Role of vertical transmission of *Toxoplasma gondii* in prevalence of infection

Geoff Hide

*Ecosystems and Environment Research Centre and Biomedical Research Centre, School of Environment and Life Sciences, University of Salford, Salford, M5 4WT, UK*

Email: g.hide@salford.ac.uk

Key words: *Toxoplasma*; vertical; humans; rodents; sheep

Abstract

The parasite, *Toxoplasma gondii*, is a highly successful pathogen that infects around 30% of the global human population. Additionally, it is able to infect all warm blooded animals with high prevalence. This is surprising as it is a parasite of the cat and can only complete its full sexual cycle in that host. This review examines the importance key routes of transmission: infective oocysts from the cat, ingestion of raw infected tissue and vertical transmission. The latter route of transmission has traditionally been thought to be rare. In this review, this assumption is examined and discussed in the light of the current literature. The available evidence points to the possibility that vertical transmission occurs frequently in natural populations of mice however the evidence in sheep is currently ambivalent and controversial. In humans, the situation appears as though vertical transmission may be rare although there is still much that is unexplained.

Keywords: *Toxoplasma*; vertical; humans; rodents; sheep

Of all parasites, *Toxoplasma gondii* is probably the one that most closely conforms to the definition of a perfect parasite. It is very widely distributed globally, infects all warm blooded animals and occurs with high prevalence in the majority of species investigated [1,2,3]. Furthermore, although it can cause significant disease in humans and animals, in the vast proportion of cases the parasite appears to cause very little harm to the host and is therefore asymptomatic. For example approximately 30 per cent of the human population globally are infected with *Toxoplasma*. However, this varies with countries like the US, China and the UK having lower prevalence levels of 10-20% while continental European, South American and other countries can have prevalence levels that are much higher than the global average [1,2,3,4]. The majority of infected individuals, if immunocompetent, are unlikely ever to realise that they have the infection. However, toxoplasmosis can be a significant cause of disease in immunocompromised individuals such as HIV sufferers or during transplant therapy. When the parasite does cause disease, the effects can cause major health problems in humans and can result in significant economic outcomes in domestic livestock [3].

The most serious disease outcome is abortion or miscarriage which is particularly significant for humans and domestic livestock. Congenital transmission, which is generally believed to occur by transplacental transmission when a mother becomes infected during pregnancy, is often associated with severe disease of the developing foetus, neonate or the cause of miscarriage. These symptoms can be wide ranging but include of neurological effects such as hydrocephalus, retinochoroiditis and intracranial calcification [5,6]. Furthermore the parasite can cause respiratory disease including pneumonia like symptoms in both neonates and adults [7]. The effects of this parasite are particularly concerning because it is extremely successful due to the broad host range, high prevalence and widespread distribution in humans and animals.

A very puzzling aspect of the biology of *T. gondii* is that despite this wide global presence, the parasite is specifically a parasite of the cat [8,9,10]. Felids are the only animals through which the parasite can complete its full sexual life cycle. It would, therefore, be expected that the distribution of the parasite should mirror that of feline populations. However as will be seen later this is not always the case.

The lifecycle of *T. gondii* [Figure 1] is well understood and three main routes of transmission are recognised [11]. However, while the stages of the life cycle are clear, the relative importance of the different routes of transmission, in natural populations of hosts, is not clear [12]. The first stage in the life cycle occurs in the cat which sheds many millions of infective oocysts per day during defecation [1,3]. This route of transmission ensures that the oocysts are distributed in the environment [watercourses, soil, animal feeds, crops for human consumption etc.] and are picked up by secondary hosts, usually by ingestion. In the case of humans, this can occur also by ingestion following, gardening, handling cat litter and eating unwashed vegetables [13]. The second key route of transmission is the consumption of undercooked infected meat. Secondary hosts become infected with the parasite by ingestion of oocysts. These oocysts develop into rapidly dividing tachyzoites which disseminate through the animal or human body [11]. At this stage the tachyzoites evade the immune system by becoming intracellular and they have the capability to invade any nucleated cell types. Many types of circulating cells, such as macrophages, are involved in the translocation of the parasite throughout the animal or human body. Eventually, the host immune system is able to lock down the parasite and the tachyzoites transform into the slow dividing bradyzoite stage [11]. At this point, the bradyzoites develop into a tissue cyst which can persist for the lifetime of the host. These cysts typically develop within brain tissue, muscle tissue, lung tissue but can infect a wide variety of other tissue types. It is consumption of infected, uncooked, meat from secondary hosts containing these tissue oocysts that form the second route of transmission. In natural transmission cycles, small rodents are thought to be a key reservoir for reinfection of cats [14,15,16]. The final route of transmission is congenital transmission. During pregnancy the parasite crosses the placenta and infects the foetus [11] presumably occurring whilst the host immune system is modified to prevent rejection of the foetus. It is possible that this may provide an opportunity for the parasite to exploit this and to cross the placenta. In the human population, a significant at risk group is pregnant mothers. In general, the prevalence of infection in this group mirrors that of the wider population in each country [4,17]. Congenital transmission is generally believed to occur when the mother becomes infected during pregnancy [18] and this is detected by seroconversion during pregnancy. An alternative possibility, is that this immune system modification enables chronically infected hosts to undergo parasite reactivation followed by transplacental transmission [19]. This possibility will be discussed later. Overall, this diversity of routes of transmission provides some clues as to how the parasite has become so widely distributed and prevalent. While, it is clear that cats are central to transmission, asexually derived parasite transmission cycles do contribute to parasite distribution which bypasses the felid hosts. There are some examples of hosts where the parasite is prevalent and yet the link to cat distribution is by no means obvious.

*T. gondii* has been reported in a very wide range of species including animals that do not come into contact with cats. For example studies have reported *Toxoplasma* infection in marine otters [20], dolphins [21], seals and other unusual marine species. Furthermore, the parasite is found in areas uninhabited by cats. For example in the Svalbard region of Northern Norway, the parasite is highly prevalent in arctic foxes despite the absence of cats on these islands [22]. Further studies on these arctic fox populations suggests that there is frequent infection and a high degree of clonality of *Toxoplasma* strains in infected animals [23].

