Ovine abortion: an overview

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Chlamydophila abortus and Toxoplasma gondii are the two leading causes of ovine abortion in Ireland, resulting in substantial losses to the sheep industry and posing a threat to human health. Surveillance and rapid reliable diagnosis of C. abortus or T. gondii infection is critical so that control measures may be put in place.
This article provides an overview of the transmissions and pathogenesis of these infections, and examines the emerging diagnostic techniques currently being developed

Several infectious agents have been implicated in the aetiology of ovine abortion which is an important cause of loss to the Irish sheep industry. The two most common infectious causes are *Chlamydophila abortus* and *Toxoplasma gondii*, both of which are obligate intracellular pathogens that cause severe placentitis leading to abortion in late gestation.

In the UK, Enzootic Abortion of Ewes (EAE) caused by *C. abortus*, is the most commonly diagnosed cause of ovine abortion and the costs to the farming industry have been estimated at £15-20 million (€16-22 million) per annum (Wood 1992), with a similar level of loss due to Toxoplasmosis likely. EAE is a notifiable disease in Ireland, where its incidence has increased dramatically in recent years (Markey *et al.* 1996). The rate of abortion in Irish ewes due to *C. abortus* and *T. gondii* is not known, but the annual reports of the Irish Regional Veterinary Laboratories reveal that EAE was diagnosed in 22% and 6% of ovine abortion submissions in 2006 and 2007, respectively and that Toxoplasmosis was diagnosed in 18% and 34% of ovine abortion submissions in 2006 and 2007 respectively (**Table 1**). The cost of toxoplasmosis to the Irish sheep industry has not been estimated and losses associated with *T. gondii* are harder to determine because, as well as causing abortion during the later stages of gestation, *T. gondii* can also cause foetal loss at earlier stages of pregnancy (Sargison 2003). The best estimate of the cost of toxoplasmosis to the UK sheep industry based on lost output (abortions/stillbirths), input costs, and the cost of control measures was £11 million (Bennet and Ijpelaar 2003).

Table 1: Figures for the diagnosis of ovine abortion from the RVL surveillance reports 2006 and 2007

Aetiology	2007 (%)	2006 (%)
Toxoplasma gondii	33.8	18.4
Chlamydophila abortus	5.8	22.4
Other bacterial	6.5	3.2
Diagnosis not reached	43	Not stated
Total number of submissions	240	250

As well as posing a threat to the viability of the national flock, both *C. abortus* and *T. gondii* are zoonotic pathogens that carry a substantial risk to human health. Infection with *C. abortus* is usually due to exposure to infected birth fluids and membranes of sheep or goats (Jorgensen 1997). Most of the cases of human infection with *C. abortus* involve pregnant women, as the infection affects both the mother and the unborn child, which often does not survive, leaving the mother in need of intense hospital care (Meijer *et al.* 2004; Jorgensen 1997). However, infection of non-pregnant individuals is usually asymptomatic or associated with mild flu-like illness (Jorgensen 1997). Exposure to *T. gondii* infected tissues or fluids at lambing also poses a risk to human health, and there is currently no vaccine to prevent Toxoplasmosis in humans (Dubey 2008). However, the majority of cases of human toxoplasmosis are acquired either by consumption of undercooked meats that contain encysted Toxoplasma bradyzoites, or ingestion of food or water contaminated with sporulated *T. gondii* oocysts from the faeces of cats. Typically, toxoplasmosis causes a mild flu-like illness or no illness and after the first few weeks the parasite rarely causes symptoms in healthy adults. Infection with *T. gondii* during pregnancy may result in foetal infections with potentially severe consequences such as encephalitis or death *in utero*. In immune-compromised patients, toxoplasmosis can be fatal. Control measures for EAE and Toxoplasmosis are available, but their application is only economically justifiable if the diagnosis has been rapidly and reliably confirmed.

