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Original

Detection of antibodies against *Neospora caninum* in canines in the urban and rural area of Cumaral, Meta, Colombia

Paula Méndez-Ramírez[1] MVZ; Julián Marín-Henao[1] MVZ; Agustín Góngora-Orjuela[2*] Dr.Sci; Jorge Parra-Arango[2] MSc; Diego Piedrahita[3] Dr Sci; Jenny Chaparro-Gutiérrez[3] Dr.Sci.

[1]Universidad de los Llanos, Escuela de Ciencias Animales, Villavicencio, Meta, Colombia.
[2]Universidad de los Llanos. Grupo de Investigación en Reproducción y Genética Animal, Villavicencio, Meta, Colombia.
[3]Universidad de Antioquia, Facultad de Ciencias Agrarias. Grupo de Investigación CIBAV, Medellín, Colombia.
[*]Correspondencia: agongora@unillanos.edu.co

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ABSTRACT

Objective. To estimate the seroprevalence of *Neospora caninum* in canines in the urban and rural area of Cumaral, Meta and determine some risk factors associated with seropositivity. **Materials and methods.** A cross-sectional study was carried out on 222 dogs (112 dogs from the urban area and 110 dogs from the rural area), the sample size was calculated using the program Epidat v. 3.1. The sera were analyzed using the Indirect Immunofluorescence technique for IgG with a commercial kit. Chi-square frequency tests were performed using SPSS v. 25.0. **Results**. The general seroprevalence was 36.9% (95% CI: 30.9-43.5%). The seropositivity between the groups was: urban (38.4%) and rural (35.5%) (p>0.05), males (36.9%) and females (36.9%) (p>0.05); in puppies (32.7%), young dogs (40.0%) and adults (37.4%) (p>0.05), in contact with livestock farms (40.7%) and without contact (35.2 %) (p>0.05). **Conclusions.** The seroprevalence observed was high in the two populations analyzed and suggests that the canines have been in contact with the parasite, possibly due to different sources of infection that need to be studied later.

Keywords: Epidemiology; fluorescent antibody technique; indirect; oocysts; abortion *(Source: DeCs).*

RESUMEN

Objetivo. Estimar la seroprevalencia a *Neospora caninum* en caninos del área urbana y rural de Cumaral, Meta y determinar algunos factores de riesgo asociados a la seropositividad. **Materiales y métodos**. Se efectuó un estudio transversal en 222 perros (112 perros del área urbana y 110 del área rural). El tamaño de la muestra fue calculado en el programa Epidat v. 3.1. Los sueros sanguíneos fueron analizados mediante la técnica de Inmunofluorescencia Indirecta para IgG con un kit comercial. Los análisis de frecuencias, chi-cuadrado, fueron realizados mediante el paquete estadístico SPSS v. 25.0 **Resultados**. La seroprevalencia general fue 36.9% (IC95%: 30.9-43.5 %). La seropositividad entre los grupos fue: urbana (38.4 %) y rural (35.5%) (p>0.05), machos (36.9%) y hembras (36.9%) (p >0.05); en cachorros (32.7%), jóvenes (40.0%) y adultos (37.4%) (p>0.05),

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en contacto con predios pecuarios (40.7%) y sin contacto (35.2%) (p>0.05), **Conclusiones**. La seroprevalencia observada fue alta en las dos poblaciones analizadas y sugiere que los caninos han estado en contacto con el parásito, posiblemente por diferentes fuentes de infección que requieren ser estudiadas posteriormente.

Palabras clave: Epidemiología; Técnica del anticuerpo fluorescente indirecta; ooquistes; aborto (*Fuente: DeCs).*

INTRODUCTION

Neospora caninum is an obligate intracellular protozoan, with phylogenetic characteristics similar to *Toxoplasma gondii*, its life cycle is characterized by the parasite's ability to form cysts in the host and cause neuromuscular disease in dogs, however, the greatest effects occur on bovine reproduction, especially abortions and neonatal mortality, which cause economic losses to global livestock production (1). Experimental studies confirm that once the parasite reaches the host, it remains in domestic dogs and cattle (2). Canines, coyotes, and grizzly bears can act as definitive hosts for the protozoan, while some birds and other mammals act as intermediate hosts (3).

