Toxoplasmosis

Dr. J.H. Vorster, BVSc, MMedVet(Path)
Vetdiagnostix Veterinary Pathology Services
PO Box 13624
Cascades, 3202
Tel no: 033 342 5104
Cell no: 082 820 5030
E-mail: hendri@telkomsa.net

Dr. P.H. Mapham, BVSc (Hon)
Veterinary House Hospital,
339 Prince Alfred Road,
Pietermaritzburg, 3201
Tel no: 033 342 4698
Cell No: 082 771 3227
E-mail: rickm@iafrica.com

Introduction

Toxoplasma gondii is a common protozoan parasite with worldwide distribution and may infect many different mammalian species as well as birds and humans. Although most infections of livestock are subclinical, toxoplasmosis is a major cause of reproductive failure primarily in sheep and also in goats. Chickens and pigs may play an important role in the epidemiology of the disease and a high level of seroprevalence has been reported in animals in South Africa, although clinical diagnosis of the disease is rare. Toxoplasmosis is an important zoonosis well-known for causing abortions and has recently been reported worldwide, particularly in immune compromised people, where toxoplasmic-encephalitis has been a frequent manifestation of the infection.

Epidemiology

Domestic cats are considered the only definitive host and play the most important role in the dissemination of T. gondii oocysts1,2,5,6 to intermediate hosts which harbor infective tissue cysts5. Shedding of oocysts in large numbers (reported to be up to 100 million) may be seen for two to three weeks following infection6 of cats by ingestion of only a few tissue cysts or bradyzoites, and starting 2-3 days after infection1,2,5,6. Most feline infections are typically subclinical5 and cats usually develop a solid immunity after primary infection and may shed far fewer oocysts upon reinfection1. The seroprevalence for T. gondii in domestic cats, worldwide, is estimated to be 30-40%5.

Sporulated oocysts are fairly resistant in the environment and can survive freezing and drying and remain infectious for more than a year1. Due to their small size they are easily spread by wind and rain1. Intake of oocysts by cats does not seem to result in significant shedding of oocysts by cats1.

Although domestic cats play the most important role in the dissemination of T. gondii oocysts, these have also been reported to be present in faeces of naturally infected jaguar (Panthera onco), ocelots (Felis pardalis), cheetah (Acinonyx jubatus), bobcats (Lynx rufus) and Canadian lynx (Lynx conadensis)1. A sylvatic cycle may exist, with wild felids the definitive hosts and their prey (rodents and birds), as the intermediate hosts. In Southern African there is an
abundance of wild felidae and prey animals and toxoplasmosis has been reported in a Cape hunting
dog and a cheetah, whilst lions, leopards and cheetahs have all tested seropositive\(^1\).

Herbivorous animals become infected from pastures, water and feed contaminated with oocysts
from cats and other felines\(^1,2,6\). Contamination of pastures seems to be of greater importance than
contamination of conserved feed\(^3\) and contamination of water reservoirs and surface water is an
important avenue of transmission. Direct contact with aborted foetuses and necrotic placentas
seem to be of lesser significance as route of infection\(^1\).

Most sheep acquire \textit{T.gondii} infection after birth, and in pregnant ewes it may result in
transplacental invasion and infection of the foetus which may lead to early embryonic death and
resorption, foetal death and mummification, abortion, stillbirth, birth of weak lambs and neonatal
death\(^1,2,6\). Ewes usually only develop disease if infected for the first time during pregnancy and are
unlikely to have infected lambs in subsequent pregnancies. Less than 4% persistently infected sheep
transmit the parasite vertically to the next generation\(^2\).

There seems to be an increasing likelihood of animals becoming seropositive with older age due to
increased post natal exposure to an environment contaminated with oocysts\(^2\) and the
seroprevalence was determined to reach 95% in six year old ewes in some flocks\(^2\). Generally it
seems that most ewes were infected before four years of age, however, one third of old ewes were
found to remain seronegative in some highly endemic flocks\(^5\). Factors associated with \textit{T.gondii}
positive serological results in sheep have been studied and the presence of cats on the farm using
surface water for drinking water, farm size and a history of abortions were identified as risk factors\(^3\).