CHLAMYDOPHILA ABORTUS

Chlamydophila abortus, formerly the ovine subtype of *Chlamydia psittaci*, is a gramme negative obligate intracellular bacterium which exists in an extracellular infectious form termed the Elementary Body (EB) and in a non-infectious intracellular form termed the Reticulate Body (RB; **Figure 1**). Between hosts, *C. abortus* exists as the biologically inactive EB, which is fairly resistant to

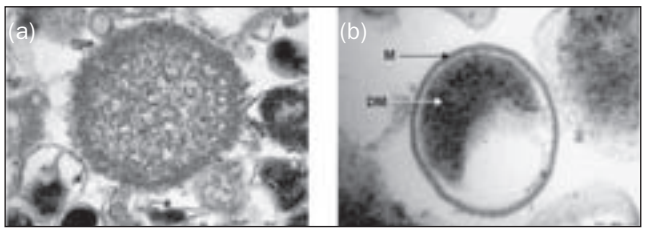

Figure 1: Transmission Electron Microscopy of purified (a) Reticulate Body (scale bar, 500 nm) and (b) Elementary Body (scale bar, 200 nm). EB are characterised by a centrally located dense mass (DM) and an outer membrane and cytoplasmic membranes (M). Photo: Patricia Marques.

environmental stresses: it can survive low environmental temperatures for weeks, and for years at less than 70ºC. The life cycle of *C. abortus* involves adhesion of the EB to the host cell outer membrane whereby it is engulfed into the cell and forms an inclusion. Within the inclusion, the EB's develop into the metabolically active RB form which undergo several cycles of proliferation, and following approximately 24-36 hours, revert back to the infectious EB form and are released upon rupture of the cell. The EB's are then able to infect new cells either in the same host or in a new host (Aitken 2000).

In sheep, *C. abortus* infection is transmitted via the oral-nasal route (Jones and Anderson 1988). Infected ewes shed large amounts of *C. abortus* in the placenta and uterine fluids at lambing, and this may be ingested by other ewes or lambs resulting in infection. Placentitis is the major typical gross pathological component of chlamydial abortion (Aitken 2000). The bacterium targets the placenta, causing tissue damage, inflammation and the resultant abortion (Kerr *et al.* 2005), and infection is associated with severe and extensive pathological changes in the foetal membranes (Stamp *et al.* 1950). Previous studies of placentitis in experimentally infected ewes have shown that chlamydial inclusions first appear in the trophoblast cells of the placentomes (Novilla and Jensen 1970; Buxton *et al.* 1990). Consequently, the chorionic surface is lost and the underlying chorioallantois undergoes severe inflammatory changes resulting in local necrosis. Infection spreads within the proximate placental tissues and, as it progresses, chlamydial inclusions may appear in the endometrial epithelium surrounding the placentomes causing an intense focal endometritis (Novilla and Jensen 1970; Buxton *et al.* 1990). Regardless of the time of infection, pathological changes are not detected in the placenta until after 90 days of gestation, and abortion does not occur until the last two or three weeks of gestation (Buxton *et al.* 1990). A characteristic feature of *C. abortus* infection is that

ewes that abort following infection do not abort again in subsequent years, in spite of evidence that the infection persists in the uterus following abortion (Stamp *et al.* 1950; Littlejohn 1950; Papp and Shewen 1996). In a recent study of the maternal immune response to *C. abortus* Sammin *et al.* (2005) demonstrated that ewes which have previously aborted following *C. abortus* infection are, to a certain degree, protected from the reoccurrence of abortion. Immunity in ewes that had previously aborted due to *C. abortus* was sufficient to reduce the acute phase response of ewes to rechallenge and to prevent abortion. However, this did not prevent the bacteria reaching the placenta. The pattern of placentitis was similar to that of the initial infection, but development may have been slower and the authors concluded that the primary outcome of maternal immunity is to prevent, or limit the numbers of *C. abortus* reaching the susceptible trophoblast (Sammin *et al.* 2005).

Late gestation abortions are usually the first signs of *C. abortus* infection within the flock, although infected ewes may have a vulval discharge and show behavioural changes up to 48 hours before abortion. The expelled foetal membranes may display varying degrees of necrosis, thickening, oedema and suppurative exudates, although the lambs are usually well developed and quite fresh, indicating foetal death has been fairly recent (**Figure 2**). Infected ewes may also give birth to live lambs which are generally weak and do not survive, although some lambs are reared successfully. In multiple births it is not uncommon for a mixture of live and dead lambs to be born (Aitken 2000).