Within the epidemiology of *N. caninum,* dogs are definitive hosts of the parasite, eliminating oocysts (4) that become the infective form for cattle and other intermediate hosts after sporulating in the environment (5). The parasite can be transmitted horizontally or vertically, for several generations, which makes the disease perpetuate over time (6). Another form of transmission is through milk contaminated with tachyzoites (1). Dogs affected by *N. caninum,* especially in *in utero* infections, develop severe neuromuscular disorders, with ascending paralysis and hyperextension of the hind limbs (7).

With regard to the general prevalence of *Neospora caninum* in cattle, values of 24% are reported in North and Central America, 18% in South America, 15% in Asia, 13% in Europe, and 8% in Africa and Oceania (8), values that are significantly lower than those reported in some places in Colombia (63-74%) (9,10). Unlike the knowledge that exists on *Neospora* in cattle in Colombia, there is little information about the importance of dogs in transmitting the parasite to cattle. This situation is aggravated by the lack of effective commercial vaccines to protect susceptible animals and prevent subsequent infection (11).

The scarce knowledge of studies in canines shows the need to estimate the prevalence of antibodies in regions with recognized dairy activity, where the reproductive problems caused by *N. caninum* could have a greater impact and therefore are unknown, as is the case of Cumaral, Meta. A rural and urban dog population of 3,788 animals has been quantified in this municipality (12).

The objective of this study was to estimate the seroprevalence of *N. caninum* in dogs in the urban and rural areas of Cumaral, Meta and to determine some factors associated with seropositivity. This information will help in the design of control programs.

MATERIALS AND METHODS

Location and sampling. A cross-sectional epidemiological study was carried out on canines in the Municipality of Cumaral, located in the Piedmont llanero region of the department of Meta, Colombia; Latitude 4º16`10"N and Longitude 73º 29' 11" W, average altitude of 452 masl, precipitation of 3000 mm/year and annual average temperature of 21◦C (Figure 1). The municipality is surrounded by tropical forests, cattle pastures, crops and native forests (13).

The sample size was determined with Epidat 3.1 (14) with the following formula:

$$n = \frac{N * Z^2 * p * q}{EE^2 * (N-1) + Z^2 * p * q}$$

Where N: estimated dog population of Cumaral 3850
p: hypothetical prevalence of *Neospora caninum* 0.19
q: 1-p; 1-0.19= 0.81
Z: 95% confidence: 1.96
EE: precision: 0.05
n: 222 sampling units

Stratified random sampling by location, rural and urban, was carried out at nine sites in the urban area and five villages in the rural area.

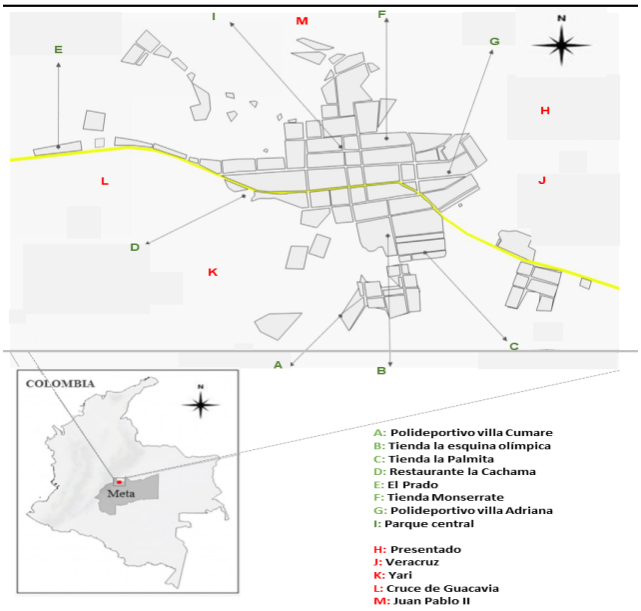

Figure 1. Location and map of the municipality of Cumaral, Meta. The letters in green correspond to the sampling sites of the urban area, and in red to the rural area.

Samples and information. 5 mL of blood were obtained from each animal by puncture of the radial vein with a 21Gx11/2 inch needle, using vacutainer tubes® (Becton Dickinson, USA) under vacuum without anticoagulant. The samples were transported along a cold chain to the Reproduction and Animal Genetics Laboratory of the Universidad de Los Llanos and centrifuged at 5000 *g* for 10 minutes. The serum was extracted with sterile pipettes, divided into 1 mL aliquots and stored at -70 °C until analysis. At the same time as the samples were taken, informed consent was obtained and an epidemiological survey was carried out by means of an interview on the sex, age, breed, size, feeding, location and reproductive status of each animal. The age groups were determined as follows: puppies <12 months of age, young dog 12-24 months, adults > 24 months.

