© Cambridge University Press 2015
ISSN 1466-2523

Animal Health Research Reviews **16**(2); 150–162
doi:10.1017/S1466252315000183

Toxoplasma gondii: history and diagnostic test development

Heidi M. Wyrosdick and John J. Schaefer*

Department of Biomedical and Diagnostic Sciences, College of Veterinary Medicine, University of Tennessee, 2407 River Dr, Knoxville, Tennessee 37996, USA

Received 8 July 2015; Accepted 24 September 2015; First published online 16 November 2015

Abstract

Toxoplasma gondii is a protozoa that causes toxoplasmosis in people and other animals. It is considered one of the most common parasitic infections in the world due to its impressive range of hosts, widespread environmental contamination and the diverse means by which animals can be infected. Despite its ubiquity and numerous ongoing research efforts into both its basic biology and clinical management, many aspects of diagnosis and management of this disease are poorly understood. The range of diagnostic options that is available for veterinary diagnostic investigators are notably more limited than those available to medical diagnosticians, making accurate interpretation of each test result critical. The current review joins other reviews on the parasite with a particular emphasis on the history and continued development of diagnostic tests that are useful for veterinary diagnostic investigations. An understanding of the strengths and shortcomings of current diagnostic techniques will assist veterinary and public health officials in formulating effective treatment and control strategies in diverse animal populations.

Keywords: *Toxoplasma gondii*, toxoplasmosis, veterinary diagnostic investigation, diagnostic test development, serology, PCR.

Toxoplasma gondii is an obligate intestinal parasite of felids and is the etiological agent of toxoplasmosis, a disease of human beings, other mammals and birds caused by tissue invasion of the parasite. While the systematic description of the organism is straightforward, being the only representative apicomplexan in the genus *Toxoplasma*, there are not many other facets of *T. gondii* and toxoplasmosis that are so easily characterized (Dubey, 2010). This has contributed to research and public interest in the organism and its associated disease, and the generation of misunderstanding and misplaced concern in the medical and veterinary communities. A singular aspect of the parasite's life cycle that is well understood is the importance of domestic and wild felids, the only known definitive hosts, in the perpetuation of *T. gondii*. Beyond that, the ability of the organism to infect new hosts through ingestion of tissue cysts in intermediate hosts, oocysts in contaminated soil, water and produce, and congenital transmission, makes evident the true complexity of the *T. gondii* life cycle. Much of the interest in toxoplasmosis relates to the severity of disease in human fetuses and immunosuppressed populations (Lindsay *et al.*, 2003). Though only an estimated 15% of human infections with *T. gondii* result in detectable clinical signs, it is the third leading cause of death from foodborne disease in the USA (Mead *et al.*, 1999), hinting at the prevalence of infection in this population. Understanding the behavior of *T. gondii* in human populations gives an incomplete picture of the parasite. Because human toxoplasmosis is a zoonosis medical and veterinary investigations should collaborate to produce reasonable control strategies. The development of new diagnostic strategies will assist veterinary investigators in their understanding of the role of toxoplasmosis in their patients, and thereby contributes to efforts to combat its perpetuation.

*Corresponding author. E-mail: jschaef6@utk.edu

History

The history of *Toxoplasma gondii* discovery, identification of hosts, description of its life cycle, diagnostic strategies and treatment have been thoroughly reviewed with ongoing research efforts and clinical experience continuing to advance our understanding of this important pathogen (Dubey, 2008, 2010; Ferguson, 2009). One investigative team in the early 20th century noted the presence of protozoal organisms in their research gundy *(Ctendactylus gundi)* in Tunisia and another in rabbits *(Oryctolagus cuniculus)* in Brazil; both initially attributed the

https://doi.org/10.1017/S1466252315000183 Published online by Cambridge University Press

observation to *Leishmania* sp., before the former group realized it was a previously undescribed species and named it *T. gondii* (Splendore, 1908; Nicolle and Manceaux, 1909). One year later, a clinical case of toxoplasmosis was described in the domestic dog (*Canis familiaris*) in Italy, an early insight into the distribution of this parasite across divergent animal species and geographic areas (Mello, 1910). Tissue cyst structures were first noted in a group of rabbits and mice and described cytologically about 20 years after the organism's initial identification (Levaditi *et al.*, 1928). Early investigators depended on microscopic work and staining techniques. The advent of serological tests and molecular diagnostic techniques led to a rapidly increased understanding of the parasite and its role in clinical disease.

Life cycle

The demonstration of successful *T. gondii* transmission by congenital, carnivorous and fecal-oral routes stimulated research interest into the parasite's life strategy that allows survival in widely diverse hosts (Weinman and Chandler, 1954; Hutchison, 1965; Frenkel *et al.*, 1970; Dubey *et al.*, 2008a, b). *T. gondii* has a complex life cycle that can only be completed in the feline definitive host (DH), as indicated in Fig. 1. The most prolific and destructive stage is the tachyzoite, formerly called the trophozoite or endozoite. This is the active stage of the parasite and the most likely stage to cause clinical disease. The second life stage is the bradyzoite, which is the slow-replicating chronic stage that is most commonly within a tissue cyst. The oocyst is the life stage that results from sexual reproduction within the intestine and is shed in the feces of the DH, the wild or domestic felid (Dabritz *et al.*, 2007a, b). In intermediate hosts (IH), sporozoites released from sporocysts penetrate through intestinal epithelial cells and migrate to the lamina propria, where numerous different types of cells are invaded, and two tachyzoites are produced by approximately 12 h post infection. Following invasion of additional tissues with tachyzoites, tissue cysts have been noted to start forming by 6 days post infection and these likely persist for the life of the animal. The life cycle in felids can follow this same course, making felids uniquely both potential intermediate and definitive hosts. The latter, due to their hosting the sexual stages of *T. gondii*, in which schizogony is noted after intestinal invasion leading to the production of sexual stages (female macrogamonts and male microgamonts) by 2 days after infection.

Following the formation of zygotes and oocyst walls, the host cell ruptures, discharging oocysts into the intestine (Dubey, 2010). Both IH and DH life cycles require evasion of the host's immune system to proceed successfully, and the same white blood cells that respond to the presence of the parasite are utilized to spread infection throughout the host (Denkers *et al.*, 2012). Each cat has the potential to shed over 1 million *T. gondii* oocysts during the initial phase of the infection (Dabritz *et al.*, 2007a, b). The oocysts become infective when they sporulate in as little as 24 h in the environment (http://www.cdc.gov/parasites/toxoplasmosis/biology). The prepatent period (PPP) between ingestion and shedding of oocysts in domestic cats varies depending on the stage of the parasite initiating infection as noted above (Frenkel *et al.* 1970). The PPP after tissue cyst ingestion is considerably shorter (3–10 days) than after sporulated oocyst ingestion (18 days) in cats (Weiss and Kim, 2007). Considering the abbreviated PPP following tissue cyst ingestion in the cat with the similarly brief time from ingestion to tissue cyst production in the IH, it is easy to surmise how quickly contamination can spread through a felid-intermediate host/prey ecosystem, e.g. felids and rodents.

Human toxoplasmosis

Toxoplasmosis was established as a human disease after observation of a case of congenital transmission in 1939 and experimental transmission to animals from this case was demonstrated (Wolf *et al.*, 1939a; Ferguson, 2009). The recognition of the potential importance of toxoplasmosis in human health precipitated development of diagnostic tests to allow antemortem detection. Sabin and Feldman recognized that tachyzoites lose their affinity for methylene blue in the presence of anti-*T. gondii* antibody, leading to the description of the first widely used serological test, the Sabin–Feldman dye test (Sabin and Feldman, 1948; Dubey, 2008). Though still considered a gold standard in serological testing, the requirement for live parasites limits the utility of the test both due to the difficulty of acquiring and storing the organism and potential risk to laboratory personnel. Multiple tests have since been developed that utilize the agglutination of killed parasite or particles coated with antigen in the presence of antibody as the basis of serological diagnosis, including the direct agglutination test, the modified agglutination test, indirect hemagglutination and latex agglutination (Desmonts and Remington, 1980; Dubey and Desmonts, 1987). These have the advantage of not requiring live organisms, but still being able to test human and animal sera equally well, due to lack of reliance on a species-specific protein conjugate for detection. A disadvantage is their reliance on subjective interpretation by the user as to a positive or negative reaction. The reliance on serological interpretation in the diagnosis of toxoplasmosis leads to difficulties for medical and veterinary clinicians in judging the significance of a positive test, i.e. is the presence of antibody indicative of a clinically significant infection or just a historical artifact of latent infection? The advent of techniques to establish the presence of the parasite in different tissues holds promise in identifying clinically significant infection, with a polymerase chain reaction (PCR) technique targeting *T. gondii* first being described over two decades ago (Burg *et al.*, 1989).

