Presence of The Neospora Caninum Parasite in Canine in The City of Valledupar, Colombia

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Accepted 20th August 2018

Abstract

In this investigation the presence of the parasite N. caninum in Canines was determined. The study that was conducted was observational, descriptive and cross-sectional, 50 canines of various races and sex were chosen, in different neighborhoods of the city of Valledupar, they were taken a blood sample and fecal material for an examination. Serological and coprological respectively. The antibodies against N. caninum were determined by the indirect immunofluorescence test (IFIT) in the laboratory Vida S.A. from the city of Valledupar and coprológicos using the Faust Test. The results showed that of the 50 sera analyzed by the IFI technique, four presented positive results to N. caninum, while none of the stool samples analyzed allowed to recognize oocysts of the parasite, but non-sporulated cysts that are eliminated by the dogs. Infected by this parasite. It is concluded that N. caninum should be considered as a sporadic etiological agent in dogs in the urban area of the city of Valledupar.

Keywords: Antibody, oocysts, parasitosis, serology, zoonoses

Introduction

For many of the inhabitants of modern urban societies, companion animals are the closest form of contact with the living animal world. The possession and care of animals of companies are historical phenomena, which can be said that, with few exceptions, they are of worldwide acceptance [1]. The positive influence of pets on the health and well-being of human beings is well recognized and includes the psychological, physiological, therapeutic and psychosocial aspects [25]. Dogs have developed a special relationship with humans, and can be considered the only species that has established a proper niche in human society [42]. Many companion dogs occupy a privileged position in our society, living closely with humans who do everything possible to provide their needs and desires [9]. Traditionally, the dog has helped man in tasks such as hunting, surveillance and as an invaluable aid in livestock grazing. However, to the extent that society has evolved from small farming communities, to increasingly larger metropolitan areas, the role of the dog has changed [12] therefore, the special relationship that dogs have developed with humans has been studied not only from the perspective of the social sciences, but also from the perspectives of psychology and human medicine [34].

Neosporosis is a disease caused by a recently recognized protozoan called N. caninum, which infects a wide range of animal species and can cause primary disease in dogs and livestock [11,19]. Domestic dogs are the only known definitive hosts for N. caninum [20]. This parasite was isolated and identified and was named Neospora caninum and was located directly in the cytoplasm of the host cell without a parasitophorous vacuole [3,17].

The morphological studies carried out with electron microscopy on the N. caninum protozoan have shown that this organism possesses a typical subcellular structure of parasites classified in the family Sarcocystidae, subclass Coccidiasina of the phylum Apicomplexa. [22,28].

Neospora caninum was observed microscopically in sections of naturally infected pups, and was isolated in cell cultures from mice and dogs inoculated with infected canine tissues. Antibodies against N. caninum were detected in sera from infected dogs by indirect immunofluorescence test [16]. There are several types of serological tests, based on the use of organisms derived from cultures or recombinant antigens of N. caninum. [18], although for some time it was discussed whether the parasites originating from dogs and bovines were similar, it coincided later in their structural and antigenic similarity. [2]. Birds, in particular, can contribute to the spread of the parasite, mainly because birds generally feed on the ground, are exposed to many pathogens and are attacked by canids, which contributes to the life cycle of the parasite [14].

It has been documented that the presence of this parasite is greater in canines from rural areas, than that described for dogs, in urban areas. [21,23,39]. Likewise, seropositivity was higher in dogs from dairy farms and cattle than in dogs from urban areas. [4, 29]. A high prevalence has also been found in stray dogs, compared with that found in house dogs [4,24]. The identification of the N. caninum oocyst by bioassay and polymerase chain reaction shows that the dog is a definitive natural host for N. caninum. [6]

Several studies have shown the prevalence of N. caninum infection in dogs from urban areas in South America, such as in Argentina [4], Colombia [40], Peru [28], Venezuela [21],
Brazil [23] and Chile [39], however, further research is needed in order to understand how to control the disease, since *N. caninum* infection has a severe economic impact on the beef and dairy cattle industries in this continent [38]. Dogs should not only be the target of deep investigations for this, but because it can cause death in these companion animals [32].

Similarly, in Colombia, there is not much information on Neosporosis in canines located in urban areas, although a clinical, serological and coprological study of *N. caninum* has been conducted, arguing that with this result it can be concluded that *N. caninum* should be considered as an important etiologic agent in dogs [40]. Currently, the scientific community is working intensively on the understanding of the biology and epidemiology of *N. caninum*, in an effort to develop diagnostic tools and establish control measures for Neosporosis [21,30,31,33,35]. The first isolation of this parasite has been developed a series of serological tests for use in dogs, livestock and a variety of other possible host species, which include the indirect immunofluorescence test, the direct agglutination test and different immunosorbent assays linked to enzymes.[7] In the city of Valledupar has not been determined the presence of this parasite, because there is no report in canines in the urban area. Considering the current importance of this parasite in public health and the limited number of investigations carried out around it, it is essential to develop a preliminary study with the general objective of identifying this parasite by detecting serum antibodies and feces in canines in the urban area of the city of Valledupar.

