



# Serological evidence of *Toxoplasma gondii* occurrence in naturally infected sheep in the Teresina microregion, Piauí, Brazil

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## ABSTRACT

Toxoplasmosis is a zoonosis of reproductive nature that is transmitted to humans mainly by ingestion of undercooked meat or drinking oocyte-contaminated water. The slaughter of small ruminants in clandestine slaughterhouses and the presence of companion animals in these locations may be epidemiologically important for the dissemination of the disease. The objective of the present study was to determine toxoplasmosis prevalence by researching anti-*Toxoplasma gondii* antibodies in meat sheep herds from farms in Teresina microregion, Piauí, Brazil. A total of 450 blood samples were collected from sheep of both sexes, belonging to 28 herds from the 14 municipalities that comprise the microregion. The samples were analyzed by the enzyme-linked immunosorbent assay (ELISA), and the presence of anti-*T. gondii* antibodies was observed in 62% (279/450) of the animals in all the municipalities and on at least one farm in each municipality. The high occurrence of positive animals is an indication of reproductive problems in these herds, characterizing a problem for both sheep rearing and public health.

**Keywords:** zoonosis; miscarriage; small ruminant.

## INTRODUCTION

In Brazil, the state of Piauí has the third largest goat herd (1.8 million) and the fifth largest sheep herd (1.6 million), which represents about 17.16% and 8.63% of the national herd, respectively (IBGE, 2019). In spite of the evolution during the last decade, sheep and goat rearing is still negatively influenced by factors including inappropriate health management. Several infectious diseases are still highly prevalent in sheep and goats, especially in the Brazilian Northeast (MESQUITA et al., 2019; RÊGO et al., 2016). Toxoplasmosis is among the main diseases that cause miscarriage in sheep and goats.

Toxoplasmosis is a widely distributed zoonosis that affects humans and warm-blooded animals, caused by the protozoan *Toxoplasma gondii* (AMENDOEIRA et al., 1999). The agent belongs to the filo Apicomplexa, family *Sarcocystidae*, subfamily *Toxoplasmatinae*, genus *Toxoplasma* (FIALHO et al., 2009; GJERDE, 2013). Its life cycle is heteroxenous and all warm-blooded animals (mammals and birds) can take part in the cycle as intermediary hosts, but felines are the definitive hosts (AMARANTE, 2014).

The disease is characterized by a reproductive infectious infirmity that causes serious losses to sheep rearing. Pregnant females infected by *T. gondii* can present embryonic death and reabsorption, fetal death, fetal mummification,

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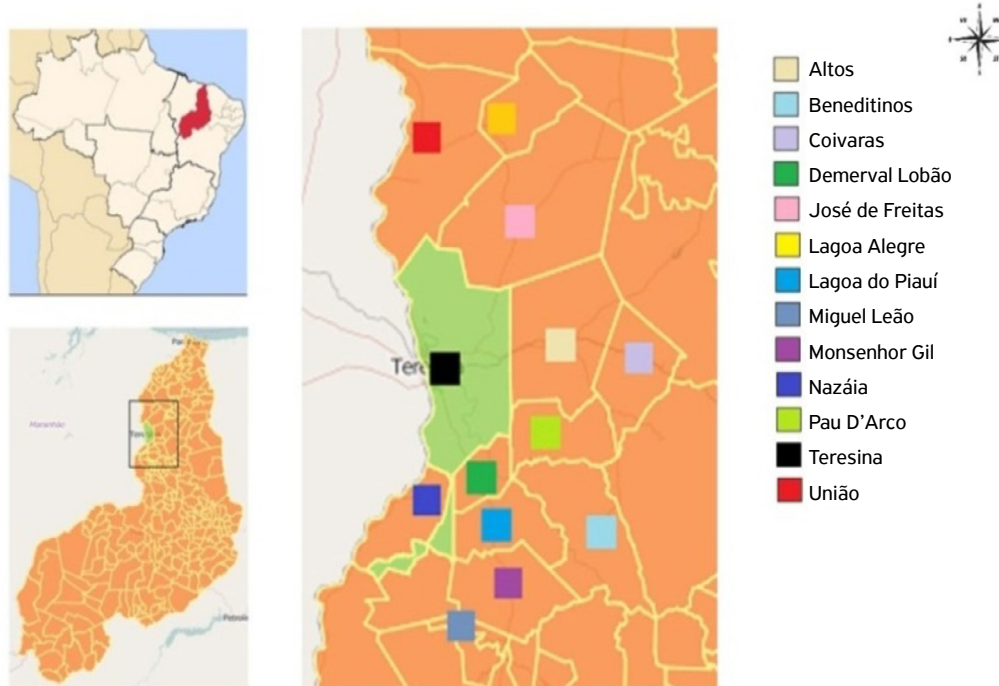
miscarriage and perinatal death (DUBEY, 2010). Miscarriage usually occurs during the last four weeks of pregnancy, and its occurrence can affect up to 58.3% pregnant ewes, that causes an important economic loss to the affected farms (MOTTA et al., 2008).

