Ovine toxoplasmosis

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SUMMARY

Congenital infection with *Toxoplasma gondii* is an important cause of abortion in sheep worldwide. The cat is the definitive host of the parasite, and infected cats may shed millions of oocysts in their faeces resulting in extensive environmental contamination and an important source of infection for grazing herbivorous animals. Studies looking at development of specific antibodies in sheep, as an indicator of exposure to *T. gondii*, have shown that there is an increase in seroprevalence associated with age indicating that most infections in sheep occur following birth. The stage of gestation when transplacental transmission of *T. gondii* to the developing foetus occurs is critical in determining the clinical outcome. The importance of endogenous transplacental transmission in persistently infected ewes and its clinical importance is a subject of current debate. Ewes infected prior to mating develop immune responses that help protect against disease in a subsequent pregnancy and also against experimental challenge administered during pregnancy. Both innate and adaptive immune responses are activated following *T. gondii* infection and experiments involving the chronic cannulation of peripheral lymph nodes in sheep have allowed the dynamics of the immune responses to be analysed in real time. A live vaccine, Toxovax® is the only commercially available vaccine worldwide to protect against congenital toxoplasmosis.

Key words: *Toxoplasma gondii*, sheep, immune responses, vaccination.

INTRODUCTION

The protozoan parasite *Toxoplasma gondii* is a major cause of infectious ovine abortion in both UK and worldwide. Current data from the Veterinary Investigation Diagnosis Analysis (VIDA) in UK shows that the three pathogens most commonly diagnosed as causing abortion in sheep are *Chlamydophila abortus* (40%), *Toxoplasma gondii* (24%) and *Campylobacter* (14%), (www.defra.gov.uk/vla/reports). The national sheep flock is estimated to be in the region of 32 million animals and although the incidence of ovine toxoplasmosis is difficult to define, a study by Blewett and Trees (1987) suggested that *T. gondii* may be responsible for 1–2% of neonatal losses annually. These losses would translate to over 0·5 million lambs in UK and 1·5 million lambs lost in Europe per year, representing a significant loss to producers and national economies. In this paper we discuss the history of the disease, parasite transmission routes, disease pathogenesis, host immunity and control strategies for ovine toxoplasmosis.

HISTORY AND BACKGROUND

Toxoplasma gondii is a fascinating parasite, discovered one hundred years ago by two different groups of scientists working in Tunisia (Nicolle and Manceaux, 1908) and in Brazil (Splendore, 1908). The parasite was named based on its morphology, *toxo* meaning arc or bow and *plasma* meaning life and the original host animal it was discovered in, a hamster like rodent, the gundi, *Ctenodactylus gundii*. Since its discovery, Toxoplasma has been found to infect all warm blooded animals including humans making it arguably the most successful parasite worldwide (Ferguson, 2009). Toxoplasma was first reported to be an important pathogen in sheep in the 1950s with a series of reports from Bill Hartley and colleagues in New Zealand where they observed *T. gondii* organisms in placental tissue from aborting sheep and within foetal tissues (Hartley, Jebson and McFarlane, 1954; Hartley and Marshall, 1957). Following this initial description, there were other reports of a similar disease in sheep occurring in Australia, UK and Europe (Dubey and Beattie, 1988). The route of transmission of the parasite to sheep was initially unclear as at this time the only two known routes of *T. gondii* transmission were via consumption of undercooked meat containing *T. gondii* tissue cysts or congenital transmission from mother to foetus (Innes, 2010). As sheep are herbivores, it was suggested that there may be another, as yet undiscovered, common route of transmission.

Work in the 1960s showed that cats were able to shed an environmentally stable form of *T. gondii* in their faeces (Hutchison, 1965). These eggs or oocysts were found to be stable in the environment and could remain infective for up to 18 months depending on climatic conditions. Favourable conditions for oocyst survival are temperate and moist, which may be why ovine toxoplasmosis is a problem in temperate

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countries such as UK, Northern Europe and New Zealand (Buxton and Rodger, 2008). Further work confirmed the cat as the definitive host of *T. gondii* (Frenkel, Dubey and Miller, 1970; Hutchison *et al.* 1970; Ferguson *et al.* 1974) and oocysts as a major source of environmental contamination and an important source of infection for grazing animals.

