	Desmonts and Remington (1980), Dubey and Desmonts (1987)
First validation of a serologic test using isolation of the parasite as standard	Dubey et al. (1995a), Dubey (1997)
PCR test developed to detect *T. gondii* DNA using B1 gene	Burg et al. (1989)
Treatment	
Sulfonamides found effective against *T. gondii*	Sabin and Warren (1942)
Pyrimethamine found synergestic with sulfonamides against dividing tachyzoites	Eyles and Coleman (1953)
Folic acid and yeast improves activity of sulfadiazine and pyrimethamine	Frenkel and Hitchings (1957)
Spiramycin found to have anti-toxoplasmic activity	Garin and Eyles (1958)
Clindamycin found to be anti-toxoplasmic	McMaster et al. (1973), Araujo and Remington (1974)
Prevention and control	
Prophylactic treatment, and screening of pregnant women initiated in Austria and France to reduce congenital toxoplasmosis	Thalhammer (1973, 1978), Desmonts and Couvreur (1974a,b)
Hygeinic measures advocated to prevent human exposure to oocysts	Frenkel and Dubey (1972)
Thermal curves to kill *T. gondii* in meat by cooking, freezing, and irradiation constructed	Dubey et al. (1986), Dubey et al. (1990), Kotula et al. (1991)
Animal production practices developed to reduce *T. gondii* infection in farm animals	Dubey et al. (1995b), Weigel et al. (1995)
Low prevalence of *T. gondii* in pigs correlated with reduction of seroprevalence of *T. gondii* in humans	Dubey et al. (2005), Jones et al. (2007)
Vaccination	
A vaccine to reduce fetal losses in sheep commercialized	Wilkins and O'Connell (1983), Buxton and Innes (1995)
Ts-4 vaccine for intermediate host	Waldeland and Frenkel (1983)
T-263 vaccine to prevent oocyst shedding by cats	Frenkel et al. (1991)

Jacobs, Remington, and Melton (1960b) used the pepsin digestion procedure to isolate viable *T. gondii* from tissues of chronically infected animals.

Dubey and Frenkel (1976) performed the first in-depth study of the development of tissue cysts and bradyzoites and described their ontogeny and morphology. They found that tissue cysts formed in mice as early as 3 days after their inoculation with tachyzoites. Cats shed oocysts with a short prepatent period (3–10 days) after ingesting tissue cysts or bradyzoites, whereas after they ingested tachyzoites or oocysts the prepatent period was longer ($\geq$ 18 days), irrespective of the number of organisms in the inocula (Dubey and Frenkel 1976; Dubey 1996, 2001, 2002b, 2006). Prepatent periods of 11–17 days are thought to result from the ingestion of transitional stages between tachyzoite and bradyzoite (Dubey 2002b, 2005). Until 1976, tissue cyst formation was considered to be mediated by host immunity. The study by Dubey and Frenkel (1976) indicated that bradyzoites and tissue cysts are an integral part of the life cycle of *T. gondii*, independent of immunity. I firmly believe that there are no strains of *T. gondii* in nature that do not form tissue cysts.

Ultrastructural studies revealed that tissue cysts develop and remain intracellular and bradyzoites differ from tachyzoites with respect to location of the nucleus (central in tachyzoites, terminal in bradyzoites), amylopectin granule (numerous in bradyzoites, absent or few in tachyzoites, contents of rhoptries honeycomb in tachyzoites, electron dense in older bradyzoites [Dubey, Lindsay, and Speer 1998; Ferguson and Hutchison 1987; Wanko, Jacobs, and Gavin 1962]).

