

# Article/Artigo

# Occurrence and risk factors associated with infection by *Toxoplasma gondii* in goats in the State of Alagoas, Brazil

Ocorrência e fatores de risco associados à infecção por *Toxoplasma gondii* em caprinos do Estado de Alagoas

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#### **ABSTRACT**

**Introduction**: Goats are considered very susceptible to infection by *Toxoplasma gondii* and when this occurs during pregnancy, it may cause fetal death with subsequent fetal resorption, abortion, mummification, stillborn or the birth of weak goats. The objective of this work was to determine the occurrence of and identify risk factors for *T. gondii* infection in goats in different mesoregions in the State of Alagoas. Methods: The research was conducted on 24 goat breeding farms in 10 municipalities. A total of 454 blood samples were examined for anti-T. gondii antibodies by indirect immunofluorescence antibody test. To evaluate the risk factors of toxoplasmosis in goats, questionnaires were applied analyzing the farm's production system and nutritional, reproductive and sanitary management. Results: Disease occurrence was 39% with 95.8% of farms presenting seropositive animals. Significant associations were observed for mesoregion (OR = 0.23; 95%CI = 0.09 - 0.57), age (OR = 0.36; 95%CI = 0.20 - 0.64), semi-intensive herd management (OR = 8.70; 95%CI = 1.87 - 40.43), access of cats to water provided for goats (OR = 3.38; 95%CI = 1.89-6.02) and cats feeding on placental remnants (OR = 2.73; 95%CI = 1.38 - 5.40). **Conclusions**: *Toxoplasma gondii* infection is disseminated in the State of Alagoas and the adoption of a program that monitors infection foci is required to reduce the risk factors identified in the present study.

Keywords: Indirect immunofluorescence. Protozoan. Epidemiology.

#### RESUMO

Introdução: Caprinos são considerados susceptíveis à infecção por Toxoplasma gondii que quando ocorre durante a gestação pode causar morte fetal com subsequente reabsorção, aborto, mumificação, natimortos ou nascimento de cabritos fracos. Objetivou-se com este estudo determinar a ocorrência e identificar os fatores de risco associados à infecção por T. gondii em caprinos nas diferentes mesorregiões do Estado de Alagoas. Métodos: A pesquisa foi realizada em 10 municípios, sendo analisadas 24 propriedades de produção caprina com aptidão mista. Foram coletadas amostras sanguíneas de 454 animais para realização da pesquisa de anticorpos anti-Toxoplasma gondii através da prova sorológica de imunofluorescência indireta. Para o estudo dos fatores de risco, foram aplicados questionários com questões referentes ao sistema de produção e manejos nutricional, reprodutivo e sanitário. **Resultados**: A ocorrência encontrada foi de 39% com 95,8% das propriedades apresentando animais positivos. Foi observada associação significativa para as variáveis: mesorregião (OR = 0,23; IC 95% = 0.09 - 0.57), idade (OR = 0.36; IC 95% = 0.20 - 0.64), sistema de criação semi-intensivo (OR = 8,70; IC 95% = 1,87 - 40,43), acesso dos gatos à água fornecida aos animais (OR = 3,38;IC 95% = 1,89 - 6,02) e gatos se alimentando de restos placentários (OR = 2,73; IC 95% = 1,38 - 5,40). Conclusões: A infecção por T. gondii está disseminada no Estado de Alagoas, sendo necessária a realização de um programa de monitoramento dos focos da infecção no Estado com objetivo de reduzir os fatores de riscos identificados no presente estudo.

Palavras-chaves: Imunofluorescência indireta. Protozoário. Epidemiologia.

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#### INTRODUCTION

Toxoplasma gondii is the most studied coccid in the world, with more than 15,000 original research articles and over 500 published revisions, since it is one of the most common agents in parasitic infections of homeothermic animals, including humans<sup>1</sup>.

Among domestic animals, the ingestion of sporulated oocysts derived from cat feces is the most important infection route. Cats are considered the principal reservoir of the cycle of toxoplasmosis, which is considered rare or nonexistent in environments without cats<sup>2</sup>.