TRANSMISSION

Oocysts in the environment

Members of the cat family are the definitive hosts of the parasite and tend to become infected for the first time when they start hunting and eating wild rodents and birds already infected with *T. gondii*. Following consumption of *T. gondii* cysts, the parasites excyst in the gut of the cat and invade and infect host cells. Sexual development of the parasite takes place in the gut of the cat resulting in the production of oocysts which are shed in the faeces. Shedding usually occurs around 3–10 days after initial infection and may continue for 2–3 weeks (Dubey and Beattie, 1988). During this period a cat may shed over 100 million oocysts and experimental studies in sheep have shown that a dose of only 200 oocysts may cause abortion in previously naïve pregnant sheep (McColgan, Buxton and Blewett, 1988). The importance of oocysts as a source of infection for sheep, has been supported by studies showing an association with infection and contamination of feed or grazing land with sporulated oocysts (Plant, Richardson and Moyle, 1974; Faull, Clarkson and Winter, 1986) and also work showing an association with cats on farms and prevalence of *T. gondii* in sheep (Skjerve *et al.* 1998). Further studies looking at development of specific antibodies in sheep, as an indicator of exposure to *T. gondii*, have shown that there is an increase in seroprevalence associated with age. This indicates that there is extensive environmental contamination with *T. gondii* oocysts and that most infections in sheep occur following exposure to the parasite after birth (Waldeland, 1977; Blewett, 1983; Lunden, Nasholm and Uggla, 1994). Recent studies have indicated that there is widespread environmental contamination with *T. gondii* oocysts (Dabritz *et al.* 2007).

Congenital transmission

Primary infection during pregnancy. As sheep are not carnivores, consumption of tissues infected with *T. gondii* bradyzoites contained within tissue cysts is not considered to be a route of transmission in these animals. The only other route of transmission is vertical from mother to foetus during pregnancy (Buxton and Rodger, 2008). The stage of pregnancy when transplacental transmission of *T. gondii* takes place is important in determining the clinical outcome. If infection occurs early in gestation, when the foetal immune system is relatively immature, foetal death is likely to occur. Infection at mid-gestation can result in birth of a stillborn or weak lamb which may have an accompanying small mummified foetus, whereas infection in later gestation may result in birth of a live, clinically normal, but infected lamb (Buxton, 1990). The birth of clinically normal but infected lambs usually occurs as a result of a primary infection in the third trimester of pregnancy, although it is also possible that transplacental transmission may occur as a result of recrudescence of an endogenous infection (Trees and Williams, 2005).

Recrudescence of an endogenous infection. While recrudescence of a persistent endogenous infection is a very common route of congenital infection with the closely related parasite *Neospora caninum* in cattle (Innes *et al.* 2005; Williams *et al.* 2009 – this special issue), it is not thought to be a significant route of transmission for *T. gondii* infection in sheep (Dubey and Beattie, 1988; Buxton and Rodger, 2008).

However, recent studies, (Duncanson *et al.* 2001; Williams *et al.* 2005, Morley *et al.* 2005, 2008), have suggested that endogenous transplacental transmission of *T. gondii* may be more important than was previously thought and that this route of transmission may be an important cause of lamb mortality. Data reported by Williams *et al.* (2005) stated that 53·7% of lambs in their test flocks had evidence of congenital *T. gondii* infection at birth with 46% of live lambs and 90% of dead lambs being positive for *T. gondii* by PCR analysis. Further work that followed ewes over successive pregnancies reported a frequency of 21% for successive *T. gondii* positive abortions, suggesting that complete protective immunity has not been acquired following a previous infection (Morley *et al.* 2008).