Enteroepithelial asexual and sexual stages. Asexual and sexual stages were reported in the intestine of cats in 1970 (Table 1). Dubey and Frenkel (1972) described the asexual and sexual development of *T. gondii* in enterocytes of the cat and designated the asexual enteroepithelial stages as types A–E, rather than as generations conventionally known as schizonts in other coccidian parasites. These stages were distinguished morphologically from tachyzoites and bradyzoites, which also occur in cat intestine. The

challenge was to distinguish different stages in the cat intestine because there was profuse multiplication of *T. gondii* 3 days post-infection. The entire cycle was completed in 66 h after feeding tissue cysts to cats (Dubey and Frenkel 1972).

TRANSMISSION

Congenital. The mechanism of transmission of *T. gondii* remained a mystery until its life cycle was discovered in 1970. Soon after the initial discovery of the organism it was found that the *C. gundi* were not infected in the wild and had acquired *T. gondii* infection in the laboratory. Initially transmission by arthropods was suspected, but this was never proven (Frenkel 1970, 1973). Congenital *T. gondii* infection in a human child was initially described by Wolf, Cowen, and Page (1939) and later found to occur in many species of animals, particularly sheep, goats, and rodents. Congenital infections can be repeated in some strains of mice with infected mice producing congenitally infected offspring for at least 10 generations (Beverley 1959).

Carnivorism. Congenital transmission occurs too rarely to explain widespread infection in man and animals worldwide. Weinman and Chandler (1954) suggested that transmission might occur through the ingestion of undercooked meat. Jacobs et al. (1960a) provided evidence to support this idea by demonstrating the resistance to proteolytic enzymes of *T. gondii* derived from cysts. They found that the cyst wall was immediately dissolved by such enzymes but the released bradyzoites survived long enough to infect the host. This hypothesis of transmission through the ingestion of infected meat was experimentally tested by Desmonts et al. (1965) in an experiment with children in a Paris sanitorium. They compared the acquisition rates of *T. gondii* infection in children before and after admission to the sanitorium. The 10% yearly acquisition rate of *T. gondii* antibody rose to 50% after adding two portions of barely cooked beef or horse meat to the daily diet and to a 100% yearly rate after the addition of barely cooked lamb chops. Because the prevalence of *T. gondii* is much higher in sheep than in horses or cattle this illustrated the importance of carnivorism in transmission of *T. gondii*. Epidemiological evidence indicates it is common in humans in some localities where raw meat is routinely eaten (Desmonts et al. 1965). Kean, Kimball, and Christenson (1969) described toxoplasmosis in a group of medical students who had eaten under-cooked hamburgers.

Fecal–oral. While congenital transmission and carnivorism can explain some of the transmission of *T. gondii* it does not explain the widespread infection in vegetarians and herbivores. Hutchison (1965), a biologist at Strathclyde University in Glasgow, first discovered *T. gondii* infectivity associated with cat feces. In a preliminary experiment, Hutchison (1965) fed *T. gondii* cysts to a cat infected with the nematode *Toxocara cati* and collected feces containing nematode ova. Feces floated in 33% zinc sulfate solution and stored in tap water for 12 mo induced toxoplasmosis in mice. This discovery was a breakthrough because, until then, both known forms of *T. gondii* (i.e. tachyzoites and bradyzoites) were killed by water. Microscopic examination of feces revealed only *T. cati* eggs and *Isospora* oocysts. In Hutchison's report, *T. gondii* infectivity was not attributed to oocysts or *T. cati* eggs. He repeated the experiment with two *T. cati*-infected and two *T. cati*-free cats. *Toxoplasma gondii* was transmitted only in association with *T. cati* infection. On this basis, Hutchison (1967) hypothesized that *T. gondii* was transmitted through nematode ova. Hutchison's (1965) report stimulated other investigators to examine fecal transmission of *T. gondii* through *T. cati* eggs. The nematode egg theory of transmission was discarded after *T. gondii* infectivity was dissociated from *T. cati* eggs (Frenkel, Dubey, and Miller 1969) and *T. gondii* infectivity was found in feces of worm-free cats fed *T. gondii* (Frenkel et al. 1969; Sheffield and Melton 1969). Finally, in 1970, knowledge of the *T. gondii* life cycle was completed by discovery of the sexual phase of the parasite in the small intestine of the cat (Table 1). *Toxoplasma gondii* oocysts, the product of schizogony and gametogony, were found in cat feces and characterized morphologically and biologically (Dubey, Miller, and Frenkel 1970a, b).