There has been a wide range of studies of *T. gondii* strains globally from many laboratories. Initially, these revealed a restricted range of clonal genotypes [24]. The predominant strains found to be infecting humans and animals fall into three categories, type I, II and III. However, more detailed analyses using a standardised multilocus approach shows this to be rather oversimplified and a much wider range of genotypes are now recognised and being discovered [25].

Studies based on feeding infected meat to experimental animals, demonstrates that carnivory [consumption of undercooked meat] very effectively initiates infection in the recipient animals [26, 27]. This method of infection is so effective that it is a commonly used bioassay technique for *T. gondii* infection [3]. There are a wide range of studies that suggest that eating undercooked meat is a risk factor for infection in humans – especially in pregnant women [13, 28]. However a recent detailed survey of human meat sources in the USA showed that there was a relatively low risk of infection from raw meat in humans. Only Seven pieces of infected meat were detected from 6282 assorted meat samples collected from supermarkets across the USA [29]. Furthermore an older study demonstrated little difference in the prevalence of *Toxoplasma* in a population of strict vegetarians compared to that in a population of non-vegetarians. [30]. While carnivory could contribute to the transmission of *Toxoplasma* in the absence of cats, it is unclear the extent of this role in natural transmission cycles.

The third route of transmission, congenital transmission, is well established. It was first reported to occur in a human child as long ago as 1939 [31]. In seminal early studies in mice [32] and sheep [33], the occurrence of congenital transmission was reported to occur over repeated generations (vertical transmission). Other examples have been reported in other species [34] and congenital transmission is now well established in humans [5]. However what is unclear in these host-parasite interactions is the quantitative importance of congenital transmission in natural populations. In humans it is an extremely important route of transmission from the perspective of causing disease as severe symptoms are often associated with this mode of transmission to the foetus [6]. However, many studies show that the frequency at which this happens is very low. For example studies have demonstrated that congenital transmission occurs at between 1-2 cases per 1000 live births in humans [2, 35] and 1- 2% in sheep [36]. The key questions in humans are whether congenital transmission occurs in a serial manner and therefore contributes to infection in future generations. Secondly, if this is the case, how frequently does it occur?

Thus, while the lifecycle of *T. gondii* is well established from a descriptive point of view, what is unclear is the relative importance of each route of transmission. It is very clear that transmission cycles which bypass the cat are significant in several animal species and probably also humans. Furthermore, questions surround the role of vertical (or serial congenital) transmission. This is an area or research that has generated some controversial and opposing views. The first aim of this review is to discuss our recent studies on the contribution of vertical transmission to the spread of *T. gondii* within natural populations of animal hosts (including humans). Secondly, to contextualise these studies in the light of the, somewhat divided, literature to identify the requirements for gaining a greater understanding of *T. gondii* transmission cycles and control.

**Vertical transmission in mice**

Vertical transmission in mice has been known for a long time. Some of the very first studies that investigated the transmission of *Toxoplasma* established that the parasite could be vertically (serially congenitally) transmitted through laboratory mice [32]. For example, Beverley demonstrated that infected mice produce congenital infected offspring for at least ten generations despite recording very high mortality in the progeny. Furthermore, cysts were observed in the infected mice suggesting that serial congenital transmission may have occurred by reactivation of these cysts. Interestingly, after conducting these experiments for up to ten generations, he found that the progeny were seronegative despite being infected [11,32]. These experiments were repeated subsequently by Jacobs [37] and it was again found that although the congenitally infected progeny contained parasites they were seronegative. Additionally, Dubey and colleagues also demonstrated that viable *T. gondii* could be isolated from seronegative naturally infected mice [15] and rats [16]. These observations of Beverley, Jacobs and Dubey could suggest that there is a difference between vertical transmission in experimental systems compared to that occurring in naturally infected populations. These multiple transgenerational infections probably more closely mimic natural infections than infections introduced in the laboratory. These observations are potentially interesting since many studies on the vertical transmission of *Toxoplasma*, in a wide range of hosts, make use of serological techniques [2, 36]. If frequent vertical transmission was a feature in natural populations of mice, and this generated congenitally infected seronegative mice, could it be that serological studies are significantly underestimating levels of vertical transmission?

In general, serological studies on the prevalence of *Toxoplasma* in natural populations of mice suggests that the prevalence is relatively low [15, 38, 39, 40] while PCR based studies generally show higher prevalence levels [41, 42, 43]. The importance of vertical transmission in rodents was demonstrated by Owen and Trees [34] who showed high frequencies of vertical transmission in captive bred house mice (*Mus musculus*) and woodmice (*Apodemus sylvaticus*). Using both PCR and serological tests, they showed that vertical transmission was occurring in 85% of the house mice litters and a 100% of the woodmice litters. Interestingly, they also showed that serological tests were less efficient at detecting vertical transmission than the PCR.

Since the early studies on rodents by Beverley, Jacobs and Dubey there have been numerous laboratory studies that have demonstrated that vertical transmission occurs. These include studies on the South American rodent, *Calomys callosus*, which has been used as a model system. In this species, congenital infection has been found to be predominantly associated with infection during pregnancy rather than reactivation of chronic infection [44] when infected with clonal isolates. However, when animals were chronically infected, challenge with atypical Brazilian strains of parasite, vertical transmission was induced but not only of the new strain but also by reactivation of the chronically infected strain [45]. The deer mouse, *Peromyscus maniculatus*, has also been used as a model system. Congenital infection has been demonstrated in this species [46, 47]. In this species, it has been shown that vertical transmission occurs frequently following ingestion of oocysts but, interestingly, in the congenitally infected offspring the serological response waned with time [48]. A recent study using inbred lines of rats suggested that host genetics also determines both susceptibility to parasite infection and also the degree to which vertical transmission occurs [49]. Despite extensive laboratory studies, there have been few studies aimed at trying to understand the frequency of vertical transmission in natural populations of rodents.

To investigate the role of vertical transmission in natural populations of rodents, we undertook a study to directly measure vertical transmission during pregnancy. A population of 200 mice was collected as part of a pest control study in Manchester in the UK [50]. When tested using a *Toxoplasma* specific, SAG1-PCR, 59% of these mice were found to be infected. Sixteen of the female mice were found to be pregnant and of those 12 were infected. A total of 78 foetuses were dissected from the 16 animals, the brains were then aseptically dissected from within these foetuses and tested using a *Toxoplasma* specific SAG1-PCR. Table 1 summarises these results. All of the foetuses from the negative mothers were, as might be expected, all uninfected. In the case of the 12 infected mothers, 47 out of 63 foetuses were found to be PCR positive. These infected foetuses were derived from all of the 12 mothers thereby representing 100% congenital transmission in infected animals. This study provided the first evidence that congenital transmission is occurring very efficiently in natural populations of mice.

