Prevalence and risk factors associated with anti-*Toxoplasma gondii* antibodies in sheep from Bahia state, Brazil

Prevalência e fatores de risco associados a anticorpos anti- *Toxoplasma gondii* em ovinos da Bahia, Brasil

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Abstract

In this study, we aimed to determine the prevalence of *Toxoplasma gondii* antibodies and identify risk factors associated with this infection in sheep from the southern region of Bahia state. Between February and December 2010, 795 sheep from 31 farms located in nine municipalities were tested. We found seroprevalence of 30.2% (240/795), with titers of 64 (38.3%), 256 (34.2%), 1,024 (18.3%), and 4,096 (9.2%) by Indirect Fluorescent Antibody Test (IFAT). Seropositive sheep were detected in all farms sampled. Univariate statistical analysis detected association between *T. gondii* seropositivity and the variables age, use of fresh food mainly, water source, stocking rate, production system, presence and number of cats on the farm, and transit of cats ($p < 0.05$). In the logistic regression model, transit of cats ($p = 0.001$), production system ($p = 0.007$), and age ($p = 0.027$) were identified as risk factors associated with *T. gondii* infection.

Keywords: Epidemiology, ovine, toxoplasmosis, zoonosis, risk factors.

Resumo

Objetivou-se com este estudo determinar a prevalência de anticorpos anti-*Toxoplasma gondii* e identificar os fatores de risco associados à infecção em ovinos no sudeste do Estado da Bahia. De fevereiro a dezembro de 2010, 795 ovinos de 31 propriedades localizadas em nove municípios foram analisados. A soroprevalência foi de 30,2% (240/795), com títulos de 64 (38,3%), 256 (34,2%), 1.024 (18,3%) e 4.096 (9,2%) pela Reação de Imunofluorescência Indireta (RIFI). Ovinos positivos foram detectados em todas as fazendas estudadas. Na análise estatística univariada detectou-se associação entre a soropositividade e idade, uso de alimentação fresca, fonte de água, sistema de produção, presença e número de gatos na fazenda e o transito de gatos ($p < 0,05$). No modelo de regressão logística, transito de gatos ($p = 0,001$), sistema de produção ($p = 0,007$) e idade ($p = 0,027$) foram identificados como fatores de risco associados à infecção por *T. gondii*.

Palavras-chaves: Epidemiologia, ovino, toxoplasmose, zoonose, fatores de risco.

Introduction

Toxoplasmosis is an important parasitic zoonosis of cosmopolitan distribution caused by the protozoan *Toxoplasma gondii*. Humans can be infected mainly through the ingestion of cysts in contaminated food (consumption of undercooked or raw meat) or sporulated oocysts in water (FERGUNSON, 2009). Cats play a fundamental role in spreading this agent because they are the definitive hosts (DUBEY; JONES, 2008; DUBEY, 2009).

The sheep industry is an economic activity in operation in every continent with different climates. The current Brazilian sheep flock is of approximately 16 million animals and 58.4% of these are in the northeastern region of the country. The state of Bahia is the second largest domestic sheep producer with 3,028,507 animals (BRASIL, 2009). *Toxoplasma gondii* is an important parasite of sheep, causing reproductive disorders such as embryonic death, abortion, stillbirth, and birth of weak animals (MASALA et al., 2003; DUBEY; JONES, 2008).

Antibodies to *T. gondii* have been found in sheep worldwide and seroprevalence rate ranges from 6.7% to 84.5% (KAMANI et al., 2010; KLUN et al., 2006). The seroprevalence of *T. gondii* infection in flocks of sheep in Brazil ranges from 7.0% to 54.6% (MOURA et al., 2007; OGAWA et al., 2003). The only study in Bahia state with sheep was carried out in the metropolitan region of Salvador and Recôncavo, with prevalence of 18.75% (GONDIM et al., 1999).

