

# REPRODUCTIVE DISORDERS CAUSED BY *Toxoplasma gondii* IN SHEEP

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**ABSTRACT:** *Toxoplasma gondii* is a cosmopolitan, intracellular, obligatory parasite protozoan of importance to public and animal health. In sheep, this species is a causative agent of reproductive disorders. The main sign produced by *T. gondii* infection in this species is abortion, which causes economic losses to sheep ranching. The main purpose of this review is to summarize the abortion outbreaks and reproductive disorders caused by *T. gondii* infection in sheep, also addressing aspects of biology, diagnosis, prevention and control of the parasite.

**KEYWORDS:** Abortion. Pathology. Reproduction. Ruminants. Toxoplasmosis.

## ALTERAÇÕES REPRODUTIVAS CAUSADAS POR *Toxoplasma gondii* EM OVINOS

**RESUMO:** *Toxoplasma gondii* é um parasito protozoário intracelular obrigatório cosmopolita de importância para saúde pública e animal. Na espécie ovina é reconhecido como agente causador de distúrbios reprodutivos. O principal quadro clínico causado pela infecção pelo *Toxoplasma* em ovinos é o abortamento, que causa perdas econômicas. O principal objetivo deste artigo é fazer um levantamento dos surtos de abortamentos e alterações reprodutivas causadas pela infecção por *T. gondii* na espécie ovina, abordando ainda aspectos da biologia, diagnóstico, prevenção e controle do parasita.

**PALAVRAS-CHAVE:** Aborto. Patologia. Reprodução. Ruminantes. Toxoplasmose.

## ALTERACIONES REPRODUCTIVAS CAUSADAS POR *Toxoplasma gondii* EN OVINOS

**RESUMEN:** *Toxoplasma gondii* es un parásito protozoario intracelular obligatorio cosmopolita, de importancia para la salud pública y animal. En la especie ovina es reconocido como agente causador de trastornos reproductivos. El principal síntoma producido por la infección con *Toxoplasma* en ovinos es el aborto, lo que provoca pérdidas económicas. Este artículo tiene como objetivo estudiar los brotes de aborto y alteraciones reproductivas causadas por la infección con *T. gondii* en la especie ovina, abordando también aspectos de la biología, diagnóstico, prevención y control del parásito.

**PALABRAS CLAVE:** Aborto. Patología. Reproducción. Rumiantes. Toxoplasmosis.

### Introduction

*Toxoplasma gondii* is an obligatory intracellular protozoan of importance to public and animal health (CENCI-GOGA et al., 2013; COSENDEY-KEZENLEITE et al., 2014; SCHLÜTER et al., 2014). Chronic toxoplasma infection affects approximately 30% of the human population (SCHLÜTER et al., 2014). In sheep, the prevalence rates range from 1.8% in Northern Tunisia (GHARBI et al., 2013) to 95.7% in Turkey (MOR; ARSLAN, 2007). The high seroprevalence of *T. gondii* in flocks is a risk factor for the transmission of toxoplasmosis to humans through undercooked meat consumption (BOUGHATTAS et al., 2014; SCHLÜTER et al., 2014).

In sheep and pregnant women, toxoplasma infection can lead to a more severe form of the disease that is congenitally transmitted (HISZCZYŃSKA-SAWICKA et al., 2014; SCHLÜTER et al., 2014).

Sheep with primary infection by *T. gondii* during pregnancy can develop reproductive disorders, such as embryonic resorption, fetal death, mummification, stillbirth,

miscarriage or birth-debilitated animals (EDWARDS; DUBEY, 2013; ESTEBAN-REDONDO; INNES, 1997).

Abortion is one of the main signs produced by *T. gondii* infection in sheep and causes economic losses in many countries due to the high prevalence of infection in this species (SILVA et al., 2013a; VAN DEN BROM et al., 2012; VERHELST et al., 2014). The main purpose of this review is to summarize the abortion outbreaks and reproductive disorders that are caused by *T. gondii* infection in sheep, also addressing aspects of the biology, diagnosis, prevention and control of the parasite.