These studies are very interesting although difficult to interpret with confidence as they rely heavily on PCR-based techniques and the methodology is not validated using supporting pathology, serological evidence or isolation of live parasites to show that the live lambs in the study were indeed congenitally infected with *T. gondii* as a result of endogenous transmission. In addition, the authors did not rule out other causes of abortion due to different pathogens on their study farm. These studies also raise the importance of the language we use to describe vertical transmission. To aid our understanding of this area it is important to define the difference between endogenous transplacental transmission and exogenous transplacental transmission as described by Trees and Williams (2005).

A recent relevant study in this area using a full range of different diagnostic techniques found that, in contrast to the studies described above, there was no significant transmission from persistently infected sheep to their offspring (Rodger *et al.* 2006). In this study, a group of sheep previously infected with

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T. gondii and a group of naïve control sheep were mated and followed through pregnancy to lambing. A full *post-mortem* was conducted on any dead lambs and placentas were examined using histopathological techniques and by *T. gondii*-specific PCR for evidence of infection. In addition, pre-colostral blood samples were collected from all the lambs to look for antibodies to *T. gondii*. The presence of *T. gondii* antibodies in pre-colostral blood samples is a good indicator that congenital transmission has occurred. The results showed that the group of 31 *T. gondii*-infected sheep gave birth to 43 live healthy lambs and 6 stillborn lambs. There was no evidence of *T. gondii* infection in any of the tissues examined using *T. gondii*-specific PCR and histopathological techniques, in addition all the foetal fluid samples from the dead lambs and the pre-colostral serum samples from the live lambs were sero-negative with the exception of one set of twin lambs born to one of the infected ewes. All the *T. gondii*-negative ewes produced live *T. gondii*-negative lambs. Therefore this more complete study using a variety of scientific techniques to confirm transmission and infection showed that the rate of congenital transmission from persistently infected ewes was very infrequent, around 3·2% (Rodger *et al*. 2006).

Data from previous published papers in this area also agree with the results of Rodger *et al*. that although endogenous transplacental transmission of *T. gondii* may occur it is very infrequent and does not pose a significant clinical risk. A study by Watson and Beverley in the UK showed that in a group of 26 ewes that were infected in a previous pregnancy with *T. gondii* and then retained and followed through a subsequent pregnancy gave birth to 24 live uninfected lambs with only one ewe aborting a pair of twins (Watson and Beverley, 1971). A larger study in Australia examined what proportion of lambs may be infected as a result of a re-activation of a previous infection and found that a group of 135 persistently infected ewes produced 178 live lambs all being pre-colostral antibody negative with evidence of only one of the ewes having an infected placenta. In addition, there was no evidence of *T. gondii* being isolated from their tissues using mouse inoculation. Therefore they concluded that congenital transmission of *T. gondii* from ewes persistently infected with the parasite is very infrequent (Munday, 1972).

OOCYSTS ARE A SIGNIFICANT SOURCE OF INFECTION FOR SHEEP AND PRIMARY INFECTION DURING PREGNANCY POSES A SIGNIFICANT CLINICAL RISK

Taken together the studies reported by Rodger *et al*. (2006), Watson and Beverley (1971) and Munday (1972) all conclude that, while endogenous transplacental transmission may occur in persistently infected ewes, it is infrequent and is unlikely to pose a significant clinical risk. Their studies used a variety of diagnostic methods including serology, histopathology, detection of *T. gondii*-specific DNA and isolation of live parasites from tissues using a mouse inoculation bioassay. These data, alongside the evidence of increased seroprevalence with age and increased seroprevalence of animals maintained in outdoor environments, suggest that the most significant route of *T. gondii* transmission for sheep is via *T. gondii* oocysts in the environment. Another very important factor in determining risk of clinical disease is the development of protective immunity in the host.

