Seroepidemiology of *Toxoplasma gondii* in domestic cattle, sheep, goats and pigs from São Tomé and Príncipe

Soroepidemiologia de Toxoplasma gondii em bovinos, ovinos, caprinos e suínos domésticos de São Tomé e Príncipe

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Abstract

Despite the global importance of the zoonotic parasite *Toxoplasma gondii*, little is known regarding its infection in the Democratic Republic of São Tomé and Príncipe (DRSTP). This is the first report of antibodies to *T. gondii* in cattle, sheep, goats and pigs from the DRSTP. Antibodies were assessed by the modified agglutination test (MAT), with a cut-off titer of 100 for cattle and 20 for sheep, goats and pigs. The present study revealed an overall seroprevalence of 55.8%; 27.1% in 48 cattle, 68.4% in 98 sheep, 70.1% in 97 goats and 43.7% in 103 pigs. The south geographical area for cattle, the central area for sheep, and adult age and living in the central region for goats were found to be risk factors for seropositivity to *T. gondii*. These results support the scenario of a considerable presence of sporulated oocysts as well as of infected intermediate hosts in the local environment. Consumption of raw or undercooked meat should be considered as an important potential source of infection for animals and humans in the DRSTP.

Keywords: Livestock, modified agglutination test, seroprevalence, toxoplasmosis.

Resumo

Apesar da importância global do parasita zoonótico *Toxoplasma gondii*, pouco se conhece sobre sua infecção na República Democrática de São Tomé e Príncipe (RDSTP). Esse é o primeiro relato de anticorpos para *T. gondii* em bovinos, ovinos, caprinos e suínos da RDSTP. Os anticorpos foram pesquisados pelo teste de aglutinação direta modificada (TADM), com um título de corte de 100 para bovinos e de 20 para ovinos, caprinos e suínos. O presente estudo revelou uma soroprevalência global de 55,8%: 27,1% em 48 bovinos, 68,4% em 98 ovinos, 70,1% em 97 caprinos e 43,7% em 103 suínos. A área geográfica sul para os bovinos, a área central para os ovinos, bem como a idade adulta e a região central para os caprinos foram considerados fatores de risco para soropositividade a *T. gondii*. Esses resultados suportam o cenário de uma considerável presença de oocistos esporulados, bem como de hospedeiros intermediários infectados no ambiente local.

O consumo de carne crua ou mal passada deve ser considerado como uma importante fonte potencial de infecção para animais e seres humanos na RDSTP.

Palavras-chave: Pecuária, teste de aglutinação direta modificada, soroprevalência, toxoplasmose.

Introduction

Toxoplasma gondii, a protozoan parasite well known in homeothermic animals, is estimated to infect one-third of the world's human population (Dubey, 2010; Halonen & Weiss, 2013). The seroprevalence of *T. gondii* varies according to the lifestyle of definitive hosts, such as the domestic cat and other felines, and intermediated hosts, including people (Defeo et al., 2002).

Among food animals, *T. gondii* has been found more prevalent in sheep, goats and pigs, than in cattle. This parasite is a major cause of infectious abortion and neonatal mortality in sheep, as well as in goats (Dubey, 2010). *Toxoplasma gondii* also causes serious illness in congenitally infected children. In addition, it is a pathogenic agent with tropism to the central nervous system, which can cause encephalitis with severe sequelae and systemic infections in immunodepressed humans (Halonen & Weiss, 2013).

Despite its global importance, little is known regarding *T. gondii* in the Democratic Republic of São Tomé and Príncipe (DRSTP). Previous reports have documented prevalence of *T. gondii* antibodies in children aged 1-5 years (21.5%), in primary school students (63.1%) and pregnant women (75.2%) in the DRSTP (Fan et al., 2006, 2012; Hung et al., 2007). Due to the shared environment of several animal species in the DRSTP, it is essential to complement and update the epidemiological status of this protozoal infection in definitive and intermediated hosts (Tenter et al., 2000).

