

Immunological determination of *Toxoplasma gondii* and *Brucella canis* in canines from shelters in Risaralda and Caldas, Coffee Region – Colombia



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Abstract

The increasing dog population in Colombia has also raised the incidence of diseases that represent human and animal health risks. *Brucella canis* is a pathogen that is the primary cause of infertility in canines. It is easily transmitted between canines through body fluids, causing abortions in females, perinatal death of puppies, epididymitis, and infertility in males. On the other hand, *Toxoplasma gondii* is an easily transmitted parasite; its transmission is the majority between canines and felines, mainly through consuming contaminated food and water, which causes a disease that commonly has a subclinical presentation in canines. However, in immunosuppressed individuals this pathogen can cause neuromus-

cular, gastrointestinal, and respiratory signs. Currently, due to the increase and geographical distribution of the canine population, it is important to gather information about the prevalence and possible risk factors associated with the presentation of these diseases in the departments of Risaralda and Caldas from Colombia. In this sense, a descriptive cross-sectional study was carried out, and a population of domestic canines was sampled ($n=93$), females $n=63$ (67.74%), and males $n=30$ (32.26%). In total, 72% of animals were positive for *Toxoplasma gondii*, while for *Brucella canis*, a prevalence of 0% was estimated.

Key words: antibodies; brucellosis; prevalence; public health; toxoplasmosis

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Introduction

The *Brucella* genus comprises a group of non-motile, non-encapsulated, non-sporulated, facultatively intracellular coccobacilli-type bacteria. Several species are known, including *Brucella canis*, whose natural reservoir is the dog, *Brucella melitensis* (sheep, goats), *Brucella ovis* (sheep), *Brucella abortus* (cattle, bison, buffalo), *Brucella neotomae* (rodents, desert rats) and *Brucella suis* (pigs) (Cosford, 2018). *Brucella* is a zoonotic bacterium, considered the main causal agent of canine brucellosis; to date, there is no vaccine, and antimicrobial therapy does not eliminate the bacteria completely, leading to reinfections (Djokic et al., 2023).

Since its first isolation in 1966, its distribution has become worldwide, causing abortions, sterility, joint disorders, uveitis, corneal opacity, discospondylitis, lymphadenitis, and epididymitis in canines (Castrillón-Salazar et al., 2013). As a zoonotic pathogen, humans can become infected by direct contact with secretions and excrement of infected dogs or by exposure in the laboratory, presenting fever, general malaise, splenomegaly, and lymphadenopathy. However, its diagnosis can go unnoticed due to the non-specificity of symptoms and the lack of serological tests to detect its presence (Van et al., 2021).

On the other hand, *Toxoplasma gondii* is a zoonotic parasite of the obligate intracellular protozoan type, belonging to the phylum Apicomplexa and family Sarcocystidae that causes toxoplasmosis, a disease with worldwide distribution (Raimundo et al., 2015). This parasite can infect most warm-blooded animals with a two-host life cycle; domestic cats and some wild felids are definitive hosts, and the rest of non-felid animals, including canines and humans, are con-

sidered intermediate hosts (Calero-Bernal & Gennari, 2019).

Canines can become infected by ingesting raw meat cysts or oocysts in food contaminated with faeces from infected cats (Oliveira et al., 2014). Infection by this agent in canines is of clinical and epidemiological relevance; oocysts ingested by canines can pass through the digestive tract and continue in an infectious state (Dubey et al., 2020). The disease includes neurological, gastrointestinal, and respiratory compromise and even disseminated infection (Nagel et al., 2013). Immunosuppression and neuromuscular disorders may occur, with older canines most likely to become infected (Salama et al., 2022). Common clinical signs of canine toxoplasmosis include encephalitis, hepatitis, and pneumonia (Rosypal et al., 2010). This study aimed to determine the presence of these two zoonotic infectious agents in canine populations residing in Risaralda and Caldas, Colombia.

