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ORIGINAL ARTICLE

OCCURRENCE OF IgG ANTIBODIES ANTI-*Toxoplasma gondii* AND *Neospora caninum* IN CATTLE RAISED IN FAMILY AGRICULTURAL PROPERTIES IN REALEZA, PARANA, BRAZIL

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ABSTRACT

This cross-sectional study evaluates the presence of antibodies against *Neospora caninum* and *Toxoplasma gondii* in cattle raised in Realeza, PR. There was a seroprevalence of *N. caninum* and *T. gondii* of 87.5% and 67.9%, respectively in the properties assessed. The frequencies of *T. gondii* and *N. caninum* in the animals were 41.1% and 55.1%, respectively. The studied coccidia are widely distributed in dairy cows reared on family farms in the municipality of Realeza, PR. Sanitary control for reproductive diseases must be adopted to prevent miscarriages and the economic damage caused by the disease. Future studies should be performed to investigate how widespread these pathogens are in cattle herds in southwestern Parana.

KEYWORDS: Toxoplasmosis; neosporosis; *Neospora caninum*; *Toxoplasma gondii*; serology, *Bos taurus*.

INTRODUCTION

Milk production is one of the main agricultural activities in Brazil, and has been gaining prominence due to its significant 62% growth over the last 11 years, overreaching the mark of 30 billion liters/year, granting the country a prominent position in the world scenario of milk production (IBGE, 2019).

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Dairy farming is very important in the State of Paraná, which is second in the national rank for dairy products. This activity is responsible for a significant amount of the monthly income of farming families (MAPA, 2017). However, milk production in Paraná could be greater, were it not for the presence of a large number of pathogens.

Several pathogens impair cattle reproduction. The parasites that most cause this problem are *Neospora* (Locatelli-Dittrich et al., 2001, 2004, 2008; Camillo et al., 2010; Martins et al., 2012; Langoni et al., 2013; Rocha et al., 2015), and *Toxoplasma* (Locatelli-Dittrich et al., 2001, 2004, 2008; Camillo et al., 2010; Martins et al., 2012; Langoni et al., 2013; Rocha et al., 2015).

Neosporosis is a major cause of reproductive failures in cattle, being associated with spontaneous abortions, decreased milk production and life expectancy in dairy cows (Cerqueira- Cézar et al., 2017; dos Santos et al., 2020). Toxoplasmosis, on the other hand, is one of the most common parasitic infections in animals and humans. It is a zoonosis caused by the protozoan *Toxoplasma gondii*, an obligate intracellular coccidia, that may infect most homeothermic species and form tissue cysts. Due to its wide scope, it has great importance in public health and animal production (OIE, 2017; dos Santos, 2020).

The complex epidemiological chain involved in the transmission of these diseases, including other domestic animals such as dogs and cats, and the different pathogenic mechanisms impair precise clinical diagnosis (Galvão et al., 2014; Gondim et al., 2017; Gomes et al., 2020). It is particularly troublesome when the farmer is not aware of these mechanisms and does not take measures regarding epidemiology and risk factors. Laboratory support is needed to identify the species involved in the parasitic infection and to reduce damage to productivity.

Considering the importance of neosporosis and toxoplasmosis to dairy cattle, the proximity of the municipality of Realeza-PR to the Argentinian border and limited studies, it is essential to know the real prevalence of these diseases so that control measures can be taken for eradication. Therefore, the objective of the research was to evaluate the occurrence of IgG antibodies against *Neospora caninum* and *T. gondii* in dairy cattle on family farms in the municipality of Realeza, PR.

MATERIAL AND METHODS

Study area and data collection

The present study was authorized and approved by CEUA (Ethics Committee on the Use of Animals) of the Federal University of Fronteira Sul (UFFS) (protocol 23205.004368/2016-11), and the free and informed consent term was signed by the farmers and researchers who participated in the study.

The sample collection was conducted in the municipality of Realeza, PR (25º46‘08‘‘ S; 53º31‘57‘‘W), from May to December 2016. The research evaluated all the family agriculture properties registered in the Southwest Milk Program in the Department of Agriculture in Realeza, Parana. These properties are located in different rural communities and are characterized by having up to 80 hectares in active use, with labor force basically from the landowning family in all the economic activities on the property; with most of the family income coming from agricultural activities developed on the farm.

Biological blood samples and serum

Animal selection was randomized and biological samples were collected from 50% of lactating cows. Samples were composed of blood from females of the following breeds: 279 Holstein, 21 Jersey/Holstein crossbreeds, 16 Jersey, 1 Girolando and 65 undefined race.

To avoid passive immunity interference, only samples from animals older than 12 months were included. Blood collection was carried out in dairy cows over one or more years of age, on 56 small farms, regardless of any history of reproductive problems in the herd.

Blood collection was performed by puncture of the coccygeal vein using a 40x12mm disposable needle and 10mL sterilized tubes with blood clot activator gel. Next, the blood samples were centrifuged at 3,000 rpm for 10 minutes to obtain the serum that was stored in micro tubes and frozen at a controlled temperature of -45°C.