Indirect immunofluorescence for antibodies (IFAT). The IFAT test was used for semi-quantitative detection of antibodies against immunoglobulin G (Fuller Laboratories, Fullerton, California, USA), following the manufacturer's instructions. Briefly, the sera were diluted in phosphate buffer solution (PBS) at a single dilution of 1:16, each well was marked with the serial number of the sample and 10 µL of serum and of the positive and negative controls were dispensed. The slides were incubated for 30 minutes in a humid chamber at 37°C, after which they were washed 3 times with PBS, shaking them to remove the excess sample, without allowing the slides to dry. One drop (10-15 µL) of conjugate (canine anti-IgG in rabbit) was added to each well and incubated for 30 minutes at 37°C in a humid chamber and dark room. The slides were washed again 3 times with PBS and 2-3 drops of mounting medium were added (glycerol 50% pH:7.2 plus thimerosal 0.0005%). The slides were mounted with cover slip and readings were started in a dark room under a microscope at 400X (Olympus IX81, USA). Samples with a positive reaction, in the single dilution 1:16, were considered positive.

Statistical analysis. Seroprevalence was calculated for the fixed factors sex, reproductive status, age group, breed and size and other factors such as: location, feeding, permanence and reproductive disorders in cattle with dogs located in livestock farms. The confidence intervals of the proportions were established using the Wilson method (15). Frequency analysis was performed by chi-square (χ^2) independence test to establish association or independence between epidemiological factors and serological response to *Neospora caninum.* Age did not present normal distribution (Kolmogorov, $p < 0.01$) with the difference between groups being evaluated with the Kruskall-Wallis (KW) test. The software used was Epidat 3.1 (14) and IBM SPSS-25.

Ethical aspects. This study was approved by the Bioethics Committee of the Universidad de los Llanos with act No. 13 of December 6, 2017.

RESULTS

The overall seroprevalence of IgG antibodies against *N. caninum* was 36.9% (82/222) (95% CI 30.9-43.5). Figures 2A and 2B present a positive and negative IFAT test for the presence of IgG antibodies against *Neospora caninum*.

There were no differences by age between seropositive and seronegative dogs (KW χ^2: 1.501; df: 1; significance 0.221), being 36 months in both groups. Seroprevalence in puppies, young dogs and adults was: 32.7, 40.0 and 37.4% respectively without significant differences between groups (Table 1).