The present study aimed at estimating the seroprevalence of *T. gondii* in cattle, sheep, goats and pigs from São Tomé, in the DRSTP, as well as at assessing the main risk factors associated with the presence of specific antibodies.

Materials and Methods

Geographical area of the study

The present study was conducted on the island of São Tomé, which is part of the DRSTP, an insular state located near the Equator line in the Gulf of Guinea and distancing 300 km from the West African coast (Figure 1). This archipelago consists of two main islands, São Tomé and Príncipe, and some adjacent islets, all totalizing an area of 1001 km^2, which makes the DRSTP one of the smallest African countries, second only to the Seychelles. According to the United Nations Population Fund (UNFPA), the country had 200,000 inhabitants in 2017 (INE, 2017).
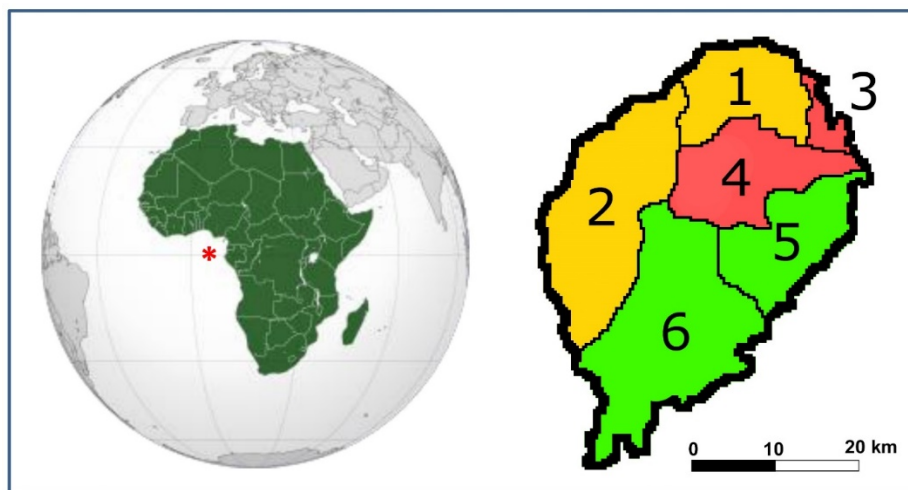
Figure 1. Location of the Democratic Republic of São Tomé and Príncipe (asterisk) in Africa. Geographical areas of São Tomé island: north (yellow), center (red), south (green); and districts: Lobata (1), Lembá (2), São Tomé (3), Mé-Zóxi (4), Cantagalo (5), Caué (6). Source: Images adapted from Wikipedia (2017).

The climate is tropical, hot and humid throughout the year, with average temperatures of around 27 °C and little daily variation. Rain fall is more abundant in the southern part of São Tomé island and scarcer in the northern part of the same island, where the capital city (São Tomé) is located. The population consists mainly of descendants of native West African individuals miscegenated with European immigrants. One third of the inhabitants live in São Tomé city and its outskirts, but many people still live in dispersed settlements. More than 40% of the population is less than 15 years of age, and another 25% is younger than 30. Life expectancy in the early 21st century was more than 65 years of age, a relatively high value for an African country and close to the world average. The main crop of São Tomé is cocoa, representing about 95% of agricultural exports. Other export crops include copra, palm kernels and coffee. The DRSTP is one the most stable and democratic countries of Africa (Costa et al., 2014; INE, 2017).

Cats, both domestic and stray, are abundant on São Tomé island and due to the tropical climatic conditions, suitable for sporulation of *T. gondii* oocysts, it is likely that exposure to sporulated oocysts is one of the most important factors associated with seropositivity to the protozoan in people (Fan et al., 2006; Hung et al., 2007). Furthermore, almost 80% of primary schoolchildren (Fan et al., 2012) and around 85% of pregnant women (Hung et al., 2007) drink unboiled water, which may be contaminated with oocysts.