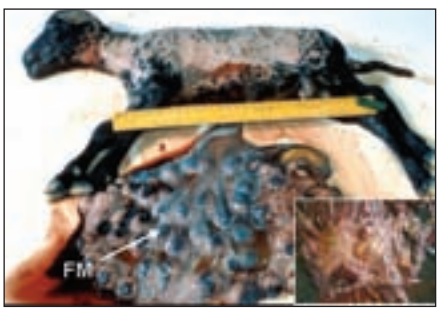

Figure 2: Lamb aborted following C. *abortus* infection. Note the oedema and thickening of the foetal membranes (FM) of the attached placenta. Inset shows placentitis due to C. *abortus*; there is leathery thickening of the intercotyledonary foetal membranes and suppurative exudate is present on cotyledons and the intercotyledonary membranes. Photo: Donal Sammin.

An initial outbreak of *C. abortus* within a flock can lead to 30% or more of ewes aborting or giving birth to stillborn or weak lambs the following year, with the incidence remaining at around 5-10% in further lambing seasons if animals are not treated (Aitken 2000). If ewes become infected earlier in gestation, the outcome is abortion or weak ill-thrifty lambs that often die (Aitken *et al.* 1990). Infection acquired by naive ewes and female lambs in the weeks peripartum through exposure to infected foetal membranes in the lambing shed can result in the development of a latent infection, where the disease is not manifested until the next lambing season (Aitken *et al.* 1990, O'Donovan *et al.* 2008). Latent infection is the mechanism by which *C. abortus* becomes enzootic in flocks.

TOXOPLASMA GONDII

T. gondii is a common infection of warm-blooded animals. A protozoan, the parasite's definitive hosts are wild and domesticated felines: in Ireland, the significant definitive host is the domestic cat. *T. gondii* has a wide range of intermediate hosts including domesticated species and terrestrial and marine mammals. Infection *in utero* with *T. gondii* can result in foetal death in humans, goats and sheep (**Figure 3**).

Oocysts of *T. gondii* are only formed in cats and are shed in the faeces. Host species, including cats, can become infected with *T. gondii* by consuming feed or water contaminated with sporulated oocysts, tissues from infected animals or by transplacental transmission. Following ingestion, sporozoites or bradyzoites escape their tissue cyst or oocyst wall, penetrate the intestine of the host, become tachyzoites and multiply locally before disseminating to other tissues via the blood or lymph. After a few multiplication cycles, the tachyzoites give rise to bradyzoites within a variety of tissues. Experimentally domestic cats are highly efficient at shedding oocysts. Dubey and Frenkel (1972) observed millions of oocysts in the faeces of cats after feeding of one *T. gondii* infected mouse. Cats shed oocysts for one to three weeks and immuno-compromised cats may be more likely to become re-infected. The prevalence of natural *T. gondii* infection and oocyst shedding in cats is unknown. In a review of published studies, up to 80% of cats worldwide had *T. gondii* antibodies, though seroprevalence varies widely between and within countries (Jones and Dubey 2009).

Figure 3: Lambs aborted following a confirmed Toxoplasma abortion. The fresher (white) lamb had a Toxoplasma SAT titre of 1/128. The co-twin exhibits signs of mummification. Foetal mummification is commonly seen in outbreaks of toxoplasma abortion, but is not pathognomonic for toxoplasmosis. Inset shows a partially autolysed foetal membrane and cotyledon.

Sheep and goats can acquire *T. gondii* infection via the ingestion of sporulated oocysts in contaminated feed, pasture or water. This is exogenous transmission. *T. gondii* infection acquired during pregnancy can result in tachyzoites infecting and multiplying within the placenta and foetus. One study reported high levels of congenital transmission of *T.gondii* in a commercial sheep flock (Duncanson *et al.* 2001). They reported detection of *T. gondii* DNA by PCR in the placenta of 42% (37 of 70) of successful pregnancies and in the tissues of lambs in 94% (18 of 19) of cases where one or more lambs were aborted or still-born. These results suggest that as well as resulting in abortion, congenital transmission of *T. gondii* to live born lambs can be a significant route of transmission.