Immunoenzymatic assay (ELISA) test for N. caninum *and* T. gondii

The collection and preparation of the samples followed the methodology applied by Sott et al., (2016); the laboratory work was carried out in the Veterinary Parasitology Laboratory at UFFS. For the immunoenzyme assay, the ELISA methodology (Imunodot Diagnostics®, Jaboticabal, Brazil) was used, according to a protocol adapted from Sott et al. (2016) to analyze animal serum samples detecting IgG antibodies against *Neospora caninum* (n = 382 animals) and *Toxoplasma* (n = 376 animals). The preparations and steps used follow the instructions in the product instruction manual. At the end of the process, the plate was read in an ELISA microplate reader (Multiskan® GO UV / VIS spectrophotometer; Thermo Scientific) with a 405 nm filter. As described in the instruction manual, the results are obtained by calculating the cut-off index, the optical density (OD) and the color of the sample. Samples that show dark yellow and OD greater than or equal to the cut-off index are considered positive, while samples that do not show dark yellow and OD less than the cut-off index are considered negative.

Statistical analysis

Confidence intervals of the proportions were calculated through the Wilson method (Thrusfield, 2005).

RESULTS AND DISCUSSION

The table presents the number of farms and animals seropositive to *N. caninum* and *T. gondii*. The seroprevalence and frequency of *N. caninum* among the properties assessed were 87.5% and 41.1%, respectively. The seroprevalence and frequency of *T. gondii* among the animals assessed were 67.9% and 55.1%, respectively.

Parasites *T. gondii* and *N. caninum* are considered the main causes of reproductive failure in domestic animals. *T. gondii* is one of the most studied pathogens in the world due to its importance for One Health.

According to Dubey (1986), detection of *T. gondii* in bovine tissues is rare and does not appear to give rise to clinical signs or abortion in pregnant cows. However, consumption of raw or undercooked beef can still be an important source of human infection (Hill and Dubey, 2002; Shaapan, 2016).

In the State of Parana, few studies are addressing the exposure of dairy cattle to the coccidian. Ogawa et al. (2005) used the indirect immunofluorescence reaction on 385 samples of dairy cow serum from 12 municipalities in northern Paraná and observed that 26% of the animals were seropositive to *T. gondii*. Antibodies against *T. gondii* using the IFAT technique were observed in 48.3% of the cows raised in Presidente Getúlio, SC, southern Brazil (Macedo et al. 2012).

The protozoan *N. caninum* is capable of parasitizing farm animals (intermediate hosts) and canids (definitive hosts) (Dubey et al., 1988; McAllister et al., 1998). Although antibodies in humans have been found in immunocompromised patients in different parts of the world (Lobato et al., 2006; Benetti et al., 200); Oshiro et al., 2015), there is no evidence of the zoonotic character of *N. caninum*. In bovine herds, the protozoan can be transmitted vertically (congenital) and horizontally (oocyst ingestion), but the vertical transmission seems to be the main route of infection with endemic abortions (Anderson et al., 1997; Locatelli-Dittrich et al., 2001, 2004; Dubey et al., 2006; Macedo et al., 2017; Diniz et al., 2019).

In a meta-analysis study related to worldwide prevalence and risk factors associated with bovine neosporosis Ribeiro et al., (2019) observed a prevalence of 24% *N. caninum* in cattle in South America. The authors reported a significant correlation between *N. caninum* infection and abortion in cows and detected higher susceptibility of dairy cattle compared to beef cattle.

Table. Seroepidemiology of *Neospora caninum* and *Toxoplasma gondii* on 56 family farms in the municipality of Realeza, State of Paraná, Brazil, 2017.

Parasite	Epidemiological parameters				Positive animals			Negative animals	
	Number of farms evaluated	Seroprevalence (%)	Number of animals examined	Frequency of IgG antibodies (%)	Reaction cutoff OD	Mean OD	Standard deviation	Mean OD	Standard deviation
Neospora caninum	56	49/56 (87.5%; 76.4%-93.8%)	382	157/382 (41.1%; 36.3%-46.1%)	0.624	0.850	0.260	0.480	0.114
Toxoplasma gondii	56	38/56 (67.9%; 54.8%-78.6%)	376	207/376 (55.1%; 50.0%-60.0%)	0.617	0.812	0.204	0.499	0.068

Optical density (OD)

Seroprevalence for *N. caninum* in Brazilian cattle ranges from 6.7 to 91.7%, depending on the geographic region and breed (Cerqueira-Cezar et al., 2017). Studies have shown that the prevalence of anti-*N. caninum* in the State of Parana ranged from 12% to 34.8% (Locatelli-Dittrich et al., 2001; Ogawa et al., 2005; Locatelli-Dittrich et al., 2008; Camillo et al., 2010; Martins et al., 2012; Langoni et al., 2013; Rocha et al., 2015; Snak et al., 2018).

The seroprevalence and frequency of antibodies in the present study was higher than that found in a study conducted previously in Parana State, proving more relevant due to the possibility of impairing reproductive and zootechnical performance of the dairy herd.

The protozoa *T. gondii* and *N. caninum* are widely distributed in dairy cows reared on family farms in the municipality of Realeza, PR. It is of paramount importance that sanitary control for reproductive diseases are reinforced to prevent miscarriages and economic damage caused by the disease. Future studies should be conducted to investigate how widespread these pathogens are in cattle herds of southwestern Paraná.

CONFLICT OF INTEREST

The authors declare no conflicts of interest

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