THE DISEASE

The disease toxoplasmosis in sheep usually manifests following a primary infection of a pregnant ewe, resulting in placental invasion and transplacental infection of the foetus. Typical clinical signs are abortion and production of stillborn or weak lambs often along with a small, mummified foetus. Placental tissue from infected ewes may also show characteristic gross white spot lesions which are visible to the naked eye and are areas of necrosis in the tissue which will limit its effective function in supporting the pregnancy (Buxton, 1990). Sheep may become infected from the consumption of sporulated oocysts. Once ingested, the oocysts excyst in the small intestine, releasing sporozoites which quickly invade and multiply within the cells of the gut differentiating into tachyzoites. The tachyzoites can be found multiplying within mesenteric lymph node cells by day 4 following infection (Dubey, 1984). Toxoplasma is an obligate intracellular parasite and uses a process of endodyogeny to multiply within host cells (Ferguson, 2009). The parasitized cells then rupture releasing tachyzoites that invade other host cells. A common clinical sign is an elevated temperature in the animal which is observed co-incident with the appearance of tachyzoites in the mesenteric lymph nodes and the fever may last for a further week, during which time tachyzoites may be detected in the circulation (Dubey and Sharma, 1980; Wastling, Nicoll and Buxton, 1993). In the pregnant ewe, the tachyzoites find their way to the placenta where they invade and multiply within the maternal caruncular septa in the placentome and from there they invade the adjoining foetal trophoblast cells (Buxton and Finlayson, 1986). The immune system of the sheep is modulated during pregnancy in order to prevent rejection of the semi-allogeneic foetus. This manifests as a damping down of the pro-inflammatory immune responses such as interleukin 2 (IL-2) and interferon gamma (IFNγ) at the maternal-foetal interface (Innes and Vermeulen, 2006; Entrican and Wheelhouse, 2006). This change in the immune environment of the placenta provides a favourable location for the parasite to establish and multiply.

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Stage of gestation

A significant factor in determining the severity of disease is the stage of gestation when infection occurs. The earlier in gestation that infection occurs the more severe the consequences for the foetus (Watson and Beverley, 1971; Hartley and Moyle, 1974; Blewett, Miller and Buxton, 1982). Infection of the placenta and foetus early in gestation is usually fatal for the foetus. Infection at mid-gestation may result in birth of a stillborn or weak lamb usually accompanied by a small mummified foetus. Infection in late gestation may result in birth of a clinically normal but infected lamb (Buxton and Rodger, 2008). Another important factor in determining severity of the disease is the ability of the foetus to mount an immune response against the parasite which increases along with foetal gestational age (Innes and Vermeulen, 2006). Infection of ewes prior to pregnancy did not result in disease during pregnancy and lambs were born healthy and uninfected (Hartley, 1961). Ewes infected prior to mating and then experimentally challenged during pregnancy produced over 90% live uninfected lambs, compared to a group of naïve ewes receiving the same challenge during pregnancy that produced only 28% live lambs (McColgan *et al*. 1988).

These experiments showed that disease mainly manifests in ewes that are infected for the first time during pregnancy and that disease severity is linked to gestational age of the foetus at the time of transplacental transmission. In addition, ewes infected in one pregnancy are unlikely to have infected lambs in subsequent pregnancies suggesting that it may be possible to immunise ewes prior to mating. Experimental infection of ewes prior to mating did indeed confer protection against a challenge administered during pregnancy suggesting that it may be possible to control the disease using vaccination.