Public health

Though felids act as the only definitive host, birds and mammals, including human beings, are known to serve as intermediate hosts (Assadi-Rad *et al.* 1995). Transmission to an IH occurs via ingestion of sporulated oocysts from feline fecal

https://doi.org/10.1017/S1466252315000183 Published online by Cambridge University Press

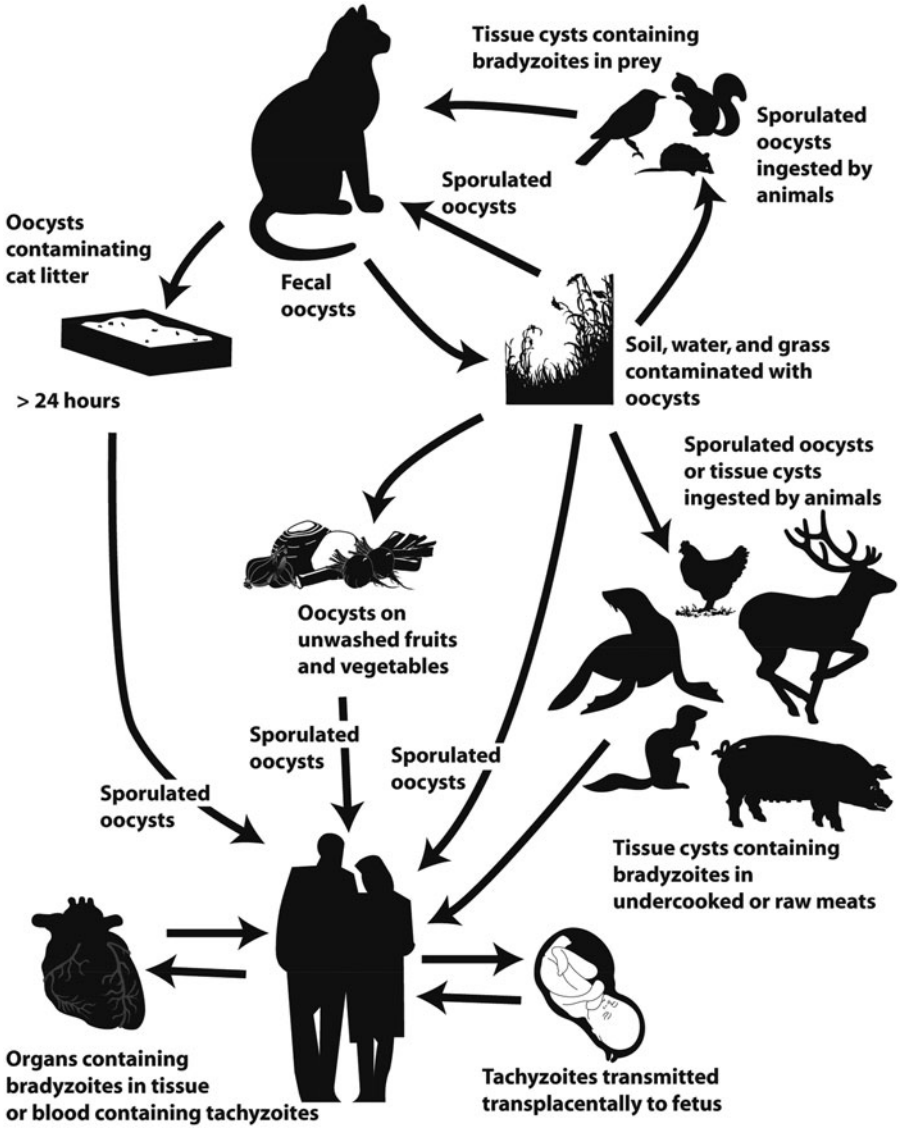


Fig. 1. The life cycle of *Toxoplasma gondii*.

contamination, ingestion of tissue cysts from raw or undercooked infected meat, or congenital transmission (transplacentally). In a European study, contact with domestic cats was not identified as a risk factor for seropositivity in people. Rather, eating undercooked meat, contact with soil and travel outside of Europe, the USA and Canada were significant. In all, 30–63% of infections were attributed to consumption of undercooked or cured meat and 6–17% to soil contact (Cook *et al.*, 2000). In the USA, parasite transmission to human adults typically occurs by ingestion of infected meats, ingestion of contaminated vegetables, or aspiration of aerosolized sporulated oocysts from cat litter boxes. In addition, there are an estimated 400–4000 new cases of congenital toxoplasmosis annually in the USA (http://www.cdc.gov/parasites/toxoplasmosis; Lopez *et al.*, 2000). The prevalence of infection in people varies widely, with a seroprevalence of 38% previously noted in the USA and a range of 4–92% noted in other countries. The fecundity of oocyst production (an average of 10,000,000 oocysts shed per infected cat has been estimated) and their environmental durability, with survival times of greater than one year noted in both natural and experimental setting, contribute to the ubiquity of the infection (Dubey, 2010). However, an estimate from the latest National Health and Nutrition Examination Survey (2009–2010) continues to recognize a trend towards decreasing rates of infection in the overall US human population with a seroprevalence of 22.5% noted (Jones *et al.*, 2014).

The pathogenicity of toxoplasmosis varies, depending on the age and species of host infected, the mode of infection, and the *T. gondii* genotype present (Assadi-Rad *et al.*, 1995). Mortality due to human toxoplasmosis occurs in very few cases, most notably due to reactive encephalitis in immunocompromised patients and abortion in naïve mothers on first exposure (Dabritz and Conrad, 2010). However, morbidity due to toxoplasmosis can take many forms, related to the diverse tissue types that can be invaded in intermediate hosts. Clinical signs and pathology in human adults range from no clinical signs to

https://doi.org/10.1017/S1466252315000183 Published online by Cambridge University Press

ocular disease, lymphadenopathy, tissue-specific lesions and localized edema, and death (Weiss and Kim, 2007). It was linked to potentially fatal central nervous system disease in AIDS patients in the late 1980s (Luft *et al.*, 1983; Tuazon, 1989; Harden and Hair, 1994). Congenital toxoplasmosis occurs when a mother is infected during pregnancy and commonly causes fetal death, though severity of disease and probability of congenital transfer depends on stage of pregnancy at time of maternal infection (Weiss and Kim, 2007). Though incidence of congenital transmission increases as pregnancy progresses, the pathogenicity of the congenital disease tends to decrease in severity (Remington and Desmonts, 1990; Lynfield and Guerina, 1997; Freeman *et al.*, 2005; Rorman *et al.*, 2006). The most commonly observed clinical and pathological signs in infants range from no clinical disease to chorioretinitis, blindness, severe hydrocephaly, meningoencephalitis, encephalomyelitis, intracerebral calcification, mental retardation, abortion, and postpartum death (Janků, 1923; Torres, 1927; Wolf *et al.*, 1939a, b; Sabin, 1942; Frenkel and Friedlander, 1951; Hogan, 1951; Remington *et al.*, 2001; Weiss and Kim, 2007). Methods to reduce human exposure include: proper cooking of meat and vegetables, hand hygiene after handling meat or soil contact, preventing predation by cats, providing litter boxes and controlling feral cat populations (Dabritz and Conrad, 2010). However, the recognition of *T. gondii* genotypes in wildlife capable of infecting people implies a sylvatic life cycle that can independently perpetuate the parasite's population (Dubey *et al.*, 2008a, b; Wendte *et al.*, 2011).

Feline toxoplasmosis

Feline toxoplasmosis was first diagnosed in a domestic cat (*Felis catus*) from Middletown, NY, in 1942 (Olafson and Monlux, 1942; Dubey, 2008). The protracted time between initial description (1908) and this first described case in what was later determined to be a definitive host of the organism is a tribute to the covert nature of *T. gondii* transmission and symptomatology in this host. It was another two decades before an investigator noted that oocysts can be isolated from feline feces (Hutchison, 1965). He further noted that they can remain infectious in the environment for at least 12 months as demonstrated by experimental transmission to mice. Feline intestinal stages, both sexual and asexual, were described in detail by Frenkel *et al.* (1970). They demonstrated that domestic cats shed oocysts following ingestion of infected mice, and further noted a discrepancy in the PPP for *T. gondii* in the feline host based on the stage of the parasite that produced the infection. Tissue cyst ingestion, which is thought to be the most common method of feline infection due to predation of intermediate hosts, revealed shedding of oocysts in 3–5 days. In contrast, ingestion of tachyzoites produced oocyst shedding in 8–10 days and ingestion of oocysts produced shedding in 21–24 days. Though oocyst shedding is intensive, with one study noting up to 13 million oocysts per gram of feces, it is temporally abbreviated, at approximately 1 week (Schares *et al.*, 2008; Dubey, 2010). This unfortunately makes direct fecal examination a diagnostic test of very low sensitivity in the definitive host, and questionable specificity due to the similar appearance of other protozoal oocysts like those of *Hammondia* and *Besnoitia* spp.

T. gondii in intermediate hosts

The search for intermediate hosts of *T. gondii* has been accomplished by numerous investigators with notable success (Dubey, 2010). Current indication that any warm-blooded animal can serve as an intermediate host for the organism and the identification of marine invertebrates as potential transport hosts significantly broadens the number of animals of potential interest (Lindsay *et al.*, 2001). The earliest animals identified as intermediate hosts of *T. gondii* were the gundy, rabbit, domestic dog and mouse (Splendore, 1908; Nicolle and Manceaux, 1909; Mello, 1910; Levaditi *et al.*, 1928). The first isolation of viable *T. gondii* was from laboratory mice and led to its characterization as an obligate intracellular parasite (Sabin and Olitsky, 1937). The lack of a requirement for a definitive host in the parasite's propagation was noted by oral infection of pigs with mouse tissue, and of mice with pork, also establishing the potential risk to human beings from meat consumption (Weinman and Chandler, 1954). Similarly, minced fetal cotyledons from suspected ovine abortion cases were demonstrated to be infectious to other pregnant ewes and mice by intraperitoneal injection (Hartley and Marshall, 1957). More recently, the surprising discovery of toxoplasmosis being a cause of significant mortality in southern sea otters (*Enhydra lutris nereis*) demonstrated how pervasive environmental contamination with oocysts is in both terrestrial and marine environments (Cole *et al.*, 2000). The ability of *T. gondii* to infect a wide host range and to have a broad geographic distribution (described from all continents) makes it one of the most common protozoal parasites in the world.

