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Seroprevalence of *Toxoplasma gondii, Neospora caninum,* and *Brucella abortus* in sheep from Toledo, Paraná, Brazil

Soroprevalência de *Toxoplasma gondii, Neospora caninum* e *Brucella abortus* em ovinos de Toledo, Paraná, Brasil

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Highlights

Currently, toxoplasmosis is the major cause of abortion in sheep.
Recently, reports of abortion due to neosporosis in sheep have increased.
Sheep is a key point in brucellosis control and eradication in Brazil.
Serological techniques are extraordinary tools for diagnosing target diseases.

Abstract

Toxoplasmosis is a cosmopolitan disease caused by *Toxoplasma gondii* and is of great significance in animal production and public health, as evidenced by the two major outbreaks of the disease that occurred in Brazil at the cities of Santa Izabel do Ivai, Paraná and Santa Maria, Rio Grande do Suu. *Neospora caninum* is the etiological agent of neosporosis, and *Brucella* spp. causes brucellosis, two of the major causes of reproductive failure in ruminants, causing problems such as abortion, and are responsible for significant economic losses in Brazilian sheep farming. Considering the scarcity of data available on the subject in the study region, the objective of our study was to evaluate seroprevalence of antibodies against *T. gondii, N. caninum,* and *B. abortus* in sheep in the municipality of Toledo, Paraná, Brazil. From August 2017 to July 2018, 22 properties were visited, and 240 blood samples were collected from females and males of reproductive age. For detecting antibodies against *T. gondii* and *N. caninum*, an Indirect Immunofluorescence technique was utilized, using cutoff points ≥64 and ≥50, respectively. For detecting antibodies against *B. abortus,* screening was conducted with Acidified Buffered Antigen and verification of reagent samples using the

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Slow Agglutination and 2-Mercaptoethanol test. Of the analyzed samples, 27.08% (65/240) had antibodies against *T. gondii,* which were distributed in 81.82% (18/22) of the properties; for *N. caninum,* there was a prevalence of 15.41% (37/240), and over 68.18% (15/22) of the properties. For *B. abortus* test results, all samples were considered negative. Our study demonstrated that most properties had animals positive for the protozoa, of which, majorly *T. gondii,* could cause abortion problems, it being one of the major agents. These epidemiological data provide a basis for strategic programs to implement monitoring and control measures to avoid economic losses in sheep farming.
Key words: Antibodies. Brucellosis. Neosporosis. Sheep farming. Toxoplasmosis.

Resumo

A toxoplasmose é uma doença cosmopolita, causada pelo *Toxoplasma gondii,* com grande importância no contexto de produção animal e saúde pública como evidenciado nos dois maiores surtos da doença que ocorreram nas cidades brasileiras de em Santa Izabel do Ivai-PR e Santa Maria-RS. Enquanto o *Neospora caninum* é o agente causador da neosporose e a *Brucella* spp. causadora da brucelose, duas das principais causas de falhas reprodutivas em ruminantes, ocasionando problemas como abortamento, e responsáveis por significativas perdas econômicas na ovinocultura brasileira. Considerando a escassez de dados disponíveis sobre o tema para a região estudada, o objetivo do presente estudo foi avaliar a soroprevalência de anticorpos contra *T. gondii, N. caninum* e *B. abortus* em ovinos do município de Toledo, Paraná, Brasil. Durante o período de agosto de 2017 a julho de 2018 foram visitadas 22 propriedades e coletadas 240 amostras de sangue de fêmeas e machos em idade reprodutiva. Para a detecção de anticorpos contra o *T. gondii* e *N. caninum* foi utilizada a técnica de Imunofluorescência Indireta empregando ponto de corte ≥64 e ≥50, respectivamente. Para a detecção de anticorpos contra *B. abortus* foi realizada a triagem com o Antígeno Acidificado Tamponado e confirmação das amostras reagentes com o teste de Soroaglutinação Lenta e do 2-Mercaptoetanol. Das amostras analisadas, 27,08% (65/240) apresentaram anticorpos contra o *T. gondii*, estes se encontravam distribuídas em 81,82% (18/22) das propriedades, para *N. caninum* observou-se uma prevalência de 15,41% (37/240), distribuídas em 68,18% (15/22) das propriedades. Quanto ao resultado dos testes da *B. abortus* todas as amostras foram consideradas negativas. O presente estudo mostrou que a grande maioria das propriedades apresentaram animais positivos para os protozoários, os quais, principalmente *T. gondii,* poderiam estar causando problemas de abortamentos. Estes dados epidemiológicos fornecem embasamento para programas estratégicos a fim de realizar medidas de monitoramento e controle, evitando perdas econômicas na ovinocultura.
Palavras-chave: Anticorpos. Brucelose. Neosporose. Ovinocultura. Toxoplasmose.