To study this phenomenon further, a closed colony of mice, originally derived from a wild collection, was tested for *Toxoplasma* infection using the SAG1-PCR technique [51]. This colony of mice had been kept isolated for over 10 years and had not been exposed to cats. Using microsatellite markers, these mice were found to be highly inbred as would be expected and it was found that 77% were infected with *Toxoplasma* as determined by PCR. This provides further supporting evidence that vertical transmission of *Toxoplasma* may be efficient in a natural, albeit closed, population of mice.

One of the implications of efficient vertical transmission is that the parasite is able to exist in animal populations in the absence of cats. To test this hypothesis, a wild population of woodmice was collected from an area relatively free of cats [52]. Surveys conducted in this area, demonstrated that the density of cats was low (<2.5 cats/km2). Two hundred and six woodmice were tested using the *Toxoplasma* specific SAG1-PCR and a prevalence of 40.78 per cent infection was measured. This high prevalence was not greatly less than the prevalence of 59% detectives in a previous study [50] where the cat density was significantly higher (>500 cats/km2). This indicated that efficient transmission was occurring in the woodmouse population despite the relative lack of cats. In this study, two of the *Apodemus* dams were found to be pregnant. Of these, one was PCR positive for *T.* *gondii* DNA and of the six foetuses within this pregnant dam, the brain tissue of two of these foetuses also contained *T.* *gondii* DNA. Although the sample sizes were small, this also directly demonstrated that vertical transmission was occurring in this population.

If vertical transmission is important in natural populations, it would be predicted that the parasite would be differentially distributed amongst different families of animals. If, on the other hand, the predominant route of infection was from oocysts within the environment or from scavenging infected animal tissue then parasite infection should be randomly distributed with respect to animal families. To test this hypothesis, a new collection of woodmice was obtained [53, 54] from the same location where low cat density was reported [55]. One hundred and twenty six mice were tested with four *Toxoplasma* specific PCR markers [SAG1, SAG2, SAG3 and GRA6] and were also genotyped using *Apodemus*  specific microsatellite markers [56]. In this study, the woodmice, from this location were found to belong to four different genetic families which were distributed in different parts of the study zone. The prevalence of infection was found to be significantly different in each of the families and was linked to host genotype rather than location of capture. This suggested that parasite infection was indeed associated with families and non-randomly distributed throughout the families [56].

Taken together, these data suggest that vertical transmission may be occurring very efficiently in mice and this raises the question as to whether this phenomenon is more generally applicable. Population studies on vertical transmission in rodents are rare and more research is needed in this area.

**Vertical transmission in sheep**

Despite the early studies by Hartley and colleagues [33], the generally accepted view of parasite transmission in sheep is that it occurs by ingestion of oocysts from the environment [57]. Several studies [36, 57, 58, 59, 60,61] suggest that vertical transmission occurs only rarely and that infection is generally by oral ingestion of oocysts. The possibility of ingestion of oocysts via water courses is thought to be significant [62]. However, studies conducted in our laboratory, using PCR, suggested that vertical transmission might be more important in sheep than previously thought [63].

To gain a better understanding of the importance of vertical transmission routes in sheep, we conducted a series of detailed studies. As the carnivory route of transmission is probably negligible in sheep, transmission and measurements should be simpler to interpret. It would only be necessary to consider two of three transmission routes (infected oocysts from the cat and congenital transmission).

Transmission of *Toxoplasma* to sheep is generally thought to be attributed to the ingestion of oocysts from past year feed hay or water which has been contaminated by cats. However, direct measurement of this mechanism of transmission is extremely difficult to contemplate. In contrast therefore, we chose to investigate the alternative hypothesis by directly measuring congenital transmission. In order to develop a non-destructive PCR detection system for *Toxoplasma* in sheep, we investigated the use of umbilical cord samples as a source of tissue for testing newborn lambs. Initial studies were aimed at confirming that umbilical cord tissue did indeed represent the infection status of lambs. Using SAG1 *Toxoplasma* specific PCR testing, we measured the infection status of umbilical cord tissue and corresponding brain tissue from naturally aborted lambs. Very good agreement was obtained and demonstrated that umbilical cord tissue was a good indicator of infection status of the lambs [63, 64, 65]. Lamb tissue (umbilical cord) tissue was collected from a total of 392 pregnancies and corresponding internal and tissues were collected from naturally aborting lambs. Congenital transmission was demonstrated to be occurring in 69% of pregnancies with a higher level (91%) in successful pregnancies (those in which at least one lamb was aborted) [63, 64]. These results are summarised in Table 2.These data support previous observations that *Toxoplasma* is associated with abortion. Interestingly though, in 65% of successful pregnancies the parasite was also found to be transmitted. This implies that apparently healthy lambs could be infected and therefore could contribute to the infection of future generations by vertical transmission.

If vertical transmission was indeed important, it would be predicted that infection rates could vary between different sheep families that were exposed to the same food/water sources. On the other hand if transmission was caused by contamination or feed or water, infection should be randomly distributed throughout different families on a single farm (i.e. exposed to the same environmental influences). To test this hypothesis, congenital transmission and abortion frequencies were measured on a single farm [66]. Records we used to construct pedigrees of different sheep families from members of a Charolais flock over the period 1992 – 2003 and to determine the frequencies of abortion in each family. During the period 2000-2003, umbilical cord samples were collected from members of these families and tested for *Toxoplasma* infectivity using the *Toxoplasma* specific SAG1-PCR. The results are presented in Table 3. Considerable variation was observed in the frequency of abortion in different families across the single farm. For example some families experienced abortion frequency is as high as 48% while other families, of comparable sizes, experienced no abortion at all over the 11 year period. These results were highly significantly different from expectations generated from the hypothesis that predicted a random abortion distribution (P<0.01). Furthermore when *Toxoplasma* infection was considered, again a highly uneven distribution was observed. For example some families were showing 100% of lambs infected while other families of a comparative size showed no infection. This was highly significantly different from the even distribution expected from random exposure to *Toxoplasma* oocysts. There was also a highly significant correlation (R= 0.89, n=27, P<0.01) between frequency of *Toxoplasma* infection and frequency of abortion in families. These data show that *Toxoplasma* infection and abortion are not randomly distributed across families and support the hypothesis that acquired infection is occurring vertically rather than by exposure to oocysts on pastures, in feed or water [66].

