

Seroprevalence, spatial distribution and risk factors associated with *Toxoplasma gondii* infection among cattle in a *quilombola* community in the Brazilian cerrado

Soroprevalência, distribuição espacial e fatores de risco associados à infecção por *Toxoplasma gondii* em bovinos de comunidade quilombola no cerrado brasileiro

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Abstract

Little is known about *Toxoplasma gondii* infection among cattle living in the Cerrado (Brazilian savanna) biome in Brazil. In particular, there is no epidemiological data relating to infection in *quilombo* lands, i.e. areas settled by Afro-descendants of escaped slaves. The aim of this study was to determine the prevalence, spatial distribution and risk factors associated with *T. gondii* infection among cattle in the Kalunga *quilombo*, in the Cerrado biome. Blood samples were collected from 1533 cattle for antibody detection using the indirect fluorescence antibody test (IFAT). The study area was subdivided into five macroregions to determine the spatial distribution of infection. An objective questionnaire was applied to the cattle owners to evaluate risk factors, which were analyzed using univariate analysis and logistic regression. The prevalence of *T. gondii* infection among cattle was 8.93% (137/1533), and antibodies were found in 49.6% of the herds (66/133), in all macroregions. The risk factors associated with *T. gondii* infection in cattle were the following: number of animals in the herd (OR: 30.56), purchase of cattle (OR: 2.57), age group (OR: 1.95) and average annual temperature (OR: 1.77). Thus, the occurrence rate, spatial distribution and risk factors associated with *T. gondii* infection among cattle in the Kalunga *quilombola* community are documented here, for the first time.

Keywords: Brazil, toxoplasmosis, cattle, epidemiology, quilombo.

Resumo

A ocorrência de anticorpos anti-*T. gondii* em bovinos, no bioma cerrado brasileiro, é pouco conhecida. Particularmente, não existem dados epidemiológicos relativos à infecção em terras quilombolas, áreas formadas por descendentes de escravos africanos refugiados. O estudo objetivou determinar a prevalência, a distribuição espacial e os fatores de risco associados à infecção por *T. gondii* em bovinos, na comunidade remanescente de quilombos Kalunga, no bioma cerrado. Amostras de sangue foram coletadas de 1.533 bovinos para detecção de anticorpos pela reação de imunofluorescência indireta (RIFI). A área em estudo foi dividida em cinco macrorregiões para determinação da distribuição espacial da infecção. Um questionário objetivo foi aplicado aos proprietários dos bovinos para a avaliação dos fatores de risco, os quais foram analisados por meio de análise univariada e regressão logística. Verificou-se ocorrência de anticorpos anti-*T. gondii* em 8,93% (137/1533) dos bovinos, com anticorpos detectados em 49,6% (66/133) dos rebanhos de todas as macrorregiões. Os fatores de risco associados

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à infecção foram: número de animais no rebanho (OR: 30,56), compra de bovinos (OR: 2,57), faixa etária dos animais (OR: 1,95) e temperatura anual média (OR: 1,77). Assim, documentam-se, de forma inédita, a ocorrência, a distribuição espacial e os fatores de risco associados à infecção por *T. gondii* em bovinos na comunidade quilombola Kalunga.

Palavras-chave: Brasil, toxoplasmose, bovino, epidemiologia, quilombo.

Introduction

Toxoplasmosis is a parasitic zoonosis of worldwide prevalence for which *Toxoplasma gondii* is the etiological agent. *T. gondii* is a coccidian that forms cysts in host tissues and has the capacity to infect most endothermic species (Tenter et al., 2000). It has been characterized as the water and food-borne parasite that has the fourth greatest impact on public health (WHO, 2014). Estimates suggest that about between 30% and 60% of human population worldwide is infected with *T. gondii*, with high heterogeneity between countries and regions (Bigna et al., 2020; CDC, 2018).

The total commercial cattle herd population of Brazil is one of the largest in the world and antibodies to *T. gondii* may be present in more than 30% of the herd of a specific farm (Azevedo et al., 2020). Thus, from a public health perspective, it is important to know the health status of cattle herds with regard to *T. gondii* infection. This is especially important in traditional rural communities, where cattle are frequently kept as a means of subsistence and water sources are shared between human and animal populations (Baiocchi, 2013).

