

## Brucellosis: A Serofrequency Study in Pets and an Approach to its Relevance to Animal Health

Brucelosis: Un estudio de serofrecuencia en mascotas y un enfoque a su relevancia para la salud animal

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### ABSTRACT

**Introduction:** Brucellosis is a significant global zoonosis. The risk of *Brucella canis* infection in humans rises with exposure to asymptomatic pets.

**Objective:** To determine the serofrequency of *Brucella canis*. and explore the factors associated with Rapid Plate Agglutination Technique with 2 $\beta$ -Mercaptoethanol (PARP-2ME) results on blood serum samples from dogs and cats in Medellín, Colombia.

**Methods:** A cross-sectional, descriptive, non-probabilistic convenience study was conducted with 50 healthy dogs and 50 healthy cats, all attending elective surgery or consultations at two veterinary centers. Blood serum samples were collected for an indirect serological test (IgG anti-*Brucella canis*) to diagnose brucellosis. Pet

owners completed a questionnaire to provide additional information. Descriptive statistics and factors associated with serological results were estimated.

**Results:** The 20% (10/50) of the dogs and 4% (2/50) of the cats tested positive for IgG anti-*Brucella canis* using the PARP-2ME test. In dogs, antibodies were associated with sex ( $p = 0.089$ ) and breed ( $p = 0.018$ ). In cats, age ( $p=0.024$ ) was associated with the outcome.

**Conclusions:** This study underscores the public health risk relevance of *Brucella canis* infection in Colombia, with a significant prevalence of IgG anti-*Brucella canis* antibodies in dogs (20%) and cats (4%) of Medellín. It highlights the need to recognize brucellosis as a zoonotic risk and identifies factors linked to seropositivity, such as sex and breed in dogs, and age and hygiene in cats. The findings emphasize the importance of preventive measures and awareness to reduce *Brucella canis* transmission between pets and its relevance in humans.

**Keywords:** brucellosis; *Brucella* spp.; Colombia; PARP-2ME; public health; zoonosis.

## RESUMEN

**Introducción:** La brucelosis es una zoonosis global significativa. El riesgo de infección por *Brucella canis* en humanos aumenta con la exposición a mascotas asintomáticas.

**Objetivo:** Determinar la serofrecuencia de *Brucella canis* y explorar los factores asociados con los resultados de la Técnica de Aglutinación Rápida en Placa con 2 $\beta$ -Mercaptoetanol (PARP-2ME) en muestras de suero sanguíneo de perros y gatos en Medellín, Colombia.

**Métodos:** Se realizó un estudio transversal, descriptivo, no probabilístico por conveniencia con 50 perros y 50 gatos sanos, todos asistiendo a cirugía electiva o consultas en dos centros veterinarios. Se recolectaron muestras de sangre para una prueba serológica indirecta (IgG anti-*B. canis*) para diagnosticar brucelosis. Los dueños completaron un cuestionario para proporcionar información adicional. Se estimaron estadísticas descriptivas y factores asociados con los resultados serológicos.

**Resultados:** El 20 % (10/50) de los perros y el 4 % (2/50) de los gatos resultaron positivos para IgG anti-*Brucella canis* utilizando la prueba PARP-2ME. En los perros, los anticuerpos se asociaron con el sexo ( $p = 0,089$ ) y la raza ( $p = 0,018$ ). En los gatos, la edad ( $p = 0,024$ ) se asoció con el resultado.

**Conclusiones:** Este estudio subraya la relevancia del riesgo para la salud pública de la infección por *Brucella canis* en Colombia, con una prevalencia significativa de anticuerpos IgG anti-*Brucella canis* en perros (20 %) y gatos (4 %) en Medellín. Destaca la necesidad de reconocer la brucelosis canina como un riesgo zoonótico e identifica factores vinculados con la seropositividad, como el sexo y la raza en perros, y la edad e higiene en gatos. Los hallazgos enfatizan la importancia de medidas preventivas y concienciación para reducir la transmisión de *B. canis* entre mascotas y su relevancia en humanos.

