5.7. *Toxoplasma*

Nevi Parameswaran[1], Adrian Wayne[1], RC Andrew Thompson[1]

[1]Murdoch University
[2]Department of Environment and Conservation

Abstract

In response to the dramatic declines in woylie numbers, a number of woylies in the Upper Warren and in control populations outside of the Upper Warren were tested for *Toxoplasma* infection. The commercially available modified agglutination test (MAT) was used to detect *Toxoplasma* antibodies in woylie sera. A number of variables were analyzed to determine if *Toxoplasma* infection is contributing to the decline in woylie numbers in the Upper Warren. The retrap rates of *Toxoplasma* seropositive woylies was observed to determine if *Toxoplasma* seropositive woylies survive. In addition, the *Toxoplasma* seroprevalence of woylies in the Upper Warren was compared with woylies located elsewhere, where populations were not declining. In March 2006, 153 woylies from the Upper Warren were tested for *Toxoplasma* antibodies, nine were seropositive for *Toxoplasma*. In July to December of the same year, 143 more woylies from the Upper Warren were tested for *Toxoplasma*, zero were seropositive. Although six out of the nine seropositive woylies were retrapped none of these woylies were re-bled in July to December 2006. All sera samples from outside of the Upper Warren, including Karakamia and Dryandra, showed a zero seroprevalence of *Toxoplasma* in woylies. Data to date suggests *Toxoplasma* might contribute to woylie deaths, however this is not conclusive. Future studies include continued monitoring of woylie populations for *Toxoplasma* antibodies and further analysis of woylie tissues for *Toxoplasma* DNA.

5.7.1. Introduction

Toxoplasma is a protozoan parasite that infects virtually all warm-blooded species, including humans and marsupials (Tenter, Heckeroth *et al.,* 2000). Infection with *Toxoplasma* can affect marsupials in several ways. Initial infection can result in acute Toxoplasmosis causing overt disease which often leads to death (Johnson, Roberts *et al.,*. 1989). Other symptoms of acute disease include lethargy, inappetence, respiratory distress and neurological disturbances. Alternatively, *Toxoplasma* can remain dormant in tissue resulting in long-term latent infection which can be reactivated during times of stress (Beveridge, 1993). Stressors which may cause reactivated Toxoplasmosis in marsupials include nutritional stresses, capture stress and transport and captivity stress (Obendorf and Munday, 1983; Beveridge, 1993). In addition, infection with *Toxoplasma* may make a marsupial more prone to predation by affecting its behaviour, movement, coordination and sight.

Felids are the only host of *Toxoplasma* that are capable of shedding oocysts (i.e. cats are the the definitive/final host). Cats shed large numbers of infective oocysts briefly during acute infection, after which it is rare for shedding to occur again. Oocysts can remain infective in soil for up to 2 years under favourable climatic conditions (Frenkel, Ruiz *et al.,*. 1975). Oocyst remain viable for longer periods of time in a warm and moist environment (Yilmaz and Hopkins, 1972). Herbivorous marsupials, may become infected with *Toxoplasma* through ingesting material contaminated with oocysts or through vertical transmission of tachyzoites. Evidence for vertical transmission in marsupials to date is anecdotal (Boorman, Kollias *et al.,* 1977; Dubey, Ott-Joslin *et al.,* 1988), however it is well established in a number of species including sheep, mice, rats, cats and humans (Johnson, 1997; Duncanson, Terry *et al.,* 2001; Marshall, Hughes *et al.,* 2004). Meat eating marsupials may also be infected via ingesting animal tissue containing *Toxoplasma bradyzoites* (cysts).

