Immune responses against *Toxoplasma* and *Sarcocystis* infections in ruminants: diagnosis and prospects for vaccination

A. UGGLA * and D. BUXTON **

Summary: Toxoplasma gondii and a variety of Sarcocystis species are common cyst-forming coccidian parasites of domestic ruminants worldwide. *T. gondii* infection is a major cause of ovine and caprine abortion and perinatal mortality, but does not appear commonly to cause significant disease in other ruminants. Sarcocystis infections are probably most important in growing ruminants in which they can cause subclinical anaemia and reduced weight gain.

The life cycles, pathogenesis and current knowledge of immunity to these protozoa, in ruminants, are reviewed. Although clinical signs may suggest a diagnosis, confirmation requires the use of specific immunological methods such as serology and immunohistochemistry. These are discussed and the necessity for using characterised antigens and antisera is emphasised. Whereas live *Toxoplasma* “vaccines” may be effective in sheep and goats, the eventual preparation of a killed vaccine capable of inducing long-term protection is of the utmost importance. To achieve this goal it will be necessary to identify the specific antigens capable of stimulating protective immunity and to conduct further research on both the pathogenesis of toxoplasmosis and fundamental aspects of the immune response of pregnant ruminants to *T. gondii*.

KEYWORDS: Abortion - Buffalo - Cattle - Control - Deer - Goat - Immunisation - Immunodiagnosis - Immunohistochemistry - Pathogenesis - Sarcocystosis - Serology - Sheep - Toxoplasmosis.

INTRODUCTION

The cyst-forming coccidian parasites *Toxoplasma gondii* and *Sarcocystis* species are intracellular protozoan organisms occurring in domestic animals and man throughout the world. The parasites are classified in the phylum Apicomplexa, class Sporozoasida, order Eucoccidiorida and family Sarcocystidae (65) and have life cycles in which carnivores (predator) are the definitive hosts and herbivores (prey) the intermediate hosts. The importance of congenital infection of women, as well as of domestic animals such as sheep, goats and pigs, by *T. gondii* has been recognised for some time. However, the clinical and subclinical effects of *Sarcocystis* infections

---

* Department of Cattle and Sheep Diseases, Faculty of Veterinary Medicine, Swedish University of Agricultural Sciences, P.O. Box 7019, S-750 07 Uppsala, Sweden.
** Animal Diseases Research Association, Moredun Institute, 408 Gilmerton Road, Edinburgh EH17 7JH, Scotland, United Kingdom.
in domestic animals have only been elucidated relatively recently, as has the zoonotic potential of two *Sarcocystis* species which require cattle and pigs as intermediate hosts.

Due to the microscopic size and intracellular localisation of the proliferative forms of these protozoan parasites and the relative difficulty in their laboratory cultivation, the development of immunodiagnostic tools such as serology and immunohistochemistry are essential in the demonstration of infection. The aim of this review is to give an up-to-date appraisal of the diagnostic methods available and to consider both the need and the prospects for vaccines against infection by these protozoa.

**TOXOPLASMA**

*Toxoplasma gondii* was first described in 1908, and in the 1950's it was recognised as a significant cause of abortion in sheep by Hartley and co-workers (49) and subsequently also in goats (77). To date however, it does not appear to cause significant clinical disease in cattle or other ruminants under field conditions. Transmission has long been known to occur both transplacentally and following ingestion of tissue cysts (carnivorism), but it was not until around 1970 that the complete life cycle, involving cats as definitive hosts, was elucidated by Hutchison and others (55). This represented a breakthrough in the understanding of the epidemiology and pathogenesis of *Toxoplasma* infection in herbivorous animals. Reviews covering different aspects of *T. gondii* and toxoplasmosis have been published by Remington and Krahenbuhl (94), Dubey and Beattie (31) and Jackson and Hutchison (55).

**Life cycle and epidemiology**

The life cycle of *T. gondii* can be divided into two parts: an asexual cycle in intermediate hosts and a sexual cycle, confined to the enteroepithelial cells of the feline definitive host, which results in the production of oocysts (Fig. 1). In the asexual cycle there are two developmental stages, the rapidly multiplying tachyzoite (endozoite, trophozoite) and the slowly multiplying bradyzoite (cystozoite). Tachyzoites actively penetrate host cells, particularly cells of the reticuloendothelial system. Each tachyzoite then becomes surrounded by a parasitophorous vacuole and multiplies until the cell ruptures and the organisms are released locally and into the bloodstream to parasitise further cells. This process continues until the host dies, or more usually it develops immunity and a chronic infection is established. Extracellular parasites are then eliminated, intracellular multiplication slows and tissue cysts containing bradyzoites develop. These microscopic cysts are found most frequently in brain and skeletal muscle and represent the quiescent stage of the parasite within the host. In some species, such as sheep, goats, pigs and man, cysts may remain for the rest of the life of the individual, while in cattle, water buffalo and deer the host may eventually become clear of infection (31, 55).

The sexual cycle is initiated when a non-immune cat ingests infective *T. gondii* either in the form of tissue cysts, tachyzoites or oocysts, but the most important sources of feline infection are birds and rodents chronically infected with tissue cysts. Bradyzoites are released from tissue cysts by gastro-intestinal proteolytic enzymes to penetrate the epithelial cells of the cat's small intestine, where subsequent sexual
reproduction results in the formation of oocysts, which are released into the gut lumen to be excreted in the faeces. The prepatent period after ingestion of tissue cysts is 3-6 days and vast numbers of oocysts may be shed continuously in the faeces from this time until about 14 days after infection. Following sporulation in the open over the next one to five days, depending upon aeration and temperature, the oocysts become infective. They are very resistant and may remain infective in the environment for a year or more. Sporulated oocysts are $11 \times 13 \, \mu m$ in diameter and contain four sporozoites in each of two sporocysts (31, 55).