**Palabras clave:** brucelosis; *Brucella* spp.; Colombia; PARP-2ME; salud pública; zoonosis.

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## Introduction

Brucellosis is one of the most prevalent zoonotic diseases globally,<sup>(1)</sup> transmitted from vertebrate animals to humans, generally through direct contact with animals or ingestion of animal-derived by-products.<sup>(2)</sup>

*Brucella* affects a wide range of animal species, including ruminants like sheep, goats, and bovines, as well as swine, dogs, equids, and wild mammals. The disease is caused by bacteria of the genus *Brucella*, with various species such as *B. melitensis*, *B. abortus*, *B. canis*, and *B. suis*, each having their preferential hosts like sheep/goats, cattle/bison, dogs, and pigs, respectively.<sup>(3)</sup> In addition, *Brucella* spp. patterns vary globally, with *B. abortus* and *B. melitensis* being the most important for livestock control.<sup>(4)</sup> Furthermore, wildlife hosts like buffalos, lions, wildebeest, impala, zebra, and hyenas have been identified as carriers of *Brucella* species, particularly *B. abortus* and *B. melitensis*, indicating a potential risk of transmission to domestic animals and humans within ecosystems.<sup>(5)</sup>

According to the World Organization for Animal Health,<sup>(2)</sup> approximately half a million cases of human brucellosis are reported annually, with a prevalence of 10/100,000. South America is considered an endemic area for human brucellosis, with significant opportunities for improvement in active surveillance, potentially impacting the ability to establish accurate incidence figures. In Colombia, the disease is distributed throughout the territory, with 25/32 provinces having a history

of it, and the provinces of Antioquia, Sucre, Córdoba, and Caquetá being considered areas of high prevalence.<sup>(6)</sup> However, the specific epidemiological information available is insufficient.

The significance of the disease in humans lies in its potential chronicity and the high costs to the healthcare system and affected individuals due to the disabilities and sequelae it generates. In addition, there is no efficient and safe vaccine, and treatment is based on prolonged antibiotic use (40-45 days), without a definitive resolution of the problem at the end of the treatment.<sup>(1)</sup> This underscores the need for detection and monitoring of *Brucella* spp. infection in susceptible animals from a public health perspective.

Several tests are commonly employed to diagnose *Brucella*-related infections based on serological implications and characterization. These tests include serum tube agglutination tests (SAT), Rose Bengal plate tests (RBPT), enzyme-linked immunosorbent assays (ELISAs), 2-mercaptoethanol tests (2 ME), complement fixation tests (CFTs), and Brucellin skin tests (BSTs).<sup>(7,8)</sup> Direct slide agglutination assays are known for their high sensitivity, cost-effectiveness, rapid results, and simplicity, allowing for efficient testing of multiple samples.<sup>(9,10)</sup> Serological tests used for diagnosing brucellosis in cattle, goats, and sheep assess common surface antigens found across various *Brucella* species and strains.<sup>(11,12)</sup> The standard agglutination test has shown a sensitivity ranging from 90.6 to 95.6% and a specificity from 94.2 to 100%, when using a standardized set of *Brucella* antigens.<sup>(13,14)</sup>

Considering the limited epidemiological information about this zoonosis, this study aims to determine the serofrequency of *B. canis* –a rough species, and explore the factors associated with the results obtained from the PARP-2ME in serum blood samples from dogs and cats residing in Medellín (Colombia). In addition, it seeks to highlight the relevance of these findings to public health in the population of interest.

## Methods

### Study design

A cross-sectional, descriptive study was conducted between February and June 2024, using nonprobabilistic convenience sampling in the geographical area of interest, with the inclusion of healthy domestic dogs (*Canis lupus familiaris*) and cats (*Felis catus*), 50 of each specie (n=100), regardless of age, weight, or breed, that attend elective medical consultation or surgery at two veterinary hospitals in the

municipality of Medellín (province of Antioquia, Colombia). This municipality is located on the Central Mountain Range at an altitude of 1,495 m.a.s.l. The study area has a tropical rainforest climate, with an average temperature of 23 °C and relative humidity ranging from 66% to 72%.<sup>(15)</sup>

### Sampling and sample analysis

Blood sample collection was carried out according to standard methods to avoid unnecessary pain or stress to the animals and under the timings and processes defined within the medical space at the two study sites. Samples were collected from the cephalic vein and placed in red-top plastic Vacutainer® tubes (without anticoagulant). The samples were transported in refrigerated containers to the laboratory, where they were centrifuged at 3,000 rpm × 5 minutes to obtain the serum, which was stored at -4°C until analysis using PARP-2ME.