### Development

#### Life cycle and pathogenesis of *Toxoplasma gondii* in sheep

Felines are the definitive hosts of *T. gondii* and shed oocysts in feces. Sheep, as most warm-blooded animals, are intermediate hosts in the toxoplasmosis transmission cycle (ESTEBAN-REDONDO; INNES, 1997). Sheep can acquire infection after oral ingestion of sporulated oocysts in the

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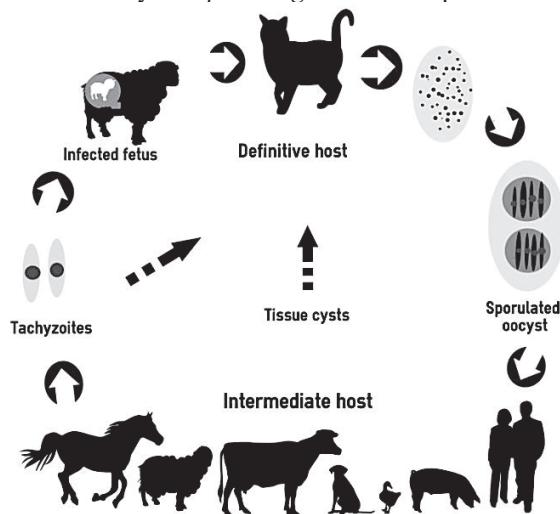
environment (soil, contaminated water or food) (DUBEY, 2010; DUBEY; JONES, 2008).

Toxoplasma infection can also occur congenitally when ewes are infected, primarily after the ingestion of oocysts. *T. gondii* rapidly multiply in the submucosa of the intestine, associated lymph nodes and other organs through the lymph and blood systems (DUBEY, 2010). Upon placental infection with tachyzoites of *T. gondii*, reproductive losses may occur in sheep, such as embryonic resorption, fetal death, mummification, stillbirth and abortion (BUXTON, 1998; EDWARDS; DUBEY, 2013). Abortion in sheep has been reported from 60 to 90 days of gestation, mainly in ewes with primary infection (DUBEY, 2010).

In a recent study of sheep that were experimentally infected with oocysts of *T. gondii* during pregnancy, abortion was reported in the acute phase between seven and 11 days after infection. However, despite the presence of thrombosis and placental infection, the researchers did not detect parasitic DNA. Thus, they suggested that the pathogenesis of abortion in the acute phase may occur as result of vascular damage caused by other mechanisms and not by the parasite replication, as proposed for classic toxoplasmosis (CASTAÑO et al., 2014).

Another form of proven transmission in sheep and goats occurs sexually (LOPES et al., 2013; WANDERLEY et al., 2015). In a study of sheep infected with oocysts and tachyzoites of *T. gondii* after natural mating in seronegative females, there was sexual transmission with subsequent vertical transmission to the lambs (LOPES et al., 2013). After the infection of the intermediate host by *T. gondii*, tissue cysts are formed (most commonly in the brain, liver, heart, muscle) and when ingested by both felines and intermediate hosts, the cycle will continue (DUBEY, 2010). Figure 1 represents the life cycle of *T. gondii*.

**Figure 1:** Life cycle of *Toxoplasma gondii*. Reproductive disorders caused by *Toxoplasma gondii* in sheep



Font: Adapted from Dubey (2010)

#### Pathological disorders resulting from toxoplasma infection in sheep

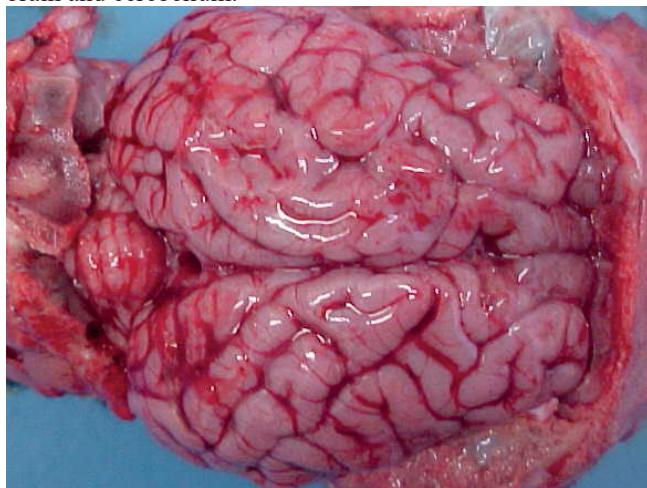
Toxoplasma infection during the pregnancy of female sheep may be associated with necrotic placentitis and