Materials and methods

Bioethical Considerations

This project obtained bioethical endorsement for research with animals by the Ethics and Animal Experimentation Committee of the Santa Rosa de Cabal University Corporation (UNISARC), as stated in protocol 001 of May 30, 2024. Our study was considered of medium risk. All procedures were carried out in the Multifunctional Laboratory of the Veterinary Medicine and Zootechnics Program facilities at the Technological University of Pereira Risaralda, Colombia.

Geographic study area

This work was a cross-sectional study carried out with dog populations from dog shelters in the municipalities of Pereira city and La Virginia municipi-

pality, Risaralda department, and in Chinchiná municipality of Caldas department, in Colombia during 2021–2022 (Figure 1). Risaralda is a department located in the central western area of Colombian territory, with annual precipitation between 1500 mm and 5000 mm, average temperature of 22°C, and altitudes between 1000 and 2500 m. In the 2019 cat and dog Rabies Vaccination Report, about 137,131 canines were registered in the Department of Risaralda (Caballero Méndez et al., 2024). On the other hand, Caldas is a department located in the central west of Colombia in the Andean

region, with annual precipitation of 2800 mm and temperatures between 13–27°C, in which all the thermal floors of the country are registered (Rivera-Pérez et al., 2022). According to the cat and dog rabies vaccination report for canines and felines for 2019, about 110,603 canines were registered in the Caldas Department (Ministerio de Salud, 2019).

Sampling

In the present study, 93 samples were taken from canines (*Canis familiaris*) in four shelters and animal foster homes. Veterinarians took the samples with pri-



Figure 1. Study locations in Risaralda and Caldas Departments in Colombia. Graphic design: Franco Montoya Luz Natalia (2024)

or informed consent from the owners. Animals were immobilised to facilitate handling and avoid trauma during sampling. Whole blood (1 mL) was collected in tubes with clot-activating serum/separating gel additive to obtain serum. The samples were obtained by puncture with a hypodermic needle of the cephalic vein after disinfection of the collection site. Samples were subsequently transferred to the multifunctional laboratory attached to the Faculty of Health Sciences, Technological University of Pereira, separated by centrifugation, and stored at 4°C for their respective analyses. Variables such as sex and origin were considered.

Sample processing

In the laboratory, the qualitative detection of IgG/IgM antibodies for *Toxoplasma gondii* was carried out using the *Toxoplasma gondii* Ac IgG+IgM Uranovet kit that consists of a lateral flow immunochromatographic assay, which includes an area for detecting specific antibodies to the *Toxoplasma gondii* parasite. The zone has a rounded well where the sample (whole blood, serum, or plasma) is added. The result zone contains lines T1 (IgG) and T2 (IgM) (test line) and line C (control line). Then, the results were interpreted according to the manufacturer's instructions. For the qualitative detection of antibodies to *Brucella canis*, the Uranovet anti-*Brucella canis* IgG Antibodies kit was used. The test consists of a lateral flow immunochromatographic assay, which includes an area for detecting specific antibodies to the *Brucella canis* bacteria. The result zone consists of a rounded well where the sample (whole blood, serum, or plasma) is added and a results zone containing the T line (test line) and the C line (control line). The results were interpreted according to the manufacturer's instructions. These tests for *Brucella* and *toxoplasma* have a sensitivity of

95.8% and a specificity of 99.7%. One drop (approximately 20 µL) of the serum sample was deposited into the test port with 2 to 3 drops (approximately 60 to 80 µL) of the test buffer. The liquid moves laterally across the surface of the test strip and reacts with the antigens already present on it. The results were interpreted after 5 to 10 minutes; after this time, a test reading was considered invalid. In the presence of anti-*T. gondii* or anti-*B. canis* antibodies, a visible line appears in the respective T zone. A line should always appear in the C zone, since this indicates a positive result. For correct interpretation of the results, a positive result consists of a line in both the C and T zones, regardless of whether the latter is clear or vague. A result is negative when only one line appears in the C zone. Finally, if a line does not appear in zone C, it is considered an invalid result, regardless of whether a line appears in zone T.