For the serological screening diagnosis, a mixture of 25 µl of serum, 25 µl of 2-β-mercaptoethanol (0,2%, SIGMA®, M6250), and 50 µl of antigen *B. canis* was prepared. The samples were observed for 2 minutes in an agglutinoscope, and those showing fine agglutination (similar to the positive control) were determined as positive.<sup>(16)</sup> The diagnostic procedures were carried out by an independent laboratory certified in testing animal-origin samples.

### Data collection

To characterize the demographic characteristics of the owners and the zoographic details of the dogs and cats in the study, a questionnaire (available as supplementary material) with 34 questions divided into three sections was used (i.e. information about the owner, pet ownership habits, aspects related to brucellosis, and the owner's knowledge about the disease) (available as supplementary material in Spanish). The instrument included an informational paragraph explaining the study's objective, the number of questions, and the estimated time for completion. In addition, a confidentiality agreement was included if the interviewee agreed to participate in the study. The questionnaire was administered personally to each owner at the time of sample collection.

The information collection questionnaire was pre-tested on a small scale to evaluate its effectiveness. Two experts in the field assessed the structure to ensure that all important topics were identified and covered. The small-scale pre-test of the questionnaire was also administered to some pet owners (n=5), aiming to identify

issues such as excessive length, poor wording, confusing questions (using technical jargon), or the possibility of subjective responses.<sup>(17)</sup>

## Statistical analysis

All data generated during the study were entered into Excel spreadsheets (Microsoft Corp., Redmond, WA, USA) and then exported to Stata v.18 (StataCorp, 2023, College Station, TX, USA) for statistical analysis. Descriptive statistics were calculated for all demographic variables of interest both owner and animal level. The associations between these variables and the dichotomous outcome of serological analysis on blood samples (positive or negative) were analyzed by Fisher's exact test when the tables were larger than 2×2; otherwise,  $\chi^2$  test p-values were calculated (significance level of  $p<0.10$ ).

## Ethical considerations

The present study has the approval of the Comité de Bioética de la Sede Investigación Universitaria (CBE-Faculty of Medicine, Universidad de Antioquia), as stated in Act 056 of October 19, 2023, and by an expedited approval by the Comité de Ética en la Experimentación Animal (CEEAA), Act 156 of December 5, 2023. This study involves working with blood samples for medical purposes, collected by trained veterinary doctors, with an informed consent signed by the owner in which the purpose and impact of the study were explained, and under the guidelines of Law 84 of 1989 of Colombia.

## Results

The 20% (10/50) of the dogs and 4% (2/50) of the cats tested positive for anti-*B. canis* antibodies using the PARP-2ME test. The 56% (28/50) of the dogs participating in the study were visiting the clinic for spay/neuter surgery (ovariohysterectomy or castration), and the other 12 were for other procedures such as cesarean section, skin mass removal, specialized consultation, sample collection, and dental cleaning. In the case of the cats, 46% (23/50) were visiting for spay/neuter surgery (ovariohysterectomy or castration), and the other 27 for specialized consultation, sample collection, and dental cleaning/extraction.

Regarding the stance on pet sterilization, three of the 100 owners expressed disagreement, although they acknowledged that it is justified as a matter of responsibility and health, and to prevent escapes. Detailed information on ownership habits and aspects related to brucellosis is presented in tables 1 and 2.

**Table 1** - Characteristics and ownership habits of the dogs and cats in the study, considering the species (n=100)

| Item                            | Dogs<br>(n=50) | Cats<br>(n=50) | General<br>(n=100) |
|---------------------------------|----------------|----------------|--------------------|
| Number of pets in the household |                |                |                    |
| 1                               | 12 (24%)       | 9 (18%)        | 21 (21%)           |
| 2                               | 17 (34%)       | 16 (32%)       | 33 (33%)           |
| 3                               | 7 (14%)        | 7 (14%)        | 14 (14%)           |
| 4                               | 5 (10%)        | 7 (14%)        | 12 (12%)           |
| >4                              | 9 (18%)        | 11 (22%)       | 20 (20%)           |
| Age (in years)                  |                |                |                    |
| <1                              | 18 (36%)       | 16 (32%)       | 34 (34%)           |
| 1-8                             | 22 (44%)       | 30 (60%)       | 52 (52%)           |
| >8                              | 10 (20%)       | 4 (8%)         | 14 (14%)           |
| Sex                             |                |                |                    |
| Female                          | 23 (46%)       | 25 (50%)       | 48 (48%)           |
| Male                            | 27 (54%)       | 25 (50%)       | 52 (52%)           |
| Purebreed                       |                |                |                    |
| Yes                             | 35 (70%)       | 7 (14%)        | 42 (42%)           |
| No                              | 15 (30%)       | 43 (86%)       | 58 (58%)           |
| Origin                          |                |                |                    |
| Breeder                         | 19 (38%)       | 7 (14%)        | 26 (26%)           |
| Adopted                         | 30 (60%)       | 42 (84%)       | 72 (72%)           |