Goats are considered very susceptible to infections by *T. gondii* and when infected during pregnancy, they present parasitemia in the first week postinfection and, consequently, in the second and third week, the placenta and fetus are infected, respectively<sup>3</sup>. Infection causes fetal death, with subsequent resorption, miscarriage, mummification, stillbirth or the birth of weak goats<sup>4</sup>.

Numerous studies of anti-*T. gondii* antibody prevalence in goats have been developed in several countries, including those conducted in the United Sates<sup>5</sup>, Venezuela<sup>6</sup>, Brazil<sup>7</sup>, Spain<sup>8</sup>, Ghana<sup>9</sup>, Uganda<sup>10</sup> and Thailand<sup>11</sup>.

Data regarding infection and the prevalence of antibodies anti-*T. gondii* in research conducted in Brazil highlighted the need to include the protozoan among the causes of reproductive losses in goat herds in the country<sup>12</sup>. However, few countries in the world regularly monitor toxoplasmosis in different hosts, including humans and particularly in animals<sup>1</sup>.

As demonstrated by several studies, the principal risk factors for infection by *T. gondii* in goats are: sex and age, in which the principal affected group is adult females; the pluviometric index, high humidity and mild temperatures in the regions where the herds are raised<sup>9,11</sup>; management systems<sup>13</sup>; common feeders and drinkers for young and adult goats; the presence of cats; and goats with reproductive disorders on the property in question<sup>4,14,15</sup>.

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The purpose of this work was to determine the occurrence of infection by *T. gondii* in goats in the State of Alagoas and identify the risk factors associated with infection.

#### **METHODS**

The State of Alagoas is located in the macroregion of northeastern Brazil between the parallels 8°48'12" and 10°30'12" south and the meridians 35°09'36" and 38°13'54" west, covering a territorial area of 27.767,7 km² (approx. 10720 mi²), corresponding to 0.3% of the national territory. Situated in the intertropical strip, the state receives a large quantity of solar energy throughout the year, varying from 2200 to 2600h of sunlight, which determines the hot climate, with annual temperatures around 22°C to 28°C¹6. The State of Alagoas is divided into three mesoregions: East, *Agreste* and *Sertão* and into 13 geographic microregions¹7.

In order to calculate the sample size, an expected prevalence of 50% was considered, to maximize the sample size, and a minimum confidence interval of 95% and statistical error of 5% was established. Thus, the working sample size (n) was determined as 358 goats  $^{18}$ ; however, a total of 454 goats (40 males and 414 females) belonging to 24 properties and from 10 municipalities were eventually studied.

The goats were subdivided into three age groups:  $\leq 12$  monthsold, 13-24 months-old and > 24 months-old. Regarding the region of origin, the study included herds from properties located in all three Alagoas mesoregions: East, *Agreste* and *Sertão*.

TABLE 1 - Risk factors according to general characteristics of the property and of the herd associated or not with infection by *Toxoplasma gondii* in goats in the State of Alagoas, 2008.

		RIFI		Univariate anal	Univariate analysis		
Variable	Number*	n	%	OR (CI 95%)	p		
Age (months)							
≤ 12	83	14	16.9	1.0	<0.001**		
between12 and 24	105	29	27.6	1.88 (0.87; 4.14)			
≥ 24	266	134	50.4	2.66 (1.59; 4.48)			
Gender							
male	40	10	25.0	1.0	0.057		
female	414	167	40.3	2.03 (0.93; 4.77)			
Region							
sertão	121	22	18.2	1.0	<0.001**		
agreste	268	112	41.2	3.23 (1.88; 5.72)			
east	65	43	66.2	2.72 (1.49; 5.05)			
Property size (ha)							
< 30	256	123	48.1	1.0	<0.001**		
between 30 and 200	65	10	15.4	0.20 (0.09; 0.41)			
above 200	133	44	33.1	2.72 (1.22; 6.54)			
Heads of goat (n)							
< 30	284	149	52.5	1.0	<0,001)**		
between 50 and 100	165	24	14.5	0.15 (0.09; 0.26)			
above 100	5	4	80.0	23.50 (2.14; 1,164.97)			
Rearing system							
intensive	34	12	35.3	1.0	<0.001**		
extensive	18	15	83.3	9.17 (1.94; 56.61)			
semi-intensive	402	150	37.3	0.12 (0.02; 0.43)			