Feline health

Toxoplasmosis in domestic cats is a topic of interest primarily due to the threat their infection poses to people and other animals, as feline hosts are typically subclinical. An extensive survey of cats that were clinically ill from any cause in the USA found a seroprevalence of 31.6% overall, with the southwestern USA having the lowest prevalence (16.1%) and the northeastern USA having the highest prevalence (43.5%). Significant factors other than geographic location that predisposed the cats to being positive included: older age, being male and being a domestic short-haired breed (Vollaire *et al.*, 2005). A study of Ohio cats brought into a mobile spay and neuter clinic revealed a 48% overall seropositivity, with a 62% prevalence noted in owner-described outdoor cats (Dubey *et al.*, 2002a, b). A survey of Pennsylvania cats from a peri-urban environment yielded a significantly lower seropositivity of 19.5% (Dubey *et al.*, 2009a, b). In the USA, the range of seropositivity survey results for cats was a low of 12.1% from Florida and a high of 80% from Iowa (Hill *et al.*, 1998; Luria *et al.*, 2004). Surveys from

https://doi.org/10.1017/S1466252315000183 Published online by Cambridge University Press

other countries produced a wider range of seroprevalence, from a low of 2.1% in a Chinese study to a high of 89.3% in a Colombian study (Shen *et al.*, 1990; Dubey, 2010). A survey of non-domestic felids in the USA found a prevalence of 43.6%, looking at both captive and free-ranging animals (Spencer *et al.*, 2003). The degree of exposure to *T. gondii* for both domestic and wild felids as demonstrated by these studies can be significant from diverse geographic and demographic areas. The role of feline predation in the *T. gondii* life cycle and the lack of an effective vaccine make the reduction of roaming cat populations a critical tool in controlling environmental contamination with oocysts. This remains a challenge in most settings, with the questionable efficacy of many sterilization and release programs potentially complicating population control efforts (Andersen *et al.*, 2004; Jessup, 2004; Nogales *et al.*, 2004; Longcore *et al.*, 2009).

Canine health

The description of toxoplasmosis in the domestic dog preceded that in the domestic cat by more than three decades (Mello, 1910; Olafson and Monlux, 1942). While the evaluation of the significance of toxoplasmosis in the dog is complicated by potential infection with *Neospora caninum*, it is clear that both parasites can cause significant diseases in dogs. An examination of stray dogs and cats in Sao Paulo, Brazil, revealed a *T. gondii* seroprevalence of 40% in cats and 50.5% in dogs, which the authors attributed to the more selective palate of cats (Meireles *et al.*, 2004). A demonstration of the broad appetite of dogs was the identification of viable oocysts in canine feces in a German study, which could have wrongly indicted dogs as an alternative definitive host if the investigators were not familiar with their coprophagic habits (Schares *et al.*, 2005). A study of stray animals in Bangkok, Thailand, revealed significantly lower levels of seroconversion than many studies, 11% in cats and 9.4% in dogs, the authors noted that this population of animals were often fed cooked fish and rice by local monks, foods expected to carry a low risk of serving as routes of infection (Jittapalapong *et al.*, 2007). A study in the USA revealed a seroprevalence of 25% in Kansas dogs, with a range of 5.2–88.5% noted in other areas of the world, the lowest value noted from urban, owned dogs in Brazil and the highest value from dogs in Mato Grosso, the Brazilian state with the lowest human population density (Lindsay *et al.*, 1990; deSouza *et al.*, 2003; Santos *et al.*, 2009). Domestic dogs, especially free roaming dogs that are more likely to participate in predation, can serve as sentinels for local *T. gondii* occurrence, as they are exposed through both oocyst and tissue cyst ingestion (Meireles *et al.*, 2004).

Large animal health

Toxoplasmosis in food animals is a topic of significant interest, both due to potential public health risk from meat and milk consumption and economic losses due to disease. One of the earliest demonstrations of the importance of toxoplasmosis in sheep production was an investigation of an abortion storm on a farm in New Zealand, in which minced cotyledons from infected ewes transmitted *T. gondii* to additional ewes and mice (Hartley and Marshall, 1957). A similar outbreak in New York revealed that 73.8% of sheep on the farm were seropositive (Dubey and Welcome, 1988). The range of seroprevalence in sheep studies in the USA is 27.1–73.8%, with a range of 3–95.7% noted from sheep studies worldwide (Zaki, 1995; Dubey *et al.*, 2008a, b; Dubey, 2010). Similar clinical effects have been noted in caprine toxoplasmosis, with US surveys noting a seroprevalence of 22.1–51.9% in goat herds, and a worldwide seroprevalence of 3.2–90.9% (McSporran *et al.*, 1985; Dubey and Adams, 1990; Patton *et al.*, 1990; Garcia-Vazques *et al.*, 1993; Dubey, 2010). Consumption of poorly cooked lamb and goat, raw milk and unpasteurized cheese should be considered potential routes of transmission (Dubey *et al.*, 2014a, b). Pork may also be a source of *T. gondii*, as reported in a Massachusetts study in which 51 of 55 pigs destined for human consumption were found to have viable tissue cysts in their heart and tongue tissue (Dubey *et al.*, 2002a, b).

The role of toxoplasmosis as a significant pathogen of cattle and horses is not well recognized. Both species can experience infection as indicated by seroprevalence and experimental infection studies, but reports of associated pathology are rare (Tassi, 2007; Dubey, 2010). These infections tend to be insignificant in both intensity and duration with a risk of misidentification due to the morphological similarity of *Neospora* spp. and *Sarcocystis* spp. The detection of viable tissue cysts and DNA in tissue samples from infected cattle and horses indicates a potential threat to human and animal health from the consumption of undercooked beef or horse meat (Dubey and Desmonts, 1987; Dubey, 2010; Papini *et al.*, 2015). Dubey *et al.* (2005) described a large survey of beef, chicken and pork purchased from retail food stores in the USA, utilizing bioassay, enzyme-linked immunosorbent assays (ELISA) and PCR analysis, to find viable *T. gondii* only in pooled pork samples. The role of unpasteurized cow's milk in transmission of *T. gondii* has been thought to be minimal, with examination of the subject continuing (Tenter *et al.*, 2000; Dehkordi *et al.*, 2013; Dubey and Jones, 2014).

Wildlife health

Many wildlife species are susceptible to *T. gondii* infection and to clinical toxoplasmosis. One species of interest as a sentinel host for domestic herbivores is the white-tailed deer *(Odocoileus virginianus*), both due to its tendency to graze alongside livestock and its wide dispersal. A study in Ohio noted a *T. gondii* seroprevalence of 44% among white-tailed deer, 6% in deer harvested in urban parks (Columbus) and 55% in more rural Hocking County (Crist *et al.*, 1999). A more recent study examined seroprevalence in deer in Northeastern Ohio and found evidence of greater exposure among urban rather than suburban populations (Ballash *et al.*, 2015). This implies that human (and by extension domestic cat) population density may not be a reliable predictor of environmental contamination by *T. gondii*.

https://doi.org/10.1017/S1466252315000183 Published online by Cambridge University Press

Surveys in other states ranged from 14.8 to 75.7% seroprevalence (Humphreys *et al.*, 1995; Vanek *et al.*, 1996; Dubey *et al.*, 2009a, b, 2014a, b; Dubey, 2010; Schaefer *et al.*, 2013). The clinical effect of toxoplasmosis on deer is poorly understood, as is the question of bias arising from an interaction between infection and the tendency to be killed by hunters, a requirement for inclusion in most of these studies.

Despite the strictly terrestrial nature of the definitive host, studies over the past 11 years have found *T. gondii* in marine settings and have attributed marine mammal morbidity and mortality to toxoplasmosis. The first major case in marine mammals occurred in sea otters off the coast of California (Miller *et al.*, 2002). These infections were linked to coastal freshwater runoff of feces from feral cat populations (Cole *et al.*, 2000; Miller *et al.*, 2002), and Shapiro *et al.* (2014) determined that further dispersal of the oocysts into the marine environment was facilitated by aquatic polymers (biofilm) and invertebrates. The diversity of the marine mammal habitats and foraging habits make it challenging to determine how many are infected with *T. gondii.* Coastal runoff may explain infection in the benthoneritic but does not explain infection in more pelagic marine mammals. A few studies have suggested the dispersal of *T. gondii* oocysts is possible to more pelagic fish-eating marine mammals via transport host or mechanical vector. Eastern oysters can filter and maintain viable *T. gondii* oocysts from the seawater within their tissues for up to 85 days (Lindsay *et al.*, 2001, 2005). Migratory filter feeding fish, including sardines and anchovies, have been implicated in the dispersal of oocysts into the marine environment by serving as transport hosts or biotic vectors. In addition to the oocysts being maintained in the alimentary tract of the fish, 30% of fish retained oocysts infective to mice (Massie *et al.*, 2010).