|                                                                                        |          |          |          |
|----------------------------------------------------------------------------------------|----------|----------|----------|
| In foster home                                                                         | 0 (0%)   | 1 (2%)   | 1 (1%)   |
| Unknown                                                                                | 1 (2%)   | 0 (0%)   | 1 (1%)   |
| Primary feed provided                                                                  |          |          |          |
| Balanced diet                                                                          | 19 (38%) | 49 (98%) | 68 (68%) |
| Homemade diet                                                                          | 7 (14%)  | 0 (0%)   | 7 (7%)   |
| B.A.R.F. diet                                                                          | 2 (4%)   | 0 (0%)   | 2 (2%)   |
| Combination of the above                                                               | 20 (40%) | 1 (2%)   | 21 (21%) |
| Other <sup>a</sup>                                                                     | 2 (4%)   | 0 (0%)   | 2 (2%)   |
| Consumption of dairy-derived products                                                  |          |          |          |
| Yes                                                                                    | 16 (32%) | 14 (28%) | 30 (30%) |
| No                                                                                     | 34 (68%) | 36 (72%) | 70 (70%) |
| Consumption of meat-derived products (raw, semi-raw, wet feed)                         |          |          |          |
| Yes                                                                                    | 37 (74%) | 45 (90%) | 82 (82%) |
| No                                                                                     | 13 (26%) | 5 (10%)  | 18 (18%) |
| Frequency of veterinary visits                                                         |          |          |          |
| 1-6 times a year                                                                       | 20 (40%) | 14 (28%) | 34 (34%) |
| Once a year                                                                            | 3 (6%)   | 6 (12%)  | 9 (9%)   |
| Only when sick                                                                         | 27 (54%) | 30 (60%) | 57 (57%) |
| Current vaccination schedule (triple vaccine and rabies)                               |          |          |          |
| Yes                                                                                    | 44 (88%) | 39 (78%) | 83 (83%) |
| No                                                                                     | 6 (12%)  | 11 (22%) | 17 (17%) |
| Regularly visits pet daycares, dog parks, or areas with concentrations of dogs or cats |          |          |          |
| Yes                                                                                    | 26 (52%) | 13 (26%) | 39 (39%) |
| No                                                                                     | 24 (48%) | 37 (74%) | 61 (61%) |
| Has ever visited animal production farms                                               |          |          |          |
| Yes <sup>b</sup>                                                                       | 20 (40%) | 4 (8%)   | 24 (24%) |
| No                                                                                     | 30 (60%) | 46 (92%) | 76 (76%) |

| Previous health problems       |          |          |          |
|--------------------------------|----------|----------|----------|
| Abortion                       | 0 (0%)   | 0 (0%)   | 0 (0%)   |
| Stillborn puppies              | 0 (0%)   | 0 (0%)   | 0 (0%)   |
| Enlarged testicles             | 0 (0%)   | 0 (0%)   | 0 (0%)   |
| Vaginal or preputial discharge | 0 (0%)   | 0 (0%)   | 0 (0%)   |
| Fever                          | 22 (44%) | 19 (38%) | 41 (41%) |
| Not applicable                 | 28 (56%) | 31 (62%) | 59 (59%) |

Legend: B.A.R.F.: raw animal-sourced feed containing meat, organs, bones, milk, or eggs; <sup>a</sup>Fruits, vegetables, cooked rice; <sup>b</sup>Beef.

