Occurrence of anti-*Toxoplasma gondii* antibody and evaluation of risk infection factors in goats raised in Sergipe state, Brazil[1]

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ABSTRACT.- Rizzo H., Jesus T.K.S., Alcântara A.M., Carvalho J.S., Pinheiro Júnior J.W., Mota R.A & Silva T.R. 2020 **Occurrence of anti-*Toxoplasma gondii* antibody and evaluation of risk infection factors in goats raised in Sergipe state, Brazil.** *Pesquisa Veterinária Brasileira 40(5):374-380*. Departamento de Medicina Veterinária, Universidade Federal Rural de Pernambuco, Rua Manuel de Medeiros s/n, Dois Irmãos, Recife, PE 52171-900, Brazil, E-mail: hubervet@gmail.com

The objective was to determine, through indirect immunofluorescence reaction (RIFI, 1:64), the occurrence of IgG antibodies to *Toxoplasma gondii* and the risk factors associated with infection in goats in the state of Sergipe. To this study were used 675 samples of blood of animals from 41 farms of the three state mesoregions from 2013 to 2014. The occurrence of seropositive goats was 30.07%, with 90.24% of farms with seropositive animals. The distribution of titers obtained was 37.93%, 11.82%, 17.24%, 18.22%, and 17.77% for 64, 128, 512 and 1024 respectively. The risk factors observed were farms that did not have facilities (p=0.000, OR=2.30, CI 95%=1.41-3.74), with flooded soils (p=0.011, OR=2.94, CI 95%=1.27-6.79), which provided feed on the ground (p=0.032, OR=1.69, CI 95%=1.04-2.74), in uncovered cages (p=0.032, OR=1.69, CI 95%=1.04-2.74), pasture-based feed (p=0.003, OR=3.52, CI 95%=1.53-8.09), with access from cats to (p=0.031, OR=1.45, CI 95%=1.03-2.04) and introduced new breeders in the last five years (p=0.036, OR=1.58, CI 95%=1.02-2.74).

INDEX TERMS: *Toxoplasma gondii*, anti-*Toxoplasma gondii*, antibody, risk infection, goats, Sergipe, Brazil, caprine, infection.

RESUMO.- [**Ocorrência de anticorpo anti-*Toxoplasma gondii* e avaliação de fatores de risco de infecção em caprinos criados no estado de Sergipe, Brasil.**] O objetivo desse estudo foi determinar, através da reação de imunofluorescência indireta (RIFI, 1:64), a ocorrência de anticorpos IgG para o *Toxoplasma gondii* e os fatores de risco associados à infecção em rebanhos caprinos do estado de Sergipe, a partir de 675 amostras de sangue de animais de 41 propriedades das três mesorregiões do estado entre os anos de 2013 e 2014. A ocorrência de caprinos soropositivos foi de 30,07% com 90,24% de propriedades com animais soropositivos. A distribuição dos títulos obtidos foi de 37,93%, 11,82%, 17,24%, 18,22% e 17,77% para 64, 128, 512 e 1024 respectivamente. Os fatores de riscos observados foram propriedades que não possuíam instalações (p=0,000, OR=2,30, IC 95%=1,41-3,74), com terrenos alagados (p=0,011, OR=2,94, IC 95%=1,27-6,79), que disponibilizam a ração no chão (p=0,032, OR=1,69, IC 95%=1,04-2,74), em cochos descobertos (p=0,000, OR=1,97, IC 95%=1,32-2,94), alimentação à base de pastagem (p=0,003, OR=3,52, IC 95%=1,53-8,09), com acesso de gatos à fonte de água (p=0,031, OR=1,45, IC 95%=1,03-2,04) e que introduziram novos reprodutores nos últimos cinco anos (p=0,036, OR=1,58, IC 95%=1,02-2,74).

TERMOS DE INDEXAÇÃO: Anticorpo, anti-*Toxoplasma gondii*, fatores de risco, infecção, caprinos, Sergipe, Brasil, infecção, *Toxoplasma gondii.*

INTRODUCTION

The most massive production of small ruminants found in Brazil is in the Northeast region (IBGE 2017). The goat farming in Sergipe is a complementary economic activity, characterized mainly by small herds without races and tested specializations in an extensive system (SEAGRI 2016).

