



Seroprevalence and risk factors for *Neospora caninum* infection in cattle from the eastern Antioquia, Colombia



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ABSTRACT

Bovine neosporosis is a parasitic disease with worldwide distribution that causes important economic losses. Because of the limited information on the occurrence of *Neospora caninum* infection in Colombia, this study aimed to determine the seroprevalence and identify the risk factors associated with this infection in cattle in Antioquia, which is the largest milk-producing state in the country. We collected 1,038 blood samples from Holstein, Jersey and crossbred cows from 31 farms. An epidemiologic questionnaire was given to all the owners. A commercial ELISA kit was used as the diagnostic technique. The occurrence of anti-*N. caninum* antibodies was determined to be 28.3% (294/1038), and 100% of the screened farms were positive, indicating that all the properties had at least one positive animal. The seropositivity within each farm ranged from 5.5% to 50%. A multivariable logistic regression model identified the following as significant risk factors: history of abortion (OR = 5.33, $p < 0.001$), replacement with cattle purchased outside the farm (OR = 1.54, $p < 0.05$), age (OR = 1.7, $p < 0.01$) and poor hygienic practices associated with manual milking (OR = 1.69, $p < 0.01$). The latter two factors suggest that horizontal transmission is an important route of infection. This study is the first to report the seroprevalence of and risk factors for *N. caninum* infection in Antioquia and allows us to conclude that *N. caninum* is widely distributed in this region.

1. Introduction

Neospora caninum is a protozoan parasite that has been reported to cause abortions, stillbirths, neonatal deaths, early foetal losses and embryo reabsorption in infected cattle (Dubey & Schares, 2011; Dubey, Schares, & Ortega-Mora, 2007). Abortions caused by *N. caninum* may be sporadic, endemic or epidemic and often occur during the second trimester of pregnancy, resulting in economic losses for the beef and dairy industries. In South America, the annual losses for the dairy industry were estimated to be \$43.6 million USD (range, \$15.62–194.41 million USD) in Argentina and \$51.3 million (range, \$35.8–111.3 million USD) in Brazil (Moore, Reichel, Spath, & Campero, 2013; Reichel et al., 2013).

The rate of vertical transmission of *N. caninum* can reach 93.7%, and this is the most important infection route in bovines (Schaes, Peters,

Wurm, Bärwald, & Conraths, 1998); however, definitive hosts such as domestic dogs, coyotes, dingoes and grey wolves (Dubey et al., 2011; Gondim, McAllister, Pitt, & Zemlicka, 2004; King et al., 2010; McAllister et al., 1998) play important roles in horizontal transmission and in maintaining infection in dairy herds.

The dairy industry is one of the main economic activities in Colombia. It has been calculated that there are 23 million bovines, of which 2.5 million are milking cows (ICA, 2013). Antioquia is the state with the highest dairy production in the country with 3.5 million liters of milk produced each day, and the microregion located in eastern Antioquia is responsible for the production of 1 million liters/day (MADR, 2012).

Although information regarding the seroprevalence of *N. caninum*, which is 17.1%–28.6% in Argentina (Moore et al., 2009, 2013), 7.6%–91.2% in Brazil (Guedes, Guimarães, Rocha, & Hirsch, 2008;

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Sousa et al., 2012), 22.4% in Chile (Patitucci, Pérez, Israel, & Rozas, 2000), 12.8% in Peru (Puray, Nidia, & Amanda, 2006), 28.8–61.3% in Uruguay (Furtado, Rosadilla, Cattáneo, Bermúdez, & Puentes, 2011; Kashiwazaki, Giannechini, Lust, & Gil, 2004) and 17.1–44% in Venezuela (Escalona, García, Mosquera, Vargas, & Corro, 2010; Obando, Bracamonte, Montoya, & Cadenas, 2010), in South America is abundant, few studies have been conducted in Colombia. Zambrano, Cotrino, Jiménez, Romero, and Guerrero (2001) first reported the occurrence of *N. caninum* in the country. They studied samples from 74 farms with reproductive problems and abortions and found that 54.1% of the animals were positive. Other studies have found seroprevalence ranging from 10.2% to 76.9% (Oviedo et al., 2007; Peña et al., 2012). However, only one study characterizing one single farm was conducted in the region of Antioquia and demonstrated 34.6% seropositive animals out of 347 cattle that were evaluated (López et al., 2007).