**Table 2** - Aspects related to brucellosis and the knowledge of the pet owners of the dogs and cats in the study about the disease, considering the species (n=100)

| Item                                                                                               | Dog<br>(n=50) | Cats<br>(n=50) | General<br>(n=100) |
|----------------------------------------------------------------------------------------------------|---------------|----------------|--------------------|
| Has heard about brucellosis or <i>Brucella</i>                                                     |               |                |                    |
| Yes                                                                                                | 13 (26%)      | 28 (56%)       | 41 (41%)           |
| No                                                                                                 | 37 (74%)      | 22 (44%)       | 59 (59%)           |
| Knows of cases of animals or people infected with the bacteria                                     |               |                |                    |
| Yes                                                                                                | 7 (14%)       | 9 (18%)        | 16 (16%)           |
| No                                                                                                 | 43 (86%)      | 41 (82%)       | 84 (84%)           |
| Recognizes that brucellosis can be transmitted to humans by dogs and cats                          |               |                |                    |
| Yes                                                                                                | 9 (18%)       | 12 (24%)       | 21 (21%)           |
| No                                                                                                 | 41 (82%)      | 38 (76%)       | 79 (79%)           |
| Use gloves or some form of protective isolation when cleaning the pet and its waste (feces, urine) |               |                |                    |
| Yes                                                                                                | 50 (100%)     | 50 (100%)      | 100 (100%)         |
| No                                                                                                 | 0 (0%)        | 0 (0%)         | 0 (0%)             |
| Sometimes                                                                                          | 0 (0%)        | 0 (0%)         | 0 (0%)             |

|                                                                                                                                                                    |          |           |          |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------|-----------|----------|
| Allows the pet to lick their face                                                                                                                                  |          |           |          |
| Yes                                                                                                                                                                | 20 (40%) | 24 (48%)  | 44 (44%) |
| No                                                                                                                                                                 | 25 (50%) | 20 (40%)  | 45 (45%) |
| Sometimes                                                                                                                                                          | 5 (10%)  | 6 (12%)   | 11 (11%) |
| Has the pet undergone tests to detect <i>Brucella</i> previously                                                                                                   |          |           |          |
| Yes                                                                                                                                                                | 1 (2%)   | 0 (0%)    | 1 (1%)   |
| No                                                                                                                                                                 | 49 (98%) | 50 (100%) | 99 (99%) |
| If the animal's origin was a breeder, does the owner know if the breeder performs tests for the detection of <i>Brucella</i>                                       |          |           |          |
| Yes                                                                                                                                                                | 0 (0%)   | 0 (0%)    | 0 (0%)   |
| No                                                                                                                                                                 | 4 (8%)   | 3 (6%)    | 7 (7%)   |
| Does not know                                                                                                                                                      | 14 (28%) | 6 (12%)   | 20 (20%) |
| Not applicable                                                                                                                                                     | 32 (64%) | 41 (82%)  | 73 (73%) |
| If the pet is female, has it had previous pregnancies                                                                                                              |          |           |          |
| Yes                                                                                                                                                                | 7 (14%)  | 0 (0%)    | 7 (7%)   |
| No                                                                                                                                                                 | 13 (26%) | 16 (32%)  | 29 (29%) |
| Does not know                                                                                                                                                      | 4 (8%)   | 2 (4%)    | 6 (6%)   |
| Not applicable                                                                                                                                                     | 26 (52%) | 32 (64%)  | 58 (58%) |
| If the answer to the previous question is yes and the owner is aware of the animal's entire life history, was the <i>Brucella</i> test performed on the male donor |          |           |          |
| Yes                                                                                                                                                                | 0 (0%)   | 0 (0%)    | 0 (0%)   |
| No                                                                                                                                                                 | 1 (2%)   | 1 (2%)    | 2 (2%)   |
| Does not know                                                                                                                                                      | 8 (16%)  | 1 (2%)    | 9 (9%)   |
| Not applicable                                                                                                                                                     | 41 (82%) | 48 (96%)  | 89 (89%) |
| If the answer to the previous question is yes and the owner is aware of the animal's entire life history, was the pregnancy carried to term                        |          |           |          |
| Yes                                                                                                                                                                | 7 (14%)  | 3 (6%)    | 10 (10%) |
| No                                                                                                                                                                 | 2 (4%)   | 2 (4%)    | 4 (4%)   |

|                                                                                                                          |           |           |            |
|--------------------------------------------------------------------------------------------------------------------------|-----------|-----------|------------|
| Not applicable                                                                                                           | 41 (82%)  | 45 (90%)  | 86 (86%)   |
| If an abortion or pregnancy loss occurred, did the same owner handle the disposal of the fetuses                         |           |           |            |
| Yes                                                                                                                      | 0 (0%)    | 0 (0%)    | 0 (0%)     |
| No                                                                                                                       | 0 (0%)    | 0 (0%)    | 0 (0%)     |
| Not applicable                                                                                                           | 50 (100%) | 50 (100%) | 100 (100%) |
| If an abortion or pregnancy loss occurred, did the animal eat the aborted remains, such as placentas, fetuses, and blood |           |           |            |
| Yes                                                                                                                      | 0 (0%)    | 0 (0%)    | 0 (0%)     |
| No                                                                                                                       | 0 (0%)    | 0 (0%)    | 0 (0%)     |
| Does not know                                                                                                            | 0 (0%)    | 0 (0%)    | 0 (0%)     |
| Not applicable                                                                                                           | 50 (100%) | 50 (100%) | 100 (100%) |