If vertical transmission of *Toxoplasma* is occurring with high frequencies and is responsible for generating high frequency aborting families, this raises questions about how we should be breeding within our sheep flocks. For example, breeding from families which have existing high levels of infection would simply propagate those infections and consequent abortions. Additionally it also raises questions about the concept of lifelong immunity which is generally associated with the infection by *Toxoplasma*. If *Toxoplasma* is associated with lifelong immunity then it would suggest that a good strategy would be to breed from ewes that have previously aborted due to *Toxoplasma*. These two situations are clearly at odds with each other. A better strategy, if vertical transmission is important, would be to breed from families with low *Toxoplasma* frequency.

To investigate this dilemma, the pedigrees of sheep families were examined to determine the frequency of infection and abortion in sequential lambings from the same ewe [67]. Using the collection of ewe data previously described [66], 29 ewes were found to have given birth to sequential lambs during the period of testing. Infected lambs were born in 31% of these sequential pregnancies of which 67% resulted in abortion (i.e. 21% overall in sequential pregnancies). These results show that prior infection and abortion does not provide protection for subsequent *Toxoplasma* pregnancies. The risk of the next lamb being infected following the birth of an infected lamb was 69% and the probability of abortion of a subsequent lamb was 55% [67]. These high risks suggest that it may be an unwise strategy to breed from infected ewes belonging to these high aborting families.

To date, the significance of vertical transmission in sheep is not universally accepted. There is a considerable body of evidence that does not support our conclusions [36, 57, 58, 59, 60, 68]. These differences of opinion could be based on the use of different approaches and techniques but further studies would need to be conducted to establish this. The implications of this research are extremely important for sheep husbandry and health and it is essential to conduct further research to reconcile these opposing views.

**Vertical Transmission in Other Animals**

There are very few other animal species where detailed studies have been carried out on natural populations. A long term study has been conducted on toxoplasmosis in sea otters in California. This is an interesting study as there is clearly no direct link between the ecology of these otters and terrestrial felids. An initial survey of southern sea otters (*Enhydra lutris nereis*) off the Californian coast showed the seroprevalence in this species of otters to be 42% for live otters and 62% for dead ones - very high considering the lack of an obvious link with cats [20]. Subsequent analyses have suggested that freshwater runoff and a complex ecology through invertebrate hosts, on which otters may feed, may have led to these levels of infection [69, 70, 71, 72, 73]. However, congenital transmission has also been shown to be occurring [69, 74]. It is possible that this latter mechanism could contribute to maintenance of infection in this species.

There are numerous individual reports of vertical transmission occurring in specific species. For example, recent studies have demonstrated vertical transmission in wild boar [75], sea lions [76] and Australian marsupials [77]. However, in these cases little is known of the extent or nature of the importance of vertical transmission.

**Vertical Transmission in humans**

It has been known for a long time that congenital transmission occurs in humans and when it occurs it can confer a high frequency of serious disease consequences. The majority of studies suggest that congenital transmission occurs at low frequency [2, 35] with rates of 1 in 1,000 to 1 in 10,000 live births reported. It is not within the scope of this review to discuss the extensive literature in this area. However, little is known about serial congenital transmission (vertical transmission) – that is, sequential transmission from generation to generation. Such studies are extremely difficult to conduct in humans due to long generation times, ethical issues and lack of robust methodologies for diagnosis of transgenerational infection.

The key issue in establishing whether vertical transmission is important in humans is that there are high prevalences of infection and yet an apparently low congenital infection rate. This suggests that either vertical transmission is not important or that we haven’t currently got robust enough methodologies for detection of this mode of transmission. Global data suggests prevalences of above 60% in some parts of the world [4] and that prevalence in pregnant women may reflect levels reported in those national statistics [4, 17]. Thus, it would seem that, in principle, a high proportion of mothers have the potential to pass the parasite to their offspring. One possibility is that congenital infection only occurs when mothers seroconvert (i.e. become infected during pregnancy). This is indeed supported by many studies including a detailed analysis of the Austrian Toxoplasmosis Register, 1992 – 2008 [78]. However, mothers that are already seropositive with a chronic infection will not be detected as seroconverting by routine tests and will not be detected as infected unless a problem occurs with the developing foetus or neonate.

Our findings in sheep that there was a high frequency of positive lambs born which were apparently healthy [63, 64] and there appeared to be transmission through sheep families with asymptomatic consequences [66, 67]. This raises some questions as to whether this could occur in humans. To investigate this possibility we used PCR testing of umbilical cord tissue, obtained at birth, to measure the infection rate in human babies. Studies in sheep showed that there was a reliable correlation between SAG1-PCR positive cord tissue and SAG1 positivity in internal organs [64]. In a study conducted in Libya [51], it was shown that 27 PCR positive samples were obtained from 276 pregnancies suggesting congenital transmission rates of 9.93%. Five of the pregnancies were unsuccessful and resulted in miscarriages but none were associated with *Toxoplasma*. Thus there is some evidence that the *Toxoplasma* positive babies are carrying *Toxoplasma* DNA despite being apparently healthy. More research is needed to investigate the longer term consequences of these data.

If vertical transmission is occurring in a “silent” manner, as could be suggested by these data, then this raises questions as to the reasons why it has not been picked up before in the many serological studies demonstrating low *T, gondii* congenital transmission rates? One possibility is that the serological detection methods are not a sufficiently reliable approach. The early studies on serial congenital transmission in mice [32] showed that after several generations the congenitally infected offspring were demonstrably infected but were seronegative. Perhaps serial congenital transmission results in a long term situation where a seronegative infection is tolerated – this would, of course, lead to an underestimation of congenital transmission if serology was the only test. More research is needed to examine this question. Additionally, if vertical transmission was as frequent as suggested in our study, it suggests that a possible mechanism of transmission could be reactivation of a chronic infection (reactivation of cysts). At present there is little evidence to suggest that this happens with high frequency however there are some indications that it does occur. Although only based on individual case studies, there have been serval reports of possible reactivation from immunocompetent mothers and subsequent congenital transmission [79, 80, 81]. Interestingly, recent studies have suggested that reactivation may be associated with modulation of T-cell immunity during pregnancy [82, 83]. Further research is required to investigate these important questions.