Considering the importance of milk production in eastern Antioquia and the lack of epidemiological studies on bovine neosporosis, the aim of this study was to evaluate the seroprevalence of *N. caninum* and to identify the risk factors associated with this infection in this region.

2. Materials and methods

2.1. Location

This study was carried out in Eastern Antioquia (longitude 75°18'–75°25'W and latitude 5°42'–6°4'N), a microregion located in western Colombia, with an altitude ranging from 2.100 to 2.500 metres above sea level, an annual average temperature ranging from 13°C to 18°C and a relative humidity between 60% and 80%. Subsistence farming and milk production characterize this region (MADR, 2012) (Fig. 1).

2.2. Sampling and serological testing

The minimum sample size was determined based on a prevalence of 30% (López et al., 2007), 95% confidence intervals and 5% absolute precision. This provided a minimum sample size of 861 animals to estimate the true prevalence with an imperfect test (Sergeant, 2017);

however, the sample size was 1038. Thirty-one farms were sampled, and at least 20 animals were sampled from each farm. Occasionally, this number corresponded to the total number of animals on the farm.

Between August and November 2012, blood samples were collected from the coccygeal vein using vacutainer tubes, and after coagulation, the samples were centrifuged at 2700 g for 10 min, and the sera were stored at –20°C until they were used.

All the analyses were carried out at the Veterinary Parasitology Center at the School of the Federal University of Goiás (UFG), Brazil. A commercial indirect ELISA Kit (CIVTEST® Hipra Laboratories S.A.; Gerona, Spain) was used to detect *N. caninum* antibodies, in accordance with the manufacturer's instructions. Sera with IRPC (relative index \times 100) values above a cut-off level of 10 were considered positive. The sensitivity and specificity of the ELISA test were determined to be 96.1% and 100%, respectively (Alvarez-Garcia et al., 2013).

2.3. Questionnaire survey

A questionnaire was completed during personal interviews with the owners or managers of each herd. The variables included as potential risk factors at the farm level were as follows: herd density, contact with a forest area, water source, breeding service, origin of replacement heifers and milking type. Other variables including parity, lactation, milk production, gestation, age of gestation, age of the animal, and breed were evaluated. Data on reproductive failures (abortion, repeated oestrus and retained foetal membranes) in the previous 24 months, as well the presence of swine, equines and poultry on the farm were also recorded.

2.4. Data analyses

The estimated seropositivity was the ratio between the number of positive tests and the total number of tests performed, with a confidence interval of 95%. A univariate analysis was performed using chi-square test (X^2). The multivariate analysis was based on the logistic regressions in a model including the variables with $p \leq 0.25$ identified in the association test. The goodness-of-fit of the model was tested by computing the Hosmer–Lemeshow statistic (HLX^2) (Hosmer, Lemeshow, & Sturdivant, 2000). All the analyses were