In dogs, the presence of IgG anti-*B. canis* antibodies was found to be associated with sex ( $p=0.089$ ) and breed ( $p=0.018$ ). In cats, age ( $p=0.024$ ) was found to be associated with the outcome. No other significant differences were found for any of the associations explored.

### Detailed description of the 10 dogs that tested positive for anti-*B. canis* antibodies using the PARP-2ME test

Four of the 10 dogs were taken to veterinary care for spay/neuter surgery. There was one case of a previous pregnancy with mummified fetuses and pyometra. In total, seven males and three females were evaluated, with ages ranging from 8 months to 9 years. One was a mixed breed, while the other nine were purebred (i.e. Pinscher, American Bully, Yorkshire Terrier, German Shepherd, English Bulldog, Labrador Retriever, Cocker Spaniel, French Poodle, German Shepherd). Of these, five came from breeders, and four were adopted. Four of the animals were fed exclusively on balanced commercial feed, while the rest had a combination of different sources, but none were on a B.A.R.F. diet (i.e. raw animal-sourced feed containing meat, organs, bones, milk, or eggs). Three consumed dairy-derived products, and eight consumed meat-derived ones. Eight of the 10 only visited the veterinarian when they were sick, and nine had an up-to-date vaccination schedule. Four visited pet daycares, and three had visited cattle farms. Regarding the owners, six had not heard

of the disease before, eight were unaware of previous cases of infection in animals or people, and seven did not know that brucellosis can be transmitted to humans by dogs and cats. Nine of them wash their hands after handling their pets' waste, six allowed their pets to lick their faces, and one of the animals had previously been tested for brucellosis.

### **Detailed description of the two cats that tested positive for anti-*B. canis* antibodies using the PARP-2ME test**

These were two females: one 2 years old (mixed breed) and the other 17 years old (Persian breed). One was taken to the veterinarian for ovariohysterectomy surgery, and the other for sample collection. Both were adopted and fed exclusively on balanced commercial feed, with no dairy-derived consumption but some meat-derived products. In addition, both had up-to-date vaccination schedules, and one of them visits a pet daycare, although neither has visited farms. In both cases, one of the owners stated they had not heard of the disease or known of any cases of infection in animals or people. Both allow their pets to lick their faces, and neither animal had previous *Brucella* tests.

## **Discussion**

The purpose of this study was to determine the serofrequency of *B. canis* in the study population as well as to identify the associated factors. Although the test used herein is considered a screening test, the immunocapture techniques-including the PARP-2ME assay, play a crucial role in aiding the diagnosis of brucellosis by offering a simple, accurate, and rapid diagnostic method. The PARP-2ME technique has been found to be superior to the standard tube agglutination test (SAT) and comparable to the Coombs test in diagnosing brucellosis, with a high sensitivity and specificity.<sup>(13,14)</sup> The antigens typically used in serological tests detect antibodies produced against smooth strains, meaning that animals or humans infected with *B. canis* would yield non-reactive results. Therefore, using a technique capable of detecting antibodies specifically produced against this species highlights the significance of our work. Also, it eliminates subjective reading errors, is reproducible, and is recommended especially for brucellosis-endemic regions, showcasing its value in clinical practice for diagnosing and monitoring brucellosis cases.<sup>(18)</sup>