Introduction

The sheep herd in Brazil has approximately 18,948,934 heads, of which 4,010,916 are reared in the southern region of Brazil, with Toledo having approximately 4,215 heads (IBGE, 2018). The per capita sheep meat consumption in Brazil remained at an average of 0.6 kg per person per year (EMBRAPA, 2019). Sheep farming in Brazil and in the world is constantly growing and consequently the production area must be concerned with the health and reproductive problems that affect small ruminants, among these concerns we can mention sheep farming as the major obstacle diseases caused by parasites (Dubey, 2010).

Toxoplasma gondii is a vastly distributed obligate intracellular protozoan, where felines, the definitive hosts, are considered the key to disease control (Dubey et al., 2004). The major source of infection for production animals is the ingestion of food or water containing sporulated oocysts (Frenkel, 1990).

Toxoplasma gondii can cause reproductive disorders in intermediate hosts including sheep (Bezerra et al., 2009). This parasite was first reported in sheep in the 1940s, and since then, several studies have demonstrated the economic significance of abortions and stillbirths in sheep (Olafson & Mon-lux, 1942). In Uruguay, a study indicated that toxoplasmosis is an important sheep herd disease, causing annual losses of US$ 1.4 to 4.7 million (Freyre et al., 1997). In sheep, *T. gondii* tissue cysts can lodge in organs and tissues that are not detectable during slaughter (Garcia et al., 1999; Moura et al., 2007; Vismarra et al., 2017), thus making the consumption of this raw meat or undercooked meat an important transmission route in humans (Soccol et al., 2009).

Neosporosis is a protozoan disease caused by *Neospora caninum* and is listed as an important cause of reproductive problems in ruminants, with cattle being the most affected (Cerqueira-Cézar et al., 2017). This protozoan was first described in dogs and later as a cause of neonatal infections in cattle, sheep, goats, and horses (Dubey, 2003). The definitive hosts for *N. caninum* are canids, which can be infected by the ingestion of cysts present in the tissues of the intermediate host (McAllister et al., 1998). Dogs are important in the biological cycle of the agent, as they may contaminate pastures through the elimination of oocysts in the feces (Cerqueira-Cézar et al., 2017). In sheep, the first report of this disease was conducted in the United Kingdom by Dubey and Lindsay (1990), wherein the agent was detected during an abortion. Since its discovery, this protozoan has been associated with reproductive problems, costs of new inseminations or coverings, reduced shelf life, and reduced animal production (Melo et al., 2006). In Argentina, a study was conducted and described for the first time on ovine abortion caused by *N. caninum,* detecting the protozoan in the placenta, central nervous system, lung, and heart of the aborted fetus (Hecker et al., 2019).

Brucellosis is an infectious disease with zoonotic potential caused by bacteria of the genus *Brucella* that affects cattle, sheep, and goats. It is considered a health and economic problem due to the reproductive disorders it causes in its hosts (Pinheiro-Junior et al., 2008). The first

isolation of a *Brucella* genus microorganism was conducted by David Bruce on the Island of Malta in humans affected by the disease known as Malta Fever. Initially, the microorganism was named *Micrococcus melitensis,* later renamed *Brucella melitensis.* Ten *Brucella* species that affect diverse animals and humans are currently known. These bacteria are not species-specific, although they have a predilection for their hosts (Martins et al., 2013).

Among the *Brucella* genus bacteria, *B. abortus* has a predilection for cattle but can affect sheep, goats, and other animals when there is an opportunity, similarly, *B. ovis,* has a predilection for sheep and can affect the same hosts (Pinheiro-Junior et al., 2008; Martins et al., 2013).

The objective of this study was to evaluate the seroprevalence of *T. gondii, N. caninum,* and *B. abortus* in sheep in the municipality of Toledo, Paraná, Brazil.

Material and Methods

This study was approved by the Committee on Ethics in the Use of Animals of this institution under protocol 1134.