Diagnostic test development

The discovery and subsequent investigation of *T. gondii* largely depended on bioassays and microscopic examination until the advent of the Sabin–Feldman dye test, which allowed highly accurate antemortem diagnosis of infection through the evaluation of serum samples (Sabin and Feldman, 1948). The dye test proved highly sensitive and accurate, but had the distinct disadvantage of requiring live tachyzoites, a potential risk to laboratory staff conducting the test (Reiter-Owona *et al.*, 1999). In medical diagnostics, a number of immunofluorescence and ELISA tests were developed, but have limited utility in veterinary diagnostics due to their requirement for species-specific protein conjugate. The development of the direct agglutination and later the modified agglutination tests, techniques that utilized formalin-killed rather than live tachyzoites and did not have a conjugate requirement, was revolutionary in the diagnosis and investigation of toxoplasmosis in animals (Desmonts and Remington, 1980; Dubey and Desmonts, 1987). These techniques have been further modified to produce additional agglutination tests that utilize the same strategy: the indirect hemagglutination test (IHA) using antigen coated sheep red blood cells and the latex agglutination test (LAT) using antigen coated latex beads. Indirect hemagglutination test results were noted to agree with Sabin–Feldman dye test results in human patients, and similarly remain positive for the life of the patient (Lunde, 1973). In a study of experimental infection using cats that were monitored for up to 950 days after inoculation, the Sabin–Feldman dye test, modified agglutination test (MAT), IHA and LAT were compared and the MAT was found to be the most sensitive of the agglutination methods (Dubey and Thulliez, 1989). The MAT and IHA were found to produce similar results in a study of dairy goats (Patton *et al.*, 1990). The MAT also had good correlation with an ELISA test developed specifically for porcine testing (Gamble *et al.*, 2005). None of the agglutination tests reliably detect IgM on known IgM positive samples, and therefore become positive later in the course of infection than the dye test (Lappin and Powell, 1991). Agglutination testing remains the most commonly used modality for the diagnosis of toxoplasmosis in animals, despite the loss of availability of some commercial kits. These diagnostic methods and those discussed below are summarized in Table 1.

Antibody testing

Diagnosis of human toxoplasmosis is largely accomplished by serological investigation using ELISAs to detect *T. gondii*-specific IgG and IgM, as the requirement for species-specific conjugate is not as burdensome as in veterinary medicine. There are numerous commercial serological tests available for the detection of infection in human beings; one study compared three ELISAs, two IHAs, one IFA and one LAT to the Center for Disease Control's reference indirect immunofluorescence test and found 100% sensitivity across the tests, with a range of specificities from 77.8 to 100%, and perfect agreement being noted by one representative of each testing method (Wilson *et al.*, 1987). The reliance on a single serum test (e.g. an IgM-specific ELISA) to determine the critical question of chronicity of infection, however, complicates the diagnostic picture, as positive results indicating acute infection can have dramatic consequences in the management of patients, especially pregnant women. Several studies have noted high sensitivities but variable specificities for commercial IgM-specific ELISAs, with a positive predictive value of only 43.3% noted in one (Wilson *et al.*, 1997; Roberts *et al.*, 2001; Kodym *et al.*, 2006). Confirmation testing is recommended in these cases, two options being paired IgG testing or IgG avidity testing (Liesenfeld *et al.*, 1997). While few IgM ELISAs are utilized for veterinary diagnostics, IgM testing can be accomplished by utilizing modified agglutination methods, but the same cautions must be noted when relying on a single serum sample for the determination of infection chronicity.

In veterinary investigation of *T. gondii*, the production of recombinant antigen and advance of molecular techniques has expanded diagnostic options, while more established agglutination techniques are becoming less commercially available. Some species-specific ELISAs have been developed and performed well when compared with reference serological techniques, notably for cats, sheep and pigs (Kimbita *et al.*, 2001;

https://doi.org/10.1017/S1466252315000183 Published online by Cambridge University Press

https://doi.org/10.1017/S1466252315000183 Published online by Cambridge University Press

Table 1. Summary of veterinary diagnostic test methods for toxoplasmosis

Diagnostic method	Evidence detected	Antigen/primer target	Advantages	Disadvantages	Animal species	Reference
Serological						
Indirect hemagglutination test (IHA)	Antibody	Tachyzoite lysate	Antemortem diagnostic method, No species-specific conjugate needed	Less sensitive and specific than MAT, no longer commercially available in the USA	All mammals and birds	Lunde (1973), Dubey and Thulliez (1989)
Latex agglutination test (LAT)	Antibody	Tachyzoite lysate	Antemortem diagnostic method, No species-specific conjugate needed, commercial kit available	Less sensitive and specific than MAT	All mammals and birds	Dubey and Thulliez (1989)
Modified antibody agglutination test (MAT)/direct agglutination test (DAT)	Antibody	Formalin fixed tachyzoites	Antemortem diagnostic method, No species-specific conjugate needed, commercial kit available from France (as DAT)	Not commercially available in the USA, requires formalin fixed tachyzoites	All mammals and birds	Desmonts and Remington (1980), Dubey and Desmonts (1987)
Indirect fluorescent antibody test (IFAT)	Antibody	Tachyzoite lysate	Antemortem diagnostic method	Requires the use of species-specific conjugate and fluorescence microscope, results depend on interpretation of microscopist	Determined by use of species specific conjugate	Silva *et al.* (2002), Shaapan *et al.* (2008)
Enzyme-linked immunosorbent assay (ELISA)	Antibody	Tachyzoite lysate or recombinant antigens (e.g. surface antigens [SAGs], dense granule antigens [GRAs] and microneme antigens [MICs])	Antemortem diagnostic method, commercial tests available, optical density results can be read using automated plate reader allowing high-throughput testing, (modification for multiple species tests described)	Limited range of utility in animal species in most formats due to conjugate requirement, some tests require automated plate reader	Cats, dogs, pigs, sheep (others as determined by conjugate)	Dabritz *et al.* (2007a, b), Eliasson *et al.* (1989), Hill *et al.* (2006), Kimbita *et al.* (2001), Klun *et al.* (2007), Mainar-Jaime and Barberan (2007), Schaefer *et al.* (2011), Shaapan *et al.* (2008), Su *et al.* (2006), Weiss and Kim (2007), Zhang *et al.* (2010)
Sabin–Feldman dye test (DT)	Antibody	Live tachyzoites	Antemortem diagnostic method, high sensitivity and specificity	Requires source of tachyzoites, hazard due to need for live tachyzoites	All mammals and birds	Sabin and Feldman (1948)
Molecular testing						
Polymerase chain reaction (PCR)	Parasite nucleic acid	B1 gene, 529 repeat sequence	Useful in confirming parasite presence in tissues, high specificity, allows quantification and genotyping using amplified sequences	Likely postmortem, DNA of parasite must be present in sample (can be difficult to locate), extremely low sensitivity depending on tissue studied, depending on sequence used and accuracy of sample containing cyst could result in false negatives	Any	Burg *et al.* (1989), Homan *et al.* (2000)

https://doi.org/10.1017/S1466252315000183 Published online by Cambridge University Press

Parasite detection					
Histology	Tissue cysts	Establishes presence of tissue cyst, allows elucidation of associated pathology	Requires necropsy or biopsy for tissue collection, histology expertise and immunohistochemistry or molecular analysis to distinguish from similar protozoal cysts	Any	Dubey (2010)
Culture	Tachyzoites	Establishes presence of viable tachyzoites	Requires laboratory animals, chick embryos or active cell cultures, hazard due to viable organism	Any	Dubey (2010)
Feline infection	Oocysts, tachyzoites or bradyzoites	Establishes presence of patent infection or viable oocysts	Requires laboratory cats, hazard due to viable organism	Any	Dubey (2010)
Fecal flotation	Oocysts	Low cost, minimal equipment required	Only for definitive hosts, poor sensitivity due to abbreviated period of shedding, poor specificity due to similar appearance of other protozoal oocysts, hazard due to viable organism	Felids	Dubey (2010)

Hill *et al.*, 2006; Dabritz *et al.*, 2007a, b; Klun *et al.*, 2007; Mainar-Jaime and Barberan, 2007; Shaapan *et al.*, 2008). Some cautions that must be noted in interpretation of these results include the potential of cross-reactivity in the presence of infections with other coccidia and the difficulty of determining true sensitivities and specificities without a reliable gold standard for comparison (Silva *et al.*, 2002; Gardner *et al.*, 2009). Most ELISA procedures require species-specific conjugates, which translates to a large number of individual ELISAs being used to test the range of animal species submitted to veterinary diagnostic laboratories, making them a poor replacement for the established agglutination techniques. Some investigators have examined the use of non-species-specific protein conjugates in veterinary diagnostic testing, e.g. proteins A, G and A/G, with some success (Eliasson *et al.*, 1989; Werre *et al.*, 2002; Bhide *et al.*, 2004; Zhang *et al.*, 2010; Schaefer *et al.*, 2011; Schaefer *et al.*, 2012; Al-Adhami and Gajadhar, 2014).