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Risk factors such as age, contact with cats, breeding system, and water supply can be highlighted and have been previously identified in ovine toxoplasmosis in Brazil (ROMANELLI et al., 2007; PINHEIRO et al., 2009; LOPES et al., 2010; ROSSI et al., 2011). Considering the importance of toxoplasmosis and the lack of epidemiological information in the state of Bahia, the aims of this study were to determine the prevalence of *T. gondii* infection in sheep flocks from southern Bahia state and identify possible risk factors associated with the infection.

Materials and Methods

Study population and blood collection

The study was conducted from February to December 2010; it involved 795 animals from 31 farms, which are distributed in nine municipalities and enrolled in the Cooperative of Sheep and Goat Producers of Southern Bahia (COOPERVINO). A sample size of 650 animals was estimated by the statistical program EPI-INFO, version 3.5.1, considering a prevalence prospect of 50%, with sampling error of 5% and confidence level of 99% for a population of 33,000 animals (BRASIL, 2009); however, a sample size larger than required was chosen.

The herd was primarily composed of animals of the Santa Inês breed or crossbreds. The age of the animals was estimated by monitoring dentition, and the animals were categorized in two age groups based on Carneiro et al. (2009): above and below 36 months. No blood samples were collected from lambs under six months of age due to the possible presence of colostral antibodies.

Blood was collected by jugular vein puncture and the serum was separated after centrifugation and stored at –20 °C until use. The proposed study was approved by the ethics committee (CEUA-UESC 031/09).

Questionnaire

In order to determine risk factors, a structured interview with objective questions was conducted regarding the general characteristics of the farm, including the total area (≥ 100 hectares or <100 hectares); breeding system (extensive or intensive); production type (family or commercial); size of the herd (≥100 animals or <100 animals); stocking rate (≥1 animal or <1 animal); cats owned by farmers (yes or no); transit of stray cats or wild felids (yes or no); number of cats (<3 or ≥3 cats); water source (trough or river/dam); source of water/food common to young and adult animals (yes or no); presence of pen (yes or no); cats with access to the animals' food and/ or water storage source (yes or no); use of fresh food mainly - grass (yes or no); gender (male or female); age (<36 months or ≥36 months); and animal breed (purebred or crossbred animals).

Detection of antibodies

Indirect fluorescence antibody test (IFAT) was performed to study anti-*T. gondii* antibodies. In this study, IFAT slides containing antigens of the RH strain of *T. gondii* were used. Negative and positive control serum (1×10^6 tachyzoites of *T. gondii* experimentally inoculated in a six-month-old lamb) were added to each slide and anti-sheep antibody conjugated to fluorescein isothiocyanate (F-7634, Sigma-Chemical, USA) were used. The IFAT cut-off value was 1:64 (FIGLIUOLO et al., 2004) and the positive samples were subjected to sequential dilutions until negative response.

Statistical analysis

To identify risk factors associated with infection by *T. gondii*, a bivariate analysis was carried out using Chi-square and Fisher's exact tests with significance level of 5%, using the statistical program EPI-INFO, version 3.5.1. All variables with $p \le 0.2$ on bivariate analysis were subjected to collinearity analysis determined by the Spearman's rank correlation test according to BioEstat 5.0. Subsequently, multivariate logistic regression analysis was performed using EPIINFO, version 3.5.1.

Results

All farms used extensive breeding programs and no artificial breeding methods. *Toxoplasma gondii* antibodies were found in 240 (30.2%) sheep, with titers of 64 (38.3%), 256 (34.2%), 1,024 (18.3%), and 4,096 (9.2%). All farms presented *T. gondii*-positive animals, with seropositivity varying from 6.3% to 100%. Among the municipalities analyzed, Camacan presented the highest frequency of seropositive animals (64.7%) and Itaju do Colônia presented the lowest frequency (9.8%) (Table 1).

Table 1. Detection and distribution of anti-*Toxoplasma gondii* antibodies in sheep in some municipalities of the microregion Ilhéus–Itabuna, State of Bahia, Brazil.