Chickens are resistant to clinical toxoplasmosis with few reported cases of confirmed toxoplasmosis
in this species\(^3\). However, chickens may be important hosts of \textit{T. gondii} infection as they may be
an efficient source of infection for cats\(^3\). Chickens are considered to be a good indicator of the rate of
environmental (soil) contamination\(^4\) with Dubey reporting prevalence rates reaching 100% in
backyard and organically raised farm chickens\(^3\). The prevalence of viable \textit{T. gondii} in commercial
indoor farmed chickens was low and ingestion of meat from chickens kept under these
circumstances was considered a low risk of transmission to humans\(^3\). While \textit{T.gondii} is rarely
excreted in chicken egg, raw eggs should not be consumed by humans as they may be infected with
\textit{T.gondii}\(^3\).

Infection with \textit{T.gondii} causes mortality in pigs and then mostly in neonatal pigs\(^4\). Most pigs are
infected postnataally after ingestion of oocysts from a contaminated environment, or after ingesting
tissue cysts from infected animals\(^4\) and a few pigs may become infected by transplacental
transmission of the parasite\(^4\). Practices such as raising pigs indoors have greatly reduced \textit{T.gondii}
infection in pigs\(^4\), however, recent trends to organic farming is likely to increase \textit{T. gondii}
infection\(^4\). Feeding of goat whey to pigs was found to be a risk factor for \textit{T.gondii} infection when
organically raised\(^4\). Infected pig meat is a source of \textit{T. gondii} infection for humans and animals in
many countries\(^4\).

Toxoplasmosis is an important zoonosis and humans may become infected following ingestion of
oocyst-contaminated soil and water, from tissue cysts in undercooked meat (sheep, pigs and
poultry), by transplantation, blood transfusion, laboratory accidents, or congenitally\(^5\). Risk factors
identified in some studies were contact with infective oocysts (primary risk factor) found in cat
faeces, contaminated soil and water, gardening and playing in sandboxes. Ingestion of undercooked pork and chicken is a common risk factor for T. gondii exposure and in contrast beef is not believed to be a major player. Inadequate washing of kitchen knives after they have been used to cut a variety of food stuffs such as raw meat, fruits and vegetables, as well as infrequent hand washing were also identified as risk factors. Abortion in pregnant women is a well-known manifestation of disease and encephalitis was the most common focal cerebral lesion in advanced clinical AIDS patients prior to the advent of anti-retroviral drugs.

**Schematic presentation: life cycle of T. gondii**

**Pathogenesis**

Following ingestion of T. gondii parasites by the intermediate host they multiply in the submucosa of the small intestine and the associated lymph nodes and the host may die at this stage due to enteritis and lymphadenitis before any other organs are affected. Parasitaemia develops during the first week, and tachyzoites are then found in lymphocytes, macrophages and granulocytes. Free forms are distributed via the lymph and blood to tissues and organs and in these tissues they actively invade, or are actively phagocytosed by the host cells where they multiply and, in the process, destroy the host cells. Cell-to-cell transmission may occur within infected organs with the net result being the development of small necrotic foci. Tissue necrosis stimulates an inflammatory response in which mononuclear cells are the predominant cell types and in most intermediate hosts...
a protective immunity may develop with the only manifestation of infection being the development of tissue cysts containing bradyzoites.

In susceptible pregnant animals, transplacental infection results in either placentitis, foetal infection or both, with abortion as the consequence. The stage of pregnancy, and therefore also the stage of the development of the foetal immune system, at which ewes become infected determines the severity of infection. The earlier infection takes place in gestation, the more severe the consequences may be. Infection of ewes may lead to early embryonic death and resorption (in early stages of gestation), abortion, foetal death and mummification, stillbirths and with infection at 70-90 days of gestation some foetuses may survive and be presented as weak lambs and or neonatal deaths. Infection of ewes in the last 30 days of gestation may result in subclinical infections of the newborn lambs.

**Immune response**

Infection in sheep stimulates the innate immune mechanisms directly and stimulation of macrophages results in production of interleukin 12 (IL-12). The latter directly stimulates natural killer (NK) cells to produce interferon gamma (IFNγ) and the first immune response detected following experimental *T.gondii* inoculation was IFNγ production within forty eight hours. Four to five days after inoculation lymphoblasts cells were detected in the efferent lymph and initially the predominant lymphoblast population consisted of CD4+T cells. The lymphoblast population peaked at nine to ten days post-inoculation when approximately fifty percent of the cells leaving the node were blast cells and at peak lymphoblast output the predominant population switched to CD8+T cells.

