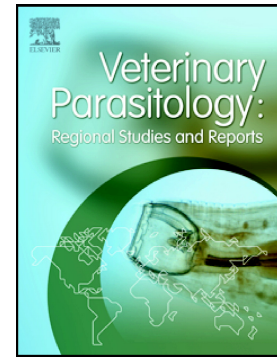


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Seroprevalence of *Neospora caninum* infection in cattle from Pereira, Colombia ***Samuel E. Idarraga-Bedoya,¹ Jaime Álvarez-Chica,¹ D. Katterine Bonilla-Aldana,^{1,2,3}****Dadin Prando Moore,⁴ Alfonso J. Rodríguez-Morales.^{2,3,4,*} arodriguez@utp.edu.co**

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Abstract

There are over 25.6 million cattle heads in Colombia being the fourth-highest herd in Latin America. This study aimed to describe the seroprevalence of *Neospora caninum* in cattle from 25 rural farms at Pereira municipality, Risaralda Department, which has a total bovine population of 43,508 animals. A cross-sectional observational study was performed in beef and dairy herds during 2017-2018. A total of 325 animals in 25 herds, with 13 animals per herd, were sampled. A commercial competitive ELISA (cELISA) kit was used to detect *N. caninum* antibodies. Associated risk factors were analyzed in two different levels: individual animals and herds. For all the independent variables, chi-square (χ^2) and Fisher tests were used to assess associations and significance. The overall estimated seropositivity was 20.6% (95%CI 16.2%-25.0%). The

seroprevalence by herds was 92.0% ranging from 0.0% to 46.2%. Noteworthy, beef herds had significantly ($p=0.0107$) higher seropositivity (50% of them above 35% of seropositivity) compared with those for milk purposes (4.8%) (OR=20.0; 95%CI 1.2-331.0). Other risk factors were not significantly ($p\geq 0.05$) associated with *N. caninum* seropositivity. Bovine neosporosis may be associated with abortions in both beef and dairy Colombian farms. More studies about the epidemiology, associated factors and consequences, as well as on tools for better diagnosis of bovine abortion, including histopathology and other ancillary tests, should be performed.

Key Words: Neosporosis; serosurvey; livestock; epidemiology; Colombia.

1. Introduction

Being the fourth highest herd in Latin America, the cattle industry is vital in Colombia. There are over 25.6 million cattle heads fed on pastures located in the tropical strip of the equatorial line. Breeds such as Brahman are ideal for beef production in these tropical conditions. Colombian cattle industry produced 932,813 tons of beef and 7,301 million litres of milk in 2019 (Federación Colombiana de Ganaderos (FEDEGAN), 2020). Essential advances in livestock health have achieved, but data about diseases affecting bovine reproductive performance still lack in Colombia (Vargas Niño et al., 2018).

Neospora caninum, a protozoan parasite belonging to the Sarcocystidae family, causes abortions and neonatal mortality in cattle worldwide (Dubey et al., 1988; Dubey and Schares, 2011). Besides, the economic losses produced by bovine neosporosis may exceed US\$1,298 billion annually (Reichel et al., 2013). However, the seroprevalence of bovine neosporosis is well known in many countries, including those in Latin America (Ribeiro et al., 2019), especially in

Brazil since 2011 (Cerqueira-Cezar et al., 2017; Martins et al., 2011; Ribeiro et al., 2019), Mexico (Arreola-Ca et al., 2012), and Argentina (Fort et al., 2015; Ribeiro et al., 2019). Recently (2020), a study in Uruguay found a neosporosis seroprevalence of 22.3% (Macchi et al., 2020). Studies in Colombia are scarce (Ribeiro et al., 2019). Only one study performed on 400 dairy cattle from the Sugamuxi province in the central region of the country reported a high seroprevalence (57.5%) (Pulido Medellín et al., 2013). To estimate the impact of *N. caninum* infection in the Colombian cattle industry, data regarding prevalence and abortion rates are needed. This study aimed to describe the seroprevalence of *N. caninum* in cattle from 25 rural farms at Pereira municipality, Risaralda Department, Colombia.