Data analysis

To determine the prevalence of *Toxoplasma gondii* and *Brucella canis* and to establish possible associated risk factors such as sex and origin, all data were tabulated on the same value scale and subjected to normality analysis. Descriptive statistics were carried out, and the frequencies of positive cases of both infectious agents were determined. Possible correlations were examined between the variables studied and the prevalence of studied infectious agents were determined by Pearson correlation analysis ($P \leq 0.05$). All data were analysed using SAS Statistical Analysis Software.

Results and discussion

The areas sampled in this study correspond to municipalities in the Risaralda Department, Colombia, with an average temperature of 22°C and annual precipita-

Table 1. *Toxoplasma gondii* and *Brucella canis* antibodies prevalence in dogs from shelters in the Coffee region, Colombia

Pathogen	Females			Males			Total prevalence
	Positive	Negative	Total females	Positive	Negative	Total males	
<i>Toxoplasma gondii</i>	44	19	63	23	7	30	72%
<i>Brucella canis</i>	0	63		0	30		0%

tion between 1500– 5000 mm, and municipalities in the Caldas Department, with a temperature between 13–27°C, yearly mean rainfall of 2800 mm, corresponding to humid tropical climates that favour the maintenance of soils and water sources contaminated with *T. gondii* oocysts, which promotes its spread (Fábrega et al., 2020). High humidity and temperatures have been reported to favour the sporulation and maintenance of oocysts in the environment (Arruda et al., 2021). Of the 93 animals sampled, 67.74% were females, and 32.26% were males; for *Toxoplasma gondii*, a seroprevalence of 72% was determined. However, 0% prevalence was observed for *B. canis* in this region (Table 1).

In Colombia, few seroprevalence studies have been carried out in canines for *T. gondii*; the last report was carried out in Bogotá in 2007 and found a seroprevalence of 16.8% (Dubey et al., 2007). In America, there was high variability reported in the antibody's prevalence to *T. gondii*: Argentina (55%), Brazil (70%), Mexico (61.7%), Panama (30%), Peru (24%), and USA (42%) (Dubey et al., 2020), reflecting the cosmopolitan behaviour of the parasite and its epidemiological importance in animal and human health (Benitez et al., 2017). Seropositivity for *T. gondii* varies between countries and even between regions of the same country and areas of the same city. This parasite is considered a zoonotic pathogen, and increasing canine

populations in urban regions and the close relationship between animals and humans facilitates its transmission, causing disease (Arruda et al., 2021). Canines have been reported as critical mechanical transmitters of the parasite. The presence of canines in homes is considered a risk factor for human infection since they spread oocysts in the environment without reproducing the parasite in the intestine, as occurs with felines (Rengifo-Herrera et al., 2017). At the same time, canines act as mechanical vectors due to their habit of eating cat faeces and rolling in excrement (Alvarado-Esquivel et al., 2014). In our study, canines were kept in shelters located in rural areas. However, they were street dogs prior to their admittance. It has been shown that dogs from rural areas and farms are associated with a greater risk of seropositivity for toxoplasmosis (Benitez et al., 2017), due to greater exposure to infected intermediate hosts (Raimundo et al., 2015), canines from urban environments due to their street habits and unrestrained movement, are epidemiologically crucial since they serve as indicators of environmental contamination and sentinels of infection by *T. gondii* (Da Silva et al., 2017). Animals that live at home or under an owner's responsibility have a lower frequency of anti-*T. gondii* antibodies compared to street or shelter populations (Arruda et al., 2021). Sex showed no significant correlation, although there was a higher pres-

entation in females than in males, which differs from other studies (Da Silva et al., 2010). The high prevalence of antibodies to *T. gondii* detected in this study can be explained by factors contributing to its spread, such as rodent predation and garbage consumption, since many dogs were rescued from the streets, and consumption of non-potable water and ponds contaminated by oocysts.