The consequences of infection of a ewe with *T. gondii* is dependent on the timing of infection with respect to stage of gestation and the number of oocysts ingested. Infection can result in barren ewes or foetal death leading to abortion or still-birth. Several studies have challenged pregnant ewes at different stages of pregnancy. Challenge with *T. gondii* oocysts before 50 days gestation can cause embryonic death and resorption causing the affected ewes to appear barren (Hartley 1961). Infection in mid-pregnancy (70 to 90 days gestation) can result in abortion or delivery of stillborn lambs, whereas challenge in later pregnancy (110 days or later) usually results in the birth of clinically normal lambs, though they may be congenitally infected (Duncanson *et al.* 2001).

The immune system of sheep is modified in pregnancy to prevent rejection of the foetus. Pregnancy suppresses the expression of certain cytokines (interleukin-2, interferon-gamma and tumour necrosis factor-alpha) that are required to mount a competent immune response to certain infectious agents including *T. gondii*. These circumstances render the placenta and foetus vulnerable in the face of primary maternal infection with *T. gondii* infection. Circulating tachyzoites in maternal circulation are therefore able to colonise the caruncular septa of the placentome and from there invade the trophoblast cells of the cotyledons and then the foetus (Buxton *et al.*2007).

In ovine abortion cases due to *T. gondii*, the main gross lesion seen is the presence of white spots in the cotyledonary tissue of the foetal membranes (**Figure 3**). This lesion is due to dystrophic mineralisation of foci of necrosis caused by tachyzoite multiplication. Microscopically inflammation is not a feature of this placental lesion (Buxton *et al.* 2007).

In aborted foeti, microscopic lesions are most frequently seen in the brain, though lesions in myocardium and skeletal muscle are also, but less commonly seen. Visualisation of intracellular tachyzoite pseudocysts or bradyzoite cysts associated with these lesions is more the exception than the rule, but where they are present, the diagnosis can be confirmed. Alternatively, if confirmation is required, immunohistochemistry can be employed to confirm the presence of *T. gondii* antigen within the brain lesions.

DIAGNOSIS

The definitive method of diagnosis of *C. abortus* or *T. gondii* infection is isolation of the pathogen from infected tissues. However, this method is labour intensive and time-consuming and relies upon the submission of fresh material to the diagnostic laboratory.

Complement-fixing (CF) serum antibodies can be detected in infected ewes following EAE (Stamp *et al.* 1952). Seroconversion occurs following the initial exposure to *C. abortus* but persistent high titre antibody only develops as a consequence of the bacteraemia that accompanies placentitis (Storz *et al.* 1968; Wilsmore *et al.* 1984, Wilsmore *et al.* 1990). The complement fixation, which is based on chlamydial lipopolysaccharide (LPS) is the most common diagnostic test, however, results can be misinterpreted due to the cross-reactivity with *Chlamydophila pecorum*, which also infects sheep (Wilson *et al.* 2009). Serological diagnosis can also be done using a variety of enzyme-linked immunosorbent assays (ELISAs) based on chlamydial antigens, LPS and outer membrane proteins (Anderson *et al.* 1995; Griffiths *et al.* 1996; Kaltenboeck *et al.* 1997; Salti-Montesanto *et al.* 1997; Longbottom *et al.* 2001). Furthermore, Markey *et al.* (1993) developed an indirect immunofluorescence assay in an attempt to improve on the detection of *C. abortus* antibodies. The serological diagnostic tests available have varying degrees of sensitivity and specificity. Furthermore, no distinction can be made between vaccinated and naturally infected sheep, thus confirmation of a negative status in vaccinated animal cannot be determined by serology (Gerber *et al.* 2007).