HOST IMMUNE RESPONSES

Following initial infection of the ewe both the innate and adaptive immune responses work together to limit multiplication of the fast replicating tachyzoite stage (Innes and Vermeulen, 2006). Toxoplasma parasites are able to stimulate innate immune mechanisms directly upon entry into the host. This direct stimulation of macrophages results in production of interleukin 12 (IL-12) which directly stimulates natural killer (NK) cells to produce interferon gamma (IFNγ) (Gazzinelli *et al*. 1993). Interferon gamma is known to be important in inhibiting the intracellular multiplication of *T. gondii* and in addition will create the appropriate cytokine microenvironment for the priming of the adaptive immune response towards a Th-1 type pro-inflammatory immune response (Innes and Vermeulen, 2006). Studies in sheep using the technique of chronic lymphatic cannulation allowed the study of the development of a primary immune response to *T. gondii* in real time (Innes and Wastling, 1995). Interestingly, the first immune response detected within 48 hours of *T. gondii* inoculation was IFNγ (Innes *et al*. 1995*a*). Previous *in vitro* studies had shown that ovine IFNγ could significantly inhibit the intracellular multiplication of *T. gondii* tachyzoites within sheep cells (Oura *et al*. 1993). Four to five days after inoculation of *T. gondii*, lymphoblast cells responding to the infection were detected in the efferent lymph. Using phenotypic markers specific for ovine lymphocytes the experiment showed that initially the predominant lymphoblast population comprised CD4+ T cells (Innes *et al*. 1995*b*). At day 9–10 post-inoculation, the lymphoblast population peaked, when around 50% of the cells leaving the node were blasting cells (Buxton *et al*. 1994). At peak lymphoblast output the predominant population switched to CD8+ T cells and *in vitro* studies showed that these activated CD8+ T cells were able to inhibit multiplication of *T. gondii* infected autologous ovine target cells directly (Innes *et al*. 1995*b*). Following the peak lymphoblast response, the parasite was no longer detected in the efferent lymph indicating that the immune system of the sheep had successfully controlled the infection (Innes and Wastling, 1995). Specific antibodies to *T. gondii* were detected from day 10–12 after inoculation indicating that cell mediated immune responses involving, CD4+, CD8+ T cells and IFNγ are important in protective immunity and recovery from a primary infection and specific antibody may play more of a role in protection against a secondary infection (Innes and Vermeulen, 2006).

These results, taken together with the work published by Watson and Beverley (1971), showing that ewes infected in one pregnancy are unlikely to have infected lambs in subsequent pregnancies, and research published by McColgan and colleagues (1988), showing that experimental infection of ewes prior to mating did indeed confer protection against a challenge administered during pregnancy, suggest that it may be possible to control the disease using vaccination.

VACCINE DEVELOPMENT

Researchers in New Zealand observed that an isolate of *T. gondii* they had recovered from an aborted lamb and maintained in the laboratory by repeated passage in mice, had lost the ability to differentiate into either bradyzoites or oocysts and had become an incomplete strain comprising the tachyzoite stage (O'Connell, Wilkins and Te Punga, 1988). This isolate, known as the S48 strain would induce a temporary infection in sheep but did not persist in the animals as it had lost the ability to differentiate into bradyzoites (Buxton, 1993). Inoculation of this

isolate into sheep prior to mating was found to afford protection against *T. gondii* induced abortion during pregnancy (O'Connell *et al*. 1988; Wilkins, O'Connell and Te Punga, 1988; Buxton, 1993). As *T. gondii* is an obligate intracellular pathogen, this live attenuated parasite was able to induce protective cell mediated immune responses as it would undergo limited multiplication within host cells, thus allowing the appropriate processing and presentation of antigens to the immune system (Buxton and Innes, 1995). When sheep previously vaccinated with the S48 isolate are then challenged with live parasites, the parasite is prevented from spreading by the host immune response (Buxton *et al*. 1994). Extrapolating these experimental findings to the situation in the field would mean that when a vaccinated pregnant sheep ingests oocysts on pasture, the sporozoites released in the gut would invade host cells and enter the mesenteric lymph nodes where the primed immune system would significantly limit the spread of the parasite. The action of the primed immune response would prevent the parasite from reaching the placenta and causing disease of the foetus.

Duration of immunity

Additional experiments examined the duration of immunity and showed that sheep vaccinated with the S48 strain of *T. gondii* and kept indoors away from any external challenge for 18 months were still immune to a challenge administered after 18 months which caused abortions in a naïve control group of sheep (Buxton *et al*. 1993). Therefore immunisation with a live, incomplete strain of *T. gondii* was highly effective in protecting sheep against congenital toxoplasmsosis and would provide long lasting immunity from a single shot.