Study and Sampling Area

The city of Toledo (24°43'11.12"S/ 53°44'35.86"W) is located in the western region of Paraná, 560 m above sea level, and has a territorial unit area of 1,196,756 km^2. The climate is subtropical, with an average annual temperature of over 16 °C and rainfall distributed throughout the year, especially in the summer. The estimated human population is 140,635 inhabitants with a 0.768 HDI and a R$ 40,433.37 GDP (IBGE, 2019).

Sampling was done using the OpenEpi program version 3.01, where a prevalence of 20% was utilized, an expected error of 5%, and a confidence interval of 95%, resulting in a minimum sample of 232 animals. The number of properties were obtained from Emater and Adapar in the municipality, and the properties were selected according to the availability of the owner, so that there was representation in the municipality, with non-probabilistic sampling.

All owners agreed to conduct the research by signing a term for consent for animal use. From August 2017 to July 2018, 22 properties were visited (Figure 1), and 240 blood samples were collected from 223 females and 17 males of reproductive age. Samples were collected from 20 animals on three farms, blood was acquired from 10 animals in 17 properties, and samples from five animals were obtained on two farms.

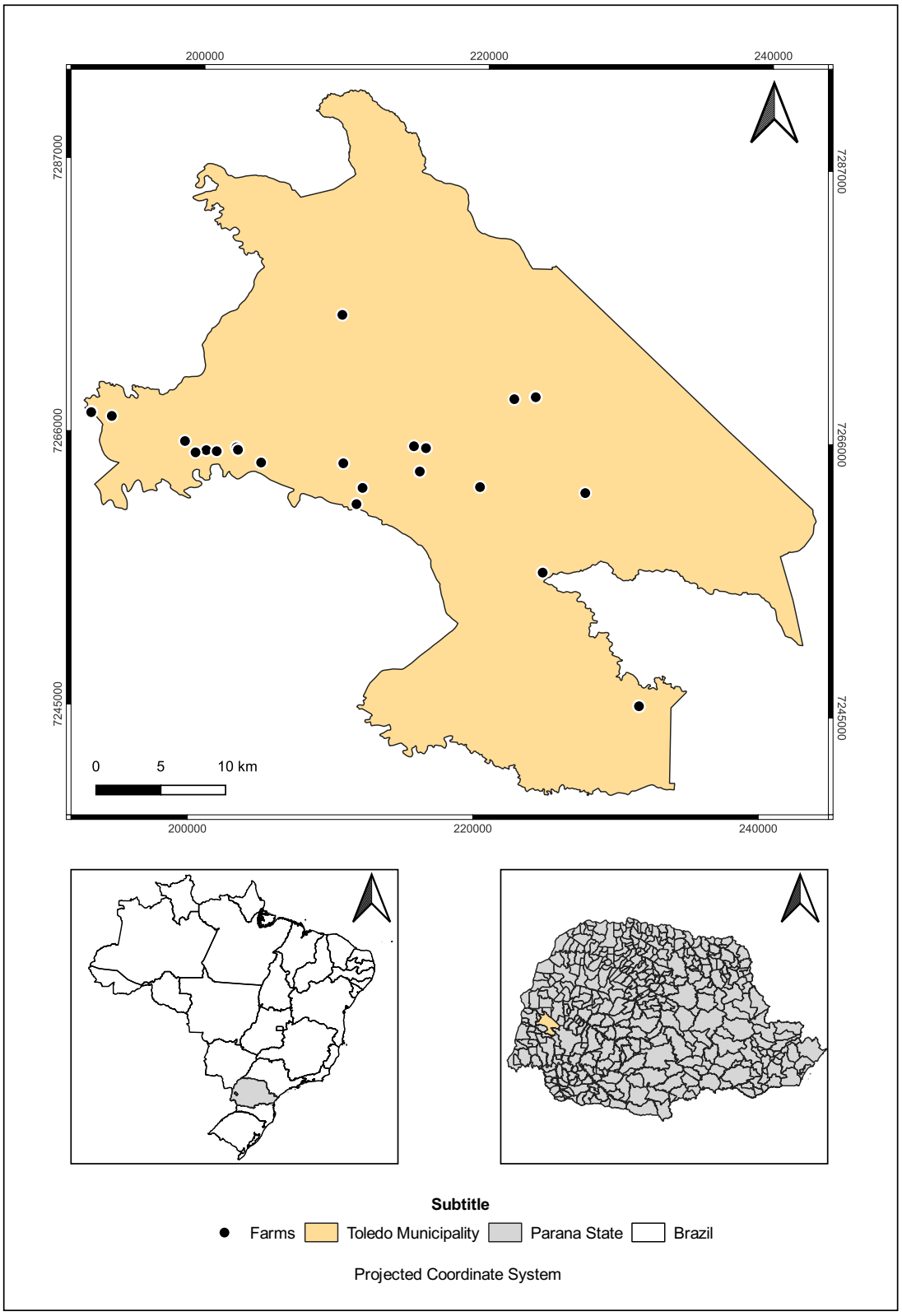

Figure 1. Sheep farms were study was performed, Toledo Municipality, Paraná, Brazil.

From the total blood collected through jugular venipuncture, after centrifugation at the Laboratory of Veterinary Parasitology, a serum sample was obtained from all animals. The serum was kept in a microtube and stored at -20 °C until serological tests were conducted.

Serological Tests

Anti-IgG antibody titers against *T. gondii* and *Neospora* spp. were determined using indirect immunofluorescence (IIF), according to Camargo (1974) and Conrad et al. (1993), respectively. RH strains for *T. gondii* and NC-1 strains for *N. caninum* were utilized as antigens. Ovine anti-IgG conjugate (FITC – Sigma) was used as the secondary antibody. The cut-off points were 64 for *T. gondii* and 50 for *Neospora* spp. Standard positive and negative controls were included for all tests. Samples considered positive were sequentially diluted in base four and two until they were negative for *T. gondii* and *N. caninum*, respectively.