2. Materials and Methods

2.1. Cross-sectional observational study and sampling

A stratified two-stage random sampling procedure was used for this study. During 2017-2018, dairy and beef herds were randomly selected from all herds in the Pereira municipality (Figure 1), which has a total bovine population of 43,508 animals. Briefly, the formula assumed 930 herds with an average of 47 animals per herd, estimating sampling 25 herds from 17 districts across the municipality. An estimated average expected prevalence of disease of 30% was used based on some previous studies (Osawa et al., 2002) in the Latin American region (Melo et al., 2006; Moore et al., 2014), an allowable error of 5% (Favero et al., 2017), and a confidence level of 95% (Moore et al., 2002). The total number of animals sampled was 325, in 25 herds with 13 animals per herd, then 0.75% of the total animals and 2.7% of the herds in the whole

municipality were sampled. The animals were selected randomly, all of them adult cattle (similar ages).

These herds met the herd-level inclusion criteria which included: the willingness to provide representative cattle for blood samplings; allow the blood to be tested for antibodies indicating exposure to *Neospora caninum*; and the ability to answer a standardized questionnaire of herd farming conditions and animal-related variables that were included in the analyses. A total of 10 mL per animal in a sterile tube without EDTA or anticoagulants was sampled.

Blood samples taken from the coccygeal vein with sterile needles (G21 0.8 × 40 mm) were centrifuged at 1,500 g for 10 min. Then the serum was harvested and stored at -20 °C until all samples were tested.

2.2. Serological test

A commercial competitive ELISA (cELISA) kit (Veterinary Medical Research & Development, VMRD, Inc. Pullman, WA, USA) was used to detect *N. caninum* antibodies. The absorbance was measured as optical density at 630 nm using a microplate reader (Multiskan RC 6.0, Labsystems, Midland, ON, Canada). The test has a sensitivity of 97.6% (Baszler et al., 1996) and a specificity of 98.6% (Baszler et al., 2001). A cow was considered to be seropositive for *N. caninum* if the inhibition percentage was $\geq 30\%$. All the serum samples were tested in duplicate.

2.3. Statistical analysis

Using Stata 14[®] IC (Stata Corp., College Station, Texas, USA), seroprevalence and associated risk factors were analyzed in two different levels: individual animals and herds. Descriptive statistics, medians, and interquartile range (IQR) were calculated for all quantitative variables. Qualitative variables were presented as relative frequencies (%). For the animal seroprevalence, a binary random-effects model and plot were used.

Association between seropositivity and independent variables such as the number of deliveries, production purpose of the cattle, milking technique (among these dairy cattle), reproductive management, abortions, retained placenta, weak calves delivered, technical assistance, herd income serological pre-test, the number of dogs around were evaluated, both at individual and herd level (Tables 1 and 2). For all the independent variables, chi-square (χ^2) and Fisher tests were used to assess associations and significance. In those significant values ($p < 0.05$), also the odds ratio with their 95% confidence interval (95% CI) was calculated.

Seroprevalence was also presented by geographical information systems (GIS)-based maps. Microsoft Access[®] software was used to design the spatial database to import incidence rates by corregiments and veredas at Pereira municipality to the GIS software. The Client GIS software open source used was Kosmo Desktop 3.0 RC1[®] (SAIG S.L., Madrid, Spain). For access to geographic data required and sharing results with institutions, support was provided by the spatial data infrastructure for the department by the Regional Information System of the Coffee-Triangle ecoregion (SIR) as standardized and reported before (Bonilla-Aldana et al., 2020; Rodriguez-Morales et al., 2017). The shapefiles of corregiments and veredas (.shp) were linked to a database through spatial joined operation to produce digital maps of the seroprevalence rates

for neosporosis for the study area. Herd data used for the GIS-based maps were derived from the geographical origin of the serosurvey, at the different geographic levels.

Finally, we estimated the seropositivity to *Neospora* by herds levels, using a random-effects model, as well as the leave-one-out forest plot and analysis to assess the impact of individual herds seroprevalence on the global seroprevalence of the study. We used the open software OpenMeta[Analyst], funded by the Agency for Healthcare Research and Quality (AHRQ), Rockville, MD, USA.