### 2.0 Materials and Methods.

#### 2.1 Place of Study.

The study was carried out in the city of Valledupar, capital of the department of Cesar - Colombia (Figure 1). It is located 10°29’ north latitude and 73°15’ west longitude. By the North it limits with the departments of the Magdalena and the Guajira, by the south with the municipalities of Sandiego, the peace and the Step; by the East with the Department of the Guajira and the municipalities of Sandiego and La Paz and by the West with the Magdalena and the municipalities of Bosconia and the Copey. The City has 6 communes where we find a total of about 175 Districts and of which we take samples in 20% of the neighborhoods of said city.

**Figure 1.** Satellite Map of the City of Valledupar, Mexico

Source: Gosur.2018

#### 2.2 Animals

In the present study, 50 dogs were used, with or without defined race, of both sexes and of different ages, without presenting any clinical symptomatology characteristic of the disease, being 30 males and 20 females respectively.

Regarding the age group, 26 dogs with ages ranging from 1 to 3 years, 16 animals aged 4 to 6 years and 8 dogs aged over 7 years were evaluated. Of the total of the studied animals belonged to the urban area of the city of Valledupar

##### 2.2.1 Collection, Preparation and Identification of Samples.

The blood samples of the animals were collected by venipuncture of the jugular or cephalic, after previous asepsis, with the help of sterile needles (30 x 8 mm), in sterile Vacutainer tubes without anticoagulant, totaling 10 ml of blood per animal. After retraction of the clot, the samples were centrifuged for ten minutes at 2000g, obtaining clear sera that were stored at -20°C in aliquots until the time of serological tests.

Likewise, the fifty patients were taken a stool sample for a coprological examination directly from the rectum using a handle for fecal matter or the center of the deposition, such sample is placed in a hermetically closed bag taking out all the air, to process it later.

##### 2.2.2 Indirect Immunofluorescence Reaction.

It was done with *N. caninum* IFA canine IgG Antibody Kit as follows. A 1:16 dilution is made by placing 150L of PBS How to Cite this Article: Rodriguez Ximena, Vizcaino Margarita & Araujo Alvaro "Presence of The Neospora Caninum Parasite in Canine in The City of Valledupar, Colombia" Weber Microbiology Research (ISSN: 2449-1606), Vol. 2(1) 2018, Article ID wmr_241, 1054-1059
with 10L of the serum to be evaluated, then the dilution is prepared for the positive control and the negative control that come in the Kit. 10L is placed in each well of the plate placing the positive control and the negative control in well 1 and 2 respectively.

In a dark petri dish with moisture, the foil was placed and taken to the incubator at 37°C for 30 minutes. Four washes are made with PBS and the conjugate of the kit is added. Subsequently, it is incubated again at 37°C for 30 minutes.

Again 4 washes are made with PBS, the sheet is dried very well. A drop is placed in each well of mounting medium and the sheet is covered with 2 coverslip slides, to be read in the fluorescent microscope at 400X. Comparing each well with the positive and negative control. Sera showing fluorescence were diluted 2 by 2 until the title was found.

2.2.3. FAUST Test.

2 grams of stool were weighed on a scale. The previously weighed stool is placed in a Bicker. 17 ml of distilled water are added. Dissolve with a spatula and sift. It is placed in a test tube and centrifuged at 2500 rpm for 5 minutes.

The supernatant is removed and the test tube is filled with a solution of 33% Zinc Sulfate (ZnSO₄) until it forms a meniscus. A cover slip is placed on top and centrifuged for 3 minutes. Later, this lamella is mounted on a sheet to be observed directly under a microscope. The film is traversed at 40X in search of *N. caninum* oocysts.

2.3 Statistical Analysis

The proportions comparison test based on the Qui-squared statistic at the 5% significance level was used. The variables analyzed correspond to the seroprevalence of dogs to *N. caninum* and the presence of *N. caninum* oocysts in fecal matter.

### 3.0 Results and Discussion

The diagnostic technique used for the determination of antibodies against *N. caninum* in the present work was the IFAT "test" that has been used in most canine serological studies [5]. The dilution of cut 1/50 allows to differentiate infected animals from those that are not [39]; so the animals that were positive in this study, are considered infected at the time of sampling. Of the 50 canines studied, only 8% (4 patients) were reactive to *N. caninum* by the indirect immunofluorescence test, and none presented oocysts in the fecal matter (Figure 2, 3). This result is higher than that found in dogs in urban areas where a general positivity of 5.17% was obtained using the serological technique of indirect immunofluorescence [21]. This result is lower than that found in populations of urban canines in Chile, where 120 canines were sampled and 15 positive animals were found evidencing the presence of antibodies against *N. caninum*. [39] Likewise, in a study conducted with sera from dogs from urban areas, with neurological signs remitted for the diagnosis of Neosporosis, in a period of 10 years, the detection of antibodies for Neosporosis in sera from dogs with compatible clinical signs was 25.6 % [44]. Likewise, studies reveal that antibodies against *N. caninum* were detected in sera from infected dogs by indirect immunofluorescence test. [26]