Animals and samples

From August 2017 to October 2017, blood samples were obtained from 346 domestic animals, comprising cattle (n = 48), sheep (n = 98), goats (n = 97) and pigs (n = 103), from the northern (Lembá and Lobata districts), central (Água Grande and Mé-Zoxi) and southern (Cantagalo and Caué) geographical areas of São Tomé island (Figure 1). All sampled animals were raised in São Tomé and were intended for human consumption.

In addition to the geographical characterization, data also included gender (female or male) and age (juvenile or adult) for all species tested and breed (mixed or defined) for cattle and pigs. Animals were classified as adult if they were ≥ 18 months for cattle, ≥ 12 months for sheep, ≥ 12 months for goats and ≥ 8 months for pigs. Defined breeds

comprised Nellore for cattle and Large White for pigs. All cattle, sheep, goats and pigs sampled came from farms where cats were present.

Blood was collected by venipuncture of manually restrained cattle, sheep and goats on farms and from pigs bled at the Central Slaughterhouse of São Tomé. After centrifugation of clotted blood, sera were separated and stored at -20 °C until serological testing at the Laboratory of Parasitology of the University of Trás-os-Montes e Alto Douro (UTAD).

Owners provided their informed consent for the inclusion of their animals in the study, which had previously been approved by the Directorate for Livestock of the Ministry of Agriculture, Fisheries and Rural Development of the DRSTP.

Serological testing

Serum samples were tested for immunoglobulin G antibodies to *T. gondii* by the modified agglutination test (MAT) using a commercial kit (Toxo-Screen DA®, bioMérieux, Lyon, France). Sera from sheep, goats and pigs were diluted at 1:20, 1:400, 1:1600 and 1:6400; and bovine sera at 1:100, 1:400, 1:1600 and 1:6400. Positive and negative controls provided with the kit were included on each testing plate. The results obtained with the MAT were expressed as an antibody titer, i.e. the reciprocal of the highest dilution at which agglutination (at least half the well diameter) is still visible after 18 h incubation at room temperature. The commercial MAT is the same as used by others (Dubey & Desmonts, 1987).

Cut-off titers of 20 for sheep, goats (Sousa et al., 2009; Lopes et al., 2013) and pigs (Dubey et al., 1995; Lopes et al., 2013) and of 100 for cattle (Dubey & Jones, 2008; Lopes et al., 2013; Jokelainen et al., 2017) were chosen to maximize sensitivity and specificity of the test. The MAT is considered the most reliable, sensitive and specific test for the detection of antibodies to *T. gondii* in various hosts and does not require species-specific reagents (Desmonts & Remington, 1980; Dubey & Desmonts, 1987; Dubey et al., 1995).

Data analysis

Assuming a default 50% seroprevalence value, a 95% confidence level and a 10% absolute error, at least 97 animals from each species were calculated to include in this study. Exceptionally for cattle, the convenient sample of 48 animals corresponds to an absolute error of approximately 14% (Thrusfield & Christley, 2018). The chi-square or Fisher exact tests were used to compare seroprevalence values. Independent variables with significant difference between categories (probability [p] value < 0.05) were selected for multiple logistic regression analysis to identify independent risk factors for seropositivity by calculating odds ratios (OR) and their 95% CI (Petrie & Watson, 2013). Statistical analyses were done with IBMS SPSS Statistics 26.0® software.