n: numbers of goats positive for RIFI, RIFI: reaction of indirect immunofluorescence, OR: *odds ratio*, CI: confidence interval. Base: 454 goats, \*number of goats tested, \*\*statistically significant.

The reaction of indirect immunofluorescence (RIFI) technique was used for serological assays, in accordance the previously described method<sup>19</sup>. The serological reactions were considered positive when total fluorescence occurred at a dilution of  $1:64^{20}$ .

To study the possible risk factors of infection, questionnaires were applied consisting of objective questions designed to elicit information regarding the breeder, general characteristics regarding the property and herd, the management system, the sanitary situation of the herd and reproductive management. From these data, identification of the risk factors associated with infection by *T. gondii* was conducted by univariate analysis of the variables of interest using Pearson's Chi square test or the Fisher exact test when necessary<sup>21</sup> and when a positive association was identified in the univariate analysis, multivariate analysis was performed using the logistic regression model, considering the serological status of the animal (positive or negative) for *T. gondii* as a dependent variable. The SAS (Statistical Analysis System) program was used to perform the statistical calculations, with significance determined as p > 0.05.

#### RESULTS

A total of 177 (39%) of the 454 blood samples analyzed were positive for anti-*Toxoplasma gondii* antibodies. Concerning titration, observation verified that 24 (13.6%) goats presented titers of 64; 31 (17.5%), 128; 73 (41.3%), 256; 40 (22.5%), 512 and 9 (5.1%) presented titers of 1,024.

Of the 24 properties analyzed in this study, the presence of goats positive for *T. gondii* was a determined on 95.8%. In addition, of the 10 municipalities analyzed, nine (90%) showed the presence of positive animals. Considering sex, predominance of females was verified, which showed 40.3% (167/414) positivity against 25% (10/40) for males.

The mesoregions East and *Agreste* showed high occurrences of 66.2% and 41.2%, respectively. The extensive management system showed the highest prevalence (83.3%) among seropositive goats; i.e., herds with more than 100 (80%) heads of goat and properties of less than 300 hectares (48.1%) (**Table 1**).

Risk factors; i.e., whether the variables sex, age, region, property size, number of animals and rearing system were associated or not with infection by *T. gondii*, were determined by univariate and multivariate analyses, as presented are in **Tables 1** and **2**. A significant association was not observed for the variable sex, despite the fact that prevalence in females was considerably higher than in males.

According to age distribution, high occurrence was observed among adult goats (27.6%, aged between 13 and 24 months-old and 50.4% for age > 24 months-old); however, no significant association was determined for this factor. In addition, the indices of occurrence verified in the mesoregions East (66.2%) and *Agreste* (41.2%) showed no association with infection by *T. gondii* in the multivariate analysis.

Considering property size, analysis verified that goats bred and raised in areas between 20 and 30ha had significantly higher chance of being infected (OR = 3.46) compared to those living on properties larger than 200ha.

Considering herd size, higher occurrence was observed in herds with more than 100 (80%) heads of goat compared with those numbering less than 50 (48%) and between 50 and 100 (15.4%). In relation to rearing system, the goats maintained using semi-intensive management showed a higher risk of infection by T. gondii (OR = 8.7) compared to extensive management.

The results of the univariate and multivariate analyses regarding the variables water source, feed, common drinkers for young and adult goats and common feeders for young and adult goats are presented in **Tables 2 and 3**. Although univariate analysis verified significant associations for the variables water source, common drinkers and common feeders for young and adult goats, these were not confirmed in the multivariate analysis.

TABLE 2 - Multivariate analysis for risk factors associated or not with infection by *Toxoplasma gondii* in goats in the State of Alagoas, 2008.