Several species of marsupial have been found to be infected with *Toxoplasma* using methods such as serology, histology and polymerase chain reaction (PCR) to detect *Toxoplasma* antibodies, organisms and DNA respectively. Outbreaks of acute toxoplasmosis causing widespread mortalities have been well reported in captive marsupials such as the long-nosed

potoroo (*Potorous tridactylus*), tammar wallaby (*Macropus eugenii*), eastern grey kangaroo (*Macropus giganteus*), red kangaroo (*Macropus rufus*), wallaroo (*Macropus robustus*) and Bennett's wallaby (*Macropus rufogriseus*) *(Boorman, Kollias et al.,* 1977; Patton, Johnson *et al*., 1986; Basso, Venturini *et al.,* 2006). The impact of *Toxoplasma* infection in wild marsupials is more difficult to determine as predation of recently infected marsupials hinders investigation into the cause of death. *Toxoplasma* antibodies have been detected in several wild marsupial populations including the koomal (*Trichosurus vulpecula*) (Eymann, Herbert *et al*., 2006), Tasmanian pademelon (*Thylogale billardierii*) and Bennett's wallaby (Johnson, Roberts *et al.,* 1988). In-depth investigations regarding the impact of *Toxoplasma* in eastern-barred bandicoots (*Perameles gunnii*) led to the conclusion that *Toxoplasma* infection is a significant cause of death among this species, both in captivity and in the wild (Obendorf, Statham *et al.,* 1996; Bettiol, Obendorf *et al.,* 2000; Miller, Mitchel *et al.,* 2000). Based on this evidence it is clear that *Toxoplasma* is an infectious agent that needs to be included in the differential diagnosis list of investigations regarding the cause of decline in wild marsupial populations.

Initial investigations into the presence of *Toxoplasma* infection in woylie populations experiencing decline began using serology. Serology detects antibodies which are often widely dispersed in the blood stream and therefore easy to detect during blood screening. However, some animals, particularly marsupials, which are known to be highly susceptible to *Toxoplasma*-related disease, may not survive initial infection and die of acute toxoplasmosis before IgG antibodies can be detected. This is one of the limitations of serology in ascertaining the impact of *Toxoplasma* infection. In addition, serology alone is unable to verify if *Toxoplasma* is causing disease in infected animals. Additional data such as retrap rates of seropositive and seronegative woylies in addition to *Toxoplasma* serology from control groups needs to be analysed to determine the impact of *Toxoplasma* infection in a population. *Toxoplasma* detection using PCR and histology detect *Toxoplasma* present within animal tissue, necessitating invasive sampling techniques or necropsy. Furthermore, during chronic infection, *Toxoplasma* is spread sparsely within tissue and is consequently difficult to detect with PCR and histology. However, histology has the benefit of examining animal tissue infected with Toxoplasma therefore determining if *Toxoplasma* contributes to disease. The Woylie Conservation Research Project (WCRP) Operations Handbook (Volume 3) established (through the guidance of the Woylie Disease Reference Council; WDRC) that serum be tested from live animals whereas tissue from recently dead woylies was to be analyzed as part of routine histology and tissue samples set aside for *Toxoplasma* PCR.

The modified agglutination test (MAT) is a popular and reliable test for the serodiagnosis of *Toxoplasma* in Australian marsupials (Dubey, Ott-Joslin *et al.,* 1988; Miller, Faulkner *et al.,* 2003). It has been used as a sensitive and specific test in humans (Desmonts and Remington 1980) and a variety of animals such as mice (Dubey, Thulliez *et al.,* 1995), pigs (Dubey, Thulliez *et al.,* 1995), sheep (Ljungstrom, Lunden *et al.,* 1994) and felids (Dubey, Lappin *et al.,* 1995; Dubey, Navarro *et al.,* 2004). It is one of the few serological tests which can be used on a range of species, and therefore can be applied to woylies without the need for time-consuming and costly optimization and validation studies. The MAT was found to be one of the most sensitive tests to detect *Toxoplasma* antibodies in kangaroo sera, when compared to the dye test, indirect agglutination test and latex agglutination test (Dubey, Ott-Joslin *et al.,* 1988).

5.7.2. Methods

In March 2006, 153 woylies from the Upper Warren were bled from the lateral tail vein as part of DEC trapping and sampling programs for the WCRP. This initial sera sample set was expanded when a further 143 woylies from the Upper Warren were bled from July to December 2006. In addition, sera from woylie populations elsewhere were also obtained. These sera samples were obtained from other declining or declined woylie populations at Dryandra (n=12), Batalling (n=17) and Venus Bay (n=14), and stable populations at Karakamia (n=81), Tutanning (n=8), St Peter Island (n=72).