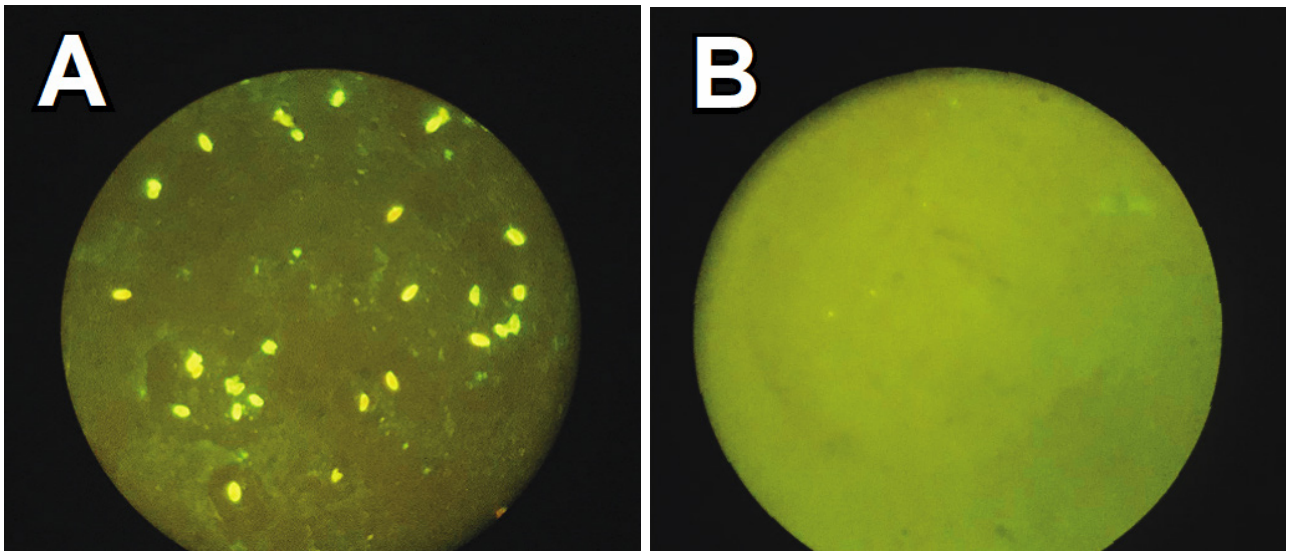

Figure 1. IFAT test, A. Positive test, note presence of fluorescent Neospora caninum tachyzoites. B. Negative control. 10X magnification.

The fixed factors: sex, age group, breed, size and reproductive status, were not found to be associated with *N. caninum* seropositivity (Table 1).

The proportions of seropositive dogs in the urban area, 38.4%, and the rural area, 35.4%, did not present significant differences between them. The same result was observed for type of feeding and the animals' permanence on the street or in a home (Table 2).

The dogs' contact with livestock farms, with cattle as the dominant domestic species, was independent of the serological status for *N. caninum*. The correct or inadequate condition of bovine placentas was independent of the dogs' serological status for *N. caninum*, there was no association between bovine abortion and dogs seropositive for *N. caninum* (Table 3).

Table 1. Fixed factors associated with *Neospora caninum* seropositivity in dogs in Cumaral, Meta.

Factor	Condition	Seropositive N. caninum	Percentage of seropositive	95% CI Seropositive	χ^2 df	Sig.
Sex	Males	41/111	36.9	28.5 – 46.2	0.000	1.000
	Females	41/111	36.9	28.5 – 46.2	1	
Age group	Puppies	16/49	32.7	21.2 – 46.6	0.599	
	Young dogs	20/50	40.0	27.6 – 53.8	2	0.741
	Adults	46/123	37.4	29.4 – 46.2		
Reproductive status	Castrated	16/36	44.0	29.5 – 60.4	0.998	0.318
	Entire	18/53	34.0	22.7 – 47.4	1	
Breed	Cross-breed	55/154	35.7	20.6 – 43.6	0.323	0.570
	Pure breed	27/68	39.7	28.9 – 51.6	1	
Size	Small	10/38	26.3	15.0 – 42.0	3.865	0.145
	Medium	58/139	41.7	33.9 – 50.0	2	
	Large	14/45	31.1	19.5 – 49.7		

CI: Confidence interval; χ^2: Chi-square; df: degrees of freedom, Sig: Significance

Table 2. Other factors associated with *Neospora caninum* seropositivity in dogs from Cumaral, Meta and associated factors.

Factor	Condition	Seropositive *N. caninum*	Percentage seropositive	95% CI Seropositive	χ^2 df	Sig.
Location	Rural	39/110	35.5	27.1 – 44.8	0.206	0.650
	Urban	43/112	38.4	29.9 – 47.6	1	
Feeding	Homemade	2/6	33.3	9.68 – 70.0	0.232	0.890
	Dry food	14/34	41.2	26.4 – 57.8	2	
	Mixed	18/49	36.7	24.7 – 50.7		
Permanence	Street	9/20	45.0	25.8 – 69.8	0.505	0.477
	Home	25/69	36.2	25.9 – 48.0	1	

CI: Confidence interval; χ^2: Chi-square; df: degrees of freedom, Sig: Significance

Table 3. Factors related to contact with cattle, associated with seropositivity to *Neospora caninum* in Cumaral dogs.

Factor	Condition	Seropositive N. caninum	Percentage of seropositive	95% CI Seropositive	χ^2 df	Sig.
Livestock farm contact	Yes	12/35	34.3	20.8 – 50.6	0.475	0.540
	No	22/54	40.7	28.7 – 54.0	1	
Bovine placenta condition	Adequate	6/14	42.9	21.4 – 67.4	0.295	0.587
	Inadequate	25/71	35.2	25.1 – 46.8	1	
Bovine Abortion	Yes	1/5	20.0	3.60 – 62.5	Prueba Fisher	0.648
	No	30/80	37.5	27.7 – 48.5		

CI: Confidence interval; χ^2: Chi-square; df: degrees of freedom, Sig: Significance

DISCUSSION

This study shows the presence of antibodies against *N. caninum* in canines in the urban and rural areas of the municipality of Cumaral, Meta, which suggests that populations have been in contact with the parasite and may represent a risk of horizontal transmission to cattle, as canines are considered the definitive hosts of the parasite. Although no differences were observed between the two populations, several studies indicate a higher seroprevalence in canines in rural areas. In dogs in Tehran (Iran) the prevalence was lower for both populations than those found in this study (16).

