Toxoplasmosis in Sheep: A Potential Risk of Infection Among Residents and Farm Workers in Lajes, Brazil

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Abstract: The *Toxoplasma gondii* protozoan is prevalent in most areas of the world, causing veterinary and medical impact. The aim of this study was to make a seroepidemiological report and identify risk factors for human toxoplasmosis among residents and workers of sheep farms in Lajes, Brazil. For diagnosis, an indirect haemagglutination test was applied; the seroprevalence was detected. An interview was conducted with each participant, obtaining information on cultural and hygiene habits, age and environmental variables suspected to affect the risk of *T. gondii*. The analysis for association with risk factors did not show significant differences. However, our dates suggest that sheep farmers are at increased risk for *Toxoplasma gondii*.

Keywords: *Toxoplasma gondii*, Sheep, Risk factors, Occupational disease, Human toxoplasmosis.

INTRODUCTION

Toxoplasma gondii is an obligate intracellular protozoan that belongs to the phylum Apicomplexa, subclass Coccidian, and can probably infect all warm-blooded animals (mammals and birds) and humans [1, 2]. Human beings can be infected with *T. gondii* by ingestion or handling of undercooked or raw meat containing tissue cysts (mainly sheep, goats and pigs) or water or food containing oocysts excreted in the faeces of infected cats [3]. Most individuals are infected inadvertently, thus the specific route of transmission cannot usually be established [4]. The seroprevalence estimates for human population vary greatly among different geographical areas within one country in accordance with cultural and hygiene habits, age and environmental factors, or other factors with epidemiologic impact [5, 6]. Thus, *T. gondii* may be transmitted from definitive to intermediate hosts, from intermediate to definitive hosts, as well as between definitive and between intermediate hosts [7-9]. Most cases of toxoplasmosis in immunocompetent humans are normally asymptomatic [8]. However, severe manifestations may occur in immunocompromised individuals [10, 11] or neonatal death as well as foetal abnormalities during pregnancy [12-14]. Moreover, only few studies have been aimed at identifying risk factors that may be associated with acquiring an infection from animals to humans. Our group, in a recent study, using an ELISA test, found that 29.41% of sheep flocks in Lajes city were seropositive [15]. Continuing an epidemiologic study of the possible animal source of infection to humans, the aim of the present study was to investigate seroprevalence in residents and workers on Sheep farms in Lajes and to identify risk factors associated with *T. gondii* seropositivity.

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MATERIALS AND METHODOLOGY

Area Description and Study Population

The area selected for sample collection was Lajes, a city located in the semi-arid region of Rio Grande do Norte state in Northeast Brazil, as it contains most of the sheep farms. Farm owners were contacted to request participation in a study of human exposure to *T. gondii* on sheep farms. Consent was obtained for 3 of the farms (LJ1, LJ2 and LJ3). Each farm was visited once during a month (October of 2006) for the purpose of obtaining human data. The subjects were included all adults (≥ 23 years of age) working or residing on the farm. An interview was conducted privately with each participant, in which information was obtained, including resident status, sex and age, handled raw meat, handled cats, consummation of raw or undercooked meat products (sheep), consummation of raw milk, eating unwashed raw vegetable or fruits, kitchen hygiene status, washing hands before eating and abortion occurrences (just for women). The procedure was approved by Ethical Committee from Federal University of Rio Grande do Norte – UFRN (CEP – 013/07). Blood samples were collected voluntarily from ten workers or residents. Approximately 5 ml of blood from a larger vein in forearm was collected. The blood samples were transported to Laboratory of Malaria Biology and Toxoplasmosis (UFRN), centrifuged (200 x g, 5 min), and serum collected, and finally stored at – 20^{o} C until analysed.

Serology

The *T. gondii*-specific IgG and IgM antibodies were detected by indirect haemagglutination test (IHA) (Toxotest kit®, Wiener Lab). Sera were serially two-fold diluted starting from 1:2 up to 1:2048 according to protocol. In parallel, all sera were treated with 2-mercaptoethanol to reduce IgM antibodies and tested. All sera reactivated at ≥ 1:16 were considered positive.