Variable (	Coefficient	p-value	Odds ratio	95%CI for OR
Region		0.006		
agreste	-0.75	0.044	0.15	[ 0.03; 0.63 ]
east	-0.36	0.070	0.23	[ 0.09; 0.57]
Age (months)		<0.001		
between 12 and 24	-1.01	< 0.0010	0,13	[ 0.06; 0.26 ]
≥ 24	-0.00	0.990	0.36	[ 0.20; 0.64 ]
Area (ha)		0.001		
between 30 and 200	0.85	< 0.001	3.46	[ 1.58; 7.56 ]
above 200	-0.46	0.196	0.92	[ 0.28; 3.04 ]
Rearing system		0.021		
extensive	-0.60	0.164	1.18	[ 0.40; 3.45 ]
semi-intensive	1.38	0.010	8.70	[ 1.87; 40.43 ]
Alimentary supplementation				
yes/no	-0.34	0.019	0.50	[ 0.28; 0.89 ]
Common feeders				
yes/no	-0.87	< 0.001	0.17	[ 0.06; 0.44 ]
Reproductive management				
natural mating/natural mating + other biotechnologies	0.93	< 0.001	6.49	[ 2.54; 16.55]
Circulation of wild animals				
yes/no	-0.62	<0,001	0.28	[ 0.16; 0.50]
Access of cats to water provided to goats				
yes/no	0.60	< 0.001	3.38	[1.89; 6.02]
Cats feeding on placental remnants				
yes/no	0.50	0.003	2.73	[1.38; 5.40]

 $TABLE\ 3-Risk factors\ according\ to\ feed\ management, associated\ or\ not\ with\ infection\ by\ \textit{Toxoplasma\ gondii}\ in\ goats\ in\ the\ State\ of\ Alagoas\ ,\ 2008.$ 

		F	RIFI Univariate analysis		
Variable	Number*	n	%	OR (95%CI)	p
Water source					
stagnant water	194	68	35.1	1.0	<0.001**
running water	142	49	34.5	0.98 (0.60; 1.58)	
stagnant + running water	118	60	50.8	1.96 (1.16; 3.34)	
Common drinkers for young and adults					
no	86	49	57.0	1.0	<0.001**
yes	368	128	34.8	2.48 (1.50; 4.13)	
Food					
without supplementation	125	50	40.0	1.0	
with supplementation	329	127	38.6	0.94 (0.61; 1.47)	0.785
Common feeders for young and adults					
no	56	40	71.4	1.0	
yes	398	137	34.4	4.76 (2.49; 9.43)	<0.001**

n: number of goats positive by RIFI, RIFI: reaction of indirect immunofluorescence, OR: odds ratio, CI: confidence interval. base: 454 goats, \*number of goats tested, \*\*statistically significant.

In relation to the reproductive management and hygienic-sanitary variables, the presence of goats with reproductive disorders, the age at which the disorders appeared and the kind of reproductive management adopted were significant in the univariate analysis (**Table 4**). However, only the variable reproductive management showed a significant association in the multivariate analysis. The adoption of a natural mating system increased the chance of infection (OR = 6.49). Of the 24 properties, 17 (70.8%) showed reproductive

disorders, with miscarriage being the most common problem observed (88.2%), followed by retention of the placenta (5.9%) and repetition of the rut (5.9%).

Analysis of the risk factors involving the definitive host of the agent verified that access to water provided for the goats by cats (OR = 3.38) and cats feeding on placental remnants (OR = 2.73) were significant variables and associated with the risk of infection by T. gondii, as demonstrated in **Table 2**.

TABLE 4 - Risk Factors according to reproductive management, associated or not with infection by *Toxoplasma gondii* in goats in the State of Alagoas, 2008.