Molecular testing

While serological detection is the primary mode of *T. gondii* diagnosis in people and other animals, molecular techniques have increasingly been used since the first identification of a gene target for PCR amplification (Burg *et al.*, 1989). PCR is now commonly used to test amniotic fluid in pregnant women with suggestive serological results, and has been used for environmental detection of oocysts and characterization of *T. gondii* genotypes in vertebrate hosts (Schwab and McDevitt, 2003; Su *et al.*, 2006). The difficulty of acquiring the appropriate tissue sample for antemortem diagnosis makes PCR a modality of questionable sensitivity but potentially excellent specificity when used in the diagnostic setting. Direct detection of the parasite is complicated in the definitive feline host by the transient appearance of oocysts in feces and in intermediate hosts by the difficulty in predicting the precise location of tissue cysts (Dubey *et al.* 1977, 2007a, b; Dubey, 1995, 2001; Dabritz and Conrad, 2010). While serology provides evidence of infection, a single sample does not allow the characterization of an acute versus a chronic infection, or the severity of the infection. The direct detection of DNA by PCR is a promising method in that a positive result establishes the presence of the organism in the tested tissue. However, the nature of the organism's complex life cycle poses a challenge in translating a successful PCR assay into a useful clinical test.

PCR

There are two commonly used targets for the detection of genomic DNA by either traditional or real time PCR, the *B1* gene and AF146527 (529-bp repeat element). The *B1* gene was initially detected as a PCR target with 35 repeats in the *T. gondii* genome by Burg *et al.* (1989), who determined a limit of detection of one organism in cell lysate and 10 organisms in a human leukocyte preparation by traditional PCR, with no evidence of detection of closely related pathogens. The *B1* gene has become a standard target for PCR detection in clinical samples and, significantly for veterinary diagnostics, does not occur in *Neospora caninum* (Schatzberg *et al.*, 2003; Chabbert *et al.*, 2004). More recently, a 529-bp target (AF146527) that is repeated 200–300 fold in the *T. gondii* genome has been described and utilized as a target for PCR detection (Homan *et al.*, 2000). No statistically significant difference in sensitivity or specificity between the *B1* gene and AF146527 targets were noted in some investigations (Filisetti *et al.*, 2003; Remington *et al.*, 2004), whereas other studies noted that assays for 529 were more sensitive for a variety of fluid and tissue samples (Homan *et al.*, 2000; Reischl *et al.*, 2003; Calderaro *et al.*, 2006; Cassaing *et al.*, 2006; Dubey, 2010; Belaz *et al.*, 2015). Investigation of sequence variation across strains from human infections noted that the AF146527 element is absent in 4.8% of cases, which may lead to false negative determinations even if the organism is present in the tissue being evaluated (Wahab *et al.*, 2010).

Both methods are used for detection of *T. gondii* in diverse tissues in human and veterinary diagnostic investigation. Samples commonly evaluated from suspected human cases include: amniotic fluid, placenta, brain, blood, cerebrospinal fluid, urine, vitreous, aqueous, bronchoalveolar lavage, pleural and peritoneal fluids (Remington *et al.*, 2004). Veterinary diagnostic laboratories offer evaluation of blood, feces, CSF, brain and other tissues, with fecal examination used to distinguish *T. gondii* oocysts from morphologically similar *Hammondia hammondi* in feline feces (Schares *et al.*, 2008). Knowledge of the parasite's life cycle is critical to increase the utility of PCR testing, e.g. blood stages are transient and expected early in infection, and detection of the parasite in tissue relies on the presence of a tissue cyst in the aliquot being tested. Even with a good sampling strategy, molecular detection must be considered a method of unproven sensitivity but good specificity, where the predictive value of a positive result is much higher than that of a negative result (Remington *et al.*, 2004). This makes molecular detection a reasonable adjunct to serological investigation, in which the opposite is true regarding positive and negative predictive values. The establishment of a reliable real-time PCR protocol for detection of *T. gondii* DNA will allow definitive diagnosis of the presence and stage of infection when serological testing produces equivocal results.

Acknowledgements

The authors would like to thank Greg Schweiger for designing Figure 1 and Aly Chapman for her contributions to its content.

References

Al-Adhami BH and Gajadhar AA (2014). A new multi-host species indirect ELISA using protein A/G conjugate for detection of anti-*Toxoplasma gondii* IgG antibodies with comparison to ELISA-IgG, agglutination assay and Western blot. *Veterinary Parasitology* **200**: 66–73.

Andersen MC, Martine BJ and Roemer GW (2004). Use of matrix population models to estimate the efficacy of euthanasia versus

https://doi.org/10.1017/S1466252315000183 Published online by Cambridge University Press

trap-neuter-return for management of free-roaming cats. *Journal of the American Veterinary Medical Association* **225**: 1871–1876.

Assadi-Rad AM, New J and Patton S (1995). Risk factors associated with transmission of *Toxoplasma gondii* to sows kept in different management systems in Tennessee. *Veterinary Parasitology* **57**: 289–297.

Ballash GA, Dubey JP, Kwok OCH, Shoben AB, Robison TL, Kraft TJ and Dennis PM (2015). Seroprevalence of *Toxoplasma gondii* in white-tailed deer (*Odocoileus virginianus*) and free-roaming cats (*Felis catus*) across a suburban to urban gradient in northeastern Ohio. *EcoHealth* **12**: 359–367.

Belaz S, Gangneux J-P, Dupretz P, Guiguen C and Robert-Gangneux F (2015). A 10-year retrospective comparison of two target sequences, REP-529 and B1, for *Toxoplasma gondii* detection by quantitative PCR. *Journal of Clinical Microbiology* **53**: 1294–1300.

Bhide MR, Curlik J, Travnicek M and Lazar P (2004). Protein A/G dependent ELISA a promising diagnostic tool in Lyme disease seroprevalence in game animals and hunting dogs. *Comparative Immunology, Microbiology & Infectious Diseases* **27**: 191–199.

Burg JL, Grover CM, Pouletty P and Boothroyd JC (1989). Direct and sensitive detection of a pathogenic protozoan, *Toxoplasma gondii*, by polymerase chain-reaction. *Journal of Clinical Microbiology* **27**: 1787–1792.

Calderaro A, Piccolo G, Gorrini C, Peruzzi S, Zerbini L, Bommezzadri S, Dettori F and Chezzi C (2006). Comparison between two real-time PCR assays and a nested-PCR for the detection of *Toxoplasma gondii*. *Acta Biomedica* **77**: 75–80.

Cassaing S, Bessieres MH, Berry A, Berrebi A, Fabre R and Magnaval JF (2006). Comparison between two amplification sets for molecular diagnosis of toxoplasmosis by real-time PCR. *Journal of Clinical Microbiology* **44**: 720–724.

Chabbert E, Lachaud L, Crobu L and Bastien P (2004). Comparison of two widely used PCR primer systems for detection of *Toxoplasma* in amniotic fluid, blood, and tissues. *Journal of Clinical Microbiology* **42**: 1719–1722.

Cole RA, Lindsay DS, Howe DK, Roderick CL, Dubey JP, Thomas NJ and Baeten LA (2000). Biological and molecular characterizations of *Toxoplasma gondii* strains obtained from southern sea otters (*Enhydra lutris nereis*). *Journal of Parasitology* **86**: 526–530.

Cook AJC, Gilbert RE, Buffolano W, Zufferey J, Petersen E, Jenum PA, Foulon W, Semprini AE, Dunn DT and On behalf of the European Research Network on Congenital Toxoplasmosis (2000). Sources of *Toxoplasma* infection in pregnant women: European multicenter case-control study. *BMJ* **321**: 142–147.

Crist SC, Stewart RL Jr, Rinehart JP and Needham GR (1999). Surveillance for *Toxoplasma gondii* in the white-tailed deer (*Odocoileus virginianus*) in Ohio. *Ohio Journal of Science* **99**: 34–37.

Dabritz HA and Conrad PA (2010). Cats and *Toxoplasma*: implications for public health. *Zoonoses and Public Health* **57**: 34–52.

Dabritz HA, Gardner IA, Miller MA, Lappin MR, Atwill ER, Packham AE, Melli AC and Conrad PA (2007a). Evaluation of two *Toxoplasma gondii* serologic tests used in a serosurvey of domestic cats in California. *Journal of Parasitology* **93**: 806–816.

Dabritz HA, Miller MA, Atwill ER, Gardner IA, Leutenegger C, Melli AC and Conrad PA (2007b). Detection of *T. gondii*-like oocysts in cat feces and estimates of the environmental oocyst burden. *Journal of the American Veterinary Medical Association* **231**: 1676–1684.

Dehkordi FS, Borujeni MR, Rahimi E and Abdizadeh R (2013). Detection of *Toxoplasma gondii* in raw caprine, ovine, buffalo, bovine and camel milk using cell cultivation, cat bioassay, capture ELISA and PCR methods in Iran. *Foodborne Pathogens and Disease* **10**: 120–125.

Denkers EY, Schneider AG, Cohen SB and Butcher BA (2012). Phagocyte responses to protozoan infection and how *Toxoplasma gondii* meets the challenge. *Public Library of Science Pathogens* **8**: e1002794.

Desmonts G and Remington JS (1980). Direct agglutination test for diagnosis of *Toxoplasma* infection: method for increasing sensitivity and specificity. *Journal of Clinical Microbiology* **11**: 562–568.

deSouza SLP, Gennari SM, Yai LEO, D'Auria SRN, Cardoso SMS, Junior JSG and Dubey JP (2003). Occurrence of *Toxoplasma gondii* antibodies in sera from dogs of the urban and rural areas from Brazil. *Revista Brasileira de Parasitologia Veterinaria* **12**: 1–3.