**Conclusions**

It is clear that the cat is an important source of infection due to the shedding of infective oocysts. However, it is also clear that other routes of transmission must play an important role. In this review, the role of vertical transmission in the dissemination of *Toxoplasma* has been discussed. The results of several studies conducted by our laboratory suggest that vertical transmission may be more important than previously thought and the aim of this review was to put these studies into context. It seems clear that vertical transmission may be a significant route of transmission in mice and, possibly, rodents in general. In sheep, the situation is much less clear and there is evidence both for and against the importance of vertical transmission. The balance of the literature is currently more against than for. However, there are some important issues raised, especially about the breeding management of sheep, which may have significant implications if vertical transmission is occurring at high frequency. In humans, the balance of data currently suggests that vertical transmission is rare. However, there is still much that is unexplained about the importance of routes of transmission of *Toxoplasma* to humans. We may not currently have the tools or methodologies to fully investigate vertical transmission in *Toxoplasma*. Furthermore, if our beliefs and understanding of the spread of *Toxoplasma* is based around the concepts of transmission being associated with ingestion of oocysts and infected tissue, we may not be asking the right questions nor developing the right methodologies to investigate vertical transmission. This mode of transmission in *Toxoplasma* may be deserving of more consideration and certainly requires more future research to address outstanding questions.

**Expert commentary**

There is a key need to understand the routes of transmission of *Toxoplasma* to its various hosts. The problem is complex and much research has already been carried out. The exhaustive contributions of scientists working on the vertical transmission of *Toxoplasma* in sheep should not be underestimated and yet there is still clear disagreement. There is a need for a robust understanding of which diagnostic methodologies are most useful for detecting congenital infection. There are surprising observations in serially congenital transmission in mice, that parasite detection by serology wanes despite the detectable presence of parasites. If vertical transmission is common in mice, which some evidence clearly suggests, then perhaps seronegativity is a feature of naturally infected animals. This might have implications for differences in transmission in natural populations compared to experimentally infected animals. Thus diagnostics need to be investigated for natural populations of animals as well as those that have been experimentally infected. In the case of research in humans and sheep, little is known about the immune status of congenitally infected neonates if vertical transmission is occurring in these species. Such research is important for gaining future perspectives in this area.

**Five year view**

To understand transmission of any infectious agent, markers are required to track the spread of the pathogen. Much research is currently on going in developing genetic markers and identifying their geographical distributions. We currently have a good and improving overview of the nature of *Toxoplasma* strains globally with strains that are associated with Europe and North America and different atypical strains rapidly being discovered in South America. However, we have little information on how strains are being spread from animal to animal or human to human within localised ecosystems. From the perspective of vertical transmission, there is a need to consider the spread of the parasite within families in natural populations. This could be done in species such as mice where large sample sizes can be collected, where generation times are short and where the use of host genotyping and parasite genotyping could be used to track the distribution of parasites through families. These types of studies are required and could, perhaps, be developed as a model system for use in larger mammals and possibly humans.

**Acknowledgements**

I would like to thank various funding agencies that have supported aspects of my work over the years: Wellcome Trust, Leverhulme Trust, Royal Society, Perry Foundation, Yorkshire Agricultural Society, British Society of Parasitology, Libyan Embassy and University of Salford. I would like to thank all my co-authors on previous papers, who although have not contributed directly to this paper, have contributed to the development of this work.

**References**

1. Dubey, J.P. and Beattie, C.P. Toxoplasmosis of Animals and Man. 1988. CRC Press, Boca Raton, Florida.

2. Tenter AM, Heckeroth AR, Weiss LM. *T. gondii*: from animals to humans. Int J Parasitol 2000; 30:1217-1258

3. Dubey JP. Toxoplasmosis of animals and humans. 2nd Edn. 2010. CRC Press, Boca Raton, Florida, USA.

4. Pappas G, Roussos N, Falagas ME. (2009). Toxoplasmosis snapshots: Global status of *Toxoplasma gondii* seroprevalence and implications for pregnancy and congenital toxoplasmosis. Int J Parasitol 2009; 39:1385-1394

5. Weiss LM, Dubey JP. Toxoplasmosis: A history of clinical observations. International J Parasitol 2009; 39:895-901.

6. Olariu TR, Remington JS, McLeod R, Alam A, Montoya JG. Severe congenital

toxoplasmosis in the United States: clinical and serologic findings in untreated

infants. Pediatr Infect Dis J. 2011; 30:1056-61

7. Guo HM, Gao JM, Luo YL, Wen YZ, Zhang YL, Hide G, Zhou WL, Ayala FJ, Lun ZR. Infection by *T. gondii,* a severe parasite of neonates and AIDS patients, causes impaired anion secretion in airway epithelia. Proc Nat Acad Sci USA 2015; 112:4435 – 4440

8. Hutchison WM, Dunachie JF, Siim JC, Work K. Life cycle of *T. gondii*. Br Med J 1969; 4:806-806

9. Dubey JP, Miller NL, Frenkel JK. The *T. gondii* oocyst from cat feces. J Exp Med 1970; 132:636-662

10. Frenkel JK, Dubey JP, Miller NL. *T. gondii* in cats: fecal stages identified as coccidian oocysts. Science 1970; 167:893-896

11. Dubey JP. History of the discovery of the life cycle of *T. gondii*. Int J Parasitol 2009; 39:877-882

12. Hide G, Morley EK, Hughes JM, Gerwash O, Elmahaishi MS, Elmahaishi KH, Thomasson D, Wright EA, Williams RH, Murphy RG, Smith JE. Evidence for high levels of vertical transmission in *T. gondii*. Parasitology 2009; 136:1877-1885