*Brucella canis* is the most common cause of canine brucellosis.<sup>(19)</sup> However, occasional infections with *B. melitensis*, *B. abortus*, or *B. suis* can occur in dogs that

come into close contact with tissues or secretions of infected livestock, especially raw milk, aborted fetuses, and placentas.<sup>(20)</sup> Our results on cats' positivity add to the limited existing literature on such findings. According to four studies reported in a previous systematic review, the individual test-wise apparent prevalence of brucellosis (according to both direct and indirect diagnostic tests) in stray/free-roaming cats worldwide ranges between 2.08 and 25% in studies from Iran and South Korea.<sup>(21)</sup> Nevertheless, and as mentioned by the authors of such review, these results should be interpreted with caution due to a low sample size ( $n = 24-48$ ) and a non-random sample selection. Previous research reported the prevalence of *B. canis* agglutinins in 170 cats in the United States, using a rapid slide agglutination test.<sup>(23)</sup> The seropositive rates among cats from the shelters were from 0 to 11.4% and for the hospitalized cats, the rates were from 0 to 8.9%. A study on exposure of jaguar populations and domestic animals to smooth *Brucella*, reported a positive result in a domestic cat by RBPT.<sup>(23)</sup> However, even though cats are not reported as a major source of infection for humans, their potential role in the transmission and spread of brucellosis, and even in the persistence of the infection within a population, should not be overlooked. Similarly to dogs, cat infections can occur through the consumption of contaminated meat and milk and abortions. Such concepts highlight the shortcomings of brucellosis control and surveillance programs concerning both domestic and wild animals.

According to a previous report, the frequency of *B. canis*-infected dogs in Colombia is of 7.7%, estimated by a weighted average of the frequency reported in each study available on PubMed and Google Scholar.<sup>(24)</sup> However, according to the researchers' experience, many reports in the country remain in the grey literature. Therefore, this frequency could be underestimating the phenomenon.

A recent study showed that *B. canis* is more elusive compared to pathogenic smooth *Brucella*,<sup>(25)</sup> which suggests that *B. canis* might also be underdiagnosed in humans. The estimated incidence of human brucellosis is around half a million new cases annually, which is likely an underestimate. Prevalence varies widely between countries and is closely linked to infection rates in domestic animals and control measures.<sup>(26,27)</sup> In addition, human brucellosis is considered an occupational disease. Thus, veterinarians, pet store employees, kennel workers and owners, dog caregivers, trainers, and laboratory technicians are at higher risk of infection due to direct contact with infected dogs, particularly through contaminated aborted fetuses or secretions.<sup>(28,29,30)</sup> The strong bond between people and their pets, with many considering their pets as family, increases the potential for pathogen transmission through saliva, urine, feces, aerosols, and skin contact.<sup>(31)</sup>

Globally, eradication plans have been established with successful outcomes; however, Latin America remains an endemic region for *Brucella* spp. Colombia lacks rigorous surveillance and control of clinical cases in humans, as active and passive surveillance is more frequently conducted in livestock. As an aggravating factor, brucellosis remains underdiagnosed in Colombia due to a lack of awareness among healthcare and veterinary professionals, the absence of accurate and reliable diagnostic tests, and the non-mandatory reporting of cases in non-production animal species to the relevant authorities.<sup>(32)</sup>

Diagnosing *B. canis* infection is highly challenging. While dogs are the most common host for *B. canis*, infections with other *Brucella* species, such as *B. suis* and *B. abortus*, can also occur in canines as well as cats and other pet species. Therefore, developing effective and accurate diagnostic methods specific to *B. canis* is crucial. Currently, preventing and controlling canine brucellosis is difficult, particularly because identifying infected dogs is challenging, which complicates efforts to prevent the spread of the disease.

In this context, it is highly desirable to develop new diagnostic methods and effective, safe vaccines. Along with these advancements, increasing awareness among healthcare professionals could significantly improve the control of canine and feline brucellosis. Practitioners need to recognize clinical signs, diagnose the disease accurately, and manage it effectively in pets and kennels. Advising pet owners on appropriate screening, treatment options, and kennel management is crucial. Specifically, heightened awareness could improve understanding of human brucellosis linked to *B. canis*, leading to more effective treatments and reducing the negative impacts of the disease on both animals and humans.

In conclusion, this study enhances the understanding of *B. canis* infection and its public health implications in Colombia, revealing an important prevalence of IgG anti-*B. canis* antibodies in dogs (20%) and, to a lesser extent, in cats (4%) living in Medellín (Colombia). Nevertheless, our results provide an approximation of what is occurring with *B. canis* in dogs and cats, based on a modest and geographically limited sample. Additionally, the study identified factors linked to seropositivity, such as sex and breed in dogs, and age in cats. These findings highlight the importance of preventive measures and public awareness to reduce the risk of *B. canis* transmission between pets and humans.

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### Conflicts of interest

The authors declare that they have no conflicts of interest.

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