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Occurrence and risk factors for *Toxoplasma gondii* infection in goats from micro-regions of the state of Piauí

Ocorrência e fatores de risco para infecção por *Toxoplasma gondii* em caprinos de microrregiões do Estado do Piauí

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Abstract

This study aimed to evaluate the seroprevalence and risk factors associated with *Toxoplasma gondii* infection in goats from micro-regions of the state of Piauí (Brazil). From six randomly selected properties, 145 goats were examined. Serum samples of the animals were analyzed by ELISA for the presence or absence of antibodies against *T. gondii*. Of these, 22% were seroreactive for *T. gondii*. Individual records were also taken for each property. These analyses showed that factors such as sex, age, water source, and presence of cats were associated with animal seropositivity. In addition, there was also a positive correlation ($p > 0.04$) between animal sex and *T. gondii* contamination. The presence of cats in the facilities and animal pasture fields appeared to be a risk factor for the contamination of goats in the assessed properties ($p > 0.03$). The parasite *Toxoplasma gondii* is present in the goat herds of southern Piauí state, and females are more likely to be contaminated.

Key words: Goats. Seroprevalence. Piauí. Toxoplasmosis.

Resumo

Este estudo teve como objetivo avaliar a soroprevalência e os fatores de risco associados a infecção por *Toxoplasma gondii* em caprinos de microrregiões do Estado do Piauí. Um total de 145 caprinos foram examinados, em seis propriedades selecionadas aleatoriamente. Amostras de soro dos caprinos foram analisadas por ELISA para investigar a presença ou ausência de anticorpos contra *T. gondii*. Assim, 22% dos caprinos foram sororeativos para *T. gondii*. Foram realizadas anotações individuais de cada propriedade, sendo observado que fatores como: sexo, idade, origem de água fornecida e presença de gatos foram associados à soropositividade encontrada nos animais, foi obtido correlação positiva ($p > 0,04$) entre o sexo dos animais e a contaminação por *T. gondii*. A presença de gatos nas instalações e ao pasto dos animais foi importante para contaminação dos caprinos nas propriedades analisadas ($p >$

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0,03). O *Toxoplasma gondii* está presente nos rebanhos caprinos do Sul do Estado do Piauí, sendo as fêmeas dos animais analisados, as mais susceptíveis ao parasito.

Palavras-chave: Caprinos. Piauí. Soroprevalência. Toxoplasmose.

Introduction

Goat rearing in northeastern Brazil has a major importance for the domestic economy, as it holds the largest herd of the country (91.4%) and makes use of its products and by-products (NOGUEIRA FILHO, 2003). The state of Piauí has the third largest herd of goats in this region (16%), surpassed only by the states of Bahia (34%) and Pernambuco (20%) (IBGE, 2008).

The first evidence of toxoplasmosis in goats was recorded by Feldman and Miller (1956), after examining herds in the state of New York (USA). Since then, various investigations have been carried out, including in Brazil.

In Brazil, the incidence of toxoplasmosis in goat herds is relevant. In south and southeast of the country, the rates average between 36.8% and 14.5% (MAINARDI et al., 2003; FIGLIUOLO et al., 2004). Whereas in northeastern states such as Bahia (UZÊDA et al., 2007), Rio Grande do Norte (LIMA et al., 2008), Ceará (CAVALCANTE et al., 2008), and Pernambuco (SILVA et al., 2003), the rates totaled 11.53%, 17.1%, 25.7%, and 10.33%, respectively. Despite these studies addressing the issue of toxoplasmosis in sheep and goats in these regions, there is still little data for the Northeast region as a whole, and especially for the state of Piauí.

Toxoplasma gondii is the causative agent of toxoplasmosis, a disease that is usually benign in immunocompetent individuals. However, it may be serious or fatal in children with congenital diseases or immunocompromised patients (DUBEY et al., 2012). Infection by this pathogen is commonly found in production animals such as sheep, goats, and pigs, leading to major economic losses through

stillbirths, miscarriages, and birth defects (UZÊDA et al., 2007; PEREIRA et al., 2012).

In addition, the transmission of *T. gondii* through food consumption is one of the most important routes of infection in humans (PEREIRA et al., 2010), which happens by intaking of cyst tissue fragments while handling or eating raw or poorly cooked meat. With the worldwide trend of large-scale meat production, *Toxoplasma* contamination has been spread currently to a larger area (JONES; DUBEY, 2012).