3. Results

The seroprevalence of *N. caninum* in cattle from 25 rural farms at Pereira municipality, Risaralda Department, Colombia, by GIS-based maps is shown in Figure 1. The overall prevalence for individual animals and herds was 20.6% (95%CI 16.2%-25.0%) and 92.0%, respectively (Figure 2). The median seropositivity rate was 15.4% (IQR 15.38-30.77%) (binary random-effects model estimate of 17.0%). Considering leaving one of the herds out, beginning with the highest seropositivity, through the one with lower, the impact on the variation of the global result, was low, ranging from 15.6% (95%CI 11.3-20.0%) to 17.8% (95%CI 13.2%-22.4%) (Figure 3).

The descriptive data regarding the risk factors obtained by the questionnaire are provided in Tables 1 and 2. There was no significant association ($p \geq 0.05$) between seropositivity and the assessed variables (Table 1). Noteworthy, regarding the production purpose of the cattle, 273 (84.0%) were for milk and 52 (16.0%) for beef. However, the last ones had significantly higher seropositivity (50% of them above 35% of seropositivity) ($p=0.011$) compared with those for

milk purposes (4.8%) (OR=20.000; 95%CI 1.209-330.952) (Table 2). The exploratory statistical analyses defined the 35% seropositivity threshold as the point with higher significant differences for the comparisons.

4. Discussion

Bovine neosporosis continues to be a neglected parasitic disease in multiple countries with significant cattle activity. And this is the case of Colombia, where there is still a lack of studies about it, them making highly relevant seroprevalence studies for this country, as well for other in the Latin American and the Caribbean region, especially in South America.

Here we provide new data regarding the seroprevalence of bovine neosporosis in Colombia. That is one of the most critical diseases affecting reproduction in cattle around the world (Dubey et al., 1988; Dubey and Schares, 2011), including Colombia. Although, economically important, bovine neosporosis has been neglected in the scientific literature, from epidemiological studies in Colombia (Ramos et al., 2017). The current prevalence is similar to the one found in a recent of the pooled prevalence of *N. caninum* in cattle, where it was 24% (95% CI, 20.0–28.0) for South America (Ribeiro et al., 2019), while in our study it was 17% (95%CI 12.3–21.6) (no significant differences).

More specifically, this study showed that neosporosis is widespread in beef and dairy cattle in the Pereira municipality, the Risaralda department. Noteworthy, the overall estimated seropositivity was 20.6%, and the seroprevalence by herds was 92.0%, which is higher than those in other Latin American regions (Reichel et al., 2013; Ribeiro et al., 2019). That may

suggest that bovine neosporosis may have a negative impact in both the Colombian beef and dairy industry, which also deserves further studies as well as appropriate interventions to reduce its potential negative implications.

Most seroepidemiological studies performed in other countries showed a higher prevalence in dairy cattle than in beef cattle (Fort et al., 2015; Koiwai et al., 2005; Moore et al., 2014). In contrast, our data showed that 50% of them were above 35% of seropositivity. This could be related to differences in management, including feeding practices and herd replacement criteria in this Colombian area. On the other hand, our finding contrasts with a previous survey done in a single farm producing both beef and dairy cattle in the Antioquia department, Colombia (López V et al., 2007), where the prevalence was significantly higher in dairy animals. Even these differences among types of production and regions, both beef and dairy farmers, must check the serostatus for neosporosis before mating or artificial insemination (Dubey et al., 1988; Dubey and Schares, 2011).

In Colombia, other studies have found a seroprevalence of neosporosis of 57.5% in Boyacá department (Pulido Medellín et al., 2013) and 10.2% in Córdoba (Oviedo S et al., 2007), 12.5% in Caquetá (Motta Giraldo et al., 2014; Motta Giraldo et al., 2012), 34.6% in Fredonia, Antioquia (López V et al., 2007), and 76.9% in Nariño (Cedeño Q and Benavides B, 2013).

Like in other important cattle industries around the world, bovine neosporosis may be causing abortions in beef and dairy Colombian farms; however, diagnosis of bovine abortion is hard to achieve. Indeed, both serology and fetal study in the absence of any other abortifacient agent are

necessary to get a final diagnosis of *N. caninum* related abortions (Dubey et al., 1988; Dubey and Schares, 2011).