Sera was tested using the commercially available MAT kit (Toxo-Screen DA, bioMerieux, REF 75 481) at two different sera dilutions; 1:40 and 1:4000 and the test undertaken according to the manufacturer's directions. The positive and negative control sera included in the kit were used in each round of samples tested, in addition to a PBS control. A sera sample was determined to be *Toxoplasma* positive when an agglutination reaction was observed at a dilution of at least 1:40, based on the manufacturer's directions.

During the *Toxoplasma* seroprevalence study of woylies, opportunistic sampling of dead or euthanized woylies for the detection of *Toxoplasma* was also undertaken. Any woylies that were submitted for routine necropsy were examined by the Duty Pathologist at Murdoch University, School of Veterinary and Biomedical Sciences, and had tissue samples removed for histology, in which *Toxoplasma* infection was screened for as a differential diagnosis. In addition, tissues including the brain, heart and skeletal muscle were set aside from each woylie necropsied to test for *Toxoplasma* DNA using PCR (Bretagne, Costa *et al.,* 1993). Samples of heart blood were also taken in necropsied woylies in which blood was deemed fresh. Sera was then obtained from this blood to test for *Toxoplasma* antibodies. In addition, some woylie pouch-young (injured, predated or orphaned) were dissected or frozen for *Toxoplasma* DNA screening.

5.7.3. Results

Toxoplasma antibodies were only detected in nine (5.8%) woylies sampled in the Upper Warren in March 2006. None of 143 woylies sampled from the Upper Warren in July-December 2006 were seropositive. The change in seroprevalence in the Upper Warren is significantly different (Fisher's exact test; p=0.0036). All other sera samples tested were classified as *Toxoplasma* seronegative and had MAT titers of <1:40 (Table 5.8.1). Assuming that the serology tests do not produce false negatives, the sample size from Karakamia (n=81) is sufficient to have 95% confidence that the actual prevalence of *Toxoplasma* within the population is 0-5%, based on point and interval estimates (Snedecor, 1956). The samples sizes at Dryandra, Tutanning and Batalling are not sufficient to confidently determine whether or not *Toxoplasma* may be present at low prevalences. For example, if the actual prevalence in a population is 5%, more than 59 samples are required to have a probability of less than 5% that all the samples are negative (SAS).

Six out of the nine *Toxoplasma* seropositive woylies were found to have been retrapped seven to 13 months after initial trapping in March 2006 (Table 5.7.2). Given that trapping intensity and frequency at the Upper Warren sites were sufficiently high, it is likely that the retrap rates have a high correlation with "loss" of those individuals from the trapped population, most likely through mortality. It is therefore probable that the three seropositive woylies that were not retrapped died. Further analysis needs to be undertaken to compare the retrap rates of seronegative and seropositive woylies, to ascertain if seropositive woylies have a higher mortality rate.

On 31 necropsies undertaken on woylies from various sites (including Karakamia, Dryandra, Tutanning and Upper Warren), there was no evidence of infection with *Toxoplasma* based on routine histology. A limited amount of tissue samples from necropsied woylies have been tested for *Toxoplasma* DNA using PCR, however out of the three tissue samples tested, no *Toxoplasma* DNA was detected. There are several tissue samples stored, from a further 28 woylies, and these samples will be tested for *Toxoplasma* DNA shortly. One out of five woylie pouch-young submitted has been tested for *Toxoplasma* DNA. *Toxoplasma* DNA was identified in a brain sample of this pouch-young. This result needs to be verified with DNA sequencing.

Table 5.7.1. *Toxoplasma* seroprevalence based on the MAT.