Dubey JP (1995). Duration of immunity to shedding of *Toxoplasma gondii* oocysts by cats. *Journal of Parasitology* **81**: 410–415.

Dubey JP (2001). Oocyst shedding by cats fed isolated bradyzoites and comparison of infectivity of bradyzoites of the VEG strain *Toxoplasma gondii* to cats and mice. *Journal of Parasitology* **87**: 215–219.

Dubey JP (2008). The history of *Toxoplasma gondii* – the first 100 years. *Journal of Eukaryotic Microbiology* **55**: 467–475.

Dubey JP (2010). *Toxoplasmosis of Animals and Humans*. 2nd edn. Boca Raton, FL: CRC Press.

Dubey JP and Adams DS (1990). Prevalence of *Toxoplasma gondii* antibodies in dairy goats from 1982 to 1984. *Journal of the American Veterinary Medical Association* **196**: 295–296.

Dubey JP and Desmonts G (1987). Serological responses of equids fed *Toxoplasma gondii* oocysts. *Equine Veterinary Journal* **19**: 337–339.

Dubey JP and Jones JL (2014). Comments on "Detection of *Toxoplasma gondii* in raw caprine, ovine, buffalo, bovine and camel milk using cell cultivation, cat bioassay, capture ELISA, and PCR methods in Iran". *Foodborne Pathogens and Disease* **11**: 500.

Dubey JP and Thulliez P (1989). Serologic diagnosis of toxoplasmosis in cats fed *Toxoplasma gondii* tissue cysts. *Journal of the American Veterinary Medical Association* **194**: 1297–1299.

Dubey JP and Welcome FL (1988). *Toxoplasma gondii*-induced abortion in sheep. *Journal of the American Veterinary Medical Association* **193**: 697–700.

Dubey JP, Christie E and Pappas PW (1977). Characterization of *Toxoplasma gondii* from the feces of naturally infected cats. *Journal of Infectious Diseases* **136**: 432–435.

Dubey JP, Gamble HR, Hill D, Sreekumar C, Romand S and Thulliez P (2002a). High prevalence of viable *Toxoplasma gondii* infection in market weight pigs from a farm in Massachusetts. *Journal of Parasitology* **88**: 1234–1238.

Dubey JP, Saville WJA, Stanek JF and Reed SM (2002b). Prevalence of *Toxoplasma gondii* antibodies in domestic cats from rural Ohio. *Journal of Parasitology* **88**: 802–803.

Dubey JP, Hill DE, Jones JL, Hightower AW, Kirkland E, Roberts JM, Marcet PL, Lehmann T, Vianna MCB, Miska K, Sreekumar C, Kwok OCH, Shen SK and Gamble HR (2005). Prevalence of viable *Toxoplasma gondii* in beef, chicken, and pork from retail meat stores in the United States: risk assessment to consumers. *Journal of Parasitology* **91**: 1082–1093.

Dubey JP, Sundar N, Hill D, Velmurugan GV, Bandini LA, Kwok OCH, Majumdar D and Su C (2008a). High prevalence and abundant atypical genotypes of *Toxoplasma gondii* isolated from lambs destined for human consumption in the USA. *International Journal of Parasitology* **38**: 999–1006.

Dubey JP, Velmurugan GV, Ulrich V, Gill J, Carstensen M, Sundar N, Kwok OCH, Thulliez P, Majumdar D and Su C (2008b). Transplacental toxoplasmosis in naturally-infected white-tailed deer: isolation and genetic characterisation of *Toxoplasma gondii* from foetuses of different gestational ages. *International Journal of Parasitology* **38**: 1057–1063.

Dubey JP, Bhatia CR, Lappin MR, Ferreira LR, Thorn A and Kwok OCH (2009a). Seroprevalence of *Toxoplasma gondii* and *Bartonella* spp. antibodies in cats from Pennsylvania. *Journal of Parasitology* **95**: 578–580.

Dubey JP, Jenkins MC, Kwok OCH, Zink RL, Michalski ML, Ulrich V, Gill J, Cartensen M and Thulliez P (2009b). Seroprevalence of *Neospora caninum* and *Toxoplasma gondii* antibodies in white tailed deer (*Odocoileus virginianus*) from Iowa and Minnesota using four serologic tests. *Veterinary Parasitology* **161**: 330–334.

Dubey JP, Dennis PM, Verma SK, Choudhary S, Ferreira LR, Oliveira S, Kwok OCH, Butler E, Carstensen M and Su C (2014a). Epidemiology of toxoplasmosis in white tailed deer (*Odocoileus virginianus*): occurrence, congenital transmission, correlates of

https://doi.org/10.1017/S1466252315000183 Published online by Cambridge University Press

infection, isolation and genetic characterization of *Toxoplasma gondii*. *Veterinary Parasitology* **202**: 270–275.

Dubey JP, Verma SK, Ferreira LR, Oliveira S, Cassinelli AB, Ying Y, Kwok OCH, Tuo W, Chiesa OA, Jones JL (2014b). Detection and survival of *Toxoplasma gondii* in milk and cheese from experimentally infected goats. *Journal of Food Protection* **77**: 1747–1753.

Eliasson M, Andersson R, Olsson A, Wigzell H and Uhlen M (1989). Differential IgG-binding characteristics of staphylococcal protein A, streptococcal protein G, and a chimeric protein AG. *Journal of Immunology* **142**: 575–581.

Ferguson DJP (2009). *Toxoplasma gondii*: 1908–2008, homage to Nicolle, Manceaux and Splendore. *Memorias do Instituto Oswaldo Cruz* **104**: 133–148.

Filisetti D, Gorcii M, Pernot-Marino E, Villard O and Candolfi E (2003). Diagnosis of congenital toxoplasmosis: comparison of targets for detection of *Toxoplasma gondii* by PCR. *Journal of Clinical Microbiology* **41**: 4826–4828.

Freeman K, Oakley L, Pollak A, Buffolano W, Petersen E, Semprini AE, Salt A, Gilbert R and European Multicentre Study on Congenital Toxoplasmosis (2005). Association between congenital toxoplasmosis and preterm birth, low birthweight and small for gestational age birth. *British Journal of Obstetrics and Gynaecology* **112**: 31–37.

Frenkel JK and Friedlander S (1951). *Pathology of Neonatal Disease, Pathogenesis, Diagnosis, and Treatment Public Health Service Publication No. 141*. Washington, DC, USA: United States Government Printing Office, Toxoplasmosis, pp. 1–150.

Frenkel JK, Dubey JP and Miller NL (1970). *Toxoplasma gondii* in cats: fecal stages identified as coccidian oocysts. *Science* **167**: 893–896.

Gamble HR, Dubey JP and Lambillotte DN (2005). Comparison of a commercial ELISA with the modified agglutination test for detection of *Toxoplasma* infection in the domestic pig. *Veterinary Parasitology* **128**: 177–181.

Garcia-Vazques Z, Rosario-Cruz R, Diaz-Garcia G and Hernandez-Baumgarten O (1993). Seroprevalence of *Toxoplasma gondii* infection in cattle, swine, and goats in four Mexican states. *Preventive Veterinary Medicine* **17**: 127–132.

Gardner IA, Greiner M and Dubey JP (2009). Statistical evaluation of test accuracy studies for *Toxoplasma gondii* in food animal intermediate hosts. *Zoonoses and Public Health* **57**: 82–94.

Harden CL and Hair LS (1994). Diagnosis of central nervous system toxoplasmosis in AIDS patients confirmed by autopsy. *AIDS* **8**: 1188–1189.

Hartley WJ and Marshall SC (1957). Toxoplasmosis as a cause of ovine perinatal mortality. *New Zealand Veterinary Journal* **5**: 119–124.

Hill DE, Chirukandoth S, Dubey JP, Lunney JK and Gamble HR (2006). Comparison of detection methods for *Toxoplasma gondii* in naturally and experimentally infected swine. *Veterinary Parasitology* **141**: 9–17.

Hill RE, Zimmerman JJ, Wills RW, Patton S and Clark WR (1998). Seroprevalence of antibodies against *Toxoplasma gondii* in free-ranging mammals in Iowa. *Journal of Wildlife Diseases* **34**: 811–815.

Hogan MJ (1951). *Ocular Toxoplasmosis*. New York: Columbia University Press.

Homan WL, Vercammen M, de Braekeleer J and Verschueren H (2000). Identification of a 200- to 300-fold repetitive 529 bp DNA fragment in *Toxoplasma gondii*, and its use for diagnostic and quantitative PCR. *International Journal of Parasitology* **30**: 67–95.

Humphreys JG, Stewart RL and Dubey JP (1995). Prevalence of *Toxoplasma gondii* antibodies in sera of hunter-killed white-tailed deer in Pennsylvania. *American Journal of Veterinary Research* **56**: 172–173.

Hutchison WM (1965). Experimental transmission of *Toxoplasma gondii*. *Nature* **206**: 961–962.

Janků J (1923). Pathogenesa a pathologická anatomie tak nazvaneho vrozeného kolombu žluté skvrany voku normálné velikem a microphthalmickém s nalezem parasitu v sítnici. Časopis lékařeů českýck [Pathogenesis and pathologic anatomy of the "congenital coloboma" of the macula lutea in an eye of normal size, with microscopic detection of parasites in the retina] 62, 1021–1027, 1052–1059, 1081–1085, 1111, 1138–1143 (For English translation, see Janků, J., 1959. Cesk. Parasitol. 6, 9–58).