The major source of *Toxoplasma* infection for susceptible herbivores is feed and water contaminated with sporulated oocysts (8). Fifty grams of infected cat faeces may contain as many as ten million oocysts (31). If, in a hypothetical situation, this was evenly dispersed throughout ten tonnes of concentrated animal feed, then each kilogram could contain between five and twenty-five sheep-infective doses (71). Similarly, pasture spread with manure and bedding from farm buildings where cats live may be a source of infection for sheep (33).

**Pathogenesis**

Abortions and neonatal mortality occur when sheep and goats suffer a primary infection during pregnancy (31). Within four days of ingestion of sporulated oocysts by susceptible pregnant sheep, organisms can be found in the mesenteric lymph nodes, where they multiply causing marked lymph node enlargement, sometimes with focal
necrosis (26). Around the fifth day tachyzoites are released to cause a parasitaemia, which may last until the twelfth day (23, 93). Coinciding with the parasitaemia the ewe displays a febrile response which can exceed 41°C around day six or seven (17). The cessation of the parasitaemia coincides with the onset of an effective maternal immune response. With the exception of the gravid uterus, the infection then persists as bradyzoites within tissue cysts.

In pregnant ruminants the gravid uterus is an “immunologically privileged” site (105). On the uterine side maternal immunological responses are locally suppressed, while the ability of the fetus, with its placenta, to recognise and respond to a pathogen such as *T. gondii* commences during the first half of gestation and develops for the remainder of pregnancy, so that fetal lambs become immunocompetent by the time they are born (15). During maternal parasitaemia, tachyzoites are able to parasitise the caruncular septa, invade the adjacent trophoblast cells of the fetal villi, and from there the rest of the fetus, between five to ten days after the onset of parasitaemia (15). Thus, the key to preventing uterine infection is to prevent a parasitaemia occurring during pregnancy. If infection does establish, however, the outcome is influenced by the stage of gestation.

Infection in early gestation is rapidly fatal (8) due to the absence of a fetal immune response to inhibit parasite multiplication (15). Subsequent resorption of the fetus can be mistaken for infertility (61). Infection in mid-gestation may also be fatal and give rise to a mummified fetus often alongside a sibling which survives to be born live, weakly or dead. Infection in late pregnancy will normally cause fetal infection but because of fetal immunocompetence by this time the lamb may be born live, infected and immune (8). When infection in the placentome is initiated, parasite multiplication causes multiple foci of placental necrosis which expand throughout the remainder of gestation until abortion or birth (15, 50), when they may be visible to the naked eye.

In the fetal brain both primary and secondary lesions develop (14, 50). Glial foci, surrounding a necrotic and sometimes mineralised centre, often associated with a mild lymphoid meningitis, represent a fetal immune response following direct damage by local parasite multiplication. Focal leukomalacia is also common (50) and is thought to be due to fetal anoxia at late gestation caused by advanced focal necrosis in the placentome preventing sufficient oxygen transfer from mother to fetus (14). Focal inflammatory lesions and associated diffuse lymphoid infiltrates may also develop in fetal liver, lung and heart and less frequently in kidneys and skeletal muscle (14). The fetal immune responses are discussed later.

**Immunity**

The development of immunity to a microbe generally involves the closely interrelated humoral and cellular arms of the immune system. Humoral immunity operates when antibodies (immunoglobulins, Ig), which can recognise and bind to specific microbial antigen(s), are secreted by B lymphocytes. In the initial stages of *T. gondii* infection IgM is secreted, and this is followed by a switch to IgG secretion during the second week of infection (94).

Antibody and complement will lyse tachyzoites *in vitro* via the classical pathway (41) and, *in vivo*, antibody attachment to extracellular organisms will make them more liable to phagocytosis (94). However, antibody alone does not produce adequate immunity to *T. gondii*, as passive transfer of specific antibody to normal mice provides
only a degree of protection against challenge with a lethal strain of the parasite (59, 73), but antibody transfer to athymic mice does not (66). Thus, while the role of antibody appears to be limited, it may still play an important part in the immune response of the host (22) and its presence is both an indication of exposure to infection and a vital diagnostic aid.

The susceptibility of athymic mice to *T. gondii* infection (12, 66) illustrates the necessity of a thymus and hence T lymphocytes, in immunity to the parasite. Phagocytic cells such as macrophages are also essential (74, 94), and protection afforded in this way is defined as cell-mediated immunity (CMI). CMI responses are initiated after antigen is taken up by antigen-presenting cells and processed and "presented" as small peptides on their surface to lymphocytes. These are in turn regulated by helper and suppressor T lymphocytes which control production and secretion of lymphokines, such as the interferons and interleukins, which not only modify the activity of other lymphocytes but macrophages as well.