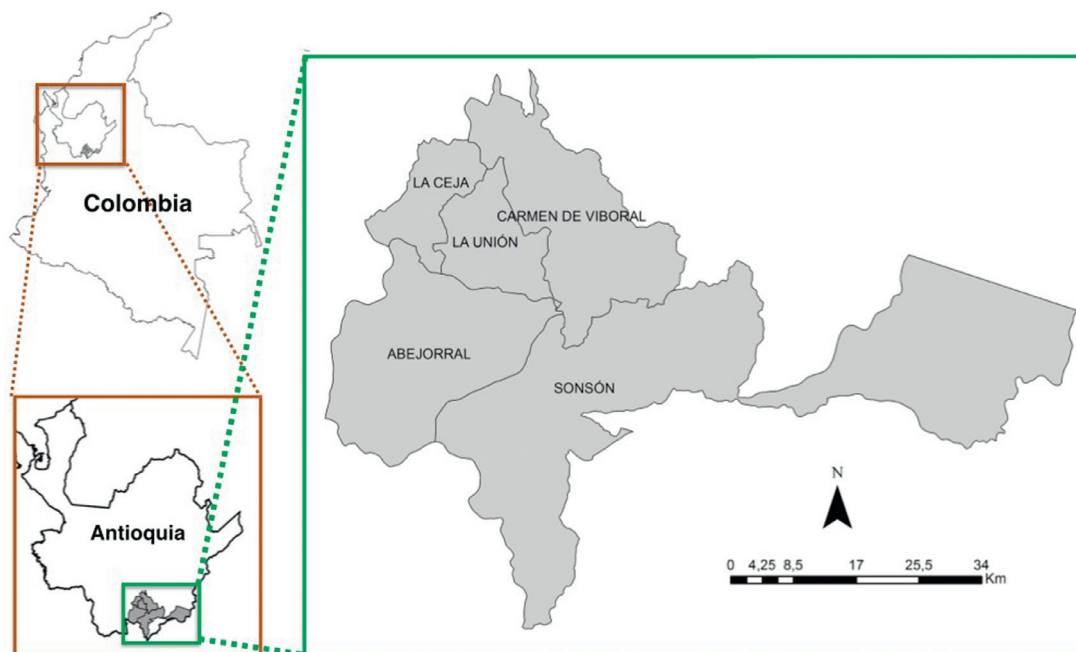


Fig. 1. Location of the municipalities where the farms and animals were sampled in Colombia.

Table 1
Seroprevalence of *Neospora caninum* infection in cattle of the municipalities of the eastern Antioquia, Colombia.

Municipalities	N° herds	N° animals		Seroprevalence (%) (95% CI)
		sampled	positives	
Abejorral	5	153	50	32.6 (25.32–40.72)
El Carmen	4	113	32	28.3 (20.24–37.56)
La Ceja	3	136	34	25.0 (17.97–33.14)
La Unión	18	608	170	27.9 (24.42–31.71)
Sonsón	1	28	8	28.5 (13.22–48.66)
TOTAL	31	1038	294	28.3 (25.59–31.17)

performed in the R language and environment for statistical computing (R Development Core Team, 2016).

3. Results

Of the 31 analyzed farms, all had at least one positive animal. Antibodies against *N. caninum* were detected in 294 of the 1038 animals tested (28.3%, 95%CI: 25.5–31.1). When the different municipalities were considered, very similar seroprevalence levels were observed. The lowest seroprevalence (25%) was observed in La Ceja, and the highest (32.6%) was observed in Abejorral; these differences were not statistically significant ($p \geq 0.05$; Table 1).

The results of the univariate analysis are summarized in Table 2. With respect to the origin of the cattle, out of the 811 homebred animals, 209 were seropositive (25.7%), and out of the 227 animals that were purchased and introduced into the herds, 85 were seropositive (37.4%; $p < 0.05$).

Regarding the milking type, the lowest prevalence was found in cows subjected to mechanical milking (20.5%) when compared with those subjected to manual milking (30.8%; $p < 0.05$).

By age, the lowest prevalence was found in animals younger than 17 months old (19.8%), and the highest prevalence was found in females older than 3 years old (31.1%; $p < 0.05$).

No significant association between the different breeds was observed ($p > 0.05$). Nevertheless, the prevalence (11.7%; $p < 0.05$) was significantly lower in crossbred animals (Holstein x Gyr) than in Holstein (29.1%) and Jersey (27.1%) cows.

There was a strong association between females with a history of abortions in last two years and the prevalence of *N. caninum*. The prevalence in this group was higher (68.6%) than in cows with no history of abortions (26.2%; $p < 0.001$). The number of females with a history of repeated oestrus was 893, of which 269 were seropositive for *N. caninum* (30.1%; $p = 0.001$); however, of the 145 females with no record of repeated oestrus, 25 were seropositive (17.2%).

No significant differences were found between the seroprevalence of *N. caninum* and independent variables, such as herd density, contact with a forest area, water source, breeding service, number of parities, lactation status, milk production, gestation and stage of gestation, retention of foetal membranes and contact with swine, equines and poultry ($p > 0.05$).

When a multiple regression analysis was performed, it revealed risk factors including the prevalence and history of abortions ($OR = 5.33$, $p < 0.001$), age ($OR = 1.7$, $p = 0.038$), replacement with cattle purchased outside the farm ($OR = 1.54$, $p = 0.008$) and poor hygienic practices associated with manual milking ($OR = 1.69$, $p = 0.0029$). The Hosmer–Lemeshow goodness-of-fit test ($p = 0.62$) indicated that the model fit was adequate (Table 3).