In retrospect the discovery and characterization of the *T. gondii* oocyst in cat feces was delayed because (1) *T. gondii* oocysts were morphologically identical to oocysts of the previously described coccidian parasite of cats and dogs (Dubey et al. 1970b) and (2) until 1970 coccidian oocysts were sporulated in 2.5% potassium dichromate. Chromation of the oocysts wall interfered with excystation of the sporozoites when oocysts were fed to mice and thus the mouse infectivity titer of the oocysts was lower than expected from number of oocysts administered (Dubey et al. 1970a). These findings led to the use of 2% sulfuric acid as the best medium for sporulation and storage of *T. gondii* oocysts (Dubey, Swan, and Frenkel, 1972). Unlike dichromate, which was difficult to wash off the oocysts, sulfuric acid could be easily neutralized and the oocysts could be injected without washing into mice (Dubey and Frenkel 1973). Unlike other coccidians, *T. gondii* oocysts were found to excyst efficiently when inoculated parenterally into mice and thus alleviated the need for oral inoculation for bioassay of oocysts (Dubey and Frenkel 1973).

Of the many species of animals experimentally infected with *T. gondii,* only felids shed *T. gondii* oocysts (Miller, Frenkel, and Dubey 1972). Oocysts shed into the environment have caused several outbreaks of disease in humans (Benenson et al. 1982; Bowie et al. 1997; de Moura et al. 2006; Teutsch et al. 1979). Sero-epidemiological studies on isolated islands in the Pacific (Wallace 1969), Australia (Munday 1972), and the United States (Dubey et al. 1997) have shown an absence of *T. gondii* on islands without cats, confirming the important role of the cat in the natural transmission of *T. gondii*. Vaccination of cats with a live mutant strain of *T. gondii* on eight pig farms in the United States reduced the transmission of *T. gondii* infection in mice and pigs (Mateus-Pinilla et al. 1999), thus supporting the role of the cat in natural transmission of *T. gondii*.

Although *T. gondii* can be transmitted in several ways, it has adapted to be transmitted most efficiently by carnivorism in the cat and by the fecal–oral (oocysts) route in other hosts. Pigs and mice (and presumably humans) can be infected by ingesting even one oocyst (Dubey et al. 1996), whereas 100 oocysts may not infect cats (Dubey 2006). Cats can shed millions of oocysts after ingesting only one bradyzoite, while ingestion of 100 bradyzoites may not infect mice orally (Dubey 2001, 2006). This information has proved very useful in conducting epidemiological studies and for the detection by feeding to cats of low numbers of *T. gondii* in large samples of meat (Dubey et al. 2005).

TOXOPLASMOSIS IN HUMANS

Congenital toxoplasmosis. Three pathologists, Wolf, Cowen, and Paige from New York, USA first conclusively identified *T. gondii* in an infant girl who was delivered full term by Caesarean section on May 23, 1938 at Babies Hospital, New York (Wolf et al. 1939). The girl developed convulsive seizures at 3 days of age and lesions were noted in the maculae of both eyes through an ophthalmoscope. She died when a month old and an autopsy was performed. At post mortem, brain, spinal cord, and right eye were removed for examination. Free and intracellular *T. gondii* were found in lesions of encephalomyelitis and retinitis of the girl and viable *T. gondii* was isolated in animals inoculated with tissues from the girl. Sabin (1942) summarized all that was known of congenital toxoplasmosis in 1942 and proposed typical clinical signs of congenital toxoplasmosis: hydrocephalus or

microcephalus, intracerebral calcification, and chorioretinitis. These signs helped in the clinical recognition of congenital toxoplasmosis.