Location		Seropositive	Total tested
Upper Warren- March	Balban	4	36
Upper Warren- March	Boyicup	0	4
Upper Warren- March	Chariup	0	4
Upper Warren- March	Corbal	2	26
Upper Warren- March	Keninup	2	34
Upper Warren- March	Warrup	0	22
Upper Warren- March	Winnejup	0	5
Upper Warren- March	Yendicup	1	8
Upper Warren- March	Misc	0	14
Summary Upper Warren - March 06		9	153
Upper Warren- Jul-Dec	Balban	0	27
Upper Warren- Jul-Dec	Boyicup	0	6
Upper Warren- Jul-Dec	Keninup	0	59
Upper Warren- Jul-Dec	Warrup	0	39
Upper Warren- Jul-Dec	Winnejup	0	12
Summary Upper Warren - Jul-Dec 06		0	143
Karakamia - Jul'06		0	81
Dryandra - Nov 06		0	12
Tutanning - Nov 06		0	8
Batalling - Nov 06		0	17

Table 5.7.2. Retrap data of *Toxoplasma* seropositive woylies

Date	Forest Block	Animal Record No.	Sex	Weight g	Age
23/03/2006*	Balban	500003370	M	1410	A
24/3/2006	Balban	500003370	M		A
4/11/2004	Balban	90000815	F	1300	A
5/11/2004	Balban	90000815	F	1260	A
18/11/2004	Balban	90000815	F	1150	A
2/11/2005	Balban	90000815	F	1340	A
3/11/2005	Balban	90000815	F	1280	A
23/03/2006*	Balban	90000815	F	1400	A
23/03/2006*	Balban	500003363	M	1570	A
27/3/2007	Balban	500003363	M	1520	A
30/3/2007	Balban	500003363	M	1540	A
17/11/2004	Balban	500003200	M	1300	A
23/03/2006*	Balban	500003200	M	1500	A
29/3/2007	Balban	500003200	M	1570	A
8/12/2005	Corbal	500002496	M	1460	A
16/03/2006*	Corbal	500002496	M	1380	A
17/3/2006	Corbal	500002496	M	1370	A
23/11/2006	Corbal	500002496	M	1550	A
17/4/2007	Corbal	500002496	M	1500	A
16/03/2006*	Corbal	500003323	M	1480	A
22/11/2006	Corbal	500003323	M	1650	A
18/4/2007	Corbal	500003323	M	1650	A
20/4/2007	Corbal	500003323	M	1700	A
28/03/2006*	Keninup	500003058	M	1250	A
31/3/2006	Keninup	500003058	M	1250	A
27/3/2007	Keninup	500003058	M	1275	A
28/3/2007	Keninup	500003058	M	1200	A
30/3/2007	Keninup	500003058	M	1225	A
9/11/2005	Keninup	500002560	M	1450	A
29/03/2006*	Keninup	500002560	M	1475	A
31/10/2006	Keninup	500002560	M	1375	A
4/04/2006*	Yendicup	500004276	M	1550	A

("*" denotes the date in which woylie sera was sampled for *Toxoplasma* antibodies, "A" under the column heading of Age, refers to "adult")

5.7.4. Discussion

The change in the Upper Warren region in the seroprevalence of *Toxoplasma* from 5.8% (n=153) to 0% (n=143) between March and July-December 2006 is statistically highly significant (Fisher's exact test; p=0.0036). The significant difference in *Toxoplasma* seroprevalence values among the same population of woylies at different time periods is epidemiologically consistent with a disease agent causing population decline. *Toxoplasma* infection in marsupials can result in acute disease and rapid death (Canfield, Hartley *et al.,* 1990). Acute disease can ensue soon after initial *Toxoplasma* infection, or after a period of chronic infection (Beveridge, 1993). Reactivated toxoplasmosis after chronic *Toxoplasma* infection has been found to occur after a "stressor" in some marsupial case studies. Therefore, there are two possible causes of widespread Toxoplasmosis in woylies in the Upper Warren; a recent increase of felids which are shedding oocysts or a recent "stressor" causing reactivated Toxoplasmosis of chronically infected woylies.

Felids have been found to shed oocysts in their faeces for only one to two weeks after initial infection with *Toxoplasma* (Dubey and Frenkel, 1972; Dubey and Frenkel, 1976). Although it is very rare for cats to reshed oocysts later in life, this has been reported in cats infected with other benign coccidian parasites, during immunosuppression or after re-exposure to *Toxoplasma* years after initial infection (Dubey and Frenkel, 1974; Dubey, 1976; Dubey, 1995). Cats can obtain *Toxoplasma* from ingesting infected meat, ingesting oocyst contaminated feed or via congenital transmission. As most cats only shed once in their life, kittens and juvenile cats are often the source of *Toxoplasma* oocysts, as opposed to mature cats.