Jessup DA (2004). The welfare of feral cats and wildlife. *Journal of the American Veterinary Medical Association* **225**: 1377–1383.

Jittapalapong S, Nimsupan B, Pinyopanuwat N, Chimnoi W, Kabeya H and Maruyama S (2007). Seroprevalence of *Toxoplasma gondii* antibodies in stray cats and dogs in the Bangkok metropolitan area, Thailand. *Veterinary Parasitology* **145**: 138–141.

Jones JL, Parise ME and Fiore AE (2014). Neglected parasitic infections in the United States: toxoplasmosis. *American Journal of Tropical Medicine and Hygiene* **90**: 794–799.

Kimbita EN, Xuan X, Huang X, Miyazawa X, Fukumoto S, Mishima M, Suzuki H, Sugimoto C, Nagaswaw H, Fujisaki K, Suzuki N, Mikami T and Igarashi I (2001). Serodiagnosis of *Toxoplasma gondii* infection in cats by enzyme-linked immunosorbent assay using recombinant SAG1. *Veterinary Parasitology* **102**: 35–44.

Klun I, Djurkovic-Djakovic O and Thulliez P (2007). Comparison of a commercial ELISA with the modified agglutination test for the detection of *Toxoplasma gondii* infection in naturally exposed sheep. *Zoonoses and Public Health* **54**: 165–168.

Kodym P, Machala L, Rohacova H, Sirocka B and Maly M (2006). Evaluation of a commercial IgE ELISA in comparison with IgA and IgM ELISAs, IgG avidity assay and complement fixation for the diagnosis of acute toxoplasmosis. *Clinical Microbiology and Infection* **13**: 40–47.

Lappin MR and Powell CC (1991). Comparison of latex agglutination, indirect hemagglutination, and ELISA techniques for the detection of *Toxoplasma gondii*-specific antibodies in the serum of cats. *Journal of Veterinary Internal Medicine* **5**: 299–301.

Levaditi C, Schoen R and Sanchis Bayarri V (1928). L'encephalo-myelite toxoplasmique chronique du lapin et de la souris. *Comptes Rendus de Societe Biologique* **99**: 37–40.

Liesenfeld O, Press C, Montoya JG, Gill R, Isaac-Renton JL, Hedman K and Remington JS (1997). False-positive results in immunoglobulin M (IgM) *Toxoplasma* antibody tests and importance of confirmatory testing: the Platelia Toxo IgM test. *Journal of Clinical Microbiology* **35**: 174–178.

Lindsay DS, Dubey JP, Upton SJ and Ridley RK (1990). Serological prevalence of *Neospora caninum* and *Toxoplasma gondii* in dogs from Kansas. *Journal of the Helminthological Society of Washington* **57**: 86–88.

Lindsay DS, Phelps KK, Smith SA, Flick G, Sumner SS and Dubey JP (2001). Removal of *Toxoplasma gondii* oocyst from sea water by eastern oysters (*Crassostrea virginica*). *Journal of Eukaryotic Microbiology* **48** (suppl.): 197S–198S.

Lindsay DS, Weiss LM and Suzuki Y (2003). Toxoplasmosis in: Richardson D & Krause P (eds.) *North American Parasitic Zoonoses*. New York: Springer US, pp. 129-150.

Lindsay DS, Collins MV, Jordan CN, Flick GJ and Dubey JP (2005). Effects of high pressure processing on infectivity of *Toxoplasma gondii* oocysts for mice. *Journal of Parasitology* **91**: 699–701.

Longcore T, Rich C, and Sullivan LM (2009). Critical assessment of claims regarding management of feral cats by trap-neuter-return. *Conservation Biology* **23**: 887–894.

Lopez A, Dietz VJ, Wilson M, Navin TR and Jones JL (2000). Preventing congenital toxoplasmosis. *MMWR. Recommendations and reports: Morbidity and mortality weekly report. Recommendations and reports/Centers for Disease Control*, **49**: 59-68.

Luft BJ, Conley F, Remington JS, Laverdiere M, Wagner KF, Levine JF, Craven PC, Strandberg DA, File TM, Rice N and Meunier-Carpentier F (1983). Outbreak of central-nervous-system toxoplasmosis in Western Europe and North America. *Lancet* **321**: 781–784.

Lunde MN (1973). Laboratory methods in the diagnosis of toxoplasmosis. *Health Laboratory Science* **10**: 319–328.

Luria BJ, Levy JK, Lappin MR, Breitschwerdt EB, Legendre AM, Hernandez JA, Gorman AP and Lee IT (2004). Prevalence of

https://doi.org/10.1017/S1466252315000183 Published online by Cambridge University Press

infectious diseases in feral cats in northern Florida. *Journal of Feline Medicine and Surgery* **6**: 287–296.

Lynfield R and Guerina NG (1997). Toxoplasmosis. *Pediatrics in Review* **18**: 75–83.

Mainar-Jaime RC and Barberan M (2007). Evaluation of the diagnostic accuracy of the modified agglutination test (MAT) and an indirect ELISA for the detection of serum antibodies against *Toxoplasma gondii* in sheep through Bayesian approaches. *Veterinary Parasitology* **148**: 122–129.

Massie GN, Ware MW, Villegas EN and Black MW (2010). Uptake and transmission of *Toxoplasma gondii* oocysts by migratory, filter-feeding fish. *Veterinary Parasitology* **169**: 296–303.

McSporran KD, McCaughan C, Currall JHS and Demsteegt A (1985). Toxoplasmosis in goats. *New Zealand Veterinary Journal* **33**: 39–40.

Mead PS, Slutsker L, Dietz V, McCaig LF, Bresee JS, Shapiro C, Griffin PM, and Tauxe RV (1999). Food-related illness and death in the United States. *Emerging Infectious Diseases* **5**: 607–624.

Meireles LR, Galisteo AJ Jr, Pompeu E and Andrade HF Jr (2004). *Toxoplasma gondii* spreading in an urban area evaluated by seroprevalence in free-living cats and dogs. *Tropical Medicine and International Health* **9**: 876–881.

Mello U (1910). Un cas de toxoplasmose du chien observe a Turin. *Bulletin de la Societe de Pathologie Exotique et de ses Filiales* **3**: 359–363.

Miller MA, Gardner IA, Kreuder C, Paradies DM, Worcester KR, Jessup DA, Dodd E, Harris MD, Ames JA, Packham AE and Conrad PA (2002). Coastal freshwater runoff is a risk factor for *Toxoplasma gondii* infection of southern sea otters (*Enhydra lutris nereis*). *International Journal of Parasitology* **32**: 997–1006.

Nicolle C and Manceaux L (1909). Sur un protozoaire nouveau du gondi. *Comptes Rendus de l'Academie des Sciences* **148**: 369–372.

Nogales M, Martin A, Tershy BR, Donlan CJ, Veitch D, Puerta N, Wood B, Alonso J (2004). A review of feral cat eradiction on Islands. *Conservation Biology* **18**: 310–319.

Olafson P and Monlux WS (1942). *Toxoplasma* infection in animals. *Cornell Veterinarian* **32**: 76–190.

Papini RA, Buzzone G, Nardoni S, Rocchigiani G and Mancianti F (2015). Seroprevalence and genotyping of *Toxoplasma gondii* in horses slaughtered for human consumption in Italy. *Journal of Equine Veterinary Science* **35**: 657–661.

Patton S, Johnson SS and Puckett K (1990). Prevalence of *Toxoplasma gondii* antibodies in nine populations of dairy goats: compared titers using modified direct agglutination and indirect hemagglutination. *Journal of Parasitology* **76**: 74–77.

Reischl U, Bretagne S, Kruger D, Ernault P and Costa J-M (2003). Comparison of two DNA targets for the diagnosis of toxoplasmosis by real-time PCR using fluorescence resonance energy transfer hybridization probes. *BMC Infectious Diseases* **3**: 7.

Reiter-Owona I, Petersen E, Joynson D, Aspock H, Darde ML, Disko R, Dreazen O, Dumon H, Grillo R, Gross U, Hayde M, Holliman R, Ho-Yen DO, Janitschke K, Jenum PA, Naser K, Olszewski M, Thulliez P and Seitz HM (1999). The past and present role of the Sabin-Feldman dye test in the serodiagnosis of toxoplasmosis. *Bulletin of the World Health Organization* **77**: 929–935.

Remington JS and Desmonts G (1990). Toxoplasmosis. In: Remington JS and Klein JO (eds) *Infectious Diseases of the Fetus and Newborn Infant*. Philadelphia, PA: Saunders, pp. 89–195.

Remington JS, McLeod R and Thulliez P (2001). Toxoplasmosis. In: Remington JS and Klein J (eds) *Infectious Diseases of the Fetus and Newborn Infant*. Philadelphia: WB Saunders, pp. 205–346.

Remington JS, Thulliez P and Montoya JG (2004). Recent developments for diagnosis of toxoplasmosis. *Journal of Clinical Microbiology* **42**: 941–945.

Roberts A, Hedman K, Luyasu V, Zufferey J, Bessieres M-H, Blatz R-M, Candolfi E, Decoster A, Enders G, Gross U, Guy E, Hayde M, Ho-Yen D, Johnson J, Lecolier B, Naessens A, Pelloux H, Thulliez P and Petersen E (2001). Multicenter evaluation of strategies for serodiagnosis of primary infection with *Toxoplasma gondii*. *The European Journal of Clinical Microbiology and Infectious Diseases* **20**: 467–474.