Acquired toxoplasmosis. Sabin (1941) reported toxoplasmosis in a 6-y-old boy from Cincinnati, OH. An asymptomatic child with initials of R.H. was hit with a baseball bat on October 22, 1937. He developed a headache 2 days later and convulsions the day after. He was admitted to the hospital on the seventh day but without obvious clinical signs. Except for lymphadenopathy and enlarged spleen, nothing abnormal was found. He then developed neurological signs and died on the 30th day of illness. The brain and spinal cord were removed for histopathological examination and bioassay. Because of the suspicion of polio virus infection a homogenate of cerebral cortex was inoculated into mice. *Toxoplasma gondii* was isolated from the inoculated mice and this isolate was given the initials of the child and became the famous RH strain. Only small lesions of non-suppurative encephalitis were found microscopically in the brain of this child without any calcification. This child most likely had acquired *T. gondii* infection recently and the blow to the head was coincidental and unrelated to the onset of symptoms. This case is historically interesting because the RH strain of *T. gondii* isolated from this boy has since 1938 been passaged in mice in many laboratories worldwide. After this prolonged passage its pathogenicity for mice has been stabilized (Dubey 1977) and it has lost the capacity to produce oocysts in cats (Frenkel, Dubey, and Hoff 1976).

Pinkerton and Weinman (1940) identified *T. gondii* in the heart, spleen and other tissues of a 22-yr-old patient who died in 1937 in Lima, Peru. Pinkerton and Henderson (1941) isolated *T. gondii* from blood and tissues of two (50- and 43-yr-old) individuals who died in St. Louis, MO. Siim (1956) drew attention to the fact that lymphadenopathy is a frequent sign of acquired toxoplasmosis in adults and these findings were confirmed by Beverley and Beattie (1958) who reported on the cases of 30 patients. A full appreciation of the clinical symptoms of acquired toxoplasmosis was achieved when outbreaks of acute toxoplasmosis were reported in adults in the United States (Teutsch et al. 1979), Canada (Bowie et al. 1997), and Brazil (de Moura et al. 2006).

Ocular disease. Before 1950, virtually all cases of ocular toxoplasmosis were considered to result from congenital transmission (Holland 2003). Wilder (1952) first identified *T. gondii* in histological sections of eyes that had been enucleated. A group of ophthalmologists from southern Brazil initially discovered ocular toxoplasmosis in siblings. Among patients with postnatally acquired toxoplamosis who did not have retinochoidal scars before, 8.3% developed retinal lesions during a 7-yr follow up (Silveira et al. 1988; Holland 2003). Ocular toxoplasmosis was diagnosed in 20 of 95 patients with acute toxoplasmosis associated with the Canadian waterborne outbreak of toxoplasmosis in 1995 (Burnett et al. 1998).

Acquired immunodeficiency syndrome (AIDS) epidemic. Before the epidemic of the acquired immunodeficiency syndrome in adults in the 1980s, neurological toxoplasmosis in adults was rarely reported and essentially limited to patients treated for tumors or those given transplants. Luft et al. (1983) reported acute toxoplasmosis-induced encephalitis that was fatal if not treated. In almost all cases clinical disease occurred as a result of reactivation of chronic infection initiated by the depression of intracellular immunity due to HIV infection. Initially, many of these cases of toxoplasmosis in AIDS patients were thought to be lymphoma.