The remaining *quilombos* are among these traditional rural communities. Originally, *quilombos* were rural settlements formed by escaped African slaves and their descendants in Brazil, as a form of resistance to captivity and exploitation (Gontijo et al., 2018). The inhabitants of these communities are called *quilombolas*. The largest remaining *quilombola* community in the country is the Kalunga Historical and Cultural Heritage Site. It is located in the central-western region of Brazil, in the Chapada dos Veadeiros microregion, which has been recognized as a world natural heritage site by UNESCO (Baiocchi, 2013; UNESCO, 2017). Because of Kalunga's historical and environmental relevance, it has been included in the Cerrado Biosphere Reserve, a priority conservation area of the Cerrado biome, i.e. the Brazilian savanna region (Brasil, 2016). Beyond the origins of the community, *quilombola* traditionality also permeates the relationship with the ecosystem. With minimal use of pesticides and maintenance of native pastures, the community survives on subsistence, and its cattle reinforces its food diversity (Baiocchi, 2013).

Despite the importance of toxoplasmosis, the Cerrado biome and the Kalunga community, little is known about *T. gondii* infection among cattle in the Cerrado. Moreover, there is no epidemiological data on the remaining *quilombola* communities in Brazil. The aim of this study was to determine the prevalence, spatial distribution and factors associated with *T. gondii* infection among cattle in the Kalunga community, in the Brazilian Cerrado region.

Material and Methods

Ethics committee

The procedures in this experiment were approved by the Research Ethics Committee (629.723) and the Animal Use Ethics Committee (008/14) of the Federal University of Goiás.

Study area and sampling

This study was conducted on samples that were collected in the area of the Kalunga Historical and Cultural Heritage Site between 2014 and 2017. This settlement is a *quilombola* community, located in the northeastern part of the state of Goiás (approximately 13°20' S, 47°20' W), in the Cerrado biome (Brazilian savanna), in Brazil (Baiocchi, 2013). Kalunga covers an area of 253,000 hectares and the local cattle herd has been estimated at 9,000 animals, distributed among approximately 433 farms (Costa, 2013).

The sample size for this study was estimated assuming an expected prevalence of *T. gondii* infection of 50% (Fajardo et al., 2013). This resulted in a minimum sample of 384 animals (considering a 95% confidence interval and 5% error) (Thrusfield, 2004). However, because of the availability of a sample bank, serum samples were tested from 1,533 cattle of different breeds (zebu, crossbreed and a local breed named Curraleiro Pé-Duro), different

ages and both sexes were tested. These cattle were kept in herds on 133 farms within the boundaries of Kalunga and were mostly raised through an extensive system.

Table 1. Prevalence of anti-*T. gondii* antibodies among cattle on farms in the Kalunga *quilombo* in the Brazilian Cerrado biome, according to the IFAT test.

Municipality	Macroregion	Positive (%)	Positive Herds (%)
Cavalcante	Engenho II	18/195 (9.23)	9/16 (56.25)
	Vão de Almas	10/281 (3.56)	6/23 (26.08)
	Vão do Moleque	30/324 (9.26)	17/30 (56.66)
Total		58/800 (7.25)	32/69 (46.37)
Monte Alegre de Goiás	Monte Alegre de Goiás*	36/389 (9.25)	15/36 (41.66)
Total		36/389 (9.25)	15/36 (41.66)
Teresina de Goiás	Teresina de Goiás*	43/344 (12.50)	19/28 (67.85)
Total		43/344 (12.50)	19/28 (67.85)
Study Total		137/1533 (8.93)	66/133 (49.6)

IFAT: indirect fluorescence antibody test. *Macroregion with the same name as the municipality.

In accordance with traditional regional divisions, the area studied here was divided into five macroregions: Engenho II (number of samples, n = 195); Vão de Almas (n = 281); Vão do Moleque (n = 324); Monte Alegre de Goiás (n = 389); and Teresina de Goiás (n = 344). The first three belong to the municipality of Cavalcante (n = 800) and the others to the municipalities of Monte Alegre de Goiás (n = 389) and Teresina de Goiás (n = 344) (Table 1).

Sample collection

Blood samples were collected from the jugular or lateral coccygeal veins by means of venipuncture. After individual identification, the material was centrifuged (10 minutes at 1,500 rpm) and the serum was aliquoted and stored at -20 °C.