Serological diagnosis can help confirm *T. gondii* infection in ewes that have aborted due to toxoplasmosis. The majority of ewes remain seropositive for at least six months following infection. Serological sampling of six or more aborted ewes is recommended. Currently a serum agglutination test is commonly used in diagnostic laboratories and titres of 1/512 or greater suggests recent infection. There are several commercial *T. gondii* antibody ELISA.

Inconclusive or non-diagnostic ovine abortion specimens are frequently reported by diagnostic veterinary laboratories despite detailed investigation and testing. The advent of a validated PCR test for *C. abortus* or *T. gondii* in ovine tissues could help identify those foeti infected with that are 'missed' due to the absence of typical histological lesions, or the absence of foetal membranes from the submission.

Molecular techniques have been emerging as a reliable method of *C. abortus* and *T. gondii* diagnosis. Because of the close genetic relationship between *Chlamydophila psittaci* and *C. abortus*, direct species identification is difficult. However, a real-time PCR assay has been designed which allows rapid, sensitive and specific detection of *C. abortus,* thus offering a new test suitable for routine diagnostic purposes (Pantchev *et al.* 2008). Ideally, placental material should be submitted for PCR diagnostics. Although the placenta is the main target organ of *C. abortus* and *T. gondii,* these pathogens have been identified in foetal organs, which would also be suitable as specimens for diagnosis by PCR. A candidate duplex PCR test that would enable lab analysis for both *C. abortus* and *T. gondii* in the same sample is currently being developed in the School of Agriculture, Food Science and Veterinary Medicine at UCD funded by the Department of Agriculture. If validation is completed satisfactorily, the test could be available for diagnostic purposes within the next year.

CONTROL MEASURES

Control measures for ovine abortion have not changed significantly in the last decade. The best way to maintain a healthy flock is to maintain a closed flock.

The control of toxoplasmosis in sheep is based on prevention of infection and, in flocks where toxoplasmosis has been confirmed, vaccination. Prevention amounts to limiting the access of cats to feed bins, baled hay, straw and pasture where possible: the aim being to limit the exposure of sheep to feed and environments contaminated with oocysts. Ewes that have aborted due to toxoplasmosis are considered immune and can be retained for breeding the following year. A commercial live vaccine is licensed for use in Ireland (Toxovax, Intervet). The vaccine contains tachyzoites of the S48 strain of *T. gondii,* and is known to provide protection for at least two lambing seasons. Timing of vaccination is important and must not occur less than three weeks prior to mating. The vaccine is live, and operators should observe the special precautions on the datasheet as self-injection can result in disease in humans.

Management of an outbreak of EAE includes rapid removal of aborting ewes and the products of abortion from the lambing shed combined with cleaning and disinfection to minimise infection of naive ewes at lambing. Antimicrobial treatment of ewes with long-acting oxytetracycline in the face of an outbreak is commonly practiced, but the benefit of such treatment is hard to quantify. Currently available vaccines against EAE are based on inactivated whole organisms (Mydiavac) and a live attenuated mutant strain (Enzovax, Intervet) which helps prevent enzootic abortion in uninfected ('clean') ewes and also reduces the spread of the disease within the flock. However, only Enzovax is licensed for use in the Republic of Ireland. Enzovax can be used at the same time as Toxovax, but the vaccines cannot be mixed and must be injected at separate sites. Vaccination is not commonly practiced as a method of infection control in many countries. Instead, farms aim to join accredited flock schemes, such as the Premium Health Scheme for Sheep in the UK, through diagnostic testing of flocks. Farms designated as being EAE-free attract premium prices in the market place. These schemes give farmers the opportunity to buy in clean ewe replacements which can then be vaccinated in an effort to prevent the introduction of EAE or to eliminate EAE from their flocks.

SUMMARY

New diagnostic measures are currently being developed for the diagnosis of the two leading causes of ovine abortion: *C. abortus* and *T. gondii*. These pathogens impact greatly on animal and human health, as well as having a substantial economic effect on the sheep industry.

If diagnosis can be rapidly and reliably confirmed, then control measures can be implemented to reduce the impact of these infections in future years and protect the economic viability of the flock.

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