Municipalities	Number of examined	Number of Positive	Prevalence (%)
Camacan	51	33	64.7
Ibicaraí	27	12	44.4
Itacaré	99	38	38.4
Ilhéus	179	60	33.5
Itapé	120	37	30.8
Canavieiras	159	38	23.9
Pau Brasil	73	12	16.4
Itabuna	26	4	15.4
Itaju do Colônia	61	6	9.8
Total	795	240	30.2

Risk factors identified by bivariate and multivariate analyses are shown in Tables 2 and 3, respectively. In the bivariate analysis, seropositivity was observed in association with age, diet based on fresh food, production system, number of cats, water source, stocking rate, and transit of stray cats or wild felids. In the multivariate analysis, age, production system, and transit of stray cats or wild felids were identified as risk factors in this study.

Regarding age, 32.6% of animals over 3 years old were identified as positive, while 25.2% of animals under 3 years old were positive (OR=1.43).

The number of cats was an important factor: farms with more than three cats presented more positive sheep (35.3%) than farms with one or two cats (27.8%) (p=0.03). The transit of cats or wild felids in farms was a determining factor, as evidenced by the

Table 2. Bivariate analysis of risk factors associated with the presence of *Toxoplasma gondii* in sheep flocks of southern Bahia.

Variables	Animals				χ^2	P value	OR	CI 95%
	Positive		Total					
	n	%	n	%				
Age group					4.17	0.041	1.43	1.02-2.00
≥03 years	175	72.9	537	67.5				
<03 years	65	27.1	258	32.5				
Breed					0.28	0.596	0.91	0.67-1.23
Purebred	128	53.3	411	51.7				
Crossbred	112	46.7	384	48.3				
Gender					0.03	0.870	0.93	0.57-1.51
Male	26	10.8	90	11.3				
Female	214	89.2	705	88.7				
Farmers have cats					1.68	0.195	1.29	0.90-1.86
Yes	190	79.2	604	76				
No	50	20.8	191	24				
Quantity of cats					3.52	0.030	1.41	1.00-1.99
≥03	104	54.7	295	48.8				
<03	86	45.3	309	51.2				
Cats with access to the water source					0.41	0.520	0.86	0.59-1.27
Yes	140	72.5	455	74.5				
No	53	27.5	156	25.5				
Transit of stray cats or wild felids						0.0001	2.14	1.56-2.94
Yes	104	43.3	250	31.4				
No	136	56.7	545	68.6				
Production system					5.34	0.020	1.62	1.09-2.42
Familiar	56	23.3	523	65.8				
Commercial	184	76.6	272	34.2				
Mostly fresh food					1.40	0.235	0.81	0.58-1.12
Yes	239	99.6	781	98.2				
No	1	0.4	14	1.8				
Presence of Pen					2.30	0.129	0.76	0.55-1.06
Yes	185	77.1	625	78.6				
No	55	22.9	170	21.4				
Stocking rate					3.61	0.057	0.72	0.52-0.99
<1	140	58.3	404	50.8				
≥1	100	41.7	391	49.2				
Water source					1.23	0.267	0.83	0.61-1.13
Trough	123	51.3	382	48.1				
River, dam	117	48.8	413	51.9				
Water source common*					0.01	0.001	1.05	0.58-1.88
Yes	223	92.9	737	92.7				
No	17	7.1	58	7.3				
Food source common*					0.01	0.001	1.05	0.58-1.88
Yes	223	92.9	737	92.7				
No	17	7.1	58	7.3				

*young and adult animals; % - percentage; χ^2 - Chi-square; OR - Odds ratio; CI - confidence interval.

Table 3. Multivariate analysis of risk factors associated with the presence of *Toxoplasma gondii* in sheep flocks of southern Bahia.

Variable	OR	CI 95%	*P* Value
Production system			
Family	1.00		
Commercial	1.50	1.02-2.21	0.0385
Transit of stray cats or wild felids			
Yes	2.18	1.58-3.01	0.0001
No	1.00		
Age group			
≥03 years	1.47	1.05-2.07	0.0267
<03 years	1.00		

Final Log-Likelihood: 941.5040; OR - Odds ratio; CI - confidence interval.

multivariate analysis, and increased the probability (OR=2.14) of sheep infection.