Limitations

Further studies in the region and the department should also consider performing molecular studies to improve and complement the detection of the infection due to *N. caninum* in cattle, and the possibilities to sequencing and phylogenetic studies for a better characterization of the parasite circulation in these animals (Reichel et al., 2020).

Conclusion

Bovine neosporosis may be causing abortions in both beef and dairy Colombian farms. More research must be done to improve the diagnosis of bovine abortion, including histopathology and other ancillary tests, but also a better knowledge of its epidemiology and factors associated is of utmost importance (Adhami et al., 2020). Finally, also better pieces of evidence regarding disease control, including interrupting the parasite cycle, improvement of farm biosecurity level, testing replacement and purchased cattle, prevention of transmission from definitive hosts, rodent control, and prevention of any immunosuppressive factors which could lead to the reactivation of chronic infection and vertical transmission in cattle, should be available with future research. Also, some reproductive management practices have been proposed to control transmission (Lagomarsino et al., 2019).

Ethical Statement

All persons gave their informed consent before their inclusion in the study. Animal procedures were performed according to standard protocols and guidelines from the Animal Ethics Committee at the Fundación Universitaria Autónoma de las Américas, Pereira, Colombia.

Conflict of Interest

The authors do not have any conflict of interest.

Acknowledgements

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Figure 1: *Neospora caninum* seroprevalence in bovine, presented by GIS-based maps in 25 rural farms from corregimientos and veredas at Pereira municipality, Risaralda Department, Colombia.