The consumption of *in nature* goat milk and its byproducts also poses risks of contamination for humans, with local cases of public health threats already reported (SIBLEY, 2003). The lack of studies and knowledge by breeders from the northeastern regions entails losses for themselves and hazards of consumption to local populations. In this context, our study is the first to correlate seroprevalence with possible risk factors for *T. gondii* infection and transmission among goat herds within the southern state of Piauí.

Thus, this study aimed to investigate *Toxoplasma gondii* occurrence in goats from farms in the south of the state of Piauí, collecting epidemiological data and correlating them to toxoplasmosis prevalence.

Material and Methods

Experiment location

The study was carried out in six goat breeding farms selected randomly. Among the evaluated properties, four are in towns within the micro-region of Alto-Médio Gurguéia, and two in municipalities within the micro-region of Chapada do Extremo Sul, all in the southern part of Piauí state, Brazil.

Animals

Data were cataloged in index cards and arranged by municipality for studies of toxoplasmosis prevalence in farms. Each card contained a questionnaire with information about the property, breeding system, and presence of pets.

Sample collection and storage

Blood samples of 3 mL were collected by jugular venous puncture from 145 goats, using 3-mL sterile syringes. The samples were stored in 5-mL sterile vacutainer tubes without anticoagulant kept at room temperature. Then, they were centrifuged at 2000 rpm for 10 minutes at room temperature, at the Laboratory of Clinical Pathology and Parasitology of the Federal University of Piauí (UFPI). The sera obtained were transferred to 1.5-mL Eppendorf tubes properly identified with the number of each animal and stored at -20°C. These were sent to the Immunology Laboratory at the Federal University of Uberlândia (UFU) for serological examination.

Enzyme-Linked Immunosorbent Assay (ELISA)

To detect IgG antibodies against *T. gondii*, an ELISA protocol was used, as previously described by Silva et al. (1997). The serum-conversion of goats was measured using polystyrene plates (INTERLAB, São Paulo, Brazil) previously covered with a *T. gondii* antigen solution (STAg), in sodium carbonate buffer (0.06 M, pH 9.6), similarly to the method by Mineo et al. (1980). After incubation for 18 hours at 4°C, wells were washed three times with PBST (PBS added to 0.01% Tween 20) and incubated with the serum samples from positive control animals, in duplicate, diluted 1:32 in PBST plus 0.5% non-fat dried milk (MOLICO – Nestlé) for 2 hours for TA. The wells were again washed with PBST and incubated with primary anti-goat antibody produced in goats (Abcam) for 1 hour for TA. Subsequently, the wells were washed and incubated with anti-goat antibody complexed with

peroxidase (R&D Systems) for 1 hour at TA. The reaction was followed by the addition of hydrogen peroxide (0.04%) and ortho-phenylenediamine (0.5mg/mL) diluted in phosphate-citrate buffer (0.1M, pH 5.0). After incubation for 15 minutes at room temperature, the reaction was stopped with H₂SO₄ (2N). Finally, the values of optical density (OD) were measured at 450nm in an ELISA reader (Titertek Multiskan Plus MKII, Flow Laboratories, McLean, USA). The levels of *anti-T. gondii* antibodies were arbitrarily expressed in ELISA index (EI), according to the formula: $EI = \text{Abs test sample} / \text{cutoff}$, where cutoff was calculated as mean OD values of negative control sera plus three standard deviations. Samples with an EI > 1.2 were considered positive for excluding positive reactivity close to EI = 1.0.

This study was developed complying with the guidelines for ethics and biosafety, and under the approval of the Ethics Committee on Animal Use (CEUA) of the Federal University of Piauí - UFPI (nº 0096/2010).

The results were subjected to Chi-square test and considered positive at ($p < 0.5$). The data were analyzed using the statistical software GraphPad Prism (GraphPad, La Jolla, CA, USA).

Results and Discussion

Among the 145 goat sera analyzed, 32 (22.07%) had reactive antibodies to *T. gondii*. Our data are consistent with those found in other Brazilian regions. In Pernambuco, Pereira et al. (2012) analyzed 167 sera samples of goats, of which 53 (31.8%) presented antibodies against the parasite. In Rio de Janeiro, Luciano et al. (2011) observed a total of 29.12% seropositive, while Mainardi et al. (2003) and Lima et al. (2008) found seroprevalence of 14.47% and 17.1%, respectively, in the state of São Paulo. In Ceará state, samples of goats, assessed by ELISA test, demonstrated that 25.1% of the goats were positive (CAVALCANTE et al., 2008). In our study, the flocks of the six evaluated

municipalities had animals contaminated by the parasite, demonstrating its dissemination throughout the south region of Piauí state. According to Table 1, the city that stood out with the highest number

of seropositive goats for *T. gondii* was Redenção do Gurguéia, followed by Currais, Bom Jesus, and Júlio Borges.