One hypothesis to explain an increased exposure of woylie populations to *Toxoplasma* is that an increased number of oocysts have been introduced to the environment. This could occur if one or more cats have ingested *Toxoplasma* infected meat. In addition, the total number of cats shedding oocysts in any given location can increase dramatically when acutely infected cats transmit *Toxoplasma* to their kittens. Therefore, there is potential for many cats to become acutely infected and consequently shed oocysts over a short period of time. Domestic cats bury their faeces in soil. Because woylies dig soil to obtain feed, this places them at a higher risk of ingesting oocyst contaminated feed compared to non-burrowing animals. An additional hypothesis as to how *Toxoplasma* can be introduced to a woylie population is that non-felid *Toxoplasma* infected species have been introduced which woylies have ingested via opportunistic scavenging.

Subclinical, chronic *Toxoplasma* infection in marsupials can become acute disease by stressors such as capture, transportation and captivity, malnourishment and extreme weather (Arundel, Barker *et al.,* 1977; Obendorf and Munday, 1983; Obendorf and Munday, 1990). Reactivated Toxoplasmosis is also commonly reported in immunosuppressed humans such as AIDS patients (Boothroyd and Grigg, 2002) and organ transplant patients (Wendum, Carbonell *et al.,* 2002). A secondary stressor has the potential to cause a reactivated, fatal Toxoplasmosis in subclinically infected woylies. A hypothesis to explain an increased level of fatal Toxoplasmosis in woylies is that *Toxoplasma* was introduced to the woylie population years prior to a secondary stressor that then caused reactivated, acute disease. Initial *Toxoplasma* infection results from ingesting infected meat or oocyst-contaminated feed and congenital infection. Given that *Toxoplasma* DNA was identified in one woylie pouch-young, this suggests that congenital transmission of *Toxoplasma* does occur in woylies. However, this result needs to be confirmed by additional tests.

The trapping records of the nine seropositive woylies captured in March 2006 showed that six were recaptured, 7 to 13 months later (Table 5.7.2). This demonstrates that some individuals can survive with *Toxoplasma*, at least in the short term. Two past seropositive woylies were rebled in 2007 trapping regimes, and these sera will soon be tested for *Toxoplasma* antibodies in order to investigate changes in immune status.

There were no seropositive results for *Toxoplasma* in woylies from all samples outside of the Upper Warren. These included populations that were not experiencing decline (i.e. Karakamia and Tutanning), and some very limited samples from populations that have declined (Dryandra, Batalling). The absence of detected *Toxoplasma* in stable woylie populations provides some (weak) comparative evidence that may implicate Toxoplasmosis as having a role in recent woylie declines. By default it also remains unknown if *Toxoplasma* can remain in a woylie population without causing (or contributing to) a dramatic decline. The strength of the evidence is greatly limited by the sample sizes collected to date, relative to the sample sizes needed to confidently assess the presence/absence of an agent that may occur at low prevalence levels. Other

populations of woylies (St Peter's Island and Venus Bay Island A) not experiencing decline have been bled to test for *Toxoplasma* antibodies, however, they remain to be analysed

Toxoplasma antibodies have been detected in other species of marsupial in the wild, including the koomal (Eymann, Herbert *et al.,* 2006), eastern-barred bandicoots (Obendorf, Statham *et al.,* 1996), Tasmanian pademelons and Bennett's wallabies (Johnson, Roberts *et al.,* 1988). Of these species, *Toxoplasma* has been confirmed as a contributor to deaths in the eastern-barred bandicoots and speculated to have caused deaths in the koomal. In addition, a case report of Toxoplasmosis in wild Tasmanian pademelons, where two carcasses were examined histologically, found *Toxoplasma* to be the cause of death (Obendorf and Munday, 1983). These two wallabies were found stumbling blindly and were subsequently euthanized. According to the land-owner where the wallabies were found, sick and dead wallabies had been observed every year, with the number of wallabies affected increasing yearly (Obendorf and Munday, 1983). From this case study it appears that *Toxoplasma* did contribute to Tasmanian pademelon deaths, however the overall population of Tasmanian pademelons in Tasmania remained relatively stable (Hocking, 2007).