Obviously the study populations of these investigations were greater than ours, so we inferred that there is no correlation between the serological findings and the coprological examinations in our study. Considering the limitations of the sampling methodology, the number of infected canines found in the present work could be considered as low, a situation similar to that reported by the different researchers worldwide where the prevalence rates in dogs from urban areas was of 26.2% in Argentina [4], 12% in England [43], as it has also been determined that dogs from urban areas were significantly less seropositive for *N. caninum* than those from rural Austria. [45].

![Figure 2. Number of Animals Positive and Negative to IFAT](image-url)
The difficulty in the search for *N. caninum* oocysts was verified in this investigation; however, we were able to verify non-sporulated oocysts in fecal stool of canines. It has been demonstrated that the identification of *N. caninum* in the feces of dogs should be based on the recovery of viable tachyzoites in cell cultures or in rodents inoculated with oocysts due to the existence of other *N. caninum* type parasites in the dog feces. [41], as the identity of oocysts is also confirmed by a bioassay [15]. This result coincides with a study in which no *N. caninum* oocysts were detected in fecal samples from 230 dogs, including 160 farm dogs. [37]

The results of our study do not coincide with investigations carried out in Colombia in order to determine the sporulation of oocysts of the *N. caninum* parasite, in which fecal samples were taken in 60 dogs of different sex, age and race and where they processed with the PCR technique, resulting that 21.6% of the total sampled animals with *N. caninum* genomic material and 78% of the samples 78% did not recognize the presence of the parasite. (10)

It is also inferior to that found by other researchers where *N. caninum* oocysts were found in canine fecal matter, by means of the centrifugal flotation technique, where the seropositivity found was 34%, as well as the prevalence for oocysts of *N. caninum* [8]. A complete study of *N. caninum* infection in dogs from Germany highlighted the difficulties in the identification of *N. caninum* oocysts. In this study, oocysts were found in 47 of 24,089 stool samples. And the oocysts were isolated from 28 of the 47 samples [27].

On the other hand, in Costa Rica to detect *N. caninum* oocysts in dog feces and determine excretion in dogs, a total of 265 samples were collected every 15 days for 7 months, such samples were examined microscopically, by PCR and by bioassay. *N. caninum* DNA was detected by PCR in four fecal samples, twice from a dog, but oocysts were not detected microscopically in these dogs [36]. However, the identification of the *N. caninum* oocyst by bioassay and polymerase chain reaction shows that the dog is a definitive natural host for *N. caninum*. [2]

When the positivity of the animals was evaluated according to age (Figure 3), a similarity of the dogs with ages ranging from 1 to more than 4 years of age was evidenced, so we assume that age is not a limitation for that the dogs become infected with this parasite. The age of the canines showed no variation in seroprevalence, which would mean that horizontal transmission would not be very important in the epidemiology of the disease. [29], however, study conducted...
in Austria showed a slight increase in seropositivity with age, indicating postnatal infection. (Four. Five).

Also in a study on the prevalence of antibodies anti- N. caninum in dogs from urban, peri-urban and rural areas in which it was shown that there were increasing levels of antibody prevalence with the increase in the age of the dogs in the three areas studied. Although this increase was not significant, it indicates a trend towards more infections with age, suggesting a postnatal exposure to N. caninum. However, a significant difference (P = 0.05) was observed in the appearance of anti-N. caninum in dogs with ages ≥ 2 years in urban (13.1% urban) versus rural (27.1% rural). Among the other age groups studied, the difference was not significant [23].

There is no known predisposition of race or differential sexual susceptibility to Neosporosis in dogs [20], but age may be a risk factor. Adult dogs shed fewer oocysts than puppies after primary exposure, and puppies can also develop reexcretion after a new challenge. (5), therefore canids are considered definitive hosts, as they can shed oocysts into the environment through their feces [14].

Conclusions

The test of choice to confirm the diagnosis of this parasite in serum by the technique of IFAT, is economical and easy to perform, also with this research confirms the difficulty to find oocysts of N. caninum in the feces of dogs and likewise we consider that this first study can elucidate many obscure aspects in relation to knowledge in our environment, this being the first step for the realization of future research, related to the presence of this parasite in the urban area of the city of Valledupar.

Acknowledgment

This work was supported by the Vice-Rector for Research, Universidad Popular del Cesar., Project 237-15 / 12/214.

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How to Cite this Article: Rodríguez Ximena, Vizcaíno Margarita & Araújo Álvaro "Presence of The Neospora Caninum Parasite in Canine in The City of Valledupar, Colombia" Weber Microbiology Research (ISSN: 2449-1606), Vol. 2(1) 2018, Article ID wmr:241, 1054-1059