infection of the fetus, leading to abortion (BUXTON, 1998; DE MORAES et al., 2011; EDWARDS; DUBEY, 2013; WEISSMANN, 2003). In animals naturally infected by *T. gondii*, abortions and placentitis have occurred (VAN DEN BROM et al., 2012). In a study in which females were infected with oocysts of *T. gondii* during pregnancy, the observed areas exhibited multifocal coagulative necrosis, marked by sharp vascular congestion, thrombosis and infarction in the cotyledons caruncular villi, leading to abortion (CASTAÑO et al., 2014).

In sheep from the State of Pernambuco, Brazil, there were observed placental abortion lesions characterized by non-suppurative inflammatory infiltrate, necrosis and mineralization, suggesting infection by *T. gondii*. The diagnosis was confirmed by detection of parasites in the placenta by nested PCR (DE MORAES et al., 2011).

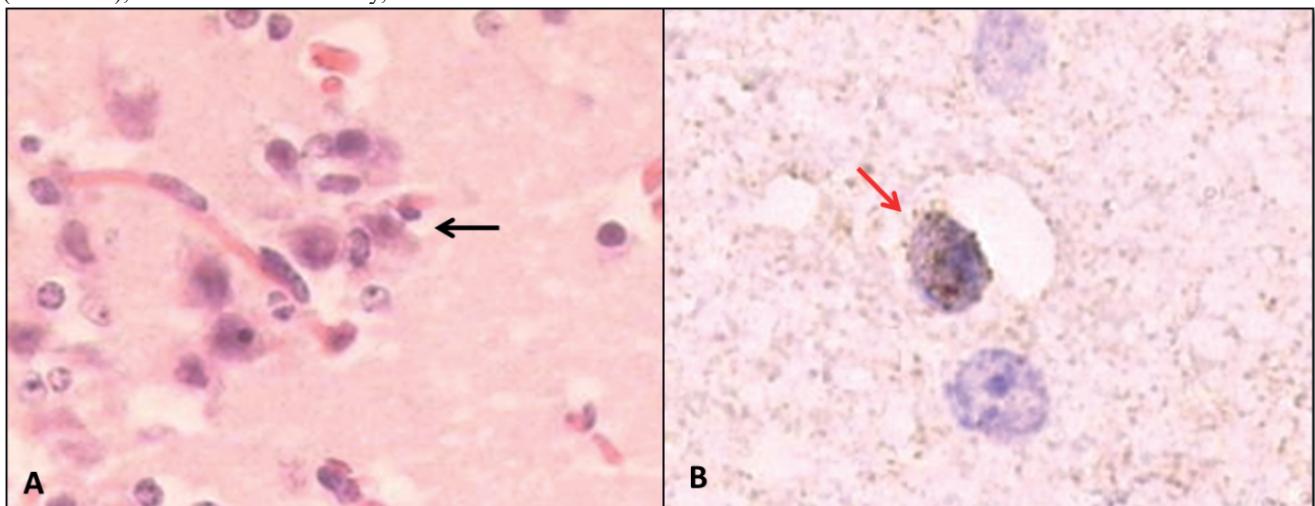
In the State of Rio Grande do Sul, Brazil, abortions due to natural infection by *T. gondii* occurred in the last month of pregnancy, affecting 58.3% of the females. Macroscopic examination of a sheep fetus showed changes as heart pallor, marked congestion in the brain and cerebellum (Figure 2), pale foci in the liver, congestion and marbled appearance in the lungs. Microscopy revealed changes suggestive of toxoplasma encephalitis characterized by severe congestion, areas of malacia with cysts, tachyzoites, microgliosis (Figure 3A), fibrinous microthrombosis and lymphocytic meningitis (DA MOTTA et al., 2008).

**Figure 2:** Brain of an aborted sheep fetus caused by toxoplasmosis infection. Severe congestion and edema in the brain and cerebellum.



Font: Figure courtesy of Dr. Adriana Costa

**Figure 3:** Photomicroscopy of the histopathological analysis of the brain from the same aborted sheep fetus caused by toxoplasmosis infection. A. Toxoplasma encephalitis. Microglial nodules (black arrow). HE 100x. B. Cysts of *Toxoplasma gondii* (red arrow), immunohistochemistry, 400x.