		RIFI		Univariate analysis	
Variable	Number*	n	%	OR (95%CI)	p
Presence of goats with reproductive disorders on the property					
no	126	31	24.6	1.0	<0.001**
yes	328	146	44.5	0.41 (0.25; 0.66)	
Problems observed+					
repetition of the rut	7	5	71.4	1.0	0.108
retention of placenta	16	10	62.5	0.67 (0.05; 6.04)	
associated abortion	305	131	43.5	0.45 (0.13; 1.42)	
Age at which reproductive disorders appeared+					
< 1 year	37	20	54.1	1.0	0.002**
between 1 and 3 years	130	70	53.8	0.99 (0.44; 2.20)	
> 3 years	161	56	34.8	0.46 (0.28; 0.75)	
Reproductive management					
natural mating	391	168	42.3	1.0	<0.001**
natural mating + other biotechnologies	63	9	14.3	0.22 (0.09; 0.47)	
Acquisition of breeder females for reposition in the last five years					
no	235	90	38.3	1.0	0.755
yes	219	87	39.7	0.94 (0.63; 1.40)	
Acquisition of breeder males for reposition in the last five years					
no	174	71	40.8	1.0	0.531
yes	280	106	37.9	1.13 (0.75; 1.70)	

n: number of goats positive by RIFI, RIFI: reaction of indirect immunofluorescence, OR: odds ratio, CI: confidence interval. +base: 328 goats, \*number of goats tested, \*\*statistically significant.

#### **DISCUSSION**

The mean infection occurrence (39%) determined in this study is above the national and regional averages of 29.3% and 27.6%, respectively. It is similar to that determined in the municipality of Goiânia (43.1%)<sup>22</sup>, in the microregion of Londrina (30.7%)<sup>23</sup> and in the States of Bahia (28.9%)<sup>24</sup>, Pernambuco (40.4%)<sup>25</sup>, Rio Grande do Norte (30.6%)<sup>13</sup> and Minas Gerais (45.8%)<sup>7</sup>. These results also diverge from other works conducted in São Paulo (14.5%<sup>26</sup> and 28.7%<sup>14</sup>), Uberlândia (19%)<sup>27</sup>, Bahia (16.4%)<sup>28</sup>, Paraíba (24.5%)<sup>29</sup> and in Ceará (25.1%)<sup>15</sup>.

Given the geographical dimensions of Brazil, significant climatic diversity can be a factor of influence in prevalence variations, as are the test used and the respective cutoff point adopted. Moreover, according to some authors<sup>14</sup>, goats maintained in suburban areas under precarious conditions of hygiene and in contact with people and their domestic animals are factors that can explain the increase in seropositivity of the herd.

Though not statistically significant in the multivariate analysis, the higher prevalence observed in females (40.4%) corroborates

some studies<sup>7,10</sup>, but is in disagreement with research in which the variable sex was a significant factor (OR =  $2.91^{11}$  and OR =  $1.73^{25}$ ) as a determinant of infection by *T. gondii*.

The hypothesis that females are more susceptible to infection, to severe forms of the disease and show higher rates of mortality by *T. gondii* has mainly been reinforced in herds in forest areas that are characteristically hot and humid, which coincides with the results of specific investigations<sup>9</sup>. Under controlled conditions in the laboratory, a dichotomy in the susceptibility between males and females is evident, revealing that physiological differences between males and females, mainly hormonal, play an important role in determining susceptibility to parasitic infections<sup>30</sup>.

The results of the multivariate analysis demonstrate a positive association for adult goats in relation to young. Similar results were described by others, who also verified that goats over one year-old presented a higher percentage of seropositivity in relation to those aged less than 12 months-old<sup>11,22</sup>. Analysis confirmed that seropositivity was directly related to age<sup>14</sup> and the age factor was significant for the occurrence of infection by *T. gondii* in goats aged over 24° and 36 months-old<sup>27</sup>. Given the time required to achieve subsistence goat breeding in the State of Alagoas, it is probable that

adult animals in the goat herds evaluated have been more frequently exposed to numerous predisposing factors or sources of infections in the environment and, therefore, present a high infection index.