One of the main diseases that affect goats is toxoplasmosis. It has clinical and economic importance as it is a congenital, abortive, and zoonotic disease (Medeiros et al. 2014, Silva et al. 2015). Toxoplasmosis is caused by *Toxoplasma gondii*, a parasite mandatory intracellular coccid whose primary host is felines, which eliminate oocysts in feces, contaminating soil, food, and water (Soares et al. 2010, Silva et al. 2015), leading to oral or aerosol infections, in addition to congenital (Silva et al. 2015) and venereal in goats (Wanderley et al. 2015). In humans, other forms of infection are the ingestion of raw milk, tissues, and organs of infected animals, direct contact with intrauterine secretions, and to a lesser extent by blood transfusion and organ transplants (Hill & Dubey 2002).

Goats are more susceptible to clinical toxoplasmosis when compared to other farm animals, causing economic losses in herds around the world due to reproductive disorders, such as abortions of mummified fetuses and births of weak puppies, which can be repeated in subsequent pregnancies (Nunes et al. 2013, Silva et al. 2015). The clinical signs are variable and unspecific for a definitive diagnosis of toxoplasmosis (Spósito Filha & Oliveira 2009). It is necessary to use complementary tests through direct methods: polymerase chain reaction (PCR), hybridization, isolation, immunohistochemistry and histological; and indirect techniques: serology, through the Sabin-Feldman test, indirect hemagglutination, complement fixation, immunoenzymatic assay (ELISA), and indirect immunofluorescence reaction (RIFI). The last one is considered the "gold standard test" due to its sensitivity in both the acute and chronic phases of the infection (Hill & Dubey 2002).

Infection with *T. gondii* has a "cosmopolitan characteristic", and in Brazil, it has been diagnosed by RIFI since 1985 in studies carried out in Minas Gerais. Since then, several kinds of research have been developed (Fialho et al. 2009). In the Northeast region, despite having the most extensive national herd, there are few surveys of goats seropositive for *T. gondii*. They are carried out in the states of Bahia (Uzêda et al. 2004), Alagoas (Anderlini et al. 2011), Pernambuco (Silva et al. 2003, Bispo et al. 2011, Pereira et al. 2012, Lúcio et al. 2016), Paraíba (Faria et al. 2007), Rio Grande do Norte (Lima et al. 2008), Ceará (Cavalcante et al. 2008), Maranhão (Soares et al. 2010, Moraes et al. 2011) and Piauí (Santos et al. 2018), which had an occurrence ranging from 4.34% to 47.56%.

Concerning the main risk factors for the spread of the disease in goat herds in the Northeast, the following can be mentioned: sex, animals of advanced age, extensive or semi-intensive breeding, properties located in hot regions, proximity to urban areas and presence of cats in herds (Anderlini et al. 2011, Bispo et al. 2011, Abu-Dalbouh et al. 2012, Garcia et al. 2012, Santos et al. 2018).

Considering the lack of epidemiological data on the presence of anti-*T. gondii* IgG antibodies in goats in the state, the present study was conducted to determine the occurrence and risk factors for parasite infection in herds in the three mesoregions of Sergipe.

MATERIALS AND METHODS

The study was carried out in the Sergipe state (9°30'49" and 11°34'05" latitude and 36°23'40" and 38°15'00" longitude), Northeast region, Brazil, comprising 75 municipalities with a total area of 21,918,493km2 divided into three edaphoclimatic mesoregions (Agreste, Leste and Sertão). It has a semi-arid climate in the west and part of the Sertão and tropical in the other regions with higher humidity on the coast (Santos et al. 2014, SEAGRI 2016).

In selecting the municipalities and properties that participated in the study, the division of the state, ease of access, convenience, and availability of producers were taken into account. The goats were randomly selected from males and females, apparently healthy, of different zootechnical patterns, and aged over six months. The sample calculation was carried out according to Thrusfield (1995), using the following parameters: population of 19,042 goats (IBGE 2017), 95% confidence interval (CI), and a percentage of sample error margin of 5%, with a prevalence of ±27.07%, (Pita Gondim et al. 1999, Silva et al 2003, Uzêda et al. 2004, Faria et al. 2007, Cavalcante et al. 2008, Lima et al. 2008, Soares et al. 2010, Anderlini et al. 2011, Bispo et al. 2011, Moraes et al. 2011, Pereira et al. 2012, Silva et al. 2015, Lúcio et al. 2016), resulting in a sample of 369 animals. The Win Episcope 2.0 software was used to calculate the number of animals sampled from the above parameters.