Font: Figure courtesy of Dr. Adriana Costa de Motta, Universidade de Passo Fundo.

Most of the microscopic changes observed in sheep fetuses infected with *T. gondii* are described in brain tissue (ANWAR et al., 2013; DA MOTTA et al., 2008; DUBEY, 2010; O'DONOVAN et al., 2012). Dubey (2010) and O'Donovan et al. (2012), highlight leukoencephalomalacia and gliosis. The areas of malacia are extensive and often observed in the periventricular white matter anterior cerebral region (DUBEY, 2010).

Inflammatory changes characterized by encephalitis, myocarditis and multifocal hepatitis are described in sheep fetuses aborted because toxoplasma infection (DA MOTTA et al., 2008; EDWARDS; DUBEY, 2013; O'DONOVAN et al., 2012; PEREIRA-BUENO et al., 2004a). In addition, tissue cysts and tachyzoites can be observed in the brain tissue of these fetuses, and tachyzoites are often observed adjacently to the cerebral vascular endothelium (ANWAR et al., 2013; DUBEY, 2010; O'DONOVAN et al., 2012). Presence of cysts (Figure 3B) of *T. gondii* in the brain of an aborted sheep fetus was confirmed by immunohistochemistry (IHC) in the State of Rio Grande do Sul (DA MOTTA et al., 2008).

#### Reproductive losses in sheep by *Toxoplasma gondii* infection

Reproductive losses in sheep due to infection by *T. gondii* have occurred in different countries. Table 1 shows abortions and stillbirths described in the past 20 years, according to the literature.

In Italy, parasitic DNA was found in 13% (21/161) of aborted sheep fetuses (CHESSA et al., 2014), and in Netherland, 10.6% (30/282) of aborted sheep fetuses were positive for *T. gondii* using IHC (VAN DEN BROM et al., 2012). Abortion caused by *T. gondii* has also been described in Spain, affecting 5.4% (4/74) of fetuses evaluated by PCR, and were observed in 10.8% (8/74) lesions associated with protozoa (MORENO et al., 2012).

In Brazil, abortions in sheep have occurred because of toxoplasmosis infection (DA MOTTA et al., 2008; DE MORAES et al., 2011), but there are few studies to date. In a study by De Moraes et al. (2011) in the State of Pernambuco Brazil, three miscarriages and two stillbirths 14.3% (5/35) caused by *T. gondii* were diagnosed by nested PCR. The agent was detected in all fetal and placental organs of those five animals, with percentages ranging from 100% in the heart and placenta to 80% in the spleen, brain, liver and lung, and 60% in the cerebellum and spinal cord.

**Table 1:** Reports of miscarriages and stillbirths in sheep due to *Toxoplasma gondii* infection diagnosed by different techniques in the last 20 years.

Location	Nº	IHC <sup>a</sup>	PCR <sup>b</sup>	Fetal Serology	Reference
Brazil	35	No	14.3%	No	(DE MORAES et al., 2011)
	74	No	5.4%	No	(MORENO et al., 2012)
Spain	53	No	16.9%	16.9%	(HURTADO et al., 2001)
	173	No	6.9%	28.3%	(PEREIRA-BUENO et al., 2004b)
Netherland	282	10.6%	No	No	(VAN DEN BROM et al., 2012)
	18	No	66%	No	(HABIBI et al., 2012)
Iran	325	No	No	5.2%	(RAZMI et al., 2010)

	161	No	13%	No	(CHESSA et al., 2014)
Italy	2421	No	11.1%	No	(MASALA et al., 2003)
	292	No	18.1%	No	(MASALA et al., 2007)
Germany	47	10.6%	10.6%	No	(STEUBER, 1995)
EUA	1.784	No	No	10.7%	(KIRKBRIDE, 1993)
	1.064	No	No	20.9%	(DUBEY; KIRKBRIDE, 1990)
Canada	163	19%	No	No	(HAZLETT et al., 2013)
Switzerland	86	19%	No	No	(CHANTON-GREUTMANN et al., 2002)

<sup>a</sup>IHC: Immunohistochemistry,<sup>b</sup>PCR: Polymerase chain reaction, No: not done

### Diagnosis of *T. gondii* abortion in sheep

Diagnosis of *T. gondii* can be performed by serological, biological, molecular and histological tests, and also by immunohistochemistry or a combination of these methods (DA MOTTA et al., 2008; DUBEY, 2010).