In the Northwest of Italy, using IFAT, the prevalence values were as follows for Rural Dogs (RD): (36.4%), (19.5%) and (9.9%) and as follows for Urban dogs (UD): (20.2%), (10.6%) and (4.8%) for the 1:40, 1:80 and 1:160 dilutions respectively. It is difficult to compare these results with the present study as it used a single 1:16 dilution. (17). Conversely, in canines that came from farms in central Italy, the prevalence was higher than 46% (18). In Brazil, dogs in rural areas were more at risk of infection than those in urban areas (19).

In Argentina, Basso et al (20) reported a higher prevalence in dogs on dairy farms (48%) and beef cattle farms (54.2%) than in dogs from urban areas (22.2%). In Japan, Sawada et al. (21), reported a prevalence of 31% in dairy farm dogs vs 7% in urban dogs. In the Netherlands, Wouda et al. (22) reported 23.6% in farm dogs versus 5.5% in dogs from urban areas

In other studies the prevalence has been lower than in the present report in both canine populations, as observed in Henan Province, central area of China RD: (18%) and UD: (11.0%) (23), in Chile in the IX region of the country RD: (26%), UD: (12.5%) ($p<0.05$) (24) and in the State of Yaracuy (Venezuela) RD: (20.6%) and UD: (5.1%) ($p<0.05$) (25). Regardless of the origin of the samples, the prevalences vary widely between regions and

countries. In Poland and Germany, using ELISA, values of 21.7% and 7.3% of seropositivity to *N. caninum* were reported, respectively (26, 27) and in Victoria (Australia), using IFAT, the prevalence was (32.9%) in domestic dogs (28).

In Colombia, a study conducted in El Rosal, Cundinamarca, examined the excrement of 60 dogs resident on 30 farms in the specialized dairy system using PCR, and found 21.6% of the dogs and 33.3% of the farms had *Neospora caninum* DNA (29).

While previous studies have shown a higher prevalence in dogs from farms or rural areas, reflecting different epidemiological conditions than in urban areas, it is possible that in this study, the lack of differences between the two populations studied can be explained by several conditions, including the high mobility of animals from rural to urban areas and vice versa, favoured by the close contiguity between the two zones; the municipality of Cumaral is surrounded by farms, especially milk producing farms, where canines can potentially roam freely, excreting fecal matter and being able to consume the remains of placentas and aborted fetuses. Another explanation could be related to the feeding of the canines in the urban area, where the inhabitants have a high meat consumption and the surplus meat is used for feeding the canines. It is known that raw or undercooked meat can contain parasite cysts and be a source of infection (30).

Another hypothesis could be related to the vertical transmission of the parasite, although no animals with clinical signs compatible with neosporosis were observed in the study. In 50 veterinary outpatient dogs at the Dover Clinic in Bogotá, which presented at least one neurological sign, 12% animals were seropositive for *Neospora caninum* using IFAT and none presented oocysts in fecal matter (31).

Similarly, in England, in a study of 373 female dogs, 13% presented titers greater than 1:50 using IFAT, 50% of the puppies were born seropositive and subsequently 25% of them developed clinical disease compatible with neosporosis; three bitches produced infected puppies in subsequent pregnancies (32).

Although the puppies of the seropositive animals were not studied, the absence of clinical forms of neosporosis in seropositive dogs may be related to low pathogenic strains circulating in the municipality of Cumaral, however, studies in this regard are scarce (6). On the other hand, there are few animal models available to test the variation in pathogenicity. In a study under the mouse model some *Neospora caninum* strains were more pathogenic (33).

Similarly, other possible sources of infection should be considered, mainly through contact with wild species, since the municipality and the region have high biodiversity (34). The presence of seropositivity to *Neospora* has previously been described in wild carnivores and other wild species including dingoes, coyotes, red foxes and birds (35).