Serological diagnosis of toxoplasmosis is based on the identification of specific anti-*T. gondii* antibodies. Various serological tests have been used, including hemagglutination (HAI), indirect immunofluorescence reaction (RIFI), modified agglutination test (MAT), agglutination by immunoabsorption (ISAGA) and immune-enzymatic test (ELISA). However, the ELISA, MAT and RIFI tests are the currently preferred methods to diagnose infection by *T. gondii* in sheep, because they ensure good specificity and sensitivity (AMARANTE; 2014).

The importance of *T. gondii* for small ruminant reproduction is well-known. On analyzing goat products suspected of infection by *T. gondii* in southern Brazil, PESCADOR et al. (2007) reported the link between reproductive losses and infection, showing the presence of both macro- and microscopic lesions (lymphoplasmacytic infiltrates in the brain and lungs, interstitial lymphoplasmacytic nephritis, necrotic lymphadenitis and periportal lymphoplasmacytic hepatitis). Considering the relevance of the problems brought on by infection by *T. gondii*, both for public health and for the sheep and goat rearing reproductive sector, the objective of the present study was to determine its prevalence in meat sheep herds on farms in the Teresina microregion, Piauí, Brazil.

## MATERIAL AND METHODS

The experimental protocol was approved by the ethics committed in animal experimentation at the Federal University of Piauí, Brazil (CONCEA-UFPI), protocol number 020/13. The research was carried in the Teresina microregion, situated in the central-north mesoregion of the state of Piauí, consisting of 14 municipalities (Altos, Beneditinos, Coivaras, Curralinhos, Demerval Lobão, José de Freitas, Lagoa Alegre, Lagoa do Piauí, Miguel Leão, Monsenhor Gil, Nazária, Pau D'Arco do Piauí, Teresina and União (Fig. 1).



**Figure 1.** Map characterizing the homogeneous microregion of Teresina, Piauí, Brazil. Source: Retrieved from REBELO et al. (2020).

Twenty-eight herds were randomly selected from all the 14 municipalities, and animals of both sexes were chosen over 6 months. The number of farms and animals chosen in the municipalities and in each herd was statistically preestablished according to THRUSFIELD (2004) and based on farm and herd registration records of the Piauí Agricultural Defense Agency. The sampling process involved 20 randomly chosen animals per farm, consisting of 60% lambing ewes, 30% young

sheep (between 6 and 12 months) and 10% adult reproducers rams. In addition to the animal's sex, the rearing system was also analyzed, and divided as intensive, semi-intensive and extensive.

A total of 450 blood samples were collected, after cleaning the region with 2% iodine alcohol, by jugular vein puncture using a vacuum system and tubes without anticoagulant. The tubes were identified with data for the municipality, farm and animal sex and number. After collection, the samples were kept at  $-20^{\circ}\text{C}$  until processing. Later, they were centrifuged for 10 min at 2,500 rpm, the serum was placed in 1.5-mL microtubes and the ELISA was applied.

The indirect ELISA tests were performed using a specific kit for in vitro diagnosis of sheep ovine toxoplasmosis (Imunoteste *Toxoplasma*, RIFI, Laboratório Imunodot Diagnósticos, Brazil), following the manufacturer's recommendations. The statistical analysis was carried out by the chi-squared test with estimated 5% error to assess the differences between the municipalities, sex and rearing systems.

## RESULTS AND DISCUSSION

A general prevalence was found in 62% (279/450) of samples for anti-*T. gondii* antibodies. There was at least one seropositive animal in all the 14 municipalities in the Teresina microregion, and specific prevalence ranged from 33.3% to 70.0%, but there was no significant difference between the municipalities ( $p > 0.05$ ) (Table 1).

**Table 1.** Total number of samples, number of positive animals and prevalence of sheep seropositive for infection by *T. gondii* by the indirect ELISA test in the Teresina/PI microregion, Brazil.

City	No. of samples	No. of positive animals	Prevalence (%)
Monsenhor Gil	79	55	70.0
José de Freitas	117	77	65.8
Pau D'Arco do Piauí	14	09	64.3
Altos	33	21	63.6
Beneditinos	52	33	63.5
Demerval Lobão	10	06	60.0
Coivaras	33	19	57.6
Lagoa Alegre	33	18	54.5
União	48	26	54.2
Teresina	15	08	53.3
Nazária	04	02	50.0
Miguel Leão	02	01	50.0
Lagoa do Piauí	07	03	42.8
Currálinhos	03	01	33.3
Total	450	279	62

Source: Elaborated by the authors.

The prevalence found in the present study was higher than that reported in some parts of Brazil. There are reports of prevalence ranging from 9.5% (Paraná) to 18.75% (Maranhão), 35.3%, and 16.9% (Pernambuco) and 38.5% (Espírito Santo) and up to 57.6% (Minas Gerais). Furthermore, it was higher than data reported for other microregions of Piauí such as the high-mid Gurguéia microregion (48.7%). (BASSI et al., 2013; MORAES et al., 2011; PEREIRA et al., 2012; RÊGO et al., 2016; ROMANELLI et al., 2007; SILVA et al., 2003; TESOLINI et al., 2012). Similar to this study, PEREIRA et al. (2012) reported animals seropositive for *T. gondii* on 100% of the farms analyzed in the state of Pernambuco, Brazil. Lack of health control, precarious conditions on many farms and the different diagnostic techniques used may have determined these differences.