Eastern-barred bandicoots have been found to be highly susceptible to death when infected with *Toxoplasma* in the wild (Obendorf, Statham *et al.,* 1996). In a study of free ranging eastern-barred bandicoots, a seroprevalence of 6.3% was found in 133 animals tested. Of the 10 seropositive animals, five were not retrapped, and of the remaining bandicoots, one was found dead in the trap with generalised toxoplasmosis (diagnosed at necropsy) while another had central nervous system disabilities consistent with Toxoplasmosis but was accidentally released and never recaptured (Obendorf, Statham *et al.,* 1996). During the period of this study, eight dead eastern-barred bandicoots were examined by necropsy and seven were confirmed as having toxoplasmosis. Based on the combination of findings, it was concluded that eastern-barred bandicoots are likely to be highly susceptible to primary *Toxoplasma* infection.

A published study of the koomal found a *Toxoplasma* seroprevalence of 6.3% in 142 animals tested. *Toxoplasma* was implicated as a contributor to deaths in these koomals based on the retrap success of the seropositive koomals. Only one out of nine seropositive animals was recaptured, however recapture success for seronegative possums declined over the sampling period from 57-35%. This reduction in koomal retrap rates may have also been influenced by other factors such as exposure to other diseases, road kill, illegal relocation and/or trap shyness (Eymann, Herbert *et al.,* 2006).

Further data analysis needs to be undertaken to compare the retrap rates of seronegative and seropositive woylies to ascertain if seropositive woylies that were not retrapped may have died from *Toxoplasma*. The ideal way to determine if *Toxoplasma* is killing woylies is to find dead or diseased woylies with signs of *Toxoplasma* infection, such as what occurred in the eastern-barred bandicoot study (Obendorf, Statham *et al.,* 1996). Toxoplasmosis has caused rapid and substantial declines in the wild in Californian sea otters (Miller *et al*., 2004) and less dramatic declines in eastern barred bandicoots (Obendorf *et al*., 1996). Diagnosis of *Toxoplasma* being the cause of decline in these species was strongly aided by the examination of dead animals via histology and, in the case of the otters, *Toxoplasma* PCR. However, a major problem that inhibits obtaining dead or dying woylies is the predation of infected woylies. In addition, when dead woylies are obtained they are often not in fresh enough condition to obtain a thorough analysis of Toxoplasmosis lesions. Although Toxoplasmosis related lesions are often evident in animals that have died peracutely as a result of the disease, identifying these lesions is difficult in carcasses that are frozen or not fresh (Canfield *et al*., 1990).The experimental infection of woylies with *Toxoplasma* in order to study the pathological effects directly is also problematic. The response of marsupials to *Toxoplasma* in captivity may bare no relation to their response in wild populations.

5.7.5. Conclusion

While there is not direct evidence, data to date is consistent with *Toxoplasma* potentially contributing to woylie deaths. Further studies, as outlined below, need to be undertaken to better identify the impact of *Toxoplasma* infection in wild woylies. The diagnosis of Toxoplasmosis in a dead or diseased woylie in the Upper Warren would add significantly to the evidence that *Toxoplasma* may be involved in the woylie declines.

5.7.6. Future work

Future studies include PCR analysis of stocks of woylie tissue from dead animals associated with the PCS Survival and mortality study (Section 4.3) and/or animals that have undergone necropsy. Sequencing of *Toxoplasma* DNA found in the brain of a woylie pouch-young needs to be undertaken to confirm the pouch-young was infected with *Toxoplasma*. Additional woylie sera samples collected from Venus Bay, St Peter's Island and the Upper Warren (2007) need to be tested for *Toxoplasma* antibodies using the MAT. Data analysis needs to be undertaken to compare the retrap rates of *Toxoplasma* seropositive and seronegative woylies.