TOXOPLASMOSIS IN OTHER ANIMALS

Mello (1910) in Turin, Italy first reported fatal toxoplasmosis in a domestic animal (a 4-mo-old dog) that died of acute visceral toxoplasmosis. Over the next 30 yr canine toxoplasmosis was reported in Cuba, France, Germany, India, Iraq, Tunisia, U.S.S.R., and the United States (Dubey and Beattie 1988). Strangely enough the first case of toxoplasmosis was not reported in a cat until 1942 when Olafson and Monlux found it in a cat from Middletown, NY, USA. Toxoplasmosis in sheep deserves special attention because of its economic impact. William Hartley, a well-known veterinary pathologist from New Zealand, and his associates J. L. Jebson and D. McFarlane discovered *T. gondii*-like organisms in the placentas and fetuses of several unexplained abortions in ewes in New Zealand. They called it New Zealand type II abortion. Hartley and Marshall (1957) finally isolated *T. gondii* from aborted fetuses. Subsequently, Jack Beverley and Bill Watson recognized epidemics of ovine abortion in the U.K. (Beverley and Watson, 1961). Dubey and Beattie (1988) summarized all that was known about toxoplasmosis in sheep and its impact on agriculture. Millions of lambs are still lost throughout the world due to this infectious disease. Dubey and Beattie (1988) reviewed the worldwide literature on toxoplasmosis in humans and other animals. The discovery and naming of two new organisms, *Neospora caninum* (Dubey et al. 1988) and *Sarcocystis neurona* (Dubey et al. 1991), that were previously thought to be *T. gondii*, resulted in new information on the host distribution of *T. gondii*. We now know that cattle and horses are resistant to clinical *T. gondii,* that *N. caninum* is a common cause of abortion in cattle worldwide (Dubey 2003), that *S. neurona* is a common cause of fatal encephalomyelitis in horses in the Americas (Dubey et al. 2001), and many cases of neosporosis in dogs were misdiagnosed as toxoplasmosis. There have been no confirmed cases of clinical toxoplasmosis in either cattle or horses (Dubey 2007).

The finding of *T. gondii* in marine mammals deserves special mention. Before the discovery of the *T. gondii* oocyst no one would have suspected that the marine environment would be contaminated with *T. gondii* and that fish-eating marine mammals would be found infected with *T. gondii* (Conrad et al. 2005; Dubey et al. 2003). Cole et al. (2000) isolated viable *T. gondii* from sea otters in the United States. Several reports of fatal toxoplasmosis in marine mammals have now appeared in the literature.

DIAGNOSIS

Sabin–Feldman dye test. Development of a novel serologic test, the dye test, in 1948 by Albert Sabin and Harry Feldman was perhaps the greatest advancement in the field of toxoplasmosis (Sabin and Feldman 1948). The dye test is highly sensitive and specific with no evidence for false results in humans. The ability to identify *T. gondii* infections based on a simple serological test opened the door for extensive epidemiological studies on the incidence of infection. It became clear that *T. gondii* infections are widely prevalent in humans in many countries.

Detection of IgM antibodies. Remington, Miller, and Brownlee (1968) first proposed the usefulness of the detection of IgM antibodies in cord blood or infant serum for the diagnosis of congenital toxoplasmosis because IgM antibodies do not cross the placenta, whereas IgG antibodies do. Remington (1969) modified the indirect fluorescent antibody test and the ELISA (Naot and Remington, 1980) to detect IgM in cord blood. Desmonts, Naot, and Remington (1981) developed a modification of IgM-ELISA, combining it with the agglutination test (IgM-ISAGA) to eliminate the necessity for an enzyme conjugate. Although IgM tests are not perfect, they have proved useful for screening programs (Remington et al. 2006).

Direct agglutination test (DAT). The development of a simple DAT has aided tremendously in the serological diagnosis of toxoplasmosis in humans and other animals. In this test no special equipment or conjugates are needed. This test was initially developed by Fulton (1965) and improved by Desmonts and Rem-

ington (1980), and Dubey and Desmonts (1987) who called it the modified agglutination test (MAT). The MAT has been used extensively for the diagnosis of toxoplasmosis in animals. The sensitivity and specificity of MAT has been validated by comparing serologic data and isolation of the parasite from naturally and experimentally infected pigs (Dubey 1997; Dubey et al. 1995a).