4. Discussion

The prevalence of positive animals with *N. caninum* found in this study was 28.3%. Worldwide prevalence has been estimated to be

between 7.6% and 76.9% in America (Cedeño, Benavides, & Pasto, 2013; Sousa et al., 2012), 10.7% and 19.6% in Africa (Ghalmi et al., 2012; Ibrahim, Elfahal, & El Hussein, 2012), 5.7% and 43% in Asia (Koiwai et al., 2006; Nazir, Maqbool, Khan, Sajjid, & Lindsay, 2013), 0.5% and 27.9% in Europe (Bartels et al., 2006; Imre et al., 2012), and 10.2% in Oceania (Hall, Reichel, & Ellis, 2005), which are comparable to the findings of the present study. Although the prevalence observed in this study was not high when compared with many others, *N. caninum*-positive animals were observed in all the evaluated farms. Similar observations in Brazil (Melo, da Silva, Ortega-Mora, Bastos, & Boaventura, 2006), Pakistan (Nazir et al., 2013) and Senegal (Kamga-Waladjo et al., 2010) have been reported.

The high individual seroprevalence and the fact that all the dairy herds had at least one positive animal, indicate that *N. caninum* infection is widely disseminated among dairy cattle in eastern Antioquia. The presence of dogs that have access to the foetuses and placentas on all of the properties may explain this. It is well known that dogs become infected after eating tissues contaminated with cysts; as a result, the shedding of faecal oocysts in the environment poses a risk to the risk of infection by a horizontal route (Dijkstra, Barkema, Eysker, Hesselink, & Wouda, 2002). In Canada, Vanleeuwen et al. (2010) confirmed that the risk of infection increases on properties that have dogs that have access to the placentas and foetuses ($OR = 2.75$) compared with properties that do not allow dogs to be in contact with these materials ($OR = 1.66$). Preventive measures are recommended in this region in order to limit dogs from eating infected bovine tissues. Other factors related to the presence of dogs on the property, such as the behavior of the dogs and the number of dogs per property, were not investigated because this information could not be obtained with precision, often due to the owner's lack of knowledge. The proximity between the farms also prevented accurate data on the presence of dogs, since in many cases, the dogs on one property visited the neighboring properties.

Studies have confirmed that the proportion of animals seropositive for *N. caninum* tends to increase with increased exposure to the sources of infection (Asmare, Regassa, Robertson, & Skjerve, 2013; Eiras et al., 2011). Moore et al. (2014) confirmed that, for each year of increase in the age of bovines and buffaloes, the probability of seropositivity increased 3.5%. Our results are consistent with the aforementioned studies and confirmed that animals older than three years of age were 70% more likely than the younger animals to have had contact with sporulated oocysts of *N. caninum*. This proportion was in agreement with that obtained by Romero, Perez, Dolz, and Frankena (2002), who found that the postnatal infection probability increased 70% in animals older than three years of age. In contrast, other studies in different countries, such as Brazil (Corbellini et al., 2006; Teixeira et al., 2010), Croatia (Beck, Marinculić, Mihaljević, Benić, & Martinković, 2010), Jordan (Talafha and Al-Majali, 2013), Pakistan (Nazir et al., 2013), Romania (Imre et al., 2012), Senegal (Kamga-Waladjo et al., 2010) and Venezuela (Escalona et al., 2010), found no association between age and infection by *N. caninum*, which suggests that for these herds, transplacental transmission is probably more important. Although it is generally accepted that vertical transmission is the largest route of transmission in bovines (de Magalhães et al., 2014; Hein et al., 2012), our results indicate that horizontal transmission also plays an important role in the epidemiology of *N. caninum* in cattle in eastern Antioquia.

When compared with properties where replacements are made with animals from the same farm, farms that have purchased replacement heifers have a higher prevalence of *N. caninum* (37.4%) and are at 54% greater risk of acquiring the infection. Beck et al. (2010) in Croatia, and Asmare et al. (2013) in Ethiopia, observed that purchasing animals for replacement raised the probability of acquiring infection by 5.2 and 2.3, respectively. This emphasizes the importance of taking biosecurity measures to prevent the introduction of infected animals into the herds. Purchasing animals or replacement animals of unknown origin and serological status is a common practice in the region studied here.

In relation to breed, the univariate analysis verified that crossbred

Table 2
Univariate analyses model for the presence of antibodies against *Neospora caninum* in cows from the eastern Antioquia, Colombia.