5.7.7. Acknowledgements

Woylie sera samples were collected by DEC and collaborative associates of the WCRP including Murdoch University Vet students and Perth Zoo vets. Woylie necropsies (including histological examinations) were done by Duty Pathologists at Murdoch University, School of Veterinary and Biomedical Sciences: Graeme Knowles, Mandy O'Hara, Phil Nicholls, Sandy McLachlan, Lucy Woolford, Mark Bennett.

5.7.8. References

Arundel, J. H., I. K. Barker, *et al.* (1977). Diseases of marsupials. The Biology of Marsupials. B. Stonehouse and D. Gilmore. London, Macmillan: 141-154.

Basso, W., M. C. Venturini, *et al.* (2006). "Toxoplasmosis in captive Bennett's wallabies (*Macropus rufogriseus*) in Argentina." Vet Parasitol.

Bettiol, S. S., D. L. Obendorf, *et al.* (2000). "Pathology of experimental toxoplasmosis in eastern barred bandicoots in Tasmania." J Wildl Dis 36(1): 141-4.

Beveridge, I. (1993). Marsupial parasitic diseases. Zoo and wild animal medicine : current therapy. M. Fowler. Philadelphia, W.B. Saunders: 288-293.

Boorman, G. A., G. V. Kollias, *et al.* (1977). "An outbreak of toxoplasmosis in wallaroos (*Macropus robustus*) in a California zoo." J Wildl Dis 13(1): 64-8.

Boothroyd, J. C. and M. E. Grigg (2002). "Population biology of *Toxoplasma gondii* and its relevance to human infection: do different strains cause different disease?" Curr Opin Microbiol 5(4): 438-42.

Bretagne, S., J. M. Costa, *et al.* (1993). "Detection of *Toxoplasma gondii* by competitive DNA amplification of bronchoalveolar lavage samples." J Infect Dis 168(6): 1585-8.

Canfield, P. J., W. J. Hartley, *et al.* (1990). "Lesions of toxoplasmosis in Australian marsupials." J Comp Pathol 103(2): 159-67.

Desmonts, G. and J. S. Remington (1980). "Direct agglutination test for diagnosis of *Toxoplasma* infection: method for increasing sensitivity and specificity." J Clin Microbiol 11(6): 562-8.

Dubey, J. P. (1976). "Reshedding of *Toxoplasma* oocysts by chronically infected cats." Nature 262(5565): 213-4.

Dubey, J. P. (1995). "Duration of immunity to shedding of *Toxoplasma gondii* oocysts by cats." J Parasitol 81(3): 410-5.

Dubey, J. P. and J. K. Frenkel (1972). "Cyst-induced toxoplasmosis in cats." J Protozool 19(1): 155-77.

Dubey, J. P. and J. K. Frenkel (1974). "Immunity to feline toxoplasmosis: modification by administration of corticosteroids." Vet Pathol 11(4): 350-79.

Dubey, J. P. and J. K. Frenkel (1976). "Feline toxoplasmosis from acutely infected mice and the development of *Toxoplasma* cysts." J Protozool 23(4): 537-46.

Dubey, J. P., M. R. Lappin, *et al.* (1995). "Diagnosis of induced toxoplasmosis in neonatal cats." J Am Vet Med Assoc 207(2): 179-85.

Dubey, J. P., I. T. Navarro, *et al.* (2004). "*Toxoplasma gondii* infections in cats from Parana, Brazil: seroprevalence, tissue distribution, and biologic and genetic characterization of isolates." J Parasitol 90(4): 721-6.

Dubey, J. P., J. Ott-Joslin, *et al.* (1988). "Toxoplasmosis in black-faced kangaroos (*Macropus fuliginosus melanops*)." Vet Parasitol 30(2): 97-105.

Dubey, J. P., P. Thulliez, *et al.* (1995). "*Toxoplasma gondii* in Iowa sows: comparison of antibody titers to isolation of *T. gondii* by bioassays in mice and cats." J Parasitol 81(1): 48-53.