Results

Analysis of the 346 samples revealed an overall seroprevalence to *T. gondii* of 55.8% (193/346; 95% CI: 50.4−61.1). Antibodies to *T. gondii* were found in 13 (27.1%) of the 48 cattle (Table 1): one had a titer of 100, four a titer of 400, four a titer of 1600 and four a titer ≥ 6400. Regarding sheep (Table 2), antibodies were found in 67 (68.4%) of the 98 animals: a titer of 20 in three, a titer of 400 in one, a titer of 1600 in six and a titer of ≥ 6400 in 57. Sixty-eight (70.1%) out of the 97 goats were seropositive (Table 3): three had a titer of 400, 11 a titer of 1600 and 53 a titer ≥ 6400. Antibodies to *T. gondii* were found in 45 (43.7%) of the 103 pigs (Table 4): eight had a titer of 20, 10 a titer of 400, 15 a titer of 1600 and 12 a titer ≥ 6400. In pairwise comparison, statistically significant

differences ($p < 0.001$) were found between sheep and pigs, sheep and cattle, goats and pigs, and goats and cattle.

Table 1. Seroprevalence of *Toxoplasma gondii* infection in cattle from São Tomé island according to gender, age group, breed and geographical area.

	Animals tested (n)	Relative distribution (%)	MAT-positive (n)	Prevalence (%)	95% CI
Gender					
Female	45	93.8	11	24.4	12.9-39.5
Male	3	6.2	2	66.7	9.4-99.2
				$p = 0.174$	
Age group					
Juvenile	12	25.0	5	41.7	15.2-72.3
Adult	36	75.0	8	22.2	10.1-39.1
				$p = 0.263$	
Breed					
Mixed	46	95.8	13	28.3	16.0-43.5
Defined	2	4.2	0	0.0	0.0-84.2
				$p = 1.000$	
Geographical area					
North	30	62.5	3	10.0[a]	2.1-26.5
Center	4	8.3	0	0.0	0.0-60.2
South	14	29.2	10	71.4[a] p = NA	41.9-91.6
Total	48	13.9	13	27.1	15.3-41.8

Bonferroni's correction (i.e. multiplying each *p* value by 3) has been incorporated: [a]*p* = 0.003 (only statistically significant differences are shown for pairwise comparisons of the geographical area category). CI = confidence interval; MAT = modified agglutination test; NA = not accounted (more than 20% of cells with expected counts less than 5).

Table 2. Seroprevalence of *Toxoplasma gondii* infection in sheep from São Tomé island according to gender, age group and geographical area.

	Animals tested (n)	Relative distribution (%)	MAT-positive (n)	Prevalence (%)	95% CI
Gender					
Female	74	75.5	59	79.7	68.8-88.2
Male	24	24.5	8	33.3	15.6-55.3
				$p < 0.001$	
Age group					
Juvenile	19	19.4	7	36.8	16.3-61.6
Adult	79	80.6	60	75.9	65.0-84.9
				$p = 0.003$	
Geographical area					
North	52	53.1	30	57.7[a]	43.2- 1.3
Center	33	33.7	31	93.9[a,b]	79.8-99.3
South	13	13.3	6	46.2[b]	19.2-74.9
				$p = 0.001$	
Total	98	28.3	67	68.4	58.2-77.4

Bonferroni's correction (i.e. multiplying each *p* value by 3) has been incorporated: [a]*p* = 0.003; [b]*p* = 0.003; (only statistically significant differences are shown for pairwise comparisons of the geographical area). CI = confidence interval; MAT = modified agglutination test.

Table 3. Seroprevalence of *Toxoplasma gondii* infection in goats from São Tomé island according to gender, age group, breed and geographical area.

	Animals tested (n)	Relative distribution (%)	MAT-positive (n)	Prevalence (%)	95% CI
Gender					
Female	67	69.1	51	76.1	64.1-85.7
Male	30	30.9	17	56.7	37.4-74.5
				$p = 0.090$	
Age group					
Juvenile	23	23.7	8	34.8	16.4-57.3
Adult	74	76.3	60	81.1	70.3-89.2
				$p < 0.001$	
Geographical area					
North	43	44.3	24	55.8[a]	39.9-70.9
Center	27	27.8	24	88.9[a]	70.8-97.6
South	27	27.8	20	74.1	53.7-88.9
				$p < 0,001$	
Total	97	29.8	68	70.1	60.0-79.0

Bonferroni's correction (i.e. multiplying each *p* value by 3) has been incorporated: [a]*p* = 0.024 (only statistically significant differences are shown for pairwise comparisons of the geographical area category). CI = confidence interval; MAT = modified agglutination test.