Macrophages are normally able to phagocytose and kill microbes, but in the case of toxoplasmosis, tachyzoites are able actively to invade macrophages and multiply (74, 81, 96). Multiplication of tachyzoites within macrophages can be inhibited by both oxygen-dependent and oxygen-independent mechanisms including a mechanism activated by gamma interferon (IFN-gamma) (69, 74, 79). The organisms appear to require the amino acid tryptophan (80, 91) and *in vivo* experiments confirm the crucial role of IFN-gamma in inhibition of parasite proliferation (102). Thus, while other aspects of CMI, including natural killer cells (51, 98), may play a role in controlling infection, it is macrophages in immune animals, possibly activated by the cytokines derived from T cells responding to the antigens of *T. gondii*, that probably represent the principal system of host protection.

**Ruminant immunity**

Although there is a vast literature on serology of ruminant toxoplasmosis, our understanding of ruminant immunity to the parasite is limited. The fact that *Toxoplasma* causes abortion and lifelong chronic infection in sheep and goats, but not in cattle and deer (31), is an indication of a fundamental, as yet undefined, immune difference between these species. However, in general terms it is likely that ruminant immunity to *T. gondii* will resemble that of other species.

Research has shown that local challenge of non-immune ovine popliteal lymph nodes with *T. gondii* can induce marked enlargement and evidence of both B and T lymphocyte responses. Acute changes included focal necrosis, due to unrestrained tachyzoite multiplication, haemorrhages and a general loss of architecture. More specific immune changes included expansion of medullary sinuses by accumulations of macrophages and lymphocytes, considerable cortical nodular development and enlargement of the paracortex by numerous plasmablasts (13). The cell output in the efferent lymph from nodes challenged in this way included a minority population of plasmablasts, which peaked seven days after challenge, and a larger group of putative T lymphoblasts which peaked at eleven days (70). In addition, live *Toxoplasma* organisms were present for a fortnight after challenge, latterly in the presence of specific antibody (13). Thus T lymphoblasts predominated in the lymph while many B lymphocytes were confined to the node.

Similar challenge of nodes in immune sheep evoked no tissue destruction, but some enlargement due to an increase in cortical follicles and numbers of paracortical plasma cells and lymphoblasts. In efferent lymph the increase in cellular output
occurred sooner, but contained a proportionally greater number of plasmablasts (70). *T. gondii* organisms were virtually absent in lymph. In this respect it is relevant that multiplication of the parasite can be prevented in cultured bovine monocytes by IFN-gamma and other lymphokines (53).

The ovine fetal immune system can respond to *T. gondii* at or soon after 60 days gestation when both humoral and cellular responses can be detected (15). The latter are seen in mesodermal tissues of the placenta where rudimentary mononuclear inflammatory cells accumulate. The humoral response is apparent as an increase in IgM-containing B lymphocytes in the spleen and lymph nodes, which reaches a peak around 20 days after maternal infection. This is followed by a similar increase in IgG-containing B lymphocytes by 30 days. Specific anti-*Toxoplasma* IgM and IgG circulating antibody, detectable in the fetus by 30 days after maternal infection, can be used in the diagnosis of *Toxoplasma* abortion (15). Since infection of pregnant and non-pregnant ewes provokes substantial maternal immunity, a uterine *Toxoplasma* infection will not develop in a future gestation (71). In goats, however, transplacental transfer of infection has been observed during subsequent pregnancies (31).

Although a great deal of research on ruminant immunity remains to be done, these observations are not inconsistent with findings in other species. Where *T. gondii* can stimulate both humoral and cellular immunity, antibody alone is insufficient to kill *Toxoplasma* organisms; macrophages, activated by T lymphocyte-derived lymphokines, play a prominent role in killing.

**SARCOCYSTIS**

*Sarcocystis* species have been known since the nineteenth century as so called Miescher's tubules or sarcosporidia in muscular tissues of rodents and farm animals. In 1972 the coccidian nature of the life cycle, involving a predator-prey relationship similar to that of *T. gondii*, was described for some *Sarcocystis* species by Rommel, Heydorn and Erber (95). Gametogony was also demonstrated *in vitro* by Fayer (34). During the following years the life cycles of most of the *Sarcocystis* species of domestic animals were elucidated. The different species of *Sarcocystis* are presently characterised by their host range, the size of the mature sarcocyst and the structure of the cyst wall (32, 65, 95).

*Sarcocystis* infections are extraordinarily common in domestic animals in all parts of the world. Ruminants may act as intermediate hosts to a variety of species, one of which has man as the definitive host (Table I) (65, 95). Most *Sarcocystis* species are regarded as essentially non-pathogenic, while a few, in particular the canine-borne species, may cause clinical as well as subclinical disease in their respective intermediate hosts. Acute sarcocystosis is however seldom recorded in the field, while the subclinical effects, including anaemia and poor weight gain in young ruminants (76), are probably the most important economic consequences of *Sarcocystis* infection. In the definitive hosts *Sarcocystis* rarely causes any obvious clinical signs, although infection in man, with for example *S. hominis* from raw beef, may cause gastro-intestinal disorders, such as nausea, stomach ache and diarrhoea. For general reviews of *Sarcocystis* and sarcocystosis, see Tadros and Laarman (103) and Dubey et al. (32).
TABLE I
Sarcocystis species in some domestic and semi-domesticated ruminants