Serological analysis

Serum samples were subjected to the indirect fluorescence antibody test (IFAT) to detect anti-*T. gondii* IgG antibodies (Camargo, 1964). The slides were coated with tachyzoites of the ME-49 strain (12 to 15 x 10³ tachyzoites/well). Anti-bovine IgG antibodies produced in rabbit (Sigma, St. Louis, USA) were used as a conjugate. A cut-off point at a dilution of 1:64 was established (Magalhães et al., 2016; Souza et al., 2016). IFAT slides were examined by means of fluorescence microscopy using an epifluorescence microscope (Olympus Bx 51, Olympus Inc., Tokyo, Japan) at 40x magnification. Samples were considered positive when *T. gondii* tachyzoites exhibited complete peripheral fluorescence. The samples were subjected to twofold dilutions. The titers were determined as the highest dilution with a positive reaction. Positive and negative controls were included in all the reactions.

Spatial distribution

The coordinates of each farm were obtained via the global positioning system (GPS) using the Garmin MIN Oregon® 450 device. From this, the spatial distribution of the herds with positive samples was determined (ArcGIS, ArcMap 10.4), thus correlating seroprevalence with the geographical locations of the herds.

Risk factors associated with *T. gondii* infection

Risk factors were evaluated by means of an objective questionnaire, which was applied to the cattle owners after obtaining their consent. This included the following objective questions: number of animals in the herd, purpose of rearing, contact with cats and wild species, water source, type of pasture, occurrences of abortion and reproductive problems, and cattle purchasing practices. Information regarding the breed, sex and age range of the animals sampled was obtained directly at the time of collecting the blood samples. Environmental data from the study area, including altitude, average annual temperature, precipitation and normalized difference vegetation index (NDVI) were collected from the database of the Laboratory of Image Processing and Geoprocessing of the Federal University of Goiás (LAPIG/UFG) and the National Institute of Meteorology (INMET).

Data analysis

Firstly, the variables were evaluated through univariate analysis, using Pearson’s chi-square test or Fisher’s exact test, when necessary, and adopting a 95% confidence interval. Those with $p \leq 0.2$ were selected for the logistic regression model, in which the serological results (positive or negative) were considered to be the dependent variable. The final model, which had the lowest AIC, was composed of the variables that presented $p < 0.05$, for which the odds ratio was estimated. The R software was used to perform the statistical calculations (R Core Team, 2018).

Results

Out of the 1,533 cattle sampled, 137 had anti-*T. gondii* antibodies, with seroprevalence of 8.93% (95% CI: 7.55% - 10.47%). Antibodies were found in 49.6% (66/133) of the herds. Antibody titers of 64, 128, 256, 512 and 1024 were observed in 57.66% (79/137), 31.38% (43/137), 8.75% (12/137), 0.73% (1/137) and 1.46% (2/137) of the seropositive animals, respectively. Among the different regions of the Kalunga community, the seroprevalence in cattle ranged from 3.55% to 12.50%. The lowest proportions of individuals and herds exposed to the parasite were observed in the Vão de Almas macroregion (municipality of Cavalcante) (3.55% and 26.08%, respectively) (Table 1).

The spatial distribution of *T. gondii* seropositive herds is shown in Figure 1. In general, infected cattle were distributed homogeneously throughout the Kalunga territory. But, except in Vão de Almas, some areas of higher seroprevalence (about 40%), called “hot zones” of infection, were observed in all macroregions. These zones