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Statistical Analysis

Risk factor were measured at categorical level, associations were examined in 2 x 2 contingency tables, 95% confidence intervals, and *P* values, using the Fisher test.

RESULTS

The presence of anti-*Toxoplasma gondii* antibodies of IgG class was found in 7 out of 10 examined person (70%) with titers ranging from 1:16 to 1:256. High specific antibody levels were most frequent at 1:32 (42.8%), whereas 14.3% were positive at 1:256 (Table **1**). None of the specific IgM antibodies were detected.

Table 1. IgG Anti-*Toxoplasma gondii* Antibodies Titers in Sera of Residents and Works of Sheep Farms in Lajes, Brazil, by an Indirect Haemagglutination Test (IHA)

Titers	Positivity	
	Nº of Individuals	%
1:16	1	14.3
1:32	3	42.8
1:64	2	28.6
1:256	1	14.3
Total	7	100,0

The results of the analysis for association of the environmental and cultural habits categorical risk factors with *T. gondii* seropositivity did not show significant differences. In relation to sex, no significant differences could be observed. Only one risk factor had a tendency to association, however, statistic differences were not found. The seroprevalence associated with age categories showed that the group ranged from 23 to 32 years was lower than the group ranged from 53-63 years (Table **2**).

DISCUSSION

The present work investigates the prevalence of toxoplasmosis in humans and the degree of its possible correlation, having contacts with sources of infection on an endemic area of ovine toxoplasmosis. This study is the first report showing an epidemiologic investigation of risk factor associated to human toxoplasmosis in residents and workers of sheep farms in Lajes. The percentage of seropositivity for *T. gondii* was 70%, with moderate IgG antibody levels, suggestive of chronic infection. Since most subjects were residents on the farm where they worked, this suggests the existence of some stability in the environmental exposure to parasite over extended periods of time. Thus, our results are comparable to the Brazil average, which is estimated between 50-83% [16-19]. Seroprevalence estimates for human populations vary greatly from one region to another, among different countries, and among different ethnic groups living in the same area [8, 20, 21]. For example, seroprevalence in Mexico, have been estimated to range between 6.1 and 7.72% [22]. Higher prevalence have been observed in other countries, such as Madagascar (83.5%) [23]. In addition, prevalence rate vary over time, with age, cultural habits and environmental factors [5]. Some authors had verified a positive association between age and seropositivity [24-26]. In our study, no significant differences could be observed to age despite of tendency verified; higher prevalence in the group with elevated age indicating a higher risk of exposure as age increases [27]. No differences were found in seroprevalence by sex, result compared to those obtained by other works [19, 26, 28]. One unexpected result obtained was the absence of cats inside the farms. Some people reported the presence of cats around the farms, this fact is very important since the cats are reservoirs for animal and human toxoplasmosis [1]. In fact, many cats in this region are feral and do not interact directly with humans.

Despite toxoplasmosis being considered an occupational and cultural disease, the other variables analysed in this study were not associated with the risk of *T. gondii* seropositivity in statistical analysis. However, the true association may have been masked by number of evaluated people.

This paper presents the first study about the prevalence and risk factors in rural population in Lajes, Rio Grande do Norte State. A limitation of this study is the small number of examined individuals, which does not allow the drawing of any firm conclusions. However, there is a necessity to develop farm management options to prevent transmission from sheeps to humans. Nevertheless, the obtained results suggest that sheep farmers are at increased risk for *Toxoplasma gondii*. Further investigations for determination of risk factors associated with infection may serve to indicate type preventive to reduce human infection.

Table 2. Anti-*Toxoplasma gondii* Antibodies in Residents and Works of Sheep Farms in Lajes, Brazil, by Age Groups

Age (Years)	IHA Test		
	Reagent N (%)	Non-reagent N (%)	Total N (%)
23-32	3 (60)	2 (40)	5 (100)
33-42	2 (66,7)	1 (33,3)	3 (100)
43-52	1 (100)	0 (0)	1 (100)
53-63	1 (100)	0 (0)	1 (100)
Total	7 (70)	3 (30)	10 (100)

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