Table 4. Seroprevalence of *Toxoplasma gondii* infection in pigs from São Tomé island according to gender, age group, breed and geographical area.

	Animals tested (n)	Relative distribution (%)	MAT-positive (n)	Prevalence (%)	95% CI
Gender					
Female	50	48.5	21	42.0	28.2-56.8
Male	53	51.5	24	45.3	31.6-59.5
				$p = 0.891$	
Age group					
Juvenile	25	24.3	15	60.0	38.7-78.9
Adult	78	75.7	30	38.5	27.7-50.2
				$p = 0.097$	
Breed					
Mixed	90	87.4	37	41.1	30.8-52.0
Defined	13	12.6	8	61.5	31.6-86.1
				$p = 0.276$	
Geographical area					
North	42	40.8	19	45.2	29.8-61.3
Center	20	19.4	10	50.0	27.2-72.8
South	41	39.8	16	39.0	24.2-55.5
				$p = 0.695$	
Total	103	29.8	45	43.7	33.9-53.8

CI = confidence interval; MAT = modified agglutination test.

Of the 193 seropositive animals, 6.7% (13/193) were cattle, 34.7% (67/193) were sheep, 35.2% (68/193) were goats and 23.3% (45/193) were swine. Of these 193 seropositive animals, 65.3% (126/193) had a titer equal to or greater than 6400.

Regarding risk factors (Table 5), cattle from the south of the island had a significantly higher seroprevalence, with a 22.5 fold higher risk of positivity (OR = 22.5; 95% CI: 4.3-118.8). Sheep from the central geographical were 11.4 times more likely to be exposed (OR = 11.4, 95% CI: 2.5-52.6) than the northern sheep. The risk factors for *T. gondii* infection in goats, in descending order, were the adult age of animals (OR = 6.7, 95% CI: 2.3-19.6) and living in the central area (OR = 4.4, 95% CI: 1.1-18.0). Gender and age group of sheep and age of goats were not confirmed as risk factors by multiple logistic regression.

Table 5. Identification of risk factors for *Toxoplasma gondii* infection in domestic animals from São Tomé island by multiple logistic regression.

	Seroprevalence (%)	OR	95% CI
Cattle (n = 48)			
Geographical area			
North	10.0	1 (Ref.)	
Center	0.0	0.0 (p = 0.999)	0.0-0.0
South	71.4	22.5 (p < 0.001)	4.3-118.8
Sheep (n = 98)			
Geographical area			
North	57.7	1 (Ref.)	
Center	93.9	11.4 (p = 0.002)	2.5-52.6
South	46.2	0.6 (p = 0.456)	0.2-2.1
Goats (n = 97)			
Age group			
Juvenile	34.8	1 (Ref.)	
Adult	81.1	6.7 (p = 0.001)	2.3-19.6
Geographical area			
North	55.8	1 (Ref.)	
Center	88.9	4.4 (p = 0.040)	1.1-18.0
South	74.1	2.3 (p = 0.158)	0.7-7.2

CI = confidence interval; OR = odds ratio; Ref. = reference category.

Discussion

The present study revealed an overall seroprevalence of *T. gondii* of 55.8%, which supports the scenario of a considerable presence of sporulated oocysts as well as of infected intermediate hosts in the local environment. This is the first report on antibodies to *T. gondii* in cattle, sheep, goats and pigs from the DRSTP.