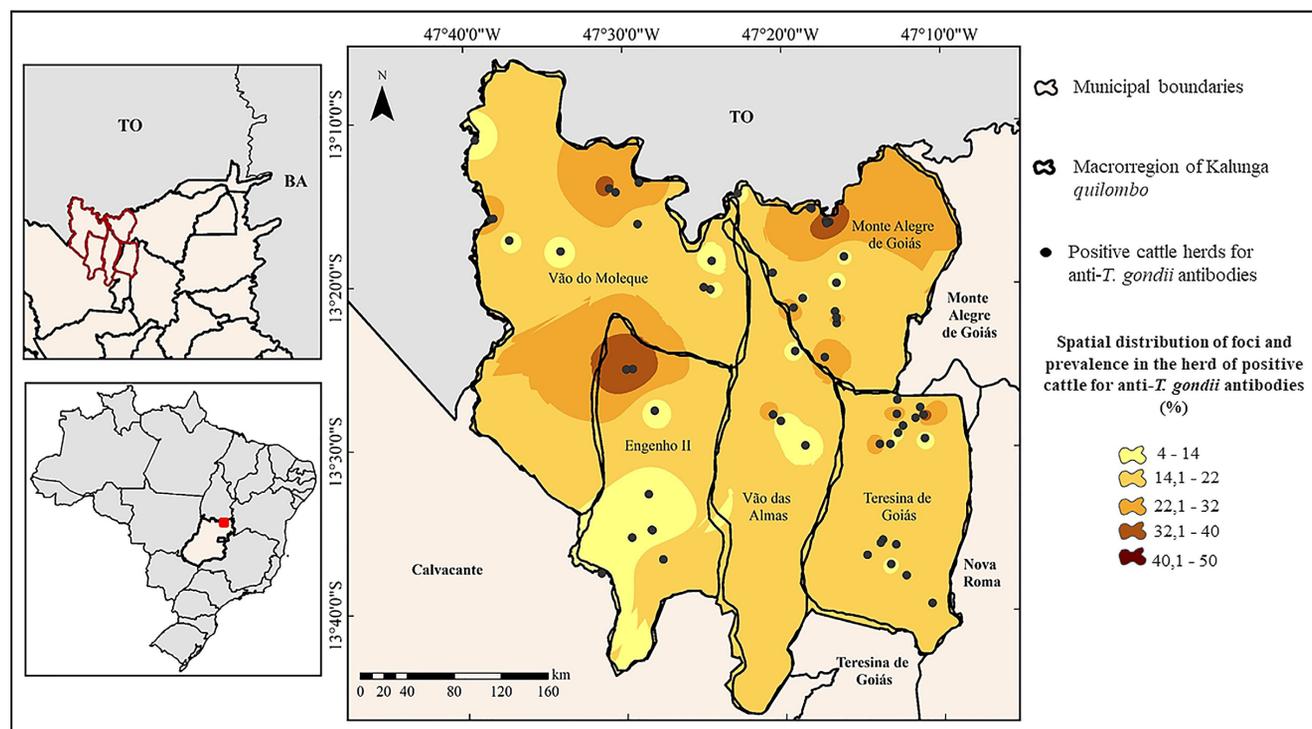


Figure 1. Spatial distribution and prevalence of anti-*T. gondii* antibodies among cattle herds in the Kalunga quilombo in the Brazilian Cerrado biome. Note: Datum WGS84.

Table 2. Variables associated with the presence of anti-*T. gondii* antibodies among cattle in the Kalunga *quilombo* in the Brazilian Cerrado biome, according to univariate analysis.

Variables	N	Positive	Univariate Analysis (χ^2 / Fisher's exact)
		Cattle (%)	p value
Age range			
>36 months	1184	88 (7.43%)	0.000*
25-36 months	185	24 (12.97%)	
0-24 months	164	25 (15.24%)	
Cattle purchase			
There is no purchase	266	15 (5.63%)	0.000*
Up to 12 months	626	43 (6.86%)	
> 12 months	641	79 (12.32%)	
Cattle in herd			
More than 100	95	1 (1.05%)	0.002*
From 11 to 100	1398	130 (9.29%)	
Up to 10	40	6 (15.00%)	
AAT			
>31.2°C	985	80 (8.12%)	0.159*
28.5 to 31.1°C	548	57 (10.40%)	
NDVI			
0.225 to 0.346	642	44 (6.85%)	0.019*
0.347 to 0.469	891	93 (10.43%)	
Breed			
Zebu	875	92 (10.51%)	0.044*
Crossbreed	587	40 (6.81%)	
Local Breed	71	5 (7.04%)	
AP			
1146 to 1417mm	488	34 (6.96%)	0.079*
1418 to 1688mm	1045	103 (9.85%)	
Type of pasture			
Formed	62	5 (8.06%)	0.095*
Native	172	23 (13.37%)	
Both	1299	109 (8.39%)	
Purpose of rearing			
Consumption	575	43 (7.47%)	0.117*
Sale	122	16 (13.11%)	
Both	836	78 (9.33%)	
Sex			
Male	183	11 (6.01%)	0.180*
Female	1350	126 (9.33%)	

AAT: average annual temperature; AP: annual precipitation; N: number of samples; NDVI: normalized difference vegetation index; χ^2 : Pearson's chi-square test; *: significant variables ($p < 0.2$) in univariate analysis, subsequently entered into the logistic regression model.

represent 22.2% (2/9) of the positive herds of Engenho II, in which 40% (8/20) of positivity were verified; 20% (3/15) of positive herds in Monte Alegre de Goiás, with 41.3% (12/29) of positivity in them; 10.5% (2/19) of positive herds in Teresina de Goiás, with 40.7% (11/27) of positivity in them and 5% (1/17) of Vão do Moleque, with 40% (4/10) of positivity in them.