Independent variable	N° animals		Seroprevalence (%) (95% CI)	Odds ratio (95% CI)	p-value ¹
	Sampled	Positives			
Farm characteristics					
<i>Density</i>					
<0.5 animal/ha	583	166	28.4 (24.8–32.3)	1	0.636
0.5–08 animal/ha	341	92	28.9 (22.3–32.0)	0.92(0.68–1.25)	0.625
>0.9 animal/ha	114	36	31.5 (23.0–40.9)	1.15(0.75–1.78)	0.503
<i>Jungle contact</i>					
No	397	107	26.9 (22.6–31.6)	1	0.483
Yes	641	187	29.1 (25.6–32.8)	1.11(0.84–1.47)	0.440
<i>Water source</i>					
Open	619	174	28.1 (24.5–31.8)	1	0.907
Public	419	120	28.6 (24.3–33.2)	1.02(0.77–1.35)	0.852
<i>Breeding service</i>					
Bull	136	34	25.0 (17.9–31.1)	1	0.411
AI	902	260	28.8 (25.8–31.9)	1.21(0.80–1.83)	0.356
<i>Origin²</i>					
Homebred	811	209	25.7 (22.7–28.9)	1	<0.001
Purchased	227	85	37.4 (31.1–44)	1.72(1.26–2.35)	<0.001
<i>Milking²</i>					
Mechanic	258	53	20.5 (15.7–25.9)	1	0.001
Manual	780	241	30.8 (27.6–34.2)	1.73(1.23–2.42)	0.001
Cattle characteristics					
<i>Parity</i>					
Primiparus	150	40	26.6 (19.7–34.4)	1	0.519
Pluriparus	605	180	29.7 (26.1–33.5)	1.16(0.77–1.74)	0.456
<i>Lactating</i>					
Lactating	483	202	41.8 (37.3–46.3)	1	0.555
Dry	76	37	48.6 (37.0–60.4)	0.85(0.56–1.31)	0.484
<i>Milk production</i>					
<10L	95	45	47.3 (37–57.8)	1	0.645
11–15 L	254	99	38.9 (32.9–45.2)	0.82(0.53–1.25)	0.367
>16 L	134	58	43.2 (34.7–52.1)	0.91(0.57–1.46)	0.706
<i>Gestation</i>					
No	547	150	27.4 (23.7–31.3)	1	0.541
Yes	491	144	29.3 (25.3–33.5)	0.91(0.69–1.19)	0.496
<i>Stage of gestation²</i>					
1–3 m	208	67	32.2 (25.9–39)	1	0.135
3–6 m	159	37	23.2 (16.9–30.6)	0.63(0.39–1.02)	0.060
>6 m	123	39	31.7(23.6–40.7)	0.97(0.60–1.57)	0.924
<i>Age²</i>					
<17 m	166	33	19.8 (14–26.7)	1	0.011
18–36 m	217	57	26.2 (20.5–32.6)	1.43(0.88–2.33)	0.143
>36 m	655	204	31.1 (27.6–34.8)	1.57 (1.01–2.44)	0.004
<i>Breed²</i>					
Holstein	889	259	29.1 (26.1–32.2)	1	0.084
Jersey	114	31	27.1 (19.2–36.3)	0.90(0.58–1.40)	0.666
Crossbred	34	4	11.7 (3.3–27.4)	0.32(0.11–0.92)	0.027
Reproductive disorders					
<i>Abortion²</i>					
No	987	259	26.2 (23.5–29.1)	1	<0.001
Yes	51	35	68.6 (54.1–80.8)	6.14(3.34–11.29)	<0.001
<i>Repetition of estrus²</i>					
No	145	25	17.2 (11.4–24.3)	1	0.001
Yes	893	269	30.1 (27.1–33.2)	2.06(1.31–3.25)	0.001
<i>Placental retention</i>					
No	650	186	28.6 (25.1–32.2)	1	0.842
Yes	388	108	27.8 (23.4–32.5)	0.96(0.72–1.04)	0.787
Contacts					
<i>Presence of swine</i>					
No	782	219	28.0 (24.8–31.2)	1	0.750
Yes	256	75	29.2 (23.7–35.2)	1.06(0.78–1.45)	0.690
<i>Presence of equine</i>					
No	154	42	27.2 (20.4–35)	1	0.828
Yes	884	252	28.5 (25.5–31.6)	1.06(0.72–1.56)	0.753
<i>Presence of poultry²</i>					
No	641	171	26.6 (23.2–30.2)	1	0.154
Yes	397	123	30.9 (26.4–35.7)	1.23(0.03–1.62)	0.134

¹ Chi-square test.