<table>
<thead>
<tr>
<th>Intermediate host</th>
<th>Sarcocystis species</th>
<th>Definitive host</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>S. cruzi (bovicanis)</td>
<td>Dog, wolf, coyote, fox</td>
</tr>
<tr>
<td></td>
<td>S. hirsuta (bovifelis)</td>
<td>Cat</td>
</tr>
<tr>
<td></td>
<td>S. hominis (bovihominis)</td>
<td>Man, other primates</td>
</tr>
<tr>
<td>Sheep</td>
<td>S. tenella (ovicanis)</td>
<td>Dog, coyote, fox</td>
</tr>
<tr>
<td></td>
<td>S. arieticanis</td>
<td>Dog</td>
</tr>
<tr>
<td></td>
<td>S. gigantea (ovifelis)</td>
<td>Cat, fox</td>
</tr>
<tr>
<td></td>
<td>S. medusiformis</td>
<td>Cat</td>
</tr>
<tr>
<td>Goat</td>
<td>S. capracanis</td>
<td>Dog, coyote, fox</td>
</tr>
<tr>
<td></td>
<td>S. hircicanis</td>
<td>Dog</td>
</tr>
<tr>
<td></td>
<td>S. moulei</td>
<td>Cat</td>
</tr>
<tr>
<td>Water buffalo</td>
<td>S. levinei</td>
<td>Dog</td>
</tr>
<tr>
<td></td>
<td>S. fusiiformis</td>
<td>Cat</td>
</tr>
<tr>
<td>Yak</td>
<td>S. poephagicanis</td>
<td>Dog</td>
</tr>
<tr>
<td></td>
<td>S. poephagii</td>
<td>Not known</td>
</tr>
<tr>
<td>Reindeer</td>
<td>S. rangi</td>
<td>Fox</td>
</tr>
<tr>
<td></td>
<td>S. tarandivulpes</td>
<td>Fox, racoon, dog</td>
</tr>
<tr>
<td></td>
<td>S. grüneri</td>
<td>Fox, racoon, dog</td>
</tr>
<tr>
<td></td>
<td>S. rangiferi</td>
<td>Not known</td>
</tr>
<tr>
<td></td>
<td>S. tarandi</td>
<td>Not known</td>
</tr>
<tr>
<td></td>
<td>S. hardangeri</td>
<td>Not known</td>
</tr>
<tr>
<td>Red deer/wapiti</td>
<td>S. cervicanis</td>
<td>Dog</td>
</tr>
<tr>
<td></td>
<td>S. sybillensis</td>
<td>Dog</td>
</tr>
<tr>
<td></td>
<td>S. wapiti</td>
<td>Dog, coyote</td>
</tr>
</tbody>
</table>

Life cycle and epidemiology

The different species of Sarcocystis have largely similar obligatory two host life cycles (Fig. 2). Ruminant intermediate hosts become infected by ingestion of oocysts or sporocysts contaminating their feed or water. The sporocysts excyst in the gut and the sporozoites released penetrate the intestinal wall and establish infections throughout the body in endothelial cells of small arteries and capillaries, where two generations of asexual reproduction take place. After entering an endothelial cell, the parasite divides repeatedly to form a meront (schizont) which then lyases to release a large number of organisms (merozoites). Generally after a second merogonous generation the merozoites are able to invade striated muscle cells, and sometimes also cells of the nervous system, where they finally initiate the formation of sarcocysts. The parasites multiply within the sarcocyst which gradually matures and increases in size until it eventually contains vast numbers of slender, crescentic cystozoites (bradyzoites). The sarcocysts can be microscopic (microcysts) or visible to the naked eye (macrocysts). Only mature sarcocysts are infective for the definitive host, and the maturation of microcysts takes 2-3 months, whereas the much larger macrocysts are generally not infective until after more than twelve months. Sarcocysts may persist in the tissues of the host for years, although a proportion may degenerate earlier (32, 103).
ASEXUAL CYCLE

Intermediate host
(herbivore)

CYSTOZOITES
(bradyzoites)
in sarcocysts
(tissue cysts)

MERozoITES
in meronts
(schizonts)

Ingestion

SEXUAL CYCLE

Definitive host
(carnivore)

gut infection

OOCYSTS

SPOROCYSTS
in faeces

Ingestion

FIG. 2
Life cycle of Sarcocystis species

The definitive hosts become infected by ingesting tissues containing sarcocysts of the appropriate species. In the gut the numerous cystozoites are released from the cysts and invade the intestinal mucosa where the parasite undergoes a sexual cycle of development resulting in the formation of oocysts. The prepatent period is generally 7-14 days after which sporulated oocysts (each containing two sporocysts) or, more commonly, free sporocysts (each containing four sporozoites) can be found in the faeces. Sporocyst shedding may continue for several weeks and since the definitive host does not become strongly immune to reinfection, shedding can recur. The sporocysts are in the size range of 7-10 × 11-16 µm, depending on species. They are very resistant and may survive in the environment for many months (32, 103).

Pathogenesis

The canine-borne microcyst Sarcocystis species, such as S. cruzi in cattle, S. tenella in sheep and S. capracaenis in goats, are frequently found to be pathogenic for their intermediate hosts, and the pathogenesis of these infections is largely similar. The severity of disease induced correlates with the number of infective sporocysts ingested, and clinical signs can include fever, anaemia, anorexia, weight loss, death and, in pregnant animals, abortion (32).

The principal pathological changes are induced during the merogonous stages of parasite multiplication in vascular endothelial cells, which are destroyed through the release of merozoites into the circulation (58, 83, 88). First generation meronts are
formed in the endothelium of arterioles in most organs and tissues 1-2 weeks after infection (83). Second generation meronts appear approx. 3-5 weeks after infection, and at this stage intravascular coagulation, perivascular inflammation, necrosis and haemorrhages are present in various tissues throughout the body (30, 83, 88). Fever is associated with the parasitaemic phases, and anaemia develops as a result of haemorrhagic diathesis possibly in combination with an extravascular haemolysis which may be of immunological origin (39). Deaths following large infective doses occur most often during the second merogonous stage (32).