The IFAT test used in the study is considered of choice for the diagnosis of *Neospora caninum* (36). By analyzing various serological tests, Campero et al (37) found high sensitivity and specificity (> 95%) and concordance between IFAT, immunoblotting and ELISA-p38. In another study the prevalence of antibodies measured by competitive ELISA and IFAT did not vary significantly between tests, with 32.9% and 29.8%, respectively (26). Although cross-reactions between *Neospora caninum* and other protozoan parasites such as *Toxoplasma gondii* are possible, in the case of IFAT they are considered minimal (38).

With respect to seropositivity according to age, the highest positivity was observed in young animals (40%) which coincides with the reported data. In dogs from Victoria, Australia, however, the highest prevalence was found in the adult group (28). Breed, sex and age were not factors associated with seroprevalence, as was observed in canines from the central area of China (23). By contrast in Poland, the prevalence was higher in females than males (28.0% and 17.3% respectively $p<0.05$). Similar results to the present study, for the variables age, sex, race and eating habits (18).

The results of this study have an important epidemiological significance, since either group of canines represent a risk for cattle. Wouda et al (22), have observed an increased risk of *Neospora* infection on farms where dogs live with livestock, suggesting ongoing surveillance for infection and disease, and the need for continuing education of professionals, estate managers, livestock and pet owners.

It is concluded that seroprevalence is high with no differences between dogs in urban and rural

areas suggesting that they have been in contact with the parasite, possibly from different sources of infection that need to be studied as the possible implications of horizontal transmission for livestock.

Conflict of interests

The authors declare no conflict of interest.

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REFERENCES

1. Dubey JP, Schares G, Ortega-Mora LM. Epidemiology and control of neosporosis and *Neospora caninum*. Clinic Microbiol Rev. 2007; 20(2):323-367. https://doi.org/10.1128/CMR.00031-06

2. Regidor-Cerrillo J, Díez-Fuertes F, García-Culebras A, Moore DP, González-Warleta M, Cuevas C, et al. Genetic diversity and geographic population structure of bovine *Neospora caninum* by microsatellite genotyping analysis. PLoS ONE. 2013; 8(8):e72678. https://doi.org/10.1371/journal.pone.0072678

3. Prandini da Costa Reis R, Crisman R, Roser M, Malik R, Slapeta J. Neonatal neosporosis in a 2-week-old Bernese mountain dog infected with multiple *Neospora caninum* strains based on MS10 microsatellite analysis. Vet Parasitol. 2016; 15(221):134–138. https://doi.org/10.1016/j.vetpar.2016.03.023

4. Nazir MM, Maqbool A, Akhtar M, Ayaz M, Ahmad AN, Ashraf K, et al. *Neospora caninum* prevalence in dogs raised under different living conditions. Vet Parasitol. 2014; 204(3–4):364–368. https://doi.org/10.1016/j.vetpar.2014.05.041

5. Arunvipas P, Inpankaew T, Jittapalapong S. Risk factors of *Neospora caninum* infection in dogs and cats in dairy farms in Western Thailand. Trop Anim Health Prod. 2012; 44(5): 1117–1121. https://doi.org/10.1007/s11250-011-0048-2

6. Dubey JP, Schares G, Ortega-Mora LM. Epidemiology and Control of Neosporosis and *Neospora caninum.* Clinic Microbiol Rev. 2007; 20(2):323–367. https://doi.org/10.1128/CMR.00031-06

7. Langoni H, Matteucci G, Medici B, Camossi LG, Richini- Pereira VB, Silva RC. Detection and molecular analysis of *Toxoplasma gondii* and *Neospora caninum* from dogs with neurological disorders. Rev Soc Bras Med Trop. 2012; 45(3):365–368. http://dx.doi.org/10.1590/S0037-86822012000300016

8. Ribeiro CM, Soares IR, Mendes RG, de Santis Bastos PA, Katagiri S, Renato Zavilenski RB. Meta-analysis of the prevalence and risk factors associated with bovine neosporosis. Tropical Animal Health and Production. 2019; 51(7):1783-1800 https://doi.org/10.1007/s11250-019-01929-8

9. Cardona JA, Martínez Y, Betancur CA. Seroepidemiología de hembras bovinas naturalmente infectadas por *Neospora caninum* en Córdoba, Colombia. Rev UDCA Act & Div Cient. 2015; 18(2):401-408 https://revistas.udca.edu.co/index.php/ruadc/article/view/166/1281