Detection of anti-*B. abortus* was conducted using the seroagglutination technique with Acidified Buffered Antigen from the Biological Institute of São Paulo, as provided by the National Program for the Control and Eradication of Brucellosis and Animal Tuberculosis. In each test plate well, 30μL serum was placed and 30μL antigen was incorporated, followed by homogenization and agitation for 4 min and a subsequent backlight test. Samples with the formation of lumps were considered reagents and those without lumps were considered non-reagents. Samples reactive to the Acidified Buffered Antigen Serum Agglutination Test were sent to the "Marcos Enrietti" Diagnostic Center for result verification by means of the Slow Agglutination Test and 2-Mercaptoethanol as established by (MAPA, 2006).

Results and Discussion

Of the total samples analyzed, 27.08% (65/240) had antibodies against *T. gondii,* 15.41% (37/240) against *Neospora* spp., and none were considered positive for *B. abortus.* From the properties where the samples were collected, 81.82% (18/22) had animals seropositive for *T. gondii,* 68.18% (15/22) for *N. caninum,* and 68.18 % (15/22) for both protozoa and 13.64% (3/22) were all seronegative animals for both agents.

In the search for an anti-*B. abortus,* 4/240 (1.66%) positive serum samples were identified by the Agglutination Test with Acidified Buffered Antigen, coming from four of the 22 properties (18.18%). These samples were tested for Slow Seroagglutination, and 2-Mercaptoethanol which were negative.

Analyzing the positivity of sex for *T. gondii,* antibodies were detected in 26.01% (58/223) of females, and in males, the prevalence was 41.18% (7/17), whereas for the *Neospora* spp., the occurrence of antibodies in females was 13.90% (31/223) and in males 35.29% (6/17) (Table 1). Already, 7.5% (18/240) had antibodies to both studied agents, and 65% (156/240) were seronegative for both protozoa.

Table 1
Analysis of indirect immunofluorescence reactions for *Toxoplasma gondii* and *Neospora caninum* from sheep of Toledo Municipality, Paraná, Brazil.

Variable	*Toxoplasma gondii*		X^2	P	*Neospora caninum*		X^2	P	Total (%)
	+(%)	- (%)			+(%)	-(%)			
Male	7 (4)	10 (96)			6 (3)	11 (97)			17 (73.3)
			0.36	0.27			2.45	0.6	
Female	58 (2.8)	165 (99.2)			31 (6.5)	192 (93.5)			223 (26.7)

X^2= qui-square.

The *T. gondii* antibody titers were 64 (39/65), 256 (24/65), and 1024 (2/65), respectively. The titers observed for *N. caninum* were 50 (16/37), 100 (7/37), 200 (3/37), 400 (4/37), and 800 (7/37), respectively.