Blood samples were collected from 2013 to 2014, totaling 675 goats (107 males and 568 females) from 41 farms in fifteen municipalities, where 4 to 21 samples per farm were obtained. The total number of goats per mesoregion was 235, 178, and 262 for the Agreste, Leste, and Sertão regions, respectively (Fig.1).

During visits to the properties, epidemiological questionnaires were applied to assess the risk factors associated with *Toxoplasma*
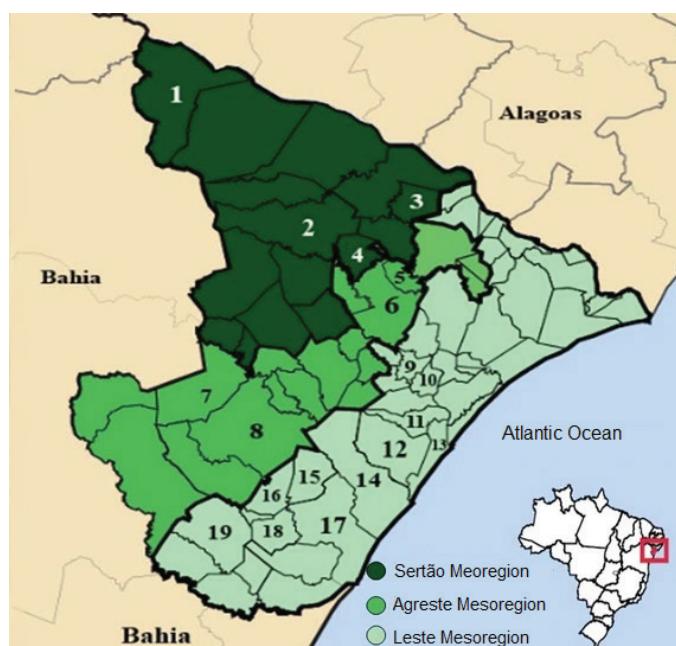
Fig.1. Map of Sergipe divided into its three mesoregions, numerically indicating the municipalities of goat herds submitted to RIFI for *Toxoplasma gondii*. Sertão Mesoregion: 1) Canindé de São Francisco, 2) Nossa Senhora da Glória, 3) Pinhão, and 4) Pedra Mole. Agreste Mesoregion: 5) Macambira, 6) Itabaiana, 7) Lagarto, and 8) Poço Verde. Leste Mesoregion: 9) Neópolis, 10) Nossa Senhora do Socorro, 11) Aracaju, 12) São Cristóvão, 13) Itaporanga D'Ajuda, 14) Salgado, and 15) Estância.

gondii infection. The questionnaires consisted of 34 closed questions, encompassing the characteristics of the herd based on history, breeding purpose, management health, nutritional and reproductive, facilities, and data on the producer.

Blood samples were collected from venipuncture of the jugular with sterile 40x12 needles in a vacuum tube (Vacutainer®), without anticoagulant, and transported under refrigeration. They were centrifuged at the Clinical Pathology Laboratory of "Hospital Veterinário Dr. Vicente Borelli", from "Faculdade Pio Décimo", Aracaju, at 1600g for 10 minutes to obtain serum, stored in Eppendorf tubes and frozen at -20°C until analysis.

Serological diagnosis for detection of anti-*T. gondii* antibodies by RIFI was carried out at the Bacteriosis Laboratory of the Department of Veterinary Medicine of the Federal Rural University of Pernambuco using the cutoff point 64 (Camargo 1964). Only reactions where the presence of fluorescence was total, around the surface of the tachyzoites (Paré et al.1995), seen through a fluorescence microscope, with a 40x objective, were considered positive.

For the execution of the statistical calculation using the EpiInfo 3.5.1 program, a descriptive statistical analysis was performed, determining the absolute and relative frequencies. For the analysis of risk factors associated with *T. gondii* infection, univariate analysis of the variables of interest was performed using Pearson's chi-square test, and when necessary, Fisher's exact test. Subsequently, logistic regression analysis was performed considering the result of the immunofluorescence antibody test (IFAT, reagent or non-reagent) as a dependent variable for *T. gondii* infection. The independent or explanatory variables considered in the model were those that presented statistical significance <0.20. This probability was stipulated so that possible risk factors were not excluded from the analysis (Hosmer & Lemeshow 1989).

The research procedures were approved by the Bioethics Committee of the "Faculdade Pio Décimo", under protocol No. 06/2011.