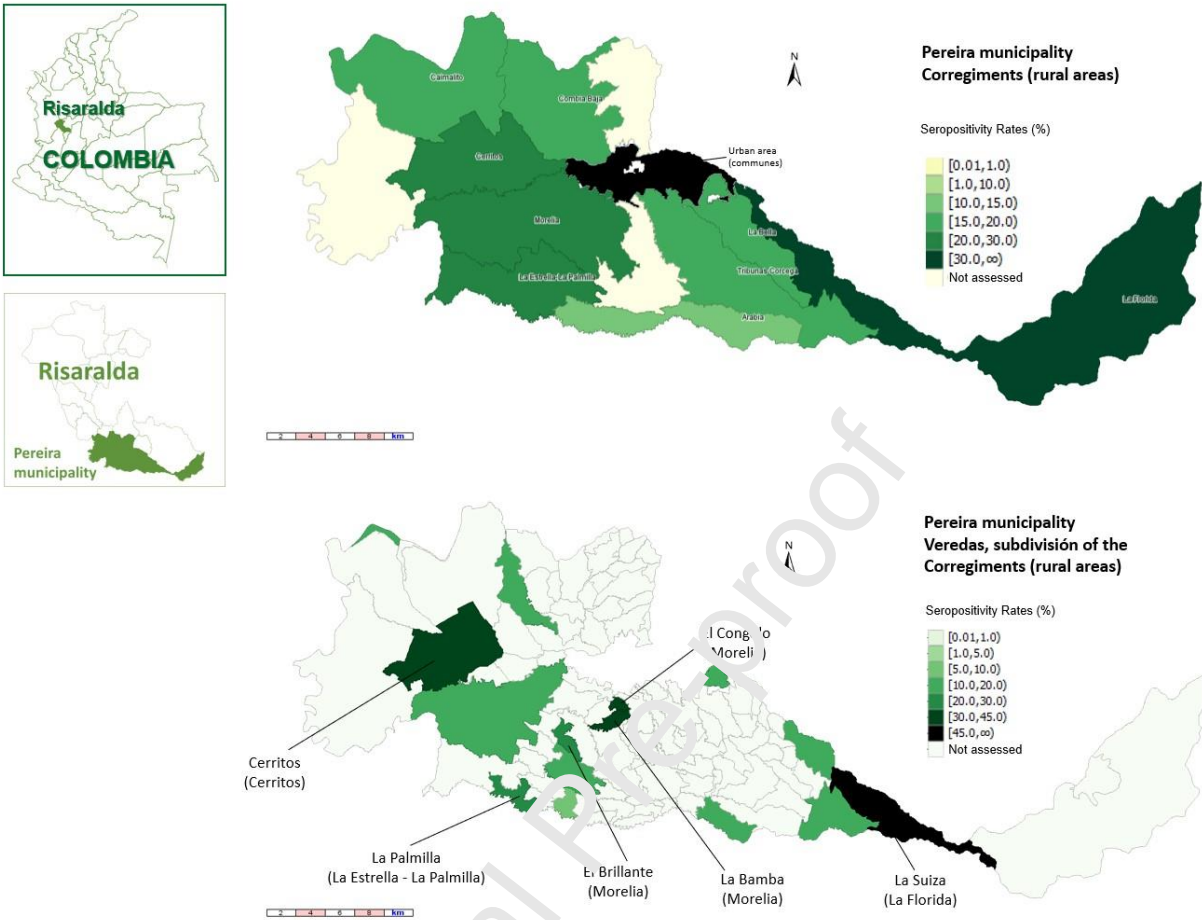


Figure 2: Binary random-effects model for the estimate of seropositivity to *Neospora caninum* by cattle herds (n=25).

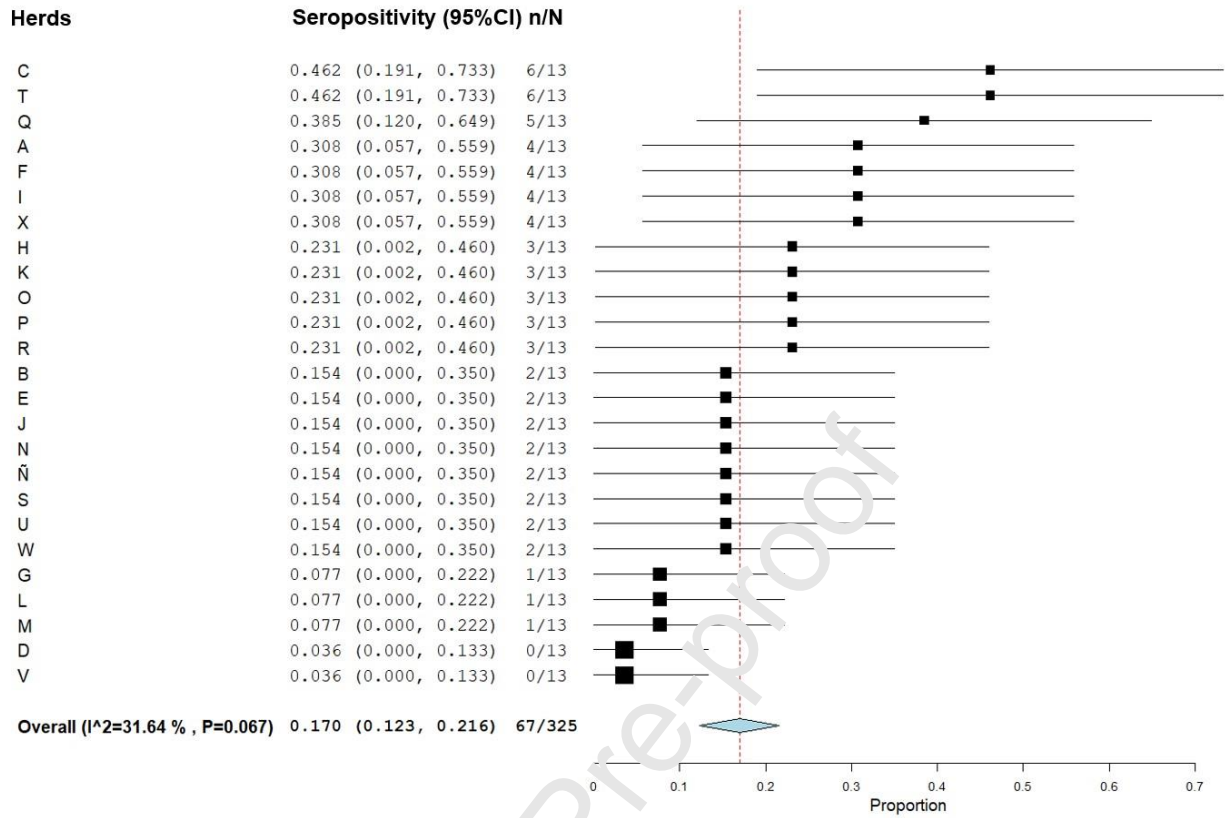


Figure 3: Leave-one-out forest plot and analysis for the estimate of seropositivity to *Neospora caninum* by cattle herds (n=25).