Dubey, J. P., P. Thulliez, *et al.* (1995). "Sensitivity and specificity of various serologic tests for detection of *Toxoplasma gondii* infection in naturally infected sows." Am J Vet Res 56(8): 1030-6.

Duncanson, P., R. S. Terry, *et al.*. (2001). "High levels of congenital transmission of *Toxoplasma gondii* in a commercial sheep flock." Int J Parasitol 31(14): 1699-703.

Eymann, J., C. A. Herbert, *et al.* (2006). "Serologic survey for *Toxoplasma gondii* and Neospora caninum in the common brushtail possum (*Trichosurus vulpecula*) from urban Sydney, Australia." J Parasitol 92(2): 267-72.

Frenkel, J. K., A. Ruiz, *et al.* (1975). "Soil survival of *Toxoplasma* oocysts in Kansas and Costa Rica." Am J Trop Med Hyg 24(3): 439-43.

Hocking, G. (2007). Tasmanian Pademelon, Department of Primary Industries and Water.

Johnson, A. M. (1997). "Speculation on possible life cycles for the clonal lineages in the genus *Toxoplasma*." Parasitol Today 13(10): 393-7.

Johnson, A. M., H. Roberts, *et al.* (1988). "Prevalence of *Toxoplasma gondii* antibody in wild macropods." Aust Vet J 65(7): 199-201.

Johnson, A. M., H. Roberts, *et al.* (1989). "Serodiagnosis of acute toxoplasmosis in macropods." Vet Parasitol 34(1-2): 25-33.

Ljungstrom, B. L., A. Lunden, *et al.* (1994). "Evaluation of a direct agglutination test for detection of antibodies against *Toxoplasma gondii* in cat, pig and sheep sera." Acta Vet Scand 35(2): 213-6.

Marshall, P. A., J. M. Hughes, *et al.* (2004). "Detection of high levels of congenital transmission of *Toxoplasma gondii* in natural urban populations of *Mus domesticus*." Parasitology 128(Pt 1): 39-42.

Miller, D. S., C. Faulkner, *et al.* (2003). "Detection of *Toxoplasma gondii* IgG antibodies in juvenile great grey kangaroos, *Macropus giganteus giganteus*." J Zoo Wildl Med 34(2): 189-93.

Miller, D. S., G. F. Mitchel, *et al.* (2000). "Agglutinating antibodies to *Toxoplasma gondii* in sera from captive eastern barred bandicoots in Australia." J Wildl Dis 36(2): 213-8.

Obendorf, D. L. and B. L. Munday (1983). "Toxoplasmosis in wild Tasmanian wallabies." Aust Vet J 60(2): 62.

Obendorf, D. L. and B. L. Munday (1990). Toxoplasmosis in wild Eastern Barred Bandicoots, *Perameles gunnii*. Bandicoots and bilbies. C. M. Seebeck, P. R. Brown, R. L. Wallis and C. M. Kemper. Sydney, Australia, Surrey Beatty in association with Australian Mammal Society: 61-66.

Obendorf, D. L., P. Statham, *et al.*. (1996). "Detection of agglutinating antibodies to *Toxoplasma gondii* in sera from free-ranging eastern barred bandicoots (Perameles gunnii)." J Wildl Dis 32(4): 623-6.

Patton, S., S. L. Johnson, *et al.*. (1986). "Epizootic of toxoplasmosis in kangaroos, wallabies, and potaroos: possible transmission via domestic cats." J Am Vet Med Assoc 189(9): 1166-9.

Tenter, A. M., A. R. Heckeroth, *et al.*. (2000). "*Toxoplasma gondii*: from animals to humans." Int J Parasitol 30(12-13): 1217-58.

Wendum, D., N. Carbonell, *et al.* (2002). "Fatal disseminated toxoplasmosis in a *Toxoplasma* seropositive liver transplant recipient." J Clin Pathol 55(8): 637.

Yilmaz, S. M. and S. H. Hopkins (1972). "Effects of different conditions on duration of infectivity of *Toxoplasma gondii* oocysts." J Parasitol 58(5): 938-9.