Following this peak lymphoblast response, parasites were no longer detected in the efferent lymph. These findings suggested that the immune system of the sheep had successfully controlled the infection at this stage and specific antibodies to *T.gondii* were detected ten to twelve days after inoculation. IgM antibodies appear before IgG after infection and disappear faster than IgG following recovery.

It therefore seems that the cell mediated immune response involving CD4 + T, CD8 + T cells and IFNγ are important in protective immunity and recovery from a primary infection and that specific antibodies may play more of a role in the protection of sheep against secondary infections.

**Clinical signs**

Systemic toxoplasmosis has been reported in most species of domestic animals and it is most prevalent in young and immunocompromised animals. Clinical signs may vary considerably and will depend on the organs affected with most consistently reported symptoms being fever, lethargy, anorexia, ocular and nasal discharges, and respiratory distress. Neurological signs include incoordination, circling, tremors, opisthotonos, convulsions, and paresis which is often associated with radiculitis and myositis. Clinical toxoplasmosis has been observed in adult goats, pigs and chickens but is considered rare.

In livestock, clinical signs are most commonly encountered after transplacental infection has occurred. Infection of sheep and goats during the early stages of pregnancy may manifest as resorption, abortion or mummification of the foetus. Infection between 50 and 120 days of
gestation may manifest in abortion or the birth of premature and weak lambs or kids, or normal births but the offspring suffering from subclinical disease\(^1\). Apart from the abortions, the affected ewes and usually not show any other clinical signs indicative of infection\(^1\). Clinical toxoplasmosis has, been observed in adult goats, pigs and chickens but is rare\(^1\). It does not seem to be of any significance in cattle and horses\(^1\).

**Pathology**

Macroscopic pathology in systemic toxoplasmosis is characterised by lesions of interstitial pneumonia, focal hepatic necrosis, lymphadenitis, myocarditis, and non-suppurative meningoencephalitis\(^1,7\). Of these pulmonary lesions seems to be the most consistently found, followed by central nervous system lesions\(^1,7\). All the macroscopic lesions observed in the various organs, in most affected species, are morphologically very similar and may vary only in degree\(^1,7\).

Pulmonary lesions may vary from irregular gray foci of necrosis on the pleural surface to hemorrhagic pneumonia with extensive involvement of the ventral regions\(^1,7\). If the liver is carefully examined areas of focal necrosis or irregular mottling and edema of the gall bladder may be seen\(^1\). The spleen and lymph nodes may be enlarged and may appear wet and often red\(^1\). Effusions into the pleural, pericardial, and peritoneal cavities occurs irregularly\(^7\). Pale areas may be seen in the myocardium and skeletal muscle\(^7\) and the pancreas may occasionally be the most severely affected organ, in which case the entire organ may appear haemorrhagic\(^7\). Yellow, small, superficial intestinal ulcers with a hyperemic border have been reported in piglets\(^7\). Large pale areas of necrosis may be present in the renal cortices, mainly in goats and kittens\(^7\).

Aborted lambs and kids usually show no significant macroscopic lesions\(^1,7\). Microscopic lesions and parasites may be seen in some cases and then in the myocardium, lung, liver and brain\(^1,7\). Irregular small to larger foci of leukoencephalomalacia and gliosis may be seen in the white matter of the brain in up to ninety percent of cases\(^1,7\).

**Focal areas of necrosis, ranging from microscopic to macroscopic, in the cotyledons of the placenta of sheep and goats are considered pathognomonic lesions of toxoplasmosis**\(^1,7\). These lesions of necrosis may be seen in approximately half of the confirmed cases of toxoplasmosis and may vary from sparse to dense, and may present in any plane of the cotyledon\(^1,7\) and may be confluent\(^1\). The cotyledon may be bright to dark red in contrast to a normal deep purple colour\(^7\) and scattered amongst the foetal villi characteristic lesions are seen as white flecks or multiple white, chalky nodules up to 2mm in diameter\(^1,7\). The villi may be oedematous with focal necrosis and desquamation of the trophoblastic epithelium\(^1,7\). The inter-cotyledonary areas are usually unaffected or show oedema only\(^1,7\). Other lesions consist of foci of caseous necrosis involving the foetal and maternal villi which may be mineralized\(^1,7\).