Goats had the highest recorded species seroprevalence (70.1%) followed by sheep (68.4%), pigs (43.7%) and cattle (27.1%). Literature generally indicates that the prevalence is higher in sheep than in goats (Hashemi-Fesharki, 1996; Sharif et al., 2007; Chikweto et al., 2011; Lopes et al., 2013), due to the selection of food. In fact, goats usually opt for taller leaves of shrubs, while sheep are pastoralists, which tend to eat grasses and clovers closer to the ground and, therefore, are more likely to contact with sporulated oocysts (Hamilton et al., 2014). The higher seroprevalence observed in small ruminants in the present work could also be due to the extensive system put into practice on the studied farms. Additionally, since the climatic conditions of the DRSTP are adequate for the sporulation of *T. gondii* oocysts, due to the hot and humid climate (Rahimi et al., 2015), it seems likely that exposure to water and food contaminated with *T. gondii* oocysts may be one of the most important factors associated with seropositivity to the protozoan in this country (Fan et al., 2012).

Animals that feed on forage, such as cattle and small ruminants, are more susceptible to *T. gondii* due to contact with the soil, which may explain the higher seroprevalence observed in small ruminants compared with swine (Hamilton et al., 2014). Pigs in the present study, fed barley bark, breadfruit, bananas, jackfruit, taro and vegetables, had an above-expected seroprevalence of 43.7%. A diet rich in raw vegetables and unwashed fruits may justify the higher prevalence observed in pigs in the present study, as well as in humans from the DRSTP in a previous report (Hung et al., 2007).

In the present work, the lowest seroprevalence was observed in cattle with 27.1%. Although cattle can be infected with *T. gondii*, infection rarely results in clinical illness, including abortion (Innes, 1997). Attempts at isolation of *T. gondii* in experimentally- or naturally-infected cattle suggest that *T. gondii* does not persist for long in bovine tissues (Dubey, 1986, 2010), a circumstance which is unlike in sheep and humans (Innes, 1997). The reasons for such variation in host susceptibility to *T. gondii* are largely unknown. Although greater resistance to the parasite has been reported in cattle (Pita Gondim et al., 1999; Sharif et al., 2007), in the present study more than 25% were seropositive.

There is a considerable debate concerning the role of beef in the epidemiology of *T. gondii* transmission to humans. The ingestion of beef has been epidemiologically linked to *T. gondii* infection in humans (Opsteegh et al., 2011) and, especially taking in consideration a country's existing eating habits, beef is not a negligible source of *T. gondii* infection for humans (Belluco et al., 2018); yet viable parasite has rarely been isolated from naturally infected cattle (Dubey, 2010). There is no agreement with respect to the cut-off titer for MAT for the detection of *T. gondii* antibodies in cattle. Using a cut-off titer of 100, as in the present study, *T. gondii* antibodies were found in 18.6% (743 of 3991) of cattle in Estonia (Jokelainen et al., 2017). Compared with the study from Estonia, the prevalence of *T. gondii* antibodies in Algeria was just 4.4% (13 of 295; cut-off titer of 25) and only two animals were positive at a titer of 100 (Khames et al., 2018). In our study, *T. gondii* antibodies were found in 27.1% (13 of 48) and nine had a titer of 400 or higher. Although our sample size is small, this is the highest level of *T. gondii* antibodies reported in cattle worldwide (Dubey, 2010).

Additional data recorded in Nigeria revealed seroprevalence values of 6.7% in sheep, 4.6% in goats (Kamani et al., 2010) and 29.1% in pigs (Onyiche & Ademola, 2015). For small ruminants, the different results obtained in northeastern Nigeria (Borno state) and the DRSTP may be due to differences in climate between these two geographical areas, since Borno is characterized by higher temperatures with a low relative humidity, which varies between 13% in the driest months and 70-80% in July and August (Kamani et al., 2010). In the DRSTP, the average temperature of the rainforest is around 26 °C, with around 75% of air relative humidity in the highest altitude areas, where it rains even in the so-called dry season (Costa et al., 2014).