² p value <0.25.

Table 3
Final multivariable logistic regression model for the presence of antibodies anti-*Neospora caninum* in cows from the eastern Antioquia, Colombia.

Dependent variables	Odds ratio (OR)	95%CI OR	p-value
<i>Abortion</i>			
No	1		
Yes	5.33	2.87–9.9	<0.001
<i>Origin</i>			
Homebred	1		
Purchased	1.54	1.11–2.13	0.008
<i>Age</i>			
<17 m	1		
18–36 m	1.42	0.86–2.34	
>36	1.70	1.11–2.60	0.038
<i>Milking</i>			
Mechanic	1		
Manual	1.69	1.19–2.43	0.0029

HLX² = 6.27; gl = 8; p = 0.6164.

animals were three times less susceptible than purebred dairy cattle to acquiring infection (OR = 0.32). Similar data were reported in Argentina (Moore et al., 2009), Venezuela (Escalona et al., 2010) and Ethiopia (Asmare et al., 2013). This fact may suggest that crossing European pure breeds with zebu may offers a protective effect for the dairy herds. This idea was reinforced by Yániz et al. (2010) in Spain, where the proportion of abortion associated with neosporosis was 4.8 and 3 times lower in Holstein cows inseminated with semen from Limousin and Belgian Blue beef bovine breeds, respectively, than those in females inseminated with semen from Holstein bulls. However, Munhoz, Pereira, Flausino, and Lopes (2009), in Brazil, found no differences between Holstein cattle versus crossbred Holstein/zebu animals. Due to the divergent findings among these different studies, these data should be interpreted with caution since the contrasting findings may be related to differences in the production systems for each breed and not to the susceptibility of the breed to the disease (Dubey et al., 2007). European pure breeds are subject to an intensive production system under high stress levels, which may favour the infection risk, while beef animals are generally kept in larger areas and with less chances of being in contact with the parasite (Moore et al., 2009).

It is well known that animals that are seropositive for *N. caninum* are more prone to abortions than seronegative animals (Dubey et al., 2007). We verified that the proportion of seropositive cows that had an abortion history (11.9%) was meaningfully higher than cows with the same clinical signs that were seronegative (2.15%), with an odds ratio of 5.3. This provides indirect evidence that *N. caninum* may be involved in abortions in cows in the studied region.

A high percentage of cows with repeated oestrus was associated with the presence of anti-*N. caninum* antibodies (30.1%; $p < 0.001$). This finding is in agreement with a study conducted in south-eastern Brazil where animals with recurrent oestrus and temporary anoestrus were 3.8 and 3.4 times more likely to be seropositive, respectively, than animals without the same clinical signs (Bruhn et al., 2013). Several authors have associated early embryonic death with *N. caninum* infection mainly due to the lack of immunological capacity of the foetus and to the lesions caused by the parasite in to the placental tissues, which justified the return to oestrus (Buxton, McAllister, & Dubey, 2002; Macaldowie et al., 2004). This idea is consistent with studies conducted in Australia and Senegal, where animals that were seropositive for *N. caninum* needed more inseminations to achieve conception, which is an occurrence that is linked to embryonic loss during the initial phases of gestation (Hall et al., 2005; Kamga-Waladjo et al., 2010).

The presence of other domestic animals was not considered a risk factor in this study. These data are consistent with studies conducted in Brazil and Colombia (Aguiar et al., 2011; Cedeño et al., 2013).

The role of wildlife in the epidemiology *N. caninum* infection in the studied region remains to be elucidated.

5. Conclusions

The seroprevalence of anti-*Neospora caninum* antibodies and their distribution in all the farms allows us to conclude that the parasite is widespread in the eastern Antioquia region. The univariate analysis indicated that Holstein and Jersey breeds were more susceptible to acquiring infections than crossbred breeds. With regard to the risk factors, a close association between abortion and seropositivity was verified, and seropositive animals were 5 times more likely to have an abortion than seronegative animals. With regard to age, animals older than three years of age were 7 times more likely than younger animals to come into contact with the parasite. Similarly, a high seroprevalence was observed in properties that performed manual milking, which was related to the poor hygienic conditions. This suggests that horizontal transmission is an important source of infection in this region of Colombia. Finally, the introduction of new animals bought from other farms into these dairy farms led to a 54% greater chance of acquiring the disease.

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Conflict of interest

The authors declare that they have no conflict of interest.

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