Table 3. Risk factors associated with the presence of anti-*T. gondii* antibodies among cattle in the Kalunga quilombo in the Brazilian Cerrado biome, according to logistic regression.

Variables	Logistic Regression		
	p value	OR ^a	CI 95% ^b
Age range			
>36 months	0.007	-	
25-36 months		1.84	1.10-2.96
0-24 months		1.95	1.16-3.19
Cattle purchase			
There is no purchase		-	
Up to 12 months	0.001	1.65	0.89-3.21
> 12 months		2.57	1.46-4.83
Cattle in herd			
More than 100		-	
From 11 to 100	0	12.71	2.69-227.43
Up to 10		30.56	4.43-617.11
AAT			
>31.2°C	0.048	-	
28.5 to 31.1°C		1.48	1.00-2.17

^aOdds ratio; ^b95% confidence interval. Complete model AIC: 904.3985. Final model AIC: 890.3248.

The variables correlated with the presence of anti-*T. gondii* through univariate analysis are described in Table 2. Then, through the logistic regression, the risk factors identified as associated with *T. gondii* infection in cattle were the following: herds composed of up to ten animals (OR: 30.56), purchase of cattle older than 12 months (OR: 2.57), age range up to 24 months (OR: 1.95) and average annual temperature from 28.5 to 31.1 °C (OR: 1.48) (Table 3). The presence of domestic and wild felids in the study area was mentioned by all the owners interviewed, although no association with the presence of anti-*T. gondii* antibodies in cattle was verified statistically.

Discussion

To the authors' knowledge, this is the first study on the prevalence of *T. gondii* infection among cattle in a quilombola community, as well as one of the few on this topic conducted in the Cerrado biome (Brazilian savanna).

The presence of anti-*T. gondii* antibodies in 8.93% (95% CI: 7.55%-10.47%) of the cattle demonstrates that the prevalence of infection in the Brazilian central Cerrado region is close to that observed in both the far western part of Brazil (11.8%) (Souza et al., 2016) and in the island portion, in the far east, in the Atlantic islands of Fernando de Noronha (10.7%) (Magalhães et al., 2016). This corroborates the copious capacity for dissemination and adaptability of the parasite (Tenter et al., 2000). On the African continent, including in savanna areas resembling the Cerrado region, similar prevalence to our study was observed, with about 12% of the cattle infected by *T. gondii* (Tonouhewa et al., 2017).

Infection with *T. gondii* was present in the herds in all five Kalunga macroregions (49.6%). Both domestic and wild felids were seen to cohabiting the Kalunga territory with cattle, and these would be the agents responsible for oocyst excretion and the massive spread of *T. gondii* in soil, water and vegetation (Dubey, 2010). This raises the risk of transmission of coccidians to the local population via oocysts in contaminated water and food (Dubey & Jones, 2008). In endemic areas, high pressure of environmental contamination with *T. gondii* oocysts favors continuous reinfection of the population (Shapiro et al., 2019).

Furthermore, because *T. gondii* infection was found in almost half of the local cattle herds (49.6%), it is prudent to consider that these ruminants may constitute an important source of infection for the Kalunga community. Although the role of beef consumption in transmission of this parasite to humans has not been completely elucidated (Opsteegh et al., 2011), this kind of meat derived from local slaughter is an important component of subsistence-based regional food (Baiocchi, 2013). It is noteworthy that the habit of eating undercooked beef is culturally disseminated in several countries and strongly present in Brazil, including in the Kalunga community, thereby favoring *T. gondii* infection in the human population (Belluco et al., 2016).

The most frequent antibody titer for *T. gondii* in these cattle was 64, and this titer was observed in 57.66% of the seropositive animals, followed by 128 in 31.38%. These results are similar to those presented by Souza et al. (2016). In cattle that are more resistant to the action of *T. gondii* and in which chronic infections are known to predominate, antibody titers less than 1,024 occur frequently (Dubey, 2010; Dubey & Thulliez, 1993). In this regard, Dubey & Thulliez (1993) demonstrated that the viability of tissue cysts can extend for more than 1,190 days in these ruminants, a period compatible with the age at which these animals are slaughtered.