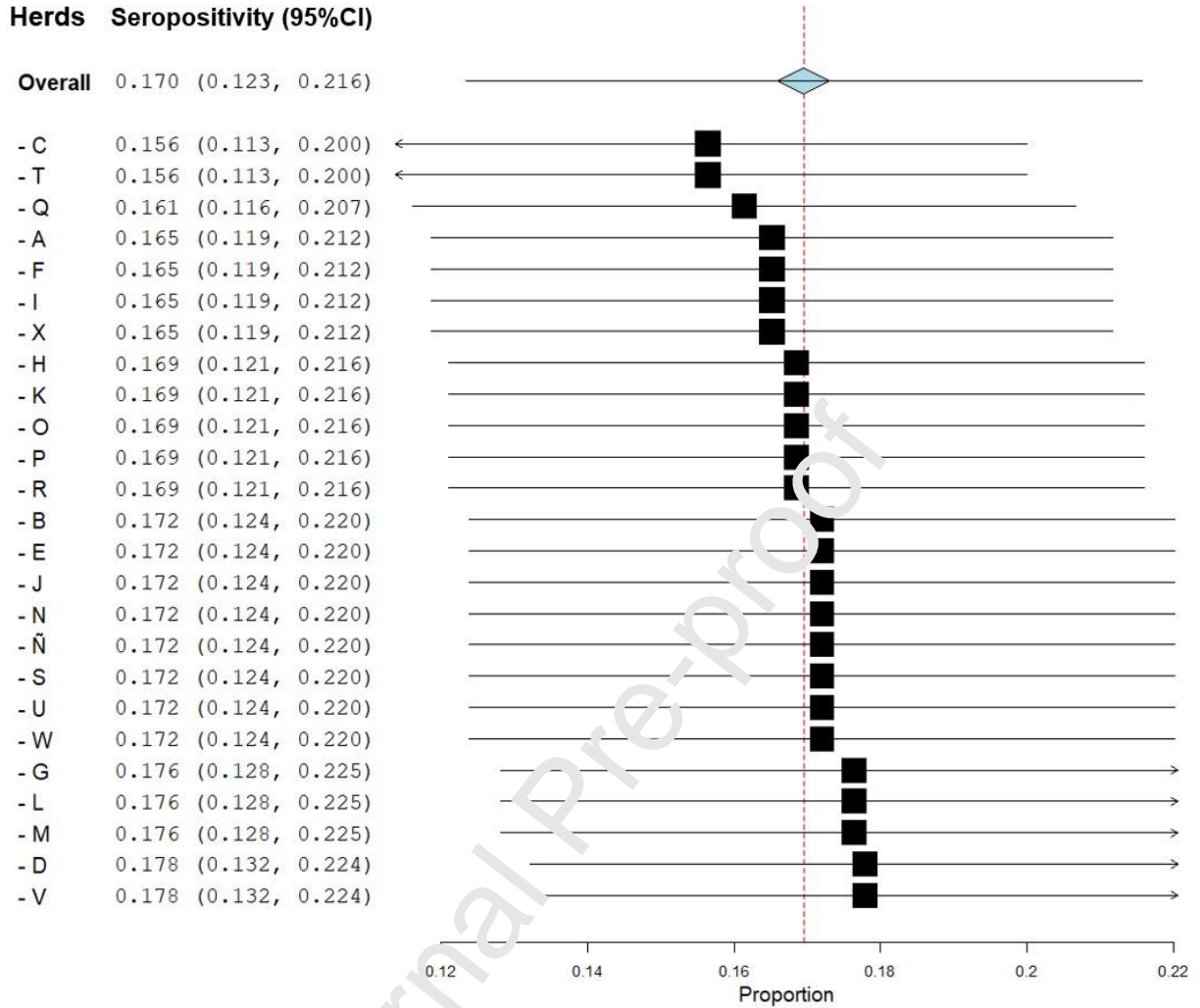


Table 1. Factors associated with the seropositivity to *Neospora caninum* at the individual level, in cattle of Pereira, Colombia.