A 63.2% prevalence was observed in the females (261/413) and 48.6% in the males (18/37), and it was statistically higher in the females ( $p < 0.05$ ) (Table 2). Working with pregnant ewes, MOTTA et al. (2008) reported that 33.3% (3/9) tested positive for anti-*T. gondii* antibodies and there was miscarriage in the last month of pregnancy in 58.3%. In spite of

this, SAKATA et al. (2012) did not report any significant differences in seroprevalence between the sexes of the affected animals, whereas SILVA et al. (2003) reported higher seroprevalence for *T. gondii* in rams than in ewes (50% vs. 31.11%). MORAES et al. (2010) reported the presence of *T. gondii* in the semen of naturally infected rams, showing the importance of the diagnosis in these individuals in the possible sexual transmission to pubescent females in the herd. In this sense, the form of data collection (privileging females) and especially the herd constitution (mostly females) may have been the determining factor in obtaining these results.

**Table 2.** Distribution of sheep seropositive for toxoplasmosis identified by the indirect ELISA test, in relation to sex and rearing system in the Teresina/PI microregion, Brazil.

Sex	Nº of animals (%)	Positive (%)	Negatives (%)
Male	37 (8.2)	18 (48.6) a	19 (51.4) a
Female	413 (91.8)	261 (63.2) b*	152 (36.8) a
<b>Total</b>	<b>450</b>	<b>279</b>	<b>171</b>
Breeding system	Nº of animals (%)	Positive (%)	Negatives (%)
Intensive	39 (8.7)	2 (5.1) a	37 (74.9) a
Semi-intensive	114 (25.3)	27 (23.7) a	87 (76.3) a
Extensive	297 (66.0)	250 (84.2) b*	47 (15.8) a
<b>Total</b>	<b>450</b>	<b>279</b>	<b>171</b>

\* Significant statistical difference.

Source: Elaborated by the authors.

Regarding the rearing system, the intensive, semi-intensive and extensive systems showed a *T. gondii* prevalence of 5.1, 23.7 and 84.2%, respectively, and significant difference was observed for the extensive system ( $p < 0.05$ ) (Table 2). This result is similar to that observed by LUCIANO et al. (2011) who reported higher seroprevalence of *T. gondii* in animals reared under the extensive system, compared to the intensive and semi-intensive systems. The difficulty in controlling environmental health for animals reared extensively, and pasture sharing with other species, especially felines, may explain the higher prevalence in this type of rearing system. Although it was not assessed in this study, cats may be present on these farms, sharing environments with the animals studied. Unlike the results of the present study, intensive rearing was considered one of the risk factors for toxoplasmosis in goats, but not in sheep (PEREIRA et al., 2012).

It was observed that in areas with a high concentration of sheep rearing, the type of rearing system has changed and the extensive system has given way to the implantation of the intensive rearing system, where infectious diseases are more easily disseminated by contact with other species and intense animal management. In this context, the study of toxoplasmosis among these animals is relevant, due to the potential occurrence of reproductive problems, considering the real possibility of congenital transmission and sheep losses (SILVA et al., 2006), that impact directly on production.

The main transmission means of *T. gondii* to man is by intake of meat, milk, water or fomites contaminated with oocytes or bradyzoites (SILVA et al., 2006). Considering that animals that are slaughtered clandestinely usually come from localities with inadequate health management, the possibility of these animals being carriers of several diseases, including toxoplasmosis, is increased. VIANA et al. (2014) reported that clandestine slaughter of small ruminants was, and still is, a reality in Teresina/PI, and showed that the precarious conditions of these clandestine slaughter locations, the environmental conditions, the professionals who perform the slaughter, and the presence of companion animals in these locations culminate in risk for several population groups.

## CONCLUSION

Infection by *T. gondii* is highly prevalent in the sheep herds in the microregion of Teresina/PI, Brazil, especially in females extensively reared. Considering the importance and representativity of the species in the local and state economy, and the risk to public health, control and health education measures should be implemented with the farmers and professionals involved, along with the general population.

## AUTHORS' CONTRIBUTIONS

**Conceptualization:** Feitosa, L.C.S.; **Writing-Review & Editing:** Ferreira, S.B.; **Formal Analysis:** Mineiro, A.L.B.B.; **Resources:** Tenório, T.G.S., Cardoso, J.F.S.; **Supervision:** Celestino, A.C.T.L., Paula, N.R.O.

## AVAILABILITY OF DATA AND MATERIAL

Data will be available upon request

## FUNDING

Not applicable.

## CONFLICTS OF INTEREST

There are no conflicts of interest.

## ETHICAL APPROVAL

The experimental protocol was approved by animal experimentation ethics committee of Universidade Federal do Piauí (AECC -FUP) under protocol number O20/2013.

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