RESULTS

The occurrence of goats seropositive to *Toxoplasma gondii* in the 41 herds evaluated in the state of Sergipe was 30.07% (203/675) with a high index of properties with positive animals, 90.24% (37/41). Concerning the mesoregions, no statistically significant difference was observed (p=0.094) with occurrences of 35.39%, 25.53%, and 30.53% in the Leste, Agreste, and Sertão respectively (Table 1).

The titers of seropositive goats varied from 64 to 1024. Of these, the titration 64 (37.93%) showed a statistically significant difference (p<0.05) from the others that show similar percentages of 11.82%, 17.24%, 18.22%, and 17.77% for 128, 256, 512 and 1024 respectively (Table 2).

Table 1. Detection of anti-*Toxoplasma gondii* antibodies determined by indirect immunofluorescence reaction (1:64) in goat serum, according to property, animals and mesoregion in the Sergipe state (2013-2014)

Municipality	Positives/Properties (%)	Caprines (%)		
		Male	Female	Positives/Total
Aracaju	3/3 (100)	10/16 (62.5)	8/34 (23.52)	18/50 (36)
Estância	1/1 (100)	2/3 (66.66)	12/17 (70.58)	14/20 (70)
Itaporanga D'Ajuda	1/1 (100)	0/4 (0)	9/16 (56.25)	9/20 (45)
Nossa Senhora do Socorro	1/1 (100)	0/3 (0)	3/15 (20)	3/18 (16.66)
Salgado	2/2 (100)	2/3 (66.66)	5/27 (18.51)	7/30 (23.33)
São Cristóvão	1/1 (100)	1/3 (33.33)	3/17 (17.64)	4/20 (20)
Neópolis	1/1 (100)	0/1 (0)	8/19 (42.1)	8/20 (40)
Total Leste mesoregion	10/10 (100)	15/33 (45.45)	48/145 (33.1)	63/178 (35.39)
Itabaiana	1/1 (100)	1/2 (50)	13/18 (72.22)	14/20 (70)
Lagarto	1/1 (100)	1/5 (20)	2/14 (14.28)	3/19 (15.78)
Macambira	2/3 (66.66)	1/9 (11.11)	10/33 (30.3)	11/42 (26.19)
Poço Verde	9/11 (81.81)	4/19 (21.05)	28/135 (20.74)	32/154 (20.77)
Total Agreste mesoregion	13/16 (81.25)	7/35 (20)	53/200 (26.5)	60/235 (25.53)
Canindé de São Francisco	6/6 (100)	5/14 (35.71)	40/105 (38.09)	45/119 (37.81)
Nossa Senhora da Glória	6/6 (100)	3/18 (16.66)	19/75 (25.33)	22/93 (23.65)
Pedra Mole	0/1 (0)	0/3 (0)	0/8 (0)	0/11 (0)
Pinhão	2/2 (100)	2/4 (50)	11/35 (31.42)	13/39 (33.33)
Total Sertão mesoregion	14/15 (93.33)	10/39 (25.64)	70/223 (31.39)	80/262 (30.53)
TOTAL	37/41 (90.24)	32/107 (29.9)	171/568 (30.1)	203/675 (30.07)

Table 2. Anti-*Toxoplasma gondii* antibody titers in 203 positive goats by indirect immunofluorescence reaction (RIFI) in the mesoregion of the Sergipe state (2013-2014)

Mesoregions	Titration (%)				
	64	128	256	512	1024
Agreste	19 (9.35)	6 (2.95)	12 (5.91)	11 (5.41)	12 (5.91)
Leste	24 (11.82)	6 (2.95)	17 (8.37)	9 (4.43)	7 (3.44)
Sertão	34 (16.74)	12 (5.91)	6 (2.95)	17 (8.37)	11 (5.41)
Total	77 (37.93)[a]	24 (11.82)[b]	35 (17.24)[b]	37 (18.22)[b]	30 (17.77)[b]

[a,b] Different lowercase letters on the same line indicate a statistically significant difference (Chi-square P<0.05).