Factor	Categories	Serology, n (%)		Total	P
		Negative	Positive		
Deliveries (n=169)*	0	33 (76.7)	10 (23.3)	43	0.889
	One or more	98 (77.8)	28 (22.2)	126	
Production (n=325)	Beef	38 (73.1)	14 (26.9)	52	0.220
	Milk	220 (80.6)	53 (19.4)	273	
Milking (n=273)	Mechanical	150 (82.0)	33 (18.0)	183	0.411
	Manual	70 (77.8)	20 (22.2)	90	
Reproductive management (n=325)	Natural breeding	99 (75.6)	32 (24.4)	131	0.163
	Artificial insemination	159 (82.0)	35 (18.0)	194	
Abortions (n=325)	Yes	180 (81.4)	41 (18.6)	221	0.180
	No	78 (75.0)	26 (25.0)	104	

Retained placenta (n=325)	Yes	175 (79.2)	46 (20.8)	221	0.897
	No	83 (79.8)	21 (20.2)	104	
Weak calves delivered (n=325)	Yes	62 (78.5)	17 (21.5)	79	0.819
	No	196 (79.7)	50 (20.3)	246	
Technical assistance (n=325)	Yes	138 (81.7)	31 (18.3)	169	0.292
	No	120 (76.9)	36 (23.1)	156	
Serological test pre-entering the herd (n=325)	Yes	208 (79.7)	53 (20.3)	261	0.781
	No	50 (78.1)	14 (21.9)	64	
Number of dogs around (n=325)	≤2	192 (77.7)	55 (22.3)	247	0.190
	≥3	66 (84.6)	12 (15.4)	78	

*In 156 animals, this variable was unknown.

Table 2. Factors associated with the seropositivity to *Neospora caninum* at the herd level, in cattle of Pereira, Colombia.

Factor, herds with	Categories	Herd seropositivity, n (%)		Total	P
		<35%	≥35%		
Production (n=25)	Beef	2 (0.0)	2 (50.0)	4	0.011
	Milk	20 (95.2)	1 (4.8)	21	
Milking (n=21)	Mechanical	14 (100.0)	0 (0.0)	14	0.147
	Manual	6 (85.7)	1 (14.3)	7	
Reproductive management (n=25)	Natural breeding	8 (80.0)	2 (20.0)	10	0.376
	Artificial insemination	14 (93.3)	1 (6.7)	15	
Abortions (n=25)	Yes	16 (94.1)	1 (5.9)	17	0.170
	No	6 (75.0)	2 (25.0)	8	
Retained placenta (n=25)	Yes	15 (88.2)	2 (11.8)	17	0.958
	No	7 (87.5)	1 (12.5)	8	
Weak calves delivered (n=25)	Yes	5 (83.3)	1 (16.7)	6	0.819
	No	17 (89.5)	2 (10.5)	19	
Technical assistance (n=25)	Yes	12 (92.3)	1 (7.7)	13	0.490
	No	10 (83.3)	2 (16.7)	12	
Serological test pre-entering the herd (n=25)	Yes	18 (90.0)	2 (10.0)	20	0.538
	No	4 (80.0)	1 (20.0)	5	
Number of dogs around (n=25)	≤2	16 (84.2)	3 (15.8)	19	0.299
	≥3	6 (100.0)	0 (0.0)	6	

Highlights

- There are over 25.6 million cattle heads in Colombia being the fourth-highest herd in Latin America.
- We describe the seroprevalence of *Neospora caninum* in cattle from 25 rural farms at Pereira municipality, Risaralda Department, Colombia.
- The overall estimated seropositivity was 20.6% (95%CI 16.23%-25.0%).
- The seroprevalence by herds was 92.0% ranging from 0.0% to 46.15%. Noteworthy, beef herds had significantly higher seropositivity (50% of them above 35% of seropositivity) compared with those for milk purposes (4.8%) (p=0.0107) (OR=20.0; 95%CI 1.209-330.952).
- Other risk factors were not significantly associated with *N. caninum* seropositivity (p≥0.05).