Table 1. Seropositive and seronegative samples for *T. gondii* of goats from the towns of Alto Médio Gurguéia and Chapada do Extremo Sul, in the southern state of Piauí (Brazil), during November and December 2011.

Town	Number of samples	ELISA	
		Reagent (%)	Non-Reagent (%)
Bom Jesus	28	8 (29)	20 (71)
Curimatá	29	1 (4)	28 (96)
Currais	12	5 (42)	7 (58)
Júlio Borges	29	6 (21)	23 (79)
Redenção do Gurguéia	17	11 (65)	6 (35)
Santa Luz	30	1 (3)	29 (97)

Upon analyzing the physical characteristics of goats, we observed a positive correlation ($p < 0.04$) between the sex of animals and contamination by *T. gondii* (Table 2). Interestingly, if compared to males, females had a higher number of positive antibodies, confirming the findings of Uzêda et al. (2007), who related the susceptibility of females to a possible immunosuppression during pregnancy and lactation. Reports in the literature correlate the age of goats to a prolonged exposure to parasites. Uzêda et al. (2007) described that in goats aged from 4 to 6 years have an increased serum-reactivity towards *T. gondii* if compared to younger animals. In the present study, the small age variation of animals (1 to 3 years) prevented us to correlate goat age with parasite contamination.

The water supplied to the flocks constituted a risk factor for the spread and contamination by *T. gondii*. Romanelli et al. (2007) and Luciano et al. (2011) found a higher infection rate in goats provided with water from weirs (46.11%), followed by those consuming water from wells (44.61%) and other water sources (24.22%). The contamination by these sources is probably due to the presence of

many seropositive animals (domestic or wild felids), which spread oocysts near these sources. However, our data showed that neither water from springs nor wells were risky for toxoplasmosis.

Cats are key hosts keys for the life cycle of *T. gondii*, being the only ones able to host the sexual form of the parasite (DUBEY et al., 1998). As expected, the presence of cats, with free access to the facilities and pasture, was relevant for the contamination of goats in the analyzed farms ($p < 0.03$). Other studies have already made this association (ROMANELLI et al., 2007; ARAUJO NETO et al., 2008; GARCIA, 2010). Therefore, it is a prominent risk factor for infection by *Toxoplasma* since oocytes in the feces of cats can remain infectious for several months to years in the environment depending on the environmental conditions (OGAWA et al., 2003). Given the importance of consumption of goat products and by-products in northeastern Brazil, our findings point to further risks of the spread of infections between goat flocks and/or to other animals within the region, but more importantly, warning for contamination of local people.

Table 2. Correlation between presence of antibodies against *T. gondii* with environmental and goat characteristics, in the southern state of Piauí, Brazil.

Variable		ELISA		Sera tested	Statistics
		Reagent (%)	Non-reagent (%)		
Sex	Male	3 (9.4%)	29 (87.6%)	32 (22.1%)	X²=3.847 p = 0.0498
	Female	29 (25.7%)	84 (74.3%)	113 (77.9%)	
	Total	32 (22.1%)	113 (77.9%)	145	
Age	From 1 to 2 years	12 (17.1%)	58 (82.9%)	70 (48.3%)	X ² =1.910 p = 0.1670
	From 1 to 3 years	20 (26.7%)	55 (73.3%)	75 (51.7%)	
	Total	32 (22.1 %)	113 (77.9%)	145	
Water source	Spring	5 (41.7%)	7 (58.3%)	12 (8.3%)	X ² = 2.922 p = 0.0874
	Artesian Well	27 (20.3%)	106 (79.7%)	133 (91.7%)	
	Total	32 (22.1 %)	133 (77.9%)	145	
Presence of cats	Presence	17 (37%)	29 (63%)	46 (31.7%)	X²=8.683 p = 0.032
	Absence	15 (15.2%)	84 (84.8%)	99 (68.3%)	
	Total	32 (22.1%)	113 (77.9%)	145	

Conclusion

Toxoplasma gondii is present in the goat herds located in the southern Piauí state, where the majority of cases was observed in the micro-region of Alto Medio Gurguéia. Goat females are more susceptible to infection than males. Cats contribute to the increasing incidence of *Toxoplasma gondii* in breeding animals. Adult animals are the most susceptible to toxoplasma infection.

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