Overall, the distribution of seropositive herds among the Engenho II, Vão do Moleque, Monte Alegre and Teresina de Goiás macroregions were homogeneous, i.e. there were no statistical differences (Table 1 and Figure 1). This was possibly due to the similar edaphoclimatic conditions in the region. The exception was in Vão de Almas, where the lowest intra-herd (3.55%) and inter-herd (26.08%) prevalences were identified. There, the average annual temperature was higher (> 31.2 °C) than those of the other macroregions (28.5 to 31.1 °C). In areas with milder temperatures, the likelihood of infection ($p = 0.048$) was 1.48-fold greater than in areas where the average temperatures were above 31.2 °C, such as Vão de Almas. According to Dubey et al. (2011), *T. gondii* sporulation capacity is markedly reduced at temperatures above 30 °C, thus reducing coccidian infectivity.

On the other hand, eight hot zones of infection with seroprevalence about 40% were observed: three in Monte Alegre de Goiás (41.3%, 12/29), two in Engenho II (40%, 8/20), two in Teresina de Goiás (10.5%, 2/19), and one in Vão do Moleque (5%, 1/17). A common factor in the herds with the highest seroprevalences was identified: livestock-rearing in wetlands near streams and rivers. It is known that *T. gondii* infection in herbivores is more prevalent in humid areas, which favor sporulation conditions and maintain the viability of oocysts on vegetation (Dubey, 2010). Also, the herds in these zones were composed of up to 10 animals each. It should be noted that the size of the herd has already been associated as a risk factor for infection by *T. gondii* in cattle (Gilot-Fromont et al., 2009; Klun et al., 2006).

Only limited information was previously available on the factors associated with *T. gondii* infection in cattle (Gilot-Fromont et al., 2009). In the present study, the final model, which has the lowest AIC value (Table 3), was considered the best fit for explaining the data (Deng et al., 2020). In this sense, it was determined that herds of up to ten animals were thirty times more likely to be infected with *T. gondii* (OR 30.56) than were herds of more than one hundred cattle. Gilot-Fromont et al. (2009) and Klun et al. (2006) similarly described higher prevalence of anti-*T. gondii* antibodies in populations smaller than one hundred animals. In small herds, because the cattle are managed closer to home, direct contact between cats and these cattle's pastures and other sources of feed and water is favored, thereby enabling the spread of oocysts (Gilot-Fromont et al., 2009; Klun et al., 2006).

Regarding the age group, it was found that cattle up to 36 months of age (OR 1.84) were at higher risk of becoming infected with *T. gondii* than were individuals over 36 months. Similarly, Gilot-Fromont et al. (2009) found that there was a reduction in seroprevalence among cattle over 30 months of age. They also reported that the presence of cats on farms changed the relationship between age and prevalence: when cats were present, seroprevalence was high among young animals and decreased later; while the reverse was observed in the absence of domestic cats (Gilot-Fromont et al., 2009). It should be noted that, in the Kalunga community, the presence of domesticated cats was reported by the cattle owners. On the other hand, Opsteegh et al. (2011) observed that seroprevalence did not increase with increasing age among cattle over 12 months of age and suggested that antibodies would not persist for life in these ruminants (Opsteegh et al., 2011). However, we considered it to be prudent to test this hypothesis through long-term individual monitoring.

Cattle purchase was also associated with exposure to *T. gondii*. In general, purchases of animals increased the risk of infection (OR 2.57), compared with herds in which no purchases were made. It is noteworthy that the serological condition of the acquired cattle was unknown to the purchasers. According to Oliveira et al. (2018), when buffaloes of unknown health status were purchased, movement of infected individuals between herds was promoted. In the Kalunga community, cattle trading among its residents predominated (Baiocchi, 2013), and this transit of seropositive animals reinforced the presence of this coccidian in and around the Kalunga area.

Conclusion

In the present study, for the first time, occurrences of *T. gondii* infection among cattle in all regions of the Kalunga quilombo community were documented and provides a warning of public health risks. Given that the risk factors are of environmental nature and difficult to control, health education, therefore, becomes important to control and prevent *T. gondii* infection. In this sense, attention should be focus to the consumption of undercooked beef, giving preference to beef subjected to cooking processes. The evaluation of cattle before the acquisition by serology for *T. gondii* could prevent the introduction of seropositive cattle in the herd. Mainly for smaller herds, raised close to homes, limiting domestic felines' access to water and livestock feed can reduce the intake of oocysts by ruminants.

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