We used immunochromatography as a diagnostic technique, with a sensitivity of 97.67% and a specificity of 97.50%. These immunochromatographic tests are high-quality, quick, easy to perform, and have proven to be cost-effective and widely used in developing countries with limited economic resources for diagnosis and treatment (Wassef and Abdel-Malek, 2019). However, techniques such as ELISA (Enzyme-linked immunosorbent assay), IFA (Indirect fluorescent antibody assay), MAT (Modified agglutination test) have been used for the quantification of antibodies, as is the case of the MAT technique that detects antibodies against the antigen surface since it uses complete tachyzoites, while the ELISA test uses soluble antigens to detect antibodies directed against these fractions (Alvarado-Esquivel et al., 2014). Serological studies are precious tools since they allow us to reveal the level of contact of the host species with *T. gondii* and interpret the epidemiological risk these animals represent for humans (Huertas-López et al., 2021). On the other hand, molecular techniques such as PCR can support the diagnosis since they have a potentially excellent specificity when identifying the presence of a gene specific to the infectious agent. The difficulty lies in acquiring the appropriate sample during the infection, which questions its sensitivity. In turn, direct detection of the parasite in intermediate hosts is difficult due to the limitation in

predicting the exact location of the tissue cyst (Wassef & Abdel-Malek, 2019).

On the other hand, in this study, the prevalence of IgG anti-*Brucella canis* antibodies in the canines analysed was 0%. Other studies have also determined a low prevalence. In Chile in 2023, a serological prevalence of 6.6% in a population of stray canines was reported (Weinborn et al., 2023). However, the prevalence rates are higher in our country. Antioquia, Colombia, reported seroprevalence of 15% in dogs from breeding farms and 9% living with humans, which indicates that this disease may be underdiagnosed (Castrillón-Salazar et al., 2013). Different laboratory tests can diagnose this microorganism, the traditional reference test being the culture of blood, urine, vaginal fluid, semen, milk, or liquids from aborted tissues or samples taken from necropsy. However, a negative culture does not rule out infection due to its low sensitivity (De Massis et al., 2022). Other tests, such as PCR, allow the precise identification of dogs with bacteremia but lack sensitivity to detect animals with chronic infection (Keid et al., 2015). On the other hand, serological tests are used as tools for early disease detection. These tests can present false negatives as a result of tests before seroconversion, and low titres of circulating antibodies in some dogs with chronic infection, or false positives due to reactions specific and non-specific cross-links with shared surface antigens in *Pseudomonas aeruginosa*, *Bordetella bronchiseptica*, *Actinobacillus equuli*, *Streptococcus*, *Staphylococcus*, *Moraxella*, and Gram-negative bacteria (Cosford, 2018).

The rapid growth of the pet and companion animal industry can cause infections by different species of *Brucella* that could become a severe public health problem. Canine brucellosis is a disease underestimated worldwide. However,

the consequences that they bring to canine health, such as contagious abortions, testicular atrophy, infertility, lymphadenitis, and reproductive problems, have added to the economic losses in dog breeders and the risk of zoonotic transmission through close contact with infected canines, constituting a concern for both animal and human health (Kang et al., 2014). Human brucellosis is considered an occupational disease; these bacteria affect veterinarians, pet store workers, laboratory personnel, and kennel workers in close contact with canines and their secretions, making canine brucellosis as an emerging urban zoonosis (Mol et al., 2020; Santos et al., 2021).

Conclusions

In Colombia, the municipalities of Pereira, La Virginia, and Chinchiná presented a high seroprevalence for *T. gondii* in dogs; this is indicative of environmental contamination in the Risaralda and Caldas Departments, and constitutes a risk for animal and human public health in the region and a greater risk of morbidity and mortality due to toxoplasmosis. It is suggested that preventive measures be established to prevent the spread of the disease. Preventing and controlling brucellosis