In the univariate analysis, twelve variables showed significance, p<0.05 (Table 3). However, when submitted to multivariate logistic regression analysis, the ones confirmed as risk factors for *T. gondii* infection were properties that: did not have facilities (p=0.000, OR=2.30, CI 95%=1.41-3.74); with flooded land (p=0.011, OR=2.94, CI 95%=1.27-6.79); which provided the feed on the ground (p=0.032, OR=1.69, CI 95%=1.04-2.74); in uncovered hods (p=0.000, OR=1.97, CI 95%=1.32-2.94); pasture-based feed (p=0.003, OR=3.52, CI 95%=1.53-8.09); with access by cats to a water source (p=0.031, OR=1.45, CI 95%=1.03-2.04) and that have introduced new breeders in the last five years (p=0.036, OR=1.58, CI 95%=1.02-2.74).

Table 3. Goats seropositive for *Toxoplasma gondii*, through RIFI (1:64), according to the studied variable, from rural properties in the Sergipe state (2013-2014)

Variable	RIFI % (soropositives/total)	OR (CI 95%)	P-value
Mesoregion			
Leste	36.51 (65/178)	-	
Agreste	25.53 (60/235)	0.63 (0.40-0.98)	0.094
Sertão	30.53 (80/262)	1.28 (0.85-1.94)	
Sex			
Female	30.21 (171/566)	1.04 (0.65-1.69)	0.478
Male	29.35 (32/109)		
Breed			
Anglo-Nubiana	28.33 (17/60)		
Boer	14.81 (8/54)		
Pardo Alpina	31.25 (5/16)	-	0.012
Saanen	26.38 (57/216)		
No defined breed	34.57 (111/321)		
Toggenburg	62.5 (5/8)		
Age of animals (years)			
1 ≤ 2	30.56 (114/373)	-	
> 2	30.51 (83/272)	1.00 (0.70-1.42)	0.468
≤ 1	20 (6/30)	0.57 (0.18-1.50)	
Total goats			
Up to 50	33.87 (84/248)	-	
Between 51 and 100	37.28 (22/59)	1.16 (0.61-2.17)	0.061
Above 100	26.35 (97/368)	0.60 (0.33-1.13)	
Breeding purpose			
Beef	32.55 (97/298)	1.23 (0.87-1.73)	0.122
Milk	28.11 (106/377)		
Breeding regime			
Extensive	35.08 (40/114)	-	
Intensive	40.74 (44/108)	1.27 (0.71-2.27)	0.006
Semi-extensive	26.32 (119/452)	0.52 (0.33-0.83)	
Reproduction biotechnologies			
No	35.56 (175/492)	3.05 (1.97-4.81)	0.000
Yes	15.3 (28/183)		
Property size (Ha)			
< 30	25.88 (95/367)	-	
Between 30 and 100	33.46 (83/248)	1.44 (1.00-2.08)	0.016
> 100	41.66 (25/60)	1.42 (0.76-2.62)	
Type of land			
Rough	27.77 (30/108)	-	
Flooded	60 (21/35)	3.75 (1.76-8.18)	0.000
Flat	28.57 (152/532)	0.27 (0.12-0.57)	

Variable	RIFI % (soropositives/total)	OR (CI 95%)	P-value
Facilities			
Masonry sheepfold	22.36 (36/161)	-	
Clay floor sheepfold	27.35 (29/106)		0.000
Slatted floor sheepfold	27.43 (62/226)		
No facilities	41.75 (76/182)		
Disinfect facilities			
No	40 (64/160)	1.80 (1.21-2.65)	0.001
Yes	26.99 (139/515)		
Feed provision			
Covered hod	21.68 (80/369)	-	
Uncovered hod	38.82 (73/188)	2.29 (1.53-3.43)	0.000
On the ground	42.37 (50/118)	1.16 (0.70-1.90)	
Feeding			
Concentrated	9.72 (7/72)	-	
Access to pasture	36.36 (160/440)	5.31 (2.35-14.02)	0.000
High volume in the hod	22.08 (36/163)	0.50 (0.32-0.76)	
Food storage			
Open location	39.62 (63/159)	1.76 (1.19-2.59)	0.002
Closed location	27.13 (140/516)		
Water supply			
Direct from source (weir/dam)	38.93 (44/113)	-	
Containers inside the facilities	28.47 (131/460)	0.62 (0.40-0.99)	0.077
Containers outside the facilities	27.45 (28/102)	0.95 (0.57-1.57)	
Presence of cats			
No	29.13 (44/151)	0.94 (0.61-1.42)	0.429
Yes	30.34 (159/524)		
Cats access to water			
No	27.16 (119/438)	0.68 (0.48-0.98)	0.019
Yes	35.16 (83/236)		
Cats access to food storage			
No	30.27 (145/479)	1.03 (0.70-1.51)	0.469
Yes	29.59 (58/196)		
Performs quarantine			
No	27.60 (106/384)	0.76 (0.54-1.07)	0.064
Yes	33.33 (97/291)		
Contact between healthy and sick animals			
No	27.2 (71/261)	0.79 (0.55-1.13)	0.113
Yes	31.88 (132/414)		
Matrix acquisition in the last five years			
No	27.14 (76/280)	0.78 (0.55-1.11)	0.094
Yes	32.15 (127/395)		
Acquisition of breeders in the last five years			
No	26.37 (96/364)	0.67 (0.48-0.94)	0.021
Yes	34.51 (107/310)		