10. Vargas-Niño A, Vargas J, Parra J, Vásquez M, Góngora A, Mogollón-Waltero EM. Estado serológico a IBR, DVB, Leucosis, Leptospira y *Neospora caninum* en hembras bovinas del Departamento de Santander, Colombia. Rev MVZ Córdoba. 2018; 23(2):6671-6680. https://doi.org/10.21897/rmvz.1341

11. Silva R, Machado G. Canine neosporosis: perspectives on pathogenesis and management. Vet Med. 2016; 26(7):59–70. https://doi.org/10.2147/VMRR.S76969

12. Ministerio de Salud. Reporte de vacunación antirrábica de perros y gatos, Colombia 2017. En http://www.minsalud.gov.co/sites/rid/Lists/BibliotecaDigital/RIDE/VS/PP/SA/nacional-municipio-2017.pdf. Revisado 5/02/2020

13. IDEAM, 2014. Atlas climatológico de Colombia. Instituto de Hidrología, Meteorología y Estudios Ambientales (IDEAM), Bogotá, Colombia. http://documentacion.ideam.gov.co/cgi-bin/koha/opac-detail.pl?biblionumber=1381&shelfbrowse_itemnumber=1465

14. EPIDAT 3.1. 2006. Programa para análisis de datos epidemiológicos tabulados. Versión 3.1. Xunta de Galicia, Conselleria de sanidade-Organización Panamericana de la salud. OPS. https://www.sergas.es/Saude-publica/Epidat-3-1-descargar-Epidat-3-1-(espanol)

15. Newcombe RG, Merino C. Intervalos de confianza para las estimaciones de proporciones y las diferencias entre ellas. Interdisciplinaria. 2006; 23 (2):141-154.

16. Haddadzadeh HR, Sadrebazzaz A. Malmasi A, Ardakani HT, Nia PK, Sadreshirazi N. Seroprevalence of *Neospora caninum* infection in dogs from rural and urban environments in Tehran, Iran. Parasitol Res. 2007; 101 (6):1563–1565. https://doi.org/10.1007/s00436-007-0678-5

17. Ferroglio E, Pasino M, Ronco F, Bena A, Trisciuoglio. A Seroprevalence of Antibodies to *Neospora caninum* in Urban and Rural Dogs in north-west Italy. Zoonoses Public Health. 2007; 54:135–139. https://doi.org/10.1111/j.1863-2378.2007.01033.x

18. Robbe D, Passarelli A, Gloria A, Di Cesare A, Capelli G, Iorio R, Traversa D. *Neospora caninum* seropositivity and reproductive risk factors in dogs. Exp Parasitol. 2016; 164:31-35. https://doi.org/10.1016/j.exppara.2016.02.003

19. Nogueira CI, Mesquita LP, Abreu CC, Nakagaki KY, Seixas JN, Bezerra PS, et al. Risk factors associated with seroprevalence of Neospora caninum in dogs from urban and rural areas of milk and coffee production in Minas Gerais State, Brazil. Epidemiol Infect. 2013; 141(11):2286–2293. https://doi.org/10.1017/S0950268813000162

20. Basso W, Venturini L, Venturini M, Moore P, Rambeau M, Unzaga J, et al. Prevalence of *Neospora caninum* infection in dogs from beef cattle farms, dairy farms, and from urban areas of Argentina. J Parasitol. 2001; 87(4):906-907. https://doi.org/10.1645/0022-3395(2001)087[0906:PONCII]2.0.CO;2

21. Sawada M, Park CH, Kondo H, Morita T, Shimada A, Yamane I, et al. Serological survey of antibody to *Neospora caninum* in Japanese dogs. J Vet Med Sci. 1998; 60(7):853-854. https://doi.org/10.1292/jvms.60.853

22. Wouda W, Dijkstra T, Kramer AM, van Maanen C, Brinkhof JM. Seroepidemiological evidence for a relationship between *Neospora caninum* infections in dogs and cattle. Int J Parasitol. 1999; 29(10):1677-1682. https://doi.org/10.1016/s0020-7519(99)00105-8

23. Wang S, Yao Z, Zhang N, Wang D, Ma J, Liu S, Zheng B, Zhang B, Liu K, Zhang H. Serological study of *Neospora caninum* infection in dogs in central China. Parasite. 2016, 23(25):1-5. https://doi.org/10.1051/parasite/2016025