By the bivariate analysis, it was possible to observe that the commercial production system used in the farms presented higher seropositivity (OR=1.61) when compared to the family production system, which is possibly due to the higher ingestion of oocysts in a contaminated environment. The family production system was characterized by only the family taking care of the animals – subsistence, while the commercial production system has employees and aims at the commercialization of animals.

Discussion

Toxoplasma gondii prevalence found in this study is similar to that described by Ueno et al. (2009), in Federal District (38.2%) and by Pinheiro et al. (2009), in Pernambuco (32.9%); higher than that found by Soares et al. (2009), in Rio Grande do Norte (20.7%) and Langoni et al. (2011), in São Paulo (18.61%); and it lower than that found by Lopes et al. (2010), in São Paulo (52%) - all Brazilian states. All studies aforementioned used the IFAT for serology investigation. The differences observed may be due to climatic variations, age, type of animals and cut-off values used in the different studies.

All farms presented *T. gondii*-positive animals, showing that *T. gondii* is widely disseminated in sheep flocks in the studied region.

Among the municipalities studied, Camacan presented the highest frequency of *T. gondii*-positive animals (64.7%), and Itaju do Colônia (9.8%) showed the lowest frequency. Camacan and Itaju do Colônia belong to the same microregion; however, Camacan municipality has higher relative humidity (above 80% in average) due to the presence of the Atlantic Forest, which is used for cacao farming, thus favoring the maintenance of oocysts in the environment. In contrast, the municipality of Itaju de Colônia has replaced cacao farming by cattle raising and is no longer forested. It is also characterized by dry season (monthly rainfall of less than 60 mm) between the months of June and September, and higher average temperatures, which could explain the lower prevalence of *T. gondii*.

By multivariate statistical analysis, it was possible to observe that commercial production presented higher seropositivity (p = 0.03) compared to the family system. Commercial systems showed greater density of animals in an environment contaminated

with oocysts and, therefore, more infected animals. Caballero-Ortega et al. (2008) found that flocks with more than 320 animals had more sheep infected with *T. gondii* compared to flocks with fewer animals.

There was no significant association between *T. gondii* infection and gender, confirming the data found by Soares et al. (2009) and Pinheiro et al. (2009). This result differs from those reported by Ramzan et al. (2009) and Van Der Puije et al. (2000), who observed that females were more affected than males, which was probably due to the lower immunologic resistance of females in some periods of their lives.

With regard to age, Pinheiro et al. (2009) and Clementino et al. (2007) found results similar to those of this study. We found that seroprevalence increases with age, suggesting that most animals acquire post-natal infection and according to Dubey and Kirkbride (1989) animals over three years of age have longer exposure to sporulated oocysts and increased risk of infection.

Stray cats and wild felids are most commonly found in almost all areas where sheep are kept, thus explaining the higher seroprevalence (p ≤ 0.0001) in farms with these animals. Similar results were found by Vesco et al. (2007), Pinheiro et al. (2009), and Lopes et al. (2010). In the studied region, the presence of stray cats and wild felids is common due to the neighboring forest area for cocoa production.

Ingestion of oocysts from the environment and congenital transmission are the two main routes of *T. gondii* transmission in sheep. The proportion of prenatal and post-natal infection in these animals is often unknown, but epidemiological data indicate that most animals are infected after birth by horizontal transmission via ingestion of sporulated oocysts (BUXTON et al., 2006). Sporulated oocysts survive for long periods of time in favorable environmental conditions, such as humid soil (DUBEY, 2009), and this is the case of most of the municipalities studied.

The results showed the presence of *T. gondii* infection in sheep flocks in the microregion of Ilhéus–Itabuna, where mutton is increasingly popular as an animal protein source. As a result, it is a potential source for human toxoplasmosis. Control and prophylactic measures such as avoiding the presence of stray cats or wild felids in areas where sheep are raised, excluding this felids from where products used in sheep feed are stored, and decreasing the stocking rate of pastures should be adopted to improve the production system. Health programs for farmers, along with the dissemination of information about the means of transmission of this parasite, should be implemented.

Acknowledgements

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