13. Cook AJC, Gilbert RE, Buffolano W, Zufferey J, Petersen E, Jenum PA, Foulon W, Semprini AE, Dunn DT. Sources of *Toxoplasma* infection in pregnant women: European multicentre case control study. Br Med J 2000; 321:142-147

14. Rifaat MA, Nasr NT, Sadek MS, Arafa MS, Mahdi AH. The role of the domestic

rat, *Rattus alexandrinus* as a reservoir host of *Toxoplasma gondii* in Egypt. J

Trop Med Hyg. 1973; 76:257-258

15. Dubey JP, Weigel RM, Siegel AM, Thulliez P, Kitron UD, Mitchell MA, Mannelli A, Mateuspinillia NE, Shen SK, Kwok OCH, Todd KS. Sources and reservoirs of *T. gondii* infection on 47 swine farms in Illinois. J Parasitol 1995; 81:723-729

16. Dubey JP, Shen SK, Kwok OC, Thulliez P. Toxoplasmosis in rats *Rattus norvegicus*: congenital transmission to first and second generation offspring and isolation *T. gondii* from seronegative rats. Parasitology 1997; **115**:9-14

17. Gao XJ, Zhao JZ, He ZH, Wang T, Yang TB, Chen XG, Shen JL, Wang Y, Lu FL, Hide G, Lun ZR. *Toxoplasma gondii* infection in pregnant women in China. Parasitol 2012; 139:139-147

18. Carlier Y, Truyens C, Deloron P, Peyron F. Congenital parasitic infections: a

review. Acta Trop 2012; 121:55-70

19. Andrade GM, Vasconcelos-Santos DV, Carellos EV, Romanelli RM, Vitor RW,

Carneiro AC, Januario JN. Congenital toxoplasmosis from a chronically infected

woman with reactivation of retinochoroiditis during pregnancy. J Pediatr Rio J.

2010; 86:85-88

20. Miller MA, Gardner IA, Kreuder C, Paradies DM, Worcester KR, Jessup DA, Dodd E, Harris MD, Ames JA, Packham AE, Conrad PA. Coastal freshwater runoff is a risk factor for *T. gondii* infection of southern sea otters *Enhydra lutris nereis*. Int J Parasitol 2002; 32:997-1006

21. Dubey JP, Zarnke R, Thomas NJ, Wong SK, Van Bonn W, Briggs M, Davis JW, Ewing R, Mensea M, Kwok OCH, Romand S, Thulliez P. *T. gondii, Neospora caninum, Sarcocystis neurona,* and *Sarcocystis canis*-like infections in marine mammals. Vet Parasitol 2003; 116:275-296

22. Prestrud KW, Dubey JP, Asbakk K, Fuglei E, Su, C. First isolate of *T. gondii* from arctic fox *Vulpes lagopus* from Svalbard. Vet Parasitol 2008; 151:110-114

23. Prestrud KW, Asbakk K, Mørk T, Fuglei E, Tryland M, Su, C. Direct high-resolution genotyping of *T. gondii* in arctic foxes *Vulpes lagopus* in the remote arctic Svalbard archipelago reveals widespread clonal Type II lineage.Vet Parasitol 2008; 158:121-128

24. Sibley LD, Boothroyd JC. Virulent strains of *T. gondii* comprise a single clonal lineage. Nature 1992; 359:82-85.

25. Shwab EK, Zhu XQ, Majumdar D, Pena HF, Gennari SM, Dubey JP, Su, C. Geographical patterns of *T. gondii* genetic diversity revealed by multilocus PCR-RFLP genotyping. Parasitol 2014; 141:453-461

26. Aspinall TV, Marlee D, Hyde JE, Sims PFG. Prevalence of *T. gondii* in commercial meat products as monitored by polymerase chain reaction – food for thought ? Int J Parasitol 2002; 32:1193-1199.

27. Su C, Evans D, Cole RH, Kissinger JC, Ajioka JW, Sibley LD. Recent expansion of *Toxoplasma* through enhanced oral transmission. Science2003; 299:414-416.

28. Boyer KM, Holfels E, Roizen N, Swisher C, Mack D, Remingto, J, Withers S, Meier P, McLeod R. Risk factors for *T. gondii* infection in mothers of infants with congenital toxoplasmosis: implications for prenatal management and screening. Am J Obstet Gynecol 2005; 192:564-571

29. 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, Gamble HR. Prevalence of viable *T. gondii* in beef, chicken and pork from retail meat stores in the United States: risk assessment to consumers. J Parasitol 2005; 91:1082-1093

30. Rawal BD. Toxoplasmosis. A dye-test on sera from vegetarians and meat

eaters in Bombay. Trans Roy Soc Trop Med Hyg 1959; 53:61-63

31. Wolf A, Cowen D, Paige B. Human toxoplasmosis occurrence in infants as an encephalomyelitis verification by transmission to animals. Science 1939; 89:226-227

32. Beverley JKA. Congenital transmission of Toxoplasmosis through successive generation of mice. Nature 1959; 183 :1348-1349

33. Hartley WJ, Marshall SC. Toxoplasmosis as a cause of ovine perinatal mortality. N Zealand Vet J 1957; 5:119-124

34. Owen MR, Trees AJ. Vertical transmission of *T. gondii* from chronically infected house *Mus musculus* and field *Apodemus sylvaticus* mice determined by polymerase chain reaction. Parasitol 1998; 116:299-304

35. Dubey JP, Jones JL. *T. gondii* infection in humans and animals in the United States. Int J Parasitol 2008; 38:1257-1278

36. Buxton D, Maley SW, Wright SE, Rodger S, Bartley P, Innes EA. *T. gondii* and ovine toxoplasmosis: New aspects of an old story. Vet Parasitol 2007; 149:25-28

37. Jacobs L. The occurrence of *Toxoplasma* infection in the absence of demonstrable antibodies. Proc First Int Congr Parasitol 1964; 1:176-177

38. Jackson MH, Hutchison WM, Siim JC. Toxoplasmosis in a wild rodent population of Central Scotland and a possible explanation of the mode of transmission. J Zool 1986; 209:549-557

39. Webster JP. Prevalence and transmission of *Toxoplasma gondii* in wild brown

rats, *Rattus norvegicus*. Parasitol. 1994; 108:407-411

40. Smith DD, Frenkel JK. Prevalence of antibodies to *T. gondii* in wild mammals of Missouri and East Central Kansas - biologic and ecologic considerations of transmission. J Wildlife Dis1995; 31:15-21