24. Patitucci AN, Phil M, Pérez MJ, Rozas MA, Israel KF. Neosporosis canine: detection of sera antibodies in rural and urban canine population of Chile. Arch Med Vet. 2001; 33 (2):227-232. http://dx.doi.org/10.4067/S0301-732X2001000200011

25. Escalona JJ, Corro AC, Suárez CE, Castillo TA, Pineda YA. Seropositividad a *Neospora caninum* en perros de áreas rurales y urbanas del estado Yaracuy, Venezuela. Rev Fac Cs Vets. UCV. 2013; 54(1):29-34. http://saber.ucv.ve/ojs/index.php/revisfcv/article/view/5146/4953

26. Gozdzik K, Wrzesien R, Wielgosz-Ostolska A, Bien J, Kozak- Ljunggren M, Cabaj W. Prevalence of antibodies against *Neospora caninum* in dogs from urban areas in Central Poland. Parasitology Research. 2011; 108(4):991–996. https://doi.org/10.1007/s00436-010-2143-0

27. Villagra-Blanco R, Angelova L, Conze T, Schares G, Bärwald A, Taubert A, Hermosilla C, Wehrend A. Seroprevalence of *Neospora caninum*-specific antibodies in German breeding bitches. Parasites & Vectors. 2018; 11(96):2-6. https://doi.org/10.1186/s13071-018-2683-1

28. Sloan S, Šlapeta J, Jabbar A, Hunnam J, De Groef B, Rawlin G, McCowan C. High seroprevalance of *Neospora caninum* in dogs in Victoria, Australia, compared to 20 years ago. Parasites & Vectors. 2017; 10(1):503. https://doi.org/10.1186/s13071-017-2464-2

29. Bulla-Blanco KD, Vanegas-Rojas PX. (2015). Identificación de neospora caninum en materia fecal de 60 caninos en hatos lecheros en el municipio el Rosal, Cundinamarca. Retrieved from https://ciencia.lasalle.edu.co/medicina_veterinaria/104

30. Cedeño D, Benavides B. Seroprevalencia y factores de riesgo asociados a *Neospora caninum* en ganado lechero en el municipio de Pasto, Colombia. Rev MVZ Córboba. 2013; 18(1):3311-3316. https://doi.org/10.21897/rmvz.193

31. Ravelo-Salcedo L. (2009). Estudio clínico, serológico y coprológico preliminar de Neospora Caninum en caninos de la clínica veterinaria Dover Bogotá - Colombia. Retrieved from https://ciencia.lasalle.edu.co/medicina_veterinaria/296

32. Barber JS, Trees AJ. Naturally ocurring vertical transmission of *Neospora caninum* in dogs. Int J Parasitol. 1998; 28:57-64. https://doi.org/10.1016/s0020-7519(97)00171-9

33. McInnes LM, Irwin P, Palmer DG, Ryan UM. In vitro isolation and characterization of the first canine *Neospora caninum* isolate in Australia. Vet Parasitol. 2006; 137:355–363. https://doi.org/10.1016/j.vetpar.2006.01.018

34. Rangel O. La biodiversidad de Colombia: significado y distribución regional. Rev Acad Colomb Cienc Ex Fis Nat. 2015; 39(151):176-200. http://dx.doi.org/10.18257/raccefyn.136

35. Donahoe SL, Lindsay SA, Krockenberger M, Phalen D, Šlapeta J. A review of neosporosis and pathologic findings of *Neospora caninum* infection in wildlife. Int J Parasitol Parasites Wildl. 2015; 4:216–38. http://doi.org/10.1016/j.ijppaw.2015.04.002

36. Atkinson R, Harper PA, Reichel MP, Ellis JT. Progress in the serodiagnosis of *Neospora caninum* infections of cattle. Parasitol Today. 2000; 16(3):110–114. https://doi.org/10.1016/s0169-4758(99)01604-x

37. Campero LM, Minke L, Moré G, Rambeaud M, Bacigalupe D, Moore DP, et al. Evaluation and comparison of serological methods for the detection of bovine neosporosis in Argentina. Rev Argent Microbiol. 2015; 47(4):295-301. https://doi.org/10.1016/j.ram.2015.07.002

38. Hemphill A, Gottstein B. A European perspective on Neospora caninum. Int J Parasitol. 2000; 30:877–924. https://doi.org/10.1016/S0020-7519(00)000722