In the Paraná state, seroprevalence varies according to the region of the state investigated. Studies done in the regions of Jaguapitã, Londrina, Guarapuava, Umuarama, and Curitiba demonstrated that the *T. gondii* seroprevalence in sheep was 51.8%, 54.6%, 51.5%, 28,3% and 27.75%, respectively (Garcia et at., 1999; Ogawa et al., 2003; Romanelli et al., 2007; Sinhorini et al., 2017; Soccol et al., 2009). The frequency of infection is extremely high in other regions of Brazil. In Santa Catarina, Sakata et al. (2012) detected an occurrence of 56.9%. Ferreira et al. (2016) reported an occurrence of 41.3% in sheep from Rio Grande do Sul. In other regions of the Brazil, including the north, northeast, and southeast, studies demonstrated a prevalence ranging from 28.2% to 52% (Pinheiro-Júnior et al., 2009; Ueno et al., 2009; Braga-Filho et al., 2010; Lopes et al., 2010; Mendonça et al., 2013; Salaberry et al., 2015).

Toxoplasmosis is of great economic significance in sheep farming due to its reproductive problems, including abortion, embryonic absorption, fetal mummification, and stillbirths. Consequently, due to its negative economic impact, seroepidemiological studies have been conducted in several countries. On the American continent, Caballero-Ortega, et al. (2008) demonstrated a frequency of antibodies against *T. gondii* of 84% in sheep from Mexico. Already, Hecker et al. (2013) in Argentina, reported a seroprevalence of 42.6% in this same species. In the old world, specifically in Europe, studying the infection in sheep, Bártová, Sedlák, and Literák (2009) in the Czech Republic reported a prevalence of 59%, and Gaffuri et al. (2006) detected a prevalence of 78% in Italy.

Infection is also important in public health, as the ingestion of infected meat is an important source of infection for humans, as the protozoan is zoonotic (Bisson et al., 2000), and epidemiological studies are considered a vital tool in understanding the infection.

In the current study, 15.41% of the samples were positive for *N. caninum*; however, there are few epidemiological studies of *Neospora caninum* in sheep in rom state of Paraná. In Guarapuava, previous studies identified seroprevalences of *N. caninum* that ranged from 3.7% and 9.5%

(Gheller et al., 2016; Romanelli et al. 2007). In the northern region of Paraná, the prevalence of anti-*N. caninum* was 13.9% (Munhoz et al., 2010). In other regions of the Brazil, studies demonstrated *N. caninum* seroprevalence ranging from 1.8% to 14.9% (Oshiro et al., 2007; Soares et al., 2009; Ueno et al., 2009; Salaberry et al., 2010; Langoni et al., 2011; Guimarãeas et al., 2015). These differences in seroprevalence may be due to environmental factors, rearing system, sample size, serological tests, and cutoff points utilized in each study (Gondim, et al., 2004; Cerqueira-Cézar et al., 2017).

In Brazil, there are few studies on *Brucella abortus,* specifically in sheep, due to the predictive behavior of this microorganism. Several studies conducted in Brazil corroborate with our results, wherein all evaluated animals were seronegative (Nozaki et al., 2004; Salabarry et al., 2011; Silva et al., 2017). However, other studies have reported the occurrence of anti-*B. abortus* antibodies in sheep, but with low prevalence levels ranging from 0.21% to 4.4% (Martins et al., 2013; Santos et al., 2016; Rizzo et al., 2019). These data suggest a low frequency of circulating *B. abortus* antibodies in this species, which may be a consequence of the bovine brucellosis control and eradication program established by the MAPA, and consequently, the bacterium circulated less frequently in the regions studied. The occurrence of brucellosis caused by *B. abortus* is predominantly related to cattle, goats, and sheep intercropping (Ocholi et al., 2005).

Brucellosis caused by *B. abortus* mostly affects cattle, which justifies the low disease incidence in sheep, since when it occurs, is probably due to infection with the placenta (Monteiro et al., 2006). Once this consortium is created, sheep farming is not the major activity on the properties, and consequently, extensive rearing of sheep with cattle, inadequate sanitary management, and periodic or non-existent technical assistance contribute to the occurrence of disease (Clementino et al., 2007).

Conclusions

The data obtained in this study demonstrated that *T. gondii* and *Neospora* spp. are circulating in sheep herds from the city of Toledo, State of Paraná. Furthermore, *B. abortus* infection was not observed in sheep evaluated during this study. Additional investigations are required to assess the economic impact of these diseases on the animals in this region.

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