First commercial vaccine protecting against congenital toxoplasmosis

The vaccine, Toxovax® produced by Intervet Schering-Plough Animal Health is available for sale in New Zealand, the UK and several other countries in Europe. It is advised to be administered prior to mating and has a meat and milk withdrawal period of six weeks following vaccination. As it is a live vaccine there are problems associated with a short shelf-life and care should be taken by those administering it as it is a zoonotic pathogen. The Toxovax® vaccine is the only commercially available vaccine against *T. gondii*, but it would not be suitable for use in people as it is live and there may be safety concerns (Innes and Vermeulen, 2006). Studies using killed vaccine preparations have not been successful in inducing protective immunity in sheep (Buxton *et al*. 1989), this may be because killed antigens are not inducing the relevant cell mediated immune responses known to be important in protective immunity (Innes and Vermeulen, 2006).

The S48 vaccine is highly effective in helping to prevent congenital toxoplasmosis in sheep by stimulating protective cell mediated immune responses prior to pregnancy. In addition, the immunity induced by the vaccine is long lasting in the absence of further challenge.

CONCLUDING REMARKS

Ovine toxoplasmosis is a significant cause of foetal loss in sheep worldwide. The weight of evidence from published scientific literature supports the theory that sporulated oocysts spread by cats into the environment are the most significant source of infection for sheep. Sheep show an increased likelihood of being seropositive to the parasite associated with age suggesting that postnatal acquisition of infection is an important route of transmission in sheep. While endogenous transplacental transmission may occur in persistently infected ewes it is infrequent and is unlikely to pose a significant clinical risk.

The work of Hide *et al*. (2009 – in this special issue) makes a contradictory case that endogenous transplacental transmission in persistently infected ewes is a significant route of *T. gondii* transmission and that this route does pose a significant clinical risk. While we know that endogenous transplacental transmission is very important in the epidemiology of bovine neosporosis, it is not thought to be as important in ovine toxoplasmosis. An issue with the studies by Hide *et al*. is that they rely heavily on PCR-based evidence to draw their conclusions and they do not verify their data using other techniques such as serology, histopathogy and isolation of live parasites. In addition, they do not investigate the role of other abortifacient agents that may be present on their study farms. Therefore, while their results do pose interesting questions that certainly warrant further research, the authors do need to further validate their findings using other standard and readily available diagnostic techniques to prove that endogenous transplacental transmission is taking place with the significant frequency they describe and show that that this represents a major clinical risk.

Our research and that of others has shown that sheep develop cell-mediated and humoral immune responses following infection with *T. gondii* that will protect against disease in a subsequent pregnancy. This protective immunity involves both the innate and adaptive immune responses in particular, CD4+, CD8+ T cells and IFNγ. As a result of sheep being able to mount effective protective immunity following infection, a live attenuated vaccine has been developed that provides effective and long-lasting protection against congenital toxoplasmosis.

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Our understanding of ovine toxoplasmosis is such that we can advise farmers that a major disease risk for sheep involves naïve pregnant animals undergoing a primary infection following consumption of sporulated oocysts present in the environment. As there is known to be widespread environmental contamination with oocysts (Dabritz *et al*. 2007), the best protection for sheep reared outdoors is for them to develop protective immunity against the parasite prior to mating. Vaccination is an effective way to help control ovine toxoplasmosis, along with good management of feed and water to prevent contamination with oocysts spread by cats.

We understand that there are still important areas of research to pursue such as whether some sheep may have a particular genetic susceptibility to *T. gondii*, understanding the immunological mechanisms involved in recrudescence of a persistent infection or whether some strains of the parasite may be more virulent than others. However, we feel it is important to validate new findings before advising farmers to adopt new practices in the management of their sheep flocks.

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