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

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

**Keywords:** Epidemiología; técnica de antígenos fluorescentes; indirecta; oocistos; aborto *(Source: DeCs).*
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:

\[
n = \frac{N \times Z^2 \times p \times q}{EE^2 \times (N-1) + Z^2 \times p \times q}
\]

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 (χ²) 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 χ²: 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).
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).

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

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

<table>
<thead>
<tr>
<th>Factor</th>
<th>Condition</th>
<th>Seropositive N. caninum</th>
<th>Percentage of seropositive</th>
<th>95% CI</th>
<th>χ² df</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Males</td>
<td>41/111</td>
<td>36.9</td>
<td>28.5 – 46.2</td>
<td>0.000</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>41/111</td>
<td>36.9</td>
<td>28.5 – 46.2</td>
<td>1</td>
<td>0.599</td>
</tr>
<tr>
<td>Age group</td>
<td>Puppies</td>
<td>16/49</td>
<td>32.7</td>
<td>21.2 – 46.6</td>
<td>2</td>
<td>0.741</td>
</tr>
<tr>
<td></td>
<td>Young dogs</td>
<td>20/50</td>
<td>40.0</td>
<td>27.6 – 53.8</td>
<td>1</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Adults</td>
<td>46/123</td>
<td>37.4</td>
<td>29.4 – 46.2</td>
<td>0.318</td>
<td></td>
</tr>
<tr>
<td>Reproductive status</td>
<td>Castrated</td>
<td>16/36</td>
<td>44.0</td>
<td>29.5 – 60.4</td>
<td>0.998</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Entire</td>
<td>18/53</td>
<td>34.0</td>
<td>22.7 – 47.4</td>
<td>1</td>
<td>0.318</td>
</tr>
<tr>
<td>Breed</td>
<td>Cross-breed</td>
<td>55/154</td>
<td>35.7</td>
<td>20.6 – 43.6</td>
<td>0.323</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pure breed</td>
<td>27/68</td>
<td>39.7</td>
<td>28.9 – 51.6</td>
<td>1</td>
<td>0.570</td>
</tr>
<tr>
<td>Size</td>
<td>Small</td>
<td>10/38</td>
<td>26.3</td>
<td>15.0 – 42.0</td>
<td>3.865</td>
<td>0.145</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>58/139</td>
<td>41.7</td>
<td>33.9 – 50.0</td>
<td>2</td>
<td>0.145</td>
</tr>
<tr>
<td></td>
<td>Large</td>
<td>14/45</td>
<td>31.1</td>
<td>19.5 – 49.7</td>
<td>1</td>
<td>0.570</td>
</tr>
</tbody>
</table>

CI: Confidence interval; χ²: 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.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Condition</th>
<th>Seropositive N. caninum</th>
<th>Percentage seropositive</th>
<th>95% CI Seropositive</th>
<th>χ² df</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location</td>
<td>Rural</td>
<td>39/110</td>
<td>35.5</td>
<td>27.1 – 44.8</td>
<td>0.206</td>
<td>0.650</td>
</tr>
<tr>
<td></td>
<td>Urban</td>
<td>43/112</td>
<td>38.4</td>
<td>29.9 – 47.6</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Feeding</td>
<td>Homemade</td>
<td>2/6</td>
<td>33.3</td>
<td>9.68 – 70.0</td>
<td>0.232</td>
<td>0.890</td>
</tr>
<tr>
<td></td>
<td>Dry food</td>
<td>14/34</td>
<td>41.2</td>
<td>26.4 – 57.8</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mixed</td>
<td>18/49</td>
<td>36.7</td>
<td>24.7 – 50.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Permanence</td>
<td>Street</td>
<td>9/20</td>
<td>45.0</td>
<td>25.8 – 69.8</td>
<td>0.505</td>
<td>0.477</td>
</tr>
<tr>
<td></td>
<td>Home</td>
<td>25/69</td>
<td>36.2</td>
<td>25.9 – 48.0</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

CI: Confidence interval; χ²: 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.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Condition</th>
<th>Seropositive N. caninum</th>
<th>Percentage seropositive</th>
<th>95% CI Seropositive</th>
<th>χ² df</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Livestock farm contact</td>
<td>Yes</td>
<td>12/35</td>
<td>34.3</td>
<td>20.8 – 50.6</td>
<td>0.475</td>
<td>0.540</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>22/54</td>
<td>40.7</td>
<td>28.7 – 54.0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Bovine placenta condition</td>
<td>Adequate</td>
<td>6/14</td>
<td>42.9</td>
<td>21.4 – 67.4</td>
<td>0.295</td>
<td>0.587</td>
</tr>
<tr>
<td></td>
<td>Inadequate</td>
<td>25/71</td>
<td>35.2</td>
<td>25.1 – 46.8</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Bovine Abortion</td>
<td>Yes</td>
<td>1/5</td>
<td>20.0</td>
<td>3.60 – 62.5</td>
<td>Prueba Fisher</td>
<td>0.648</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>30/80</td>
<td>37.5</td>
<td>27.7 – 48.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CI: Confidence interval; χ²: 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 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.
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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