DISCUSSION

The rate of 30.07% of goats seroreagent to *Toxoplasma gondii* in the state of Sergipe was similar to that described by Pereira et al. (2012) 31.73% (53/167) in Pernambuco, but lower than the 47.56% (78/164) reported by Bispo et al. (2011) in the same state and 38.98% (177/454) described by Anderlini et al. (2011) in Alagoas. Lower rates in the Northeast were observed in São Luís in Maranhão (Moraes et al. 2011),

Bahia (Uzêda et al. 2004), Mossoró in Rio Grande do Norte (Lima et al. 2008), southern Piauí (Santos et al. 2018) and Paraíba (Faria et al. 2007) presenting 4.34% (2/46), 16.35% (61/373), 17.06% (65/381), 22.07% (32/145) and 24.50% (75/306), respectively.

Regarding the outbreaks of the 41 properties studied, 37 (90.24%) presented at least one positive animal, demonstrating the dissemination of the agent in Sergipe's herds, as observed in Pernambuco's herds with 100% (10/10) and 66.66% (4/6) and in Rio Grande do Norte with 92.85% (13/14) of outbreaks (Silva et al. 2003, Cavalcante et al. 2008, Pereira et al. 2012).

There was no significant difference in the mesoregions, even in the Leste, with a humid and hot climate in Agreste and Sertão; a condition that favors the maintenance and sporulation of the oocyst in the environment. Similar results were obtained by Silva et al. (2003) in Pernambuco when comparing Zona da Mata and Agreste mesoregions (47.88% x 52.11%). Anderlini et al. (2011), when analyzing the same variables (Agreste, Leste, and Sertão) in Alagoas, observed that the Leste mesoregion showed a significant result with 66.15% (43/65) of seropositive animals. Also, this demonstrated the favorable climatic condition for the parasite's biological cycle since the values obtained in the Sertão mesoregion were only 18.18% (22/121) and 41.49% (112/268) in the Agreste.

The highest occurrence of goats showing titers of 64 in this study is similar to the results of Uzêda et al. (2004) and Pereira et al. (2012) with low titers. However, with a cutoff of 16, the highest titers presented by both were, respectively, 14.75% (9/61) for titers over 256, and 0.59% (1/167) for 4,096 titers. The higher occurrence of low titers suggests that these animals were infected (Maley et al. 1997).

As for the availability of food, both breeding systems had significance in the values obtained. They are properties belonging to small producers, subsistence breedings, whose sanitary measures and management practices are inadequate, which favors contact with contaminated sources of infection.

Anderlini et al. (2011) reported that in intensive regimes, there is a greater chance of high HIV-positive rates due to the higher concentration of animals, exposing them even more to contaminated food. The acquisition of new animals, the lack of installation, and the animal's access to the pasture had significant results, corroborating the deficiency of management on the property.

The type of flooded terrain showed a higher rate of animals reactive to the infectious agent when compared to flat and rugged terrain, probably since they present better conditions for the sporulation of oocysts and their greater permanence in the environment. The cat's access to water is fundamental in the spread of the disease since it occurs through the ingestion of oocysts of the parasite released in the soil. Due to its potential for expansion higher than the evacuation site, it can contaminate unprotected water sources (springs, artesian wells, distribution network) and pastures, especially in rainy periods, making the agent able to conclude their biological cycle in the organism of the intermediate host (goats) (Dubey 2010, Santos et al. 2018).

CONCLUSION

The occurrence of *Toxoplasma gondii* in goats in Sergipe is correlated mainly with deficient sanitary management. The results show characteristics that favor the maintenance of the agent in the environment.

Conflict of interest statement.- The authors declare that they have no conflict of interest.

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