*T. gondii* fetal antibodies can be measured using the Modified Agglutination Test (MAT) and Indirect Immunofluorescence Assay (IFA) in sheep fetuses (DUBEY; DESMONTS, 1987). In cases of suspected toxoplasmosis abortion, the placenta and fetus, with special focus on the brain, are sent to the laboratory for the diagnosis of parasitosis (WEISSMANN, 2003). The agent can be isolated from the inoculation placental and fetal tissues in mice (DUBEY, 2010; EDWARDS; DUBEY, 2013; LOPES et al., 2013). Fetal tissue samples (brain, heart, spleen, liver, lung, and spinal cord) and the placenta can be used for molecular diagnosis (DE MORAES et al., 2011).

When associated with pathology and clinical history, IHC allows a definitive diagnosis of toxoplasmosis (DAGLEISH; BENAVIDES; CHIANINI, 2010; DA MOTTA et al., 2008).

According to Silva et al. (2013b), IHC was marked with an anti-*T. gondii* antibody in liver sections, heart and brain in adult sheep infected with *T. gondii*. In these organs, the marking was found around blood vessels, in some cases on the inside thereof, and in parenchymal cells with a rounded shape. In the same study, the heart was the organ that showed a greater significant difference among the three organs compared with high-titer groups. However, it is noteworthy that in addition to the heart and liver, the brain is an elective organ for the detection of *T. gondii* through the IHC technique (DA MOTTA et al., 2008).

### 2.5 Reducing losses: control, prevention and treatment

Monitoring of toxoplasmosis is an important measure because the disease causes losses to farmers and is a zoonosis (LUCIANO et al., 2011). Laboratory diagnosis, when properly conducted, is efficient, making it a measure of control that should be used to confirm the disease in the herd and thereby reduce the economic impact of infection in the sheep industry (DUBEY, 2010).

The presence of cats is a risk factor for toxoplasmosis transmission in sheep (ANDRADE et al., 2013; COSENDEY-KEZENLEITE et al., 2014). The control of these animals was described as a preventive measure of disease in a US study with pigs, revealing that when cats are removed from direct contact with animals, the chances of infection by

*T. gondii* decrease (DUBEY et al., 1995).

The therapeutic control of toxoplasmosis is indicated only for abortion outbreaks in which we performed the laboratory diagnosis. In this case, antibiotic administration is recommended as potentiated sulfonamides (sulfa-trimethoprim) and antiparasitics, such as decoquinate and monensin, the latter of which are especially toxic and unpalatable for sheep and goats and can be incorporated into the diet (BUXTON et al., 1996; WEISSMANN, 2003).

It is noteworthy that more important than treatment is the prevention of the disease. Control measures and the prevention of toxoplasmosis are essential for animals and humans. The health management of animals can avoid losses caused by abortion due to the disease (SILVA et al., 2013a).

Therefore, control of cats through spaying and population control, control of rodents in rural environments, proper storage of raw materials and food, improved hygiene for facilities, tools and personnel are simple preventive measures for the control of toxoplasmosis (PAVLOVIC; IVANOVIC 2005).

In addition, the prevention of ovine toxoplasmosis can also be carried out by herd vaccination before the start of the mating period. The first commercial vaccine that was used was a less cystogenic live vaccine (Toxovax®, Intervet Shering-Plough), used in France, New Zealand and England to reduce neonatal mortality rates (BUXTON; INNES, 1995).

### Conclusions

*T. gondii* infection in sheep causes economic losses associated with abortion; in addition, toxoplasmosis is a zoonotic disease. However, the diagnosis of abortion in field-raised animals is difficult because only a few aborted fetuses are subjected to diagnosis, and they are often autolysed. Moreover, in addition to necropsy, it is necessary to perform sequential tests such as IHC to obtain a definitive diagnosis. Finally, this study highlights the need of adequate prevention and early diagnosis for effective control of *T. gondii* infection in sheep to avoid losses by abortion and prevent the transmission of toxoplasmosis to humans.

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