Rorman E, Zamir CS, Rilkis I and Ben-David H (2006). Congenital toxoplasmosis – prenatal aspects of *Toxoplasma gondii* infection. *Reproductive Toxicology* **4**: 458–472.

Sabin AB (1942). Toxoplasmosis. A recently recognized disease of human beings. *Advances in Pediatrics* **1**: 1–53.

Sabin AB and Feldman HA (1948). Dyes as microchemical indicators of a new immunity phenomenon affecting a protozoan parasite (*Toxoplasma*). *Science* **108**: 660–663.

Sabin AB and Olitsky PK (1937). *Toxoplasma* and obligate intracellular parasitism. *Science* **85**: 336–338.

Santos TR, Costa AJ, Toniollo GH, Luvizotto MC, Benetti AH, Santos RR, Matta DH, Lopes WD, Oliveira JA and Oliveira GP (2009). Prevalence of anti-*Toxoplasma gondii* antibodies in dairy cattle, dogs, and humans from the Jauru micro-region, Mato Grosso state, Brazil. *Veterinary Parasitology* **161**: 324–326.

Schaefer JJ, White HA, Schaaf SL, Mohammed HO and Wade SE (2011). Modification of a commercial *Toxoplasma gondii* immunoglobulin G enzyme-linked immunosorbent assay for use in multiple animal species. *Journal of Veterinary Diagnostic Investigation* **23**: 297–301.

Schaefer JJ, White HA, Schaaf SL, Mohammed HO and Wade SE (2012). Chimeric protein A/G conjugate for detection of anti-*Toxoplasma gondii* immunoglobulin G in multiple animal species. *Journal of Veterinary Diagnostic Investigation* **24**: 572–575.

Schaefer JJ, Kirchgessner MS, Whipps CM, Mohammed HO, Bunting EM and Wade SE (2013). Prevalence of antibodies to *Toxoplasma gondii* in white-tailed deer (*Odocoileus virginianus*) in New York State, USA. *Journal of Wildlife Diseases* **49**: 940–945.

Schares G, Pantchev N, Barutzki D, Heydorn AO, Bauer C and Conraths FJ (2005). Oocysts of *Neospora caninum, Hammondia heydorni, Toxoplasma gondii* and *Hammondia hammondi* in faeces collected from dogs in Germany. *International Journal of Parasitology* **35**: 1525–1537.

Schares G, Vrhovec MG, Pantchev N, Herrmann DC and Conraths FJ (2008). Occurrence of *Toxoplasma gondii* and *Hammondia hammondi* oocysts in the faeces of cats from Germany and other European countries. *Veterinary Parasitology* **152**: 34–45.

Schatzberg SJ, Haley NJ, Barr SC, deLahunta A, Olby N, Munana K and Sharp NJH (2003). Use of a multiplex polymerase chain reaction assay in the antemortem diagnosis of toxoplasmosis and neosporosis in the central nervous system of cats and dogs. *American Journal of Veterinary Research* **64**: 1507–1513.

Schwab DJ and McDevitt JJ (2003). Development of a PCR-enzyme immunoassay oligoprobe detection method for *Toxoplasma gondii* oocysts, incorporating PCR controls. *Applied Environnebtak Microbiology* **69**: 5819–5825.

Shaapan RM, El-Nawawi FA and Tawfik FAA (2008). Sensitivity and specificity of various serological tests for the detection of *Toxoplasma gondii* infection in naturally infected sheep. *Veterinary Parasitology* **153**: 359–362.

Shapiro K, Krusor C, Mazzillo FFM, Conrad PA, Largier JL, Mazet JAK and Silver MW (2014). Aquatic polymers can drive pathogen transmission in coastal ecosystems. *Proceedings of the Royal Society of London B: Biological Sciences* **281**: 20141287. http://dx.doi.org/10.1098/rspb.2014.1287

Shen L, Zhichung L, Biaucheng Z and Huayuan Y (1990). Prevalence of *Toxoplasma gondii* infection in man and animals in Guangdong, Peoples' Republic of China. *Veterinary Parasitology* **34**: 357–360.

Silva NM, Lourenco EV, Silva DAO and Mineo JR (2002). Optimisation of cut-off titres in *Toxoplasma gondii* specific ELISA and IFAT in dog sera using immunoreactivity to SAG-1 antigen as a molecular marker of infection. *Veterinary Journal* **163**: 94–98.

Spencer JA, Higginbotham MJ and Blagburn BL (2003). Seroprevalence of *Neospora caninum* and *Toxoplasma gondii* in captive and free-ranging nondomestic felids in the United States. *Journal of Zoo and Wildlife Medicine* **34**: 246–249.

Splendore A (1908). Un nuovo protozoa parassita de' conigli, incontrato nelle lesion anatomiche d'une malattia che ricorda in molti punti il

https://doi.org/10.1017/S1466252315000183 Published online by Cambridge University Press

Kala-azar dell' uomo. *Nota preliminare pel. Rev Soc Scient Sao Paulo* **3**: 109–112.

Su C, Zhang X and Dubey JP (2006). Genotyping of *Toxoplasma gondii* by multilocus PCR-RFLP markers: a high resolution and simple method for identification of parasites. *International Journal of Parasitology* **36**: 841–848.

Tassi P (2007). *Toxoplasma gondii* infection in horses. A review. *Parassitologia* **49**: 7–15.

Tenter AM, Heckeroth AR and Weiss LM (2000). *Toxoplasma gondii*: from animals to humans. *International Journal of Parasitology* **30**: 1217–1258.

Torres CM (1927). Morphologie d'un nouveau parasite de l'homme, *Encephalitozoon chagasi*, N. sp., observe dans un cas de meningo-encephalo-myelite congenitale avec myosite et myocardite. *Comptes Rendus Societe de Biologie* **97**: 1787–1790.

Toxoplasmosis. Centers for Disease Control and Prevention, 10 Jan. 2013. Web. 01 July 2015.

Tuazon CU (1989). Toxoplasmosis in AIDS patients. *Journal of Antimicrobial Chemotherapy* **23** (suppl. A): 77–82.

Vanek JA, Dubey JP, Thulliez P, Riggs MR and Stromberg BE (1996). Prevalence of *Toxoplasma gondii* antibodies in hunter-killed white-tailed deer (*Odocoileus virginianus*) in four regions of Minnesota. *Journal of Parasitology* **82**: 41–44.

Vollaire MR, Radecki SV and Lappin MR (2005). Seroprevalence of *Toxoplasma gondii* antibodies in clinically ill cats in the United States. *American Journal of Veterinary Research* **66**: 874–877.

Wahab T, Edvinsson B, Palm D and Lindh J (2010). Comparison of the AF146527 and B1 repeated elements, two real-time PCR targets used for detection of *Toxoplasma gondii*. *Journal of Clinical Microbiology* **48**: 591–592.

Weinman D and Chandler AH (1954). Toxoplasmosis in swine and rodents. Reciprocal oral infection and potential human hazard. *Proceedings of the Society for Experimental Biology and Medicine* **87**: 211–216.

Weiss LM and Kim K (2007). *Toxoplasma gondii: the model apicomplexan: perspectives and methods*. Amsterdam; Boston, Elsevier/Academic Press ISBN: 9780123695420.

Wendte JM, Gibson AK and Grigg ME (2011). Population genetics of *Toxoplasma gondii*: new perspectives from parasite genotypes in wildlife. *Veterinary Parasitology* **182**: 96–111.

Werre SR, Jacobson RH, Bowman DD, Dubey JP and Mohammed HO (2002). Evaluation of kinetics and single-read enzyme-linked immunoassays for detection of *Toxoplasma gondii* antibodies in sheep. *Journal of Veterinary Diagnostic Investigation* **14**: 225–230.

Wilson M, Ware DA and Walls KW (1987). Evaluation of commercial serodiagnostic kits for toxoplasmosis. *Journal of Clinical Microbiology* **25**: 2262–2265.

Wilson M, Remington JS, Clavet C, Varney G, Press C, Ware D and The FDA Toxoplasmosis ad hoc Working Group (1997). Evaluation of six commercial kits for detection of human immunoglobulin M antibodies to *Toxoplasma gondii*. *Journal of Clinical Microbiology* **35**: 3112–3115.

Wolf A, Cohen D and Paige BH (1939a). Human toxoplasmosis: occurrence in infants as an encephalomyelitis verification by transmission to animals. *Science* **89**: 226–227.

Wolf A, Cowen D, Paige BH (1939b). Toxoplasmic encephalomyelitis III. A new case of granulomatous encephalomyelitis due to a protozoon. *American Journal of Pathology* **15**: 657–694.

Zaki M (1995). Seroprevalence of *Toxoplasma gondii* in domestic animals in Pakistan. *Journal of the Pakistan Medical Association* **45**: 4–5.

Zhang D, Wang Z, Fang R, Nie H, Feng H, Zhou Y and Zhao J (2010). Use of protein AG in ELISA for serodiagnosis of *Toxoplasma gondii* infection in four species of animals. *Clinical and Vaccine Immunology* **17**: 485–486.

https://doi.org/10.1017/S1466252315000183 Published online by Cambridge University Press