Compared with beef, pork is more likely to be infected with *T. gondii*, due to the high susceptibility of pigs to infection (Dubey, 2009; Hill & Dubey, 2013). Tissue cysts may persist in pork over a long period of time, with *T. gondii* being identified as one of the most relevant biological risks in the context of meat inspection of pigs (EFSA, 2017). There is no testing of pork for *T. gondii* at slaughter anywhere in the world (Dorny et al., 2009; Blagojevic & Antic, 2014; Herrero et al., 2016). In Italy, a literature data-based risk assessment model estimated that beef plays a more important role in human *T. gondii* infections than pork (0.034% versus 0.019%; excluding pork cured products), due to different cooking habits, even when cattle have a lower seroprevalence of the parasite (Belluco et al., 2018).

In goats, adult age group was identified as a risk factor for seropositivity to *T. gondii*, with adult goats 5.2 times more likely to be exposed than young animals. These results are probably related to oral transmission, with older animals being exposed to the infective forms of the protozoan for longer periods of time (Lundén et al., 1994).

In the present work, seroprevalence according to geographical area was 45.5% in the north, 76.5% in the center and 54.7% in the south. Depending on the species studied, a higher seroprevalence was observed in sheep, goats and pigs from the central area, and in cattle from the southern one, with statistically significant differences detected in ruminants. Cattle from the south presented a 22.5 times higher risk of seropositivity than cattle living in the northern area. Likewise, sheep and goats from the central area were more likely to be seropositive in comparison with their northern counterparts. These figures may be a reflection of more visible poverty markers in the south of the country (Silva, 2014). Local potential sources of transmission include the quality of water supplied, hygienic management as well as contact with domestic cats (Tenter et al., 2000; Hung et al., 2007; Fan et al., 2012).

As stated earlier, there is limited data on *T. gondii* in humans in the DRSTP. Antibodies to *T. gondii* were reported in 21.5% of 121 pre-school children (Fan et al., 2006), in 63.1% of 255 students attending primary school (Fan et al., 2012) and in 75.2% of 499 pregnant women (Hung et al., 2007), but these surveys are more than a decade old. The seroprevalence values observed are higher than the ones reported in countries geographically close to the DRSTP, such as Nigeria, with 32.0% (Ohiolei & Isaac, 2016), Gabon, with 56.0% (Mickoto et al., 2010), and Cameroon, with 70.0% (Njunda et al., 2011). In addition to the climatic variations, there are still sociocultural and dietary differences, which may explain such prevalence variations (Fan et al., 2006; Njunda et al., 2011; Rahimi et al., 2015; Wam et al., 2016). Access to food depends on family income. Meat and fish represent a large part of the total household expenditure, so that none of the families surveyed by Silva (2014) consumed beef because it is the most expensive and less available food in the DRSTP. Chevon and lamb are also of high cost and difficult access for the families of São Tomé. The most consumed product of animal origin is fish, followed by chicken and giant land snails (Silva, 2014). The DRSTP is often a tourist destination. In the present study, a high seroprevalence of *T. gondii* in food animals was found on São Tomé island. Risk groups should avoid eating undercooked meat, raw vegetables and unwashed fruits and also avoid direct contact with soil.

Conclusions

Seropositivity to *T. gondii* occurs in domestic animals of São Tomé and, although no clinical cases of toxoplasmosis have been reported in any animal species, this parasite should be considered in the list of differential diagnoses in reproductive, neurological or other pathological conditions. The source of *T. gondii* infection in the DRSTP may be associated with exposure to cat feces and contact with soil or water contaminated by oocysts, leading to a higher probability of parasite ingestion in raw foods such as vegetables and fruits and contaminated water or soil. This is the first study on the prevalence of antibodies to *T. gondii* in domestic animals of the DRSTP. The results will be of interest to biologists, parasitologists, and public health workers.

Acknowledgements

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