Detection of *Toxoplasma gondii* DNA. Burg et al. (1989) first reported detection of *T. gondii* DNA from a single tachyzoite using the B1 gene in a polymerase chain reaction (PCR). Several subsequent PCR tests have been developed using different gene targets. Overall, this technique has proven very useful in the diagnosis of clinical toxoplasmosis.

TREATMENT

Sabin and Warren (1942) reported the effectiveness of sulfonamides against murine toxoplasmosis and Eyles and Coleman (1953) discovered the synergistic effect of combined therapy with sulfonamides and pyrimethamine; the latter is the standard therapy for toxoplasmosis in humans (Remington et al. 2006). Garin and Eyles (1958) found spiramycin to have antitoxoplasmic activity of in mice. Because spiramycin is non-toxic and does not cross the placenta it has been used prophylactically in women during pregnancy to reduce transmission of the parasite from mother to fetus (Desmonts and Couvreur 1974b). The discovery of clindamycin having anti-toxoplasmal activity provided another drug to treat toxoplasmosis, especially in patients allergic to sulphonamides (Araujo and Remington 1974; McMaster et al. 1973).

PREVENTION AND CONTROL

Serologic screening during pregnancy. Georges Desmonts initiated studies in Paris, France in the 1960s looking at seroconversion in women during pregnancy and the transmission of *T. gondii* to the fetus (Desmonts and Couvreur 1974a, b). Blood was obtained at the first visit, at 7 mo, and at the time of parturition. Desmonts initiated prophylactic treatment of women who seroconverted during pregnancy. Results of the 15-yr study demonstrated that (1) infection acquired during the first two trimesters was most damaging to the fetus; (2) not all women that acquired infection transmitted it to the fetus; (3) women seropositive before pregnancy did not transmit infection to the fetus; and (4) treatment with spiramycin reduced congenital transmission, but not clinical disease in infants (Desmonts and Couvreur 1974a,b). At about the same time Otto Thalhammer initiated a similar screening program for pregnant women in Austria (Thalhammer 1973, 1978). In addition to scientific knowledge, these screening programs have helped to disseminate information for the prevention of toxoplasmosis.

A neonatal serological screening and early treatment for congenital *T. gondii* infection was initiated in MA, USA in the 1980s (Guerina et al. 1994). The efficacy of treatment of *T. gondii* infection in the fetus and newborn is not fully delineated, and many issues related to the cost and benefit of screening and treatment in pregnancy and in newborns remain to be examined (Dubey and Jones 2008).

HYGIENE MEASURES

After the discovery of the life cycle of *T. gondii* in 1970 it became possible to advise pregnant women and other susceptible populations on avoiding contact with oocysts (Frenkel and Dubey 1972). Studies were conducted to construct thermal curves showing temperatures required to kill *T. gondii* in infected meat by freezing (Kotula et al. 1991), cooking (Dubey et al. 1990), and by γ irradiation (Dubey et al. 1986). These data are now used by regulatory agencies to advise consumers about the safety of meat. Freezing of meat overnight in a household freezer before human or animal consumption remains the easiest and most economical method of reducing transmission of *T. gondii* through meat.

ANIMAL PRODUCTION PRACTICES

Extensive epidemiological studies on pig farms in the United States in 1990s concluded that keeping cats out of the pig barns and raising pigs indoors can reduce *T. gondii* infection in pigs (Dubey et al. 1995b; Weigel et al. 1995). As a result of changes in pig husbandry prevalence of viable *T. gondii* in pigs is reduced to $< 1\%$ (Dubey et al. 2005). Because ingestion of infected pork is considered the main meat source of *T. gondii* for humans (at least in the United States), it is tempting to speculate that it has led to decline of seroprevalence in humans in the United States (Dubey and Jones 2008; Jones et al. 2007).

VACCINATION

Vaccination of sheep with a live cyst-less strain of *T. gondii* reduces neonatal mortality in lambs and this vaccine is available commercially (Buxton and Innes 1995; Wilkins and O'Connel 1983). To date there is no vaccine suitable for human use.

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