41. Zhang SY, Jiang SF, He YY, Pan CE, Zhu M, Wei MX. Serologic prevalence of *T. gondii* in field mice, *Microtus fortis*, from Yuanjiang, Hunan province, People's Republic of China. J Parasitol 2004; 90:437-438

42. Kijlstr, A, Meerburg B, Cornelissen J, De Craeye S, Vereijken P, Jongert, E. The role of rodents and shrews in the transmission of *T. gondii* to pigs. Vet Parasitol 2008; 156:183-190

43. Vujanić M, Ivović V, Kataranovski M, Nikolić A, Bobić B, Klun I, Villena I, Kataranovski D, Djurković-Djaković O. Toxoplasmosis in naturally infected rodents in Belgrade, Serbia. Vec Borne Zoonot Dis 2012; 11:1209-1211

44. Franco PS, Silva DA, Costa IN, Gomes AO, Silva AL, Pena JD, Mineo JR, Ferro

EA. Evaluation of vertical transmission of *Toxoplasma gondii* in *Calomys callosus*

model after reinfection with heterologous and virulent strain. Placenta 2011; 32:116-120

45. Franco PS, da Silva NM, de Freitas Barbosa B, de Oliveira Gomes A, Letta F,

Shwab EK, Su C, Mineo JR, Ferro EA. *Calomys callosus* chronically infected by

*Toxoplasma gondii* clonal type II strain and reinfected by Brazilian strains is

not able to prevent vertical transmission. Front Microbiol. 2015 6:181

46. Dabritz HA, Miller MA, Packham AE, Rejmanek D, Leutenegger CM, Gardner IA,

Atwill ER, Patricia A C. Experimental infection of *Peromyscus californicus* with

*Toxoplasma gondii*. J Parasitol 2007; 93:1360-1364

47. Dabritz HA, Miller MA, Gardner IA, Packham AE, Atwill ER, Conrad PA. Risk

factors for *Toxoplasma gondii* infection in wild rodents from central coastal

California and a review of *T. gondii* prevalence in rodents. J Parasitol 2008;

94:675-683

48. Rejmanek D, Vanwormer E, Mazet JA, Packham AE, Aguilar B, Conrad PA.

Congenital transmission of *Toxoplasma gondii* in deer mice (*Peromyscus*

*maniculatus*) after oral oocyst infection. J Parasitol 2010; 96:516-520

49. Gao JM, Yi SQ, Wu MS, Geng GQ, Shen JL, Lu FL, Hide G, Lai DH, Lun ZR.

Investigation of infectivity of neonates and adults from different rat strains to

*Toxoplasma gondii* Prugniaud shows both variation which correlates with iNOS and

Arginase-1 activity and increased susceptibility of neonates to infection. Exp

Parasitol 2015; 149:47-53

50. Marshall PA, Hughes JM, Williams RH, Smith JE, Murphy RG, Hide G. Detection of high levels of congenital transmission of *Toxoplasma gondii* in natural urban

populations of *Mus domesticus*. Parasitol 2004; 128:39-42.

51. Hide G, Gerwash O, Abushahma M, Hughes JM, Thomasson D, Wright EA, Murphy RG, Dodd N, Haq SZH, Elmahaishi KH, Elmahaishi MS. Do high levels of vertical transmission explain the high prevalence and ubiquity of *Toxoplasma gondii*? Proc Twelfth Int Cong Parasitol 2010;12:83 - 90 .

52. Thomasson D, Wright EA, Hughes JM, Dodd NS, Cox AP, Boyce K, Gerwash O,

Abushahma M, Lun ZR, Murphy RG, Rogan MT, Hide G. Prevalence and co-infection of *Toxoplasma gondii* and *Neospora caninum* in *Apodemus sylvaticus* in an area relatively free of cats. Parasitol 2011; 138:1117-1123.

53. Boyce K, Hide G, Craig PS, Harris PD, Reynolds C, Pickles A, Rogan MT.

Identification of a new species of digenean *Notocotylus malhamensis n. sp.*

(Digenea: Notocotylidae) from the bank vole (*Myodes glareolus*) and the field vole

(*Microtus agrestis*). Parasitol 2012;139:1630-1639.

54. Boyce K, Hide G, Craig PS, Reynolds C, Hussain M, Bodell AJ, Bradshaw H,

Pickles A, Rogan MT. A molecular and ecological analysis of the trematode

*Plagiorchis elegans* in the wood mouse *Apodemus sylvaticus* from a periaquatic

ecosystem in the UK. J Helminthol 2014; 88:310-20.

55. Hughes JM, Thomasson D, Craig PS, Georgin S, Pickles A, Hide G. *Neospora*

*caninum*: detection in wild rabbits and investigation of co-infection with

*Toxoplasma gondii* by PCR analysis. Exp Parasitol 2008;120:255-260.

56. Bajnok J, Boyce K, Rogan MT, Craig PS, Lun ZR, Hide G. Prevalence of

*Toxoplasma gondii* in localized populations of *Apodemus sylvaticus* is linked to

population genotype not to population location. Parasitol 2015;142:680-690.

57. Innes EA, Bartley PM, Buxton D, Katzer F. Ovine toxoplasmosis. Parasitol

2009; 136:1887-1894

58. Buxton D, Rodger SM, Maley SW, Wright SE. Toxoplasmosis: The possibility of vertical transmission. Small Ruminant Res 2006; 62:43-46

59. Rodger SM, Maley SW, Wright SE, Mackellar A, Wesley F, Sales J, Buxton D. Role of endogenous transplacental transmission in toxoplasmosis in sheep. Vet Rec

2006; 159:768-72

60. Dubey JP. Toxoplasmosis in sheep -The last 20 years. Vet Parasitol 2009; 163: 1-14

61. Katzer F, Brülisauer F, Collantes-Fernández E, Bartley PM, Burrells A, Gunn G,

Maley SW, Cousens C, Innes EA. Increased *Toxoplasma gondii* positivity relative to

age in 125 Scottish sheep flocks; evidence of frequent acquired infection. Vet

Res 2011; 42:121

62. Wells B, Shaw H, Innocent G, Guido S, Hotchkiss E, Parigi M, Opsteegh M, Green J, Gillespie S, Innes EA, Katzer F. Molecular detection of *Toxoplasma gondii* in water samples from Scotland and a comparison between the 529bp real-time PCR and ITS1 nested PCR. Water Res 2015; 87:175-181