From around six weeks of infection, sarcocysts are formed in muscular tissues and, with some species, in the central nervous system as well (30, 83, 88). During sarcocyst formation local inflammation is common, but when the sarcocysts mature, no adjacent cell reaction is present (30). A proportion of sarcocysts later undergo degeneration (19, 25, 88), during which accumulations of macrophages and lymphohistiocytic cells are found in association with degenerating muscle fibres and parasitic cysts (86, 88). Sarcocyst degeneration can be triggered by homologous reinfection (57, 86).

Eosinophilic myositis of cattle and sheep has been attributed to *Sarcocystis* infection (46), but absolute evidence for an association is yet to be presented.

Fetal death and abortion may result from infection of non-immune pregnant animals with *Sarcocystis* species of canine origin. The exact mechanisms behind *Sarcocystis*-induced abortion are not known, and organisms are rarely observed in placental or fetal tissues of experimentally infected animals (32).

**Immunity**

Little is known of the specific immune processes triggered in *Sarcocystis* infections. It seems likely, however, that mechanisms of immunity to *Sarcocystis* and *Toxoplasma* infections are similar, with CMI being important. It has been suggested that *Sarcocystis*-infected endothelium may function as antigen-presenting cells during the vascular phase of parasite proliferation (100).

Immunity to one species of *Sarcocystis* does not seem to give rise to significant protection against another species (35, 78). However, sheep, goats and cattle surviving a primary *Sarcocystis* infection have been found to be protected against lethal or acute clinical disease after homologous challenge some months later (24, 25, 35, 37, 86). In this case the acquired immunity does not eliminate the parasites, nor does it prevent further establishment of sarcocysts following the reinfection (86). Also, anticoccidial drug treatment given during a primary infection has been shown to prevent clinical disease, but sarcocysts became established and the animals were protected against illness and death following subsequent challenge (35, 64). Long-term natural immunity in *Sarcocystis* infections may therefore be dependent on the persistence of a viable, although quiescent, infection providing constant immunological stimulus to the host. The duration of such immunity is unknown, but it seems probable that in the field it may be maintained by recurrent exposure to infection (35, 37, 86).

As in toxoplasmosis and other protozoan infections, suppression of immune responses to unrelated antigens as well as increased susceptibility to secondary infections has been recorded (25, 44). Depletion of both T and B lymphocytes was evident in lymphoid organs of cattle exposed to lethal doses of parasite (38). However,
in mice given low doses of *S. muris*, marked splenomegaly due to an increase in numbers of T and B lymphocytes and macrophages was seen and the changes were most pronounced twenty days after inoculation (45).

**DIAGNOSIS**

**Clinical signs**

The vast majority of natural *Toxoplasma* and *Sarcocystis* infections in domestic animals are subclinical. Clinical signs, when present, are generally vague and unspecific and may for *T. gondii* include a period of fever, anorexia, respiratory distress and sometimes diarrhoea. Central nervous disorders are rarely recorded (31, 32).

The most obvious and serious clinical manifestations of toxoplasmosis in domestic animals are those seen during infection of pregnant ewes and goats, described above, although the ewe or nanny goat rarely shows any signs of illness during the infection. Reproductive disorders due to toxoplasmosis are not known in cows and other ruminants under field conditions (31, 55).

Clinical features of sarcocystosis in ruminants can include a normocytic and normochromic anaemia, fever, anorexia and weight loss (32). Myositis may be reflected in increased serum concentrations of enzymes such as creatine kinase (57, 92). Subclinical anaemia and hypoalbuminaemia may also be detected (92).

**Pathological findings**

Following *Toxoplasma* abortion in sheep and goats, white foci of necrosis 2 to 3 mm in diameter may be present in placental cotyledons but intercotyledonary membranes will appear normal (5, 50). Aborted fetuses may be fresh, autolysed with bloodstained subcutaneous oedema or mummified. In less autolytic cases the characteristic histological lesions, already described, may be seen. *T. gondii* tissue cysts can be hard to find by histology although they may be isolated from fresh brain (7).

Isolation of *T. gondii* from infected tissues or body fluids can be performed by mouse inoculation (31), although the method is slow and does not distinguish between acute and latent infections. *Toxoplasma* can grow in virtually any mammalian cell line, but cell culture is rarely used for routine isolation of the parasite (31). *Sarcocystis* organisms have also been successfully grown in cell culture (11, 34).

*Sarcocystis* microcysts in tissues are readily recognised by histology with standard stains, but to determine the particular species, electron microscopy may be required. The presence of *Sarcocystis* in muscle can also be established by microscopical demonstration of cystozoites in enzymatically digested tissue (32).

In animals dead from acute sarcocystosis, widespread capillary haemorrhages may be macroscopically visible, giving a mottled appearance to tissue, particularly tongue, heart and skeletal muscle (unpublished observations). Inflammatory lesions may be seen in many tissues. Encephalitis can be marked, with haemorrhages and focal gliosis, occasionally with central necrosis and often associated with protozoal meronts in adjacent capillary endothelium. Heart, tongue and liver may show marked
mononuclear cell infiltrations, whereas inflammation in skeletal muscle, pulmonary interstitium and renal cortical parenchyma is generally milder. Protozoal meronts are most frequently seen in brain, but occasionally also in capillaries of tongue and cardiac muscles and in renal glomeruli (30, 88).

In Sarcocystis-induced abortion, inflammatory lesions in fetal membranes and tissues are often mild and unspecific, and parasites are not regularly demonstrable. However, meronts with associated mononuclear cell infiltrations, haemorrhages and foci of necrosis can be present in the maternal tissues of the placentome (32).

Immunohistochemistry

To overcome problems of visualisation and species identification of protozoan parasites found in histological sections, immunohistochemical staining methods have become increasingly important. Fluorescent antibody techniques used to demonstrate T. gondii or Sarcocystis organisms in smears and histological sections (2, 83) have been replaced by methods involving enzyme labelled antibodies. The latter methods require only an ordinary light microscope; counterstaining aids examination and slides are permanent. Furthermore, as antigens of T. gondii and Sarcocystis spp. appear to be well preserved in formalin-fixed paraffin-embedded tissues, retrospective studies can be carried out (47; unpublished).

With immunoperoxidase staining T. gondii has been demonstrated in ovine material and aborted tissues (13, 56, 109), as has Sarcocystis in sheep (56, 99) and cattle (unpublished). As sera from rabbits immunised with crude Toxoplasma tachyzoite or Sarcocystis cystozoite antigen may cross-react with each other at low serum dilutions (unpublished), their respective specificity should be checked. Polyclonal antisera to ovine or bovine Sarcocystis microcyst cystozoites also cross-react with one another and will also stain cystozoites of other Sarcocystis species (unpublished) as well as second generation merozoites present during clinical sarcocystosis (83, 99; unpublished). The use of specific monoclonal antibodies could eliminate cross-reactions (87).

Techniques such as the polymerase chain reaction (PCR) for identifying and amplifying specific lengths of DNA, have recently been developed for T. gondii (10). PCR and related techniques involving nucleic acid probes still need to be evaluated in veterinary protozoology but may become important diagnostic tools in the future.

Serology

Many serological methods for the diagnosis of toxoplasmosis have been established over the years. The first methods to be developed were the dye test (DT) of Sabin and Feldman and the complement fixation test (CFT) (31). The DT remains the reference test for Toxoplasma antibodies and is well suited for use in sheep (31, 116) and goats (27), but its specificity in bovines, particularly with low DT titres, has been questioned (28, 107, 123).

The indirect fluorescent antibody test (IFAT) for Toxoplasma antibodies correlates well with the DT and has been extensively used for analysis of ovine (75, 108) and caprine (77) as well as bovine sera (20, 106). Since the DT, IFAT and direct agglutination test (DAT) employ intact T. gondii tachyzoites as antigen, they mainly
detect antibodies directed against the cell surface of the parasite, thought to be the 
most specific ones. The DAT has been used for detection of IgG antibodies to T. 
gondii in sheep (29), goats (27), cattle (28) and red deer (122).

Among serological assays employing soluble extracts of disrupted T. gondii 
organisms as antigen are the indirect haemagglutination test (IHAT), the latex 
agglutination test (LAT) and the enzyme-linked immunosorbent assay (ELISA). As 
with other agglutination tests, the IHAT and LAT do not require species-specific 
antisera or conjugates. Since test kits have been commercially available, they have 
become popular for serodiagnosis of ovine toxoplasmosis (29, 54). However, 
antibodies detected by the IHAT and LAT may appear later in infection than those 
detected by tests such as the DT, and their sensitivity may therefore be low, especially 
in acute infections (6, 27). Thus, the correlation between results of the IHAT and 
the DT may be poor in sheep (54), cattle (28, 101) and red deer (122). In experimentally 
inoculated buffalo calves, IHAT antibodies reached only low levels and were difficult 
to detect after a few months (43).

ELISA for T. gondii antibodies has been adapted for use in most domestic animals 
including sheep (17, 67, 90, 112), goats (3) and cattle (107, 112). When using 
Toxoplasma antigen prepared by freeze-thawing and/or ultrasonication of tachyzoites, 
cross-reactions with Sarcocystis antibodies, present during the acute phase of infections 
with S. cruzi in calves (107) and S. tenella in lambs (Uggla and Blewett, unpublished), 
have been observed. To enhance specificity, attempts have been made to increase 
the yield of cell membrane components in the antigen preparation (52). Toxoplasma 
"iscom" particles consisting of detergent-extracted membrane proteins of T. gondii 
tachyzoites have been tested as assay antigen in ELISA and have proved more specific 
than conventionally prepared soluble Toxoplasma antigen (67, 107).

Less commonly used serological assays for Toxoplasma antibodies in animals are 
the radio-immunoassay (RIA) (36), the carbon immunoassay (CIA) (118), the 
diffusion-in-gel ELISA (DIG-ELISA) (108) and the dot-ELISA techniques (89).

In meat-producing animals, serology has been widely used to assess the prevalence 
of Toxoplasma infection, since in most of these animals, with the important exception 
of cattle, there is an adequate correlation between the presence of antibodies to T. 
gondii and demonstrable parasites in tissues of the animal (31, 75, 115, 123). In fact, 
serology has been suggested as a tool to certify meat as being free of Toxoplasma 
(112). Methods to detect T. gondii antigen in homogenised tissue (113) or antibodies 
in tissue extracts of animals (48) have also been investigated. During chronic or latent 
infection, meat-producing animals like sheep often display serum antibody levels 
equivalent to DT or IFAT titres of 1:20 - 1:80 (31, 106, 123).

Serology is also an important tool in the diagnosis of ovine Toxoplasma abortion. 
On a flock basis, high anti-Toxoplasma Ig or IgG levels, equivalent to 1:1000 or more 
with the IFAT or DT, in sera from ewes which have aborted or produced stillbirths 
and which were sampled within a few weeks of the outbreak, are suggestive of T. 
gondii as being the causative agent (31, 117). The presence of specific antibody in 
serum or tissue fluid from stillborn lambs or in precolostral serum from live lambs 
or goat kids indicates uterine infection (15, 27).

Measurement of specific IgM indicates a recent T. gondii infection (6, 90), but 
for routine diagnosis of ovine Toxoplasma abortion, IgM determination is generally 
not needed.
For the serological diagnosis of *Sarcocystis* infections similar assays have been developed. Of these, the IFAT (103, 104, 119) and ELISA (42, 84, 99, 103, 104, 119) have been the most commonly used, but the IHAT (68, 119), CFT (37) and dot-ELISA (104) have also been used. Assay antigens can be readily produced from *Sarcocystis* cystozoites, and since these generally exhibit strong cross-reactivity between *Sarcocystis* species (42, 82, 84, 119), antigens from heterologous species can be employed, although homologous antigens usually give rise to stronger reactions (84, 87). During experimental *T. gondii* infection in mice free of *Sarcocystis* infection, cross-reacting antibodies were recorded with serological assays employing crude *Sarcocystis* antigen (104).

Transient *Sarcocystis* IgM responses generally lasting for a couple of months were evident from around 30 days onwards after experimental infections in sheep (84, 86, 99) and cattle (42). A persistent and often slowly rising IgG response was also found to appear from around 30 days after infection and reached its peak about three months later (19, 42, 84, 86). The presence of specific IgM thus correlates with clinical sarcocystosis in sheep and cattle, and could be used to confirm diagnosis of the latter, whereas detection of specific IgG would be more useful for the diagnosis of latent or chronic infections.

The apparent lack of antibody response to the first merogonous generation of *Sarcocystis* proliferation may be due to the fact that most tests use cystozoite antigen, which may not detect antibodies directed against the earliest stage of the parasite. In support of this suggestion, marked differences have been detected between the antigens of first generation merozoites on the one hand and second generation merozoites and cystozoites on the other (83), although common antigens are also present (11, 82). Thus, for detection of early humoral immune responses, assays employing sporozoite or, if possible, first generation merozoite antigens may be preferable.

Assays for detection of circulating *Toxoplasma* antigen and immune complexes have been developed (111, 114), but to date have not been applied to infection in farm animals. *Sarcocystis* antigenaemia has been demonstrated in acutely infected pigs (82).

**Detection of cellular immunity**

As discussed above, CMI is important in *Toxoplasma* and *Sarcocystis* infections. The delayed type hypersensitivity (DTH) or skin test enables a crude assessment of a CMI response to a specific antigen deposited intradermally. However, *Toxoplasma* skin tests (40) have not been applied routinely in veterinary diagnostics. The same is true for the *Toxoplasma* lymphocyte transformation (blastogenesis) test (63) which measures the ability of lymphocytes to recognise *T. gondii* antigen in *vitro*. Both DTH and lymphocyte transformation can be detected in chronic *Toxoplasma* infections in man and mouse, often months after the onset of infection (63, 94).

Lymphocyte transformation reactions have been studied also in *Sarcocystis* infections of cattle and sheep, but findings appeared inconclusive (38, 42, 86). However, in diagnosis of *Sarcocystis* infection, measurement of specific cellular immunity is probably of limited practical value.

**VACCINATION**

Attempts at immunising pigs against clinical sarcocystosis using antigenic preparations derived from homologous *Sarcocystis* cystozoites did not induce
protective immunity (85). However, since the immunising effects of low-dose live sporozoite inoculations seem adequate for induction of protection against clinical disease (32), and since the requirement for an immunisation scheme for sarcocystosis is not yet established, at present there seems to be no great impetus for the development of a Sarcocystis vaccine. In contrast, an effective vaccine against Toxoplasma abortion for use in sheep and goats would be highly desirable.

Many experiments on the immunogenicity of different antigenic preparations, as well as attenuated strains or mutants of T. gondii, have been performed in mice (1, 72, 94), but only a few using sheep or goats. Following infection, ewes and most nanny goats develop protective immunity to challenge with T. gondii in subsequent pregnancies (31). A successful vaccine would need to mimic a live infection and so prevent a parasitaemia occurring in a pregnant sheep which ingested sporulated Toxoplasma oocysts. It would therefore have to prevent sporozoites penetrating the intestinal epithelium and/or prevent subsequent multiplication of the tachyzoite stage of the parasite in the mesenteric lymph nodes. However, neither a killed whole Toxoplasma tachyzoite vaccine (4) nor one in Freund’s incomplete adjuvant (120) protected sheep against experimental challenge with T. gondii.

The antigenic structure of T. gondii is quite complex. While most research has investigated tachyzoites there are both antigenic similarities and differences between them, bradyzoites and sporozoites (62). All three forms consist of an outer water-insoluble cell envelope surrounding somatic (cytoplasmic) components. Various workers have identified over twenty tachyzoite antigens (60), but it is generally agreed that there are up to five major antigens on the surface of the tachyzoite membrane, which seem important for inducing immunity (21, 60), as do certain non-membranous components (97) and excreted/secreted antigens (22).

Recently a Toxoplasma membrane ‘iscom’ vaccine (67) was found to elicit significant cellular as well as humoral immune responses and considerable protection against lethal challenge in mice (110). In sheep it induced a substantial antibody response and, following challenge, lamb mortality was 36% after a mean gestation of 141 days compared with 65% after a mean gestation of 132 days in unvaccinated challenged ewes, but the differences were not statistically significant for the group sizes tested (18).

Research with an attenuated strain of T. gondii in New Zealand has been sufficiently encouraging for it to be marketed commercially as a vaccine for sheep and goats in that country (121). Live tachyzoites of this strain are injected into sheep where they induce a short-lived infection. Thus, while sheep are protected they do not appear to be left chronically infected, so avoiding the potential public health hazard of infected meat being eaten. The disadvantages of the vaccine are that it has a very short shelf-life and could be a hazard to those using it or handling meat from animals killed soon after vaccination.

**CONCLUSIONS**

The diagnosis of toxoplasmosis and sarcocystosis is well established, with numerous diverse immunological techniques suitable for many situations. To standardise and further increase their specificity, defined antigens and antisera are required. In the
future, early diagnosis of Toxoplasma infection in sheep and goats with developing placental pathology could allow the employment of chemoprophylactic and chemotherapeutic regimes (9, 16, 17). The detection of parasite DNA by nucleic acid probe techniques also needs to be evaluated. Their specificity and high sensitivity will ensure them an important role in veterinary diagnostics.

A vaccine for sarcocystosis may not currently be thought important, but one to prevent Toxoplasma-induced abortion in sheep and goats is required. The ideal candidate, a stable, killed formulation capable of inducing long-term solid immunity, is not yet available. To reach this goal the specific ovine and caprine immune responses to T. gondii need to be analysed so as to identify those antigens which, when presented in the correct manner, will induce protection.

**RÉPONSE IMMUNITAIRE À LA TOXOPLASMOSSE ET À LA SARCOCYSTOSE CHEZ LES RUMINANTS : DIAGNOSTIC ET PERSPECTIVES DE VACCINATION. – A. Uggla et D. Buxton.**

Résumé: Toxoplasma gondii, et diverses espèces de Sarcocystis, sont des parasites des ruminants domestiques communs et cosmopolites. Ces parasites sont des coccidies qui conduisent à la formation de kystes. L'infection par T. gondii est l'une des principales causes d'avortement et de mortalité périnatale chez les ovin et caprin, mais elle ne semble pas provoquer de maladies graves chez les autres ruminants. Quant à l'infection par Sarcocystis, sa conséquence la plus notable est, probablement, de provoquer chez les jeunes ruminants une anémie discrète et un retard de croissance.

Les auteurs passent en revue les cycles évolutifs, la pathogénie et les connaissances actuelles sur les défenses immunitaires des ruminants vis-à-vis de ces protozoaires. Même si le diagnostic peut parfois être établi sur des bases cliniques, sa confirmation impose le recours à des méthodes immunologiques spécifiques, telles que la sérologie et l'immunocytochimie, qui font l'objet de la discussion. La nécessité d'utiliser des antigènes et des antisérums spécifiques est soulignée. Même si les «vaccins» vivants contre Toxoplasma peuvent être efficaces chez les ovin et caprin, la mise au point de vaccins inactivés, capables d'induire une protection durable, revêt une importance primordiale. Pour atteindre cet objectif, il est nécessaire d'identifier, d'une part, les antigènes spécifiques capables de stimuler les défenses immunitaires, et de développer, d'autre part, les recherches sur la pathogénie de la toxoplasmose, et sur les aspects fondamentaux des réactions immunitaires vis-à-vis de T. gondii chez les femelles gravides.


**RESPUESTA INMUNITARIA A LA TOXOPLASMOSIS Y A LA SARCOCISTOSIS EN LOS RUMINANTES: DIAGNÓSTICO Y PERSPECTIVAS DE VACUNACIÓN. – A. Uggla y D. Buxton.**

Resumen: Toxoplasma gondii y varias especies de Sarcocystis son parásitos de los ruminantes domésticos comunes y de distribución mundial; son coccidios que
generan la formación de quistes. La infección por T. gondii es una de las principales causas de aborto y de mortalidad perinatal en ovinos y caprinos, pero no parece provocar enfermedades graves en los demás rumiantes. La consecuencia de mayor importancia de las infecciones por Sarcocystis consiste probablemente en provocar en los animales jóvenes anemia subclínica y retardos en el crecimiento.

Los autores pasan revista a los ciclos evolutivos, la patogenia y el estado actual de los conocimientos sobre las defensas inmunitarias de los rumiantes respecto de estos protozoarios. Aun cuando se pueda a veces establecer el diagnóstico a partir de bases clínicas, se impone para su confirmación recurrir a métodos inmunológicos específicos, tales como la serología o la inmunohistoquímica, que se exponen. Se subraya la necesidad de utilizar antígenos y antisueros específicos. Si bien las «vacunas» vivas contra Toxoplasma pueden ser eficaces en ovinos y caprinos, es fundamental obtener vacunas inactivadas, que puedan inducir una protección duradera. Para llevar a cabo este objetivo, es necesario identificar, por una parte, los antígenos específicos capaces de estimular las defensas inmunitarias y, por otra parte, desarrollar la investigación sobre la patogenia de la toxoplasmosis y sobre los aspectos fundamentales de las reacciones inmunitarias ante T. gondii en las hembras preñadas.


*  *

REFERENCES