Although no significant findings were determined in the multivariate analysis, among the mesoregions studied, East and Agreste showed significant associations with infection by T. gondii in the univariate analysis. The Agreste region of Alagoas is a climatic transition zone between the East and the Sertão, it has a semiarid climate that is considerably humid and the volume of rainfall and the period of humidity is around 2.5-fold higher than the Sertão<sup>31</sup>. This could be a relevant factor for higher rates of infection determined in this study, in agreement with previous studies in the State of Bahia in Brazil<sup>24</sup> and in countries like Spain<sup>8</sup> and Thailand<sup>11</sup>, which showed that regions of high humidity and milder temperatures favor higher prevalences. However, in Africa, it would be a mistake to attribute differences in prevalence to higher pluviometric indices, such that attention should be focused on the kind of soil and its vegetable cover, which affect local humidity and provide microclimates favorable to the maintenance of the oocysts<sup>10</sup>.

In East Alagoas, the climate is humid, coastal and tropical and the mean annual temperature of around 25°C, typical of the Atlantic Forest, is propitious for the maintenance of oocysts of *T. gondii*, in agreement with results reported for the State of Pernambuco<sup>25</sup>, Brazil, which has a climate similar to Alagoas. Most notable are comparisons between the Zona da Mata in Pernambuco and the East region of Alagoas, where higher indices of seropositivity were registered for both.

Concerning the size of the properties studied, those between 30 and 200 hectares showed a significant association with infection (OR = 3.46) in the multivariate analysis. In these kinds of properties, the goats generally pasture at greater distances, facilitating their contact with areas that can be frequented by domestic or wild feline and contamination by sporulated oocysts.

The management of a property oriented by animal production constitutes a fundamental role in prophylaxis or as potential factor regarding the dissemination of disease in the herd. In this study, the semi-intensive management system proved to be a risk factor that was significantly associated with infection by  $T.\ gondii$  (OR = 8.7). This rearing system permits contact between goats and oocysts excreted in the feces of the wild or domestic felines, thus increasing the probability of infection. Similarly, in the State of Rio Grande do Norte, it was observed that the system of extensive management (OR=1.18) was associated with infection 13, also previously demonstrated in the State of Ceará 15, particularly in regions with poor socioeconomic conditions, where goat raising consists subsistence breeding and where it is common for the goats to be confined overnight.

The presence of goats with reproductive disorders and aged between one and three years-old on the properties showed a significant association in the univariate analysis, even though this was not confirmed in the multivariate analysis. Females of this age are more susceptible and reproductive disorders in this phase become more evident in the herd<sup>9,27</sup>. In most herds, fetal losses occur in females of similar age, particularly among those over three years-old<sup>4</sup>.

The property variable regarding the adoption of a natural mating system increased the risk of infection (OR = 6.49) by T. gondii. Even in the light of this result, is not possible to definitively conclude that venereal transmission occurred in this study, although one study involving goats experimentally infected with T. gondii verified that males can excrete the parasite in semen for up to  $52 \text{ days}^{33}$ .

Although the mere presence of cats on the property did not constitute a risk factor in this study, certain characteristics associated with the habitual behavior of cats were significant, including access to water consumed by the herd (OR = 3.38) and the fact that cats feed on the placental remnants (OR = 2.73). The presence of cats has been considered a risk factor for toxoplasmosis in goats <sup>15</sup>. In a study on goats in Texas, USA, nondetection of anti-T.gondii antibodies was attributed to the absence of cats on the properties of that region <sup>33</sup>. In other similar studies, the higher prevalences verified in goat herds was associated with the presence of a high density population of cats, pets, rodents and humans on the properties, providing high availability of definitive intermediate hosts for the parasite, thus increasing the chances of infection <sup>10,15,24</sup>.

The occurrence of antibodies against *T. gondii* in goats in the State of Alagoas was reported for the first time. Based on the risk factors identified, a state-wide monitoring program that focus on preventing infection is required and should include determination of the serological status of all herds and the development of educational projects emphasizing the risk factors identified in this study, in order to promote better sanitary conditions for goat herds in Alagoas.

## **CONFLICT OF INTEREST**

The authors declare that there is no conflict of interest.

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