63. Duncanson P, Terry RS, Smith JE, Hide G. High levels of congenital

transmission of *Toxoplasma gondii* in a commercial sheep flock. Int J Parasitol

2001;31:1699-1703

64. Williams RH, Morley EK, Hughes JM, Duncanson P, Terry RS, Smith JE, Hide G.

High levels of congenital transmission of *Toxoplasma gondii* in longitudinal and

cross-sectional studies on sheep farms provides evidence of vertical transmission

in ovine hosts. Parasitol 2005;130:301-307

65. Hughes JM, Williams RH, Morley EK, Cook DA, Terry RS, Murphy RG, Smith JE,

Hide G. The prevalence of *Neospora caninum* and co-infection with *Toxoplasma*

*gondii* by PCR analysis in naturally occurring mammal populations. Parasitol 2006;132:29-36

66. Morley EK, Williams RH, Hughes JM, Terry RS, Duncanson P, Smith JE, Hide G.

Significant familial differences in the frequency of abortion and *Toxoplasma*

*gondii* infection within a flock of Charollais sheep. Parasitol 2005;131:181-185.

67. Morley EK, Williams RH, Hughes JM, Thomasson D, Terry RS, Duncanson P, Smith JE, Hide G. Evidence that primary infection of Charollais sheep with *Toxoplasma gondii* may not prevent foetal infection and abortion in subsequent lambings. Parasitol 2008;135:169-173

68. Buxton D, Maley SW, Wright SE, Rodger S, Bartley P, Innes EA. Ovine

toxoplasmosis: transmission, clinical outcome and control. Parassitologia 2007; 49:219-221

69. Miller M, Conrad P, James ER, Packham A, Toy-Choutka S, Murray MJ, Jessup D, Grigg M. Transplacental toxoplasmosis in a wild southern sea otter (*Enhydra*

*lutris nereis*). Vet Parasitol 2008; 153:12-8.

70. Shapiro K, Conrad PA, Mazet JA, Wallender WW, Miller WA, Largier JL. Effect of

estuarine wetland degradation on transport of *Toxoplasma gondii* surrogates from

land to sea. Appl Environ Microbiol 2010;76:6821-6828

71. Krusor C, Smith WA, Tinker MT, Silver M, Conrad PA, Shapiro K. Concentration

and retention of *Toxoplasma gondii* oocysts by marine snails demonstrate a novel

mechanism for transmission of terrestrial zoonotic pathogens in coastal

ecosystems. Environ Microbiol 2015;17:4527-4537

72. Shapiro K, VanWormer E, Aguilar B, Conrad PA. Surveillance for *Toxoplasma*

*gondii* in California mussels (*Mytilus californianus*) reveals transmission of

atypical genotypes from land to sea. Environ Microbiol 2015;17:4177-4188.

73. Mazzillo FF, Shapiro K, Silver MW. A new pathogen transmission mechanism in

the ocean: the case of sea otter exposure to the land-parasite *Toxoplasma gondii*.

PLoS One 2013;8:e82477

74. Shapiro K, Miller MA, Packham AE, Aguilar B, Conrad PA, Vanwormer E, Murray

MJ. Dual congenital transmission of *Toxoplasma gondii* and *Sarcocystis neurona* in

a late-term aborted pup from a chronically infected southern sea otter (*Enhydra*

*lutris nereis*). Parasitol 2015 Oct:1-13. [Epub ahead of print]

75. Calero-Bernal R, Gómez-Gordo L, Saugar JM, Frontera E, Pérez-Martín JE, Reina D, Serrano FJ, Fuentes I. Congenital toxoplasmosis in wild boar (*Sus scrofa*) and identification of the *Toxoplasma gondii* types involved. J Wildl Dis 2013; 49:1019-1023

76. Carlson-Bremer D, Colegrove KM, Gulland FM, Conrad PA, Mazet JA, Johnson CK. Epidemiology and pathology of *Toxoplasma gondii* in free-ranging California sea

lions (*Zalophus californianus*). J Wildl Dis 2015;51:362-373

77. Parameswaran N, O'Handley RM, Grigg ME, Wayne A, Thompson RC. Vertical

transmission of *Toxoplasma gondii* in Australian marsupials. Parasitol 2009;136:939-944

78. Prusa AR, Kasper DC, Pollak A, Gleiss A, Waldhoer T, Hayde M. The Austrian

Toxoplasmosis Register, 1992-2008. Clin Infect Dis 2015;60:e4-e10.

79. Ladas ID, Rallatos CL, Kanaki CS, Damanakis AG, Zafirakis PK, Rallatos G.

Presumed congenital ocular toxoplasmosis in two successive siblings.

Ophthalmologica 1999; 213:320-322

80. Kodjikian L, Hoigne I, Adam O, Jacquier P, Aebi-Ochsner C, Aebi C, Garweg JG.

Vertical transmission of toxoplasmosis from a chronically infected immunocompetent woman. Pediatr Infect Dis J 2004; 23:272-274

81. Valdès V, Legagneur H, Watrin V, Paris L, Hascoët JM. Congenital

toxoplasmosis due to maternal reinfection during pregnancy. Arch Pediatr 2011;18:761-763.

82. Gigley JP, Bhadra R, Khan IA. CD8 T Cells and *Toxoplasma gondii*: A new

paradigm. J Parasitol Res 2011; 2011:243796

83. Gigley JP, Bhadra R, Moretto MM, Khan IA. T cell exhaustion in protozoan

disease. Trends Parasitol 2012; 28:377-384

**Figure Legends**

Figure 1 Lifecycle of *T. gondii*. [Adapted from 12]. Three routes of transmission are recognised: ingestion of infective oocysts produced by the cat, consumption of undercooked/raw infected meat (carnivory) and congenital transmission.

Figure 2

Example pedigree showing a high frequency of abortion.

Symbols are as follows: female; Aborted female;

 male; Aborted male; Sex not recorded

Figure 3

Example pedigree showing a low frequency of abortion.

See Figure 2 legend for key to symbols.