Cotyledons may not all be affected to the same degree and close inspection may be required to detect such lesions\(^1\). A practical way to expose the deeper situated lesions, which may easily be overlooked, is to wash the cotyledons with saline solution\(^1\).

Placental lesions have also been described in toxoplasmosis in cats and pigs as numerous pale foci of necrosis in the allantochorion of the cats, and multiple foci of necrosis of the allantochorion associated with numerous tachyzoites in pigs\(^1\).
Figure 1:
Aborted foetus - no significant macroscopic seen in unopened foetus.

Figure 2:
Placenta - note the distinct and numerous variably sized to coalescing white foci of necrosis restricted to the cotyledons. Mild oedema of the intercotyledonary.

**Diagnosis**

A diagnosis of toxoplasmosis cannot be based solely on the presenting clinical signs\(^1\) and has to be confirmed by biological, serological or histological methods and in most cases by a combination of these\(^1\). All foetuses should therefore be routinely subjected to a comprehensive diagnostic work up to exclude all the other possible causes of abortions. A rapid and presumptive diagnosis at post mortem may be possible following microscopic examination of impression smears of lesions stained with Giemsa stains\(^1\).
Serological procedures available for the detection of humoral antibodies include the indirect haemagglutination test (IHT), indirect fluorescent antibody test (IFT), direct agglutination test, latex agglutination tests, enzyme – linked immunoabsorbent assay (ELISA), and immunoabsorbent agglutination assay test (IAAT)\(^1\).

Demonstration of antibodies to \textit{T. gondii} in a single serum sample will only confirm that an animal has been infected at some time in the past and paired serum sample two to four weeks after the first is required to demonstrate the four - fold increase in antibody titre suggestive of acute infection\(^1\). Some animals may show a high antibody titre which may persist for months after infection and a rising antibody titre may not necessarily be associated with clinical signs\(^5\). Persistent titres following clinical recovery may, in many cases, complicate interpretation of serological test results. Sheep can detect very high levels of \textit{T.gondii} antibodies during acute infection and high IgG antibodies can persist for months or years\(^1\).

Ewes are usually serologically positive at the time of abortion, and therefore a negative test will in general exclude toxoplasmosis as a cause of abortion\(^2\). A positive result may not necessarily be diagnostic as high titres may persist between consecutive breeding seasons\(^1\). Detection of \textit{T.gondii} antibodies in foetal fluids or serum is useful in the diagnosis of ovine abortion and is an aid in confirming congenital infection as maternal antibodies do not cross the placenta\(^1\).

Isolation of \textit{T. gondii} is possible from infected tissues, secretions, excretions and body fluids\(^1\). Such samples may be collected either from live animals or at post mortem and inoculated into laboratory animals or tissue cultures\(^1\). These samples will also be suitable for the polymerase chain reaction (PCR) test to detect DNA of this parasite\(^1\). In both the Spanish surveys discussed below under economic importance, DNA detection was the highest using muscle and brain samples of the foetuses\(^2\). Other organs in which the parasites could be identified included abomasums, livers and spleens\(^2\).

As most \textit{T.gondii} parasites present in typical histologic lesions are degenerate they are difficult to demonstrate in haematoxylin and eosin-stained sections\(^1,2\). Immunohistochemical staining to confirm their presence has proved practical, reliable and affordable.

**Economic importance**

In a recent review article Dubey remarks that the actual loss is difficult to estimate\(^2\). This is ascribed to various possible factors including the sporadic nature of the disease, the small number of aborted lambs submitted for diagnosis, the inadequate examination of those submitted, the collection of unsuitable material for diagnosis, serologic tests which may be non-specific and, lastly, the fact that toxoplasmosis does not produce clinical disease in the ewe (therefore this disease does not alert owners as much as some of the other bacterial and viral infections may do)\(^2\).

In the same review Dubey reported that over a period of 20 years \textit{T.gondii}, or DNA of \textit{T.gondii} was detected in up to 23% of aborted foetuses worldwide for the countries with available data\(^2\). In investigations of aborted foetuses in Spain \textit{T.gondii} DNA was detected in 23.1% of 173 foetuses submitted to a specific diagnostic laboratory\(^2\). \textit{T.gondii} aborted foetuses submitted for examination in this investigation were either in the mid (60%) or last (40%) term of gestation\(^2\).
**T. gondii** was detected by means of PCR in the tissues of aborted sheep in Sardinia, Italy during 1999-2002 in 271 of 2471 (11.1%) foetuses and in 42 of 133 (31.5%) placentas. During the period of 2003 to 2005, **T. gondii** DNA was found in 53 of 292 (18.1%) of foetuses and in 10 of 76 (13.15) placentas. The second survey was conducted in sheep from 98 farms.

In the review by Innes *et al.*, it is reported that toxoplasmosis may be responsible for 1-2% of neonatal losses annually and by estimation be a loss of 0.5 million lambs in the UK and 1.5 million lambs in Europe. In the UK the three most commonly implicated pathogens in ovine abortion are *Chlamydophila abortus* (40%), *T. gondii* (24%) and *Campylobacter sp* (14%). Data of a similar nature is not readily available for Southern Africa where currently Rift Valley Fever may be a leading cause of reproductive failure and while it may be speculated that the predominantly drier environmental conditions may be more restrictive on oocyst survival, wet seasons may change this.

**Prevention and control**

The control of toxoplasmosis in livestock should be aimed at preventing infection in pregnant animals by disrupting the parasites life cycle by excluding the definitive hosts (cats) and the intermediate hosts (rodents) from areas where livestock are fed, pastured, watered or where their feed is stored. Swill fed to pigs should be cooked and cannibalism should be eliminated.

Prophylactic treatment of ewes with monensin has been reported to reduce foetal mortality due to toxoplasmosis from 55.2% to 16.7%. Similar results were obtained by feeding sulfamethazine and pyrimethamine or decoquinate, but lasalocid did not prevent foetal loss due to toxoplasmosis.

A live vaccine is commercially available in some countries abroad for reducing losses from congenital toxoplasmosis. Vaccination three weeks before mating is recommended and it is reported that one subcutaneous injection provides protective immunity for at least eighteen months. The short comings of this live vaccine are a short shelf life and safety concerns for the people handling and administering it. Efforts are continuing to provide an improved product.

**References**


**Multiple choice questions for CPD**

- choose the option that best completes each statement below
1. The most important stage of *T. gondii* transmitted by cats is...
   a. larvae
   b. oocysts
   c. tachyzoites
   d. bradyzoites
   e. eggs

2. High numbers of oocysts are shed in cats faeces...
   a. 1-3 days after infection.
   b. 3-5 days after infection.
   c. 1 week after infection
   d. 2-3 weeks after infection
   e. 1-3 months after infection.

3. Most sheep acquire infection...
   a. prenatal
   b. post natal
   c. vertically from persistently infected animals
   d. during the first half of gestation
   e. during the second half of gestation

4. Chickens are known to be...
   a. highly susceptible to clinical toxoplasmosis
   b. of no consequence in the epidemiology
   c. an efficient source of infection for cats
   d. of no risk in transmitting disease to humans
   e. the most commonly clinically affected species in Southern Africa

5. Systemic toxoplasmosis is...
   a. only seen in humans
   b. common in adult goats, pigs and chickens
   c. characterized by nervous signs
   d. mostly seen in older animals
   e. mostly seen in young and immune compromised animals

6. The most characteristic macroscopic lesions in ovine toxoplasmosis is...
   a. lymphadenitis
   b. myocarditis
   c. necrotic placentitis
   d. meningio encephalitis
   e. pneumonia

7. Following stimulation of the ovine immune system parasites were no longer detected at...
   a. the lymphoblastic response at 2-3 days
   b. the peak of interferon production at 2 days
c. the peak of lymphoblastic response of CD8+ T cells at 9-10 days
d. 9-10 days when IgG appeared
e. 9-10 days when IgM appeared

8. Following on infection of ewes...
   a. IgM antibodies appear before IgE antibodies
   b. IgE antibodies appear before IgM antibodies
   c. IgM and IgE antibodies appear simultaneously
   d. IgM antibodies do not appear at all
   e. IgE antibodies do not appear at all

9. In order to control toxoplasmosis...
   a. swill fed to pigs should not be cooked
   b. definitive hosts should have unlimited access on the property
   c. ewes should be vaccinated after mating
   d. intermediate hosts should be allowed to cannibalise each other
   e. ewes should be treated with antibiotics and/or monensin

10. A risk factor for transmitting disease to humans is...
    a. playing with cats
    b. playing in cat sandboxes
    c. eating well cooked pork meat
    d. eating well cooked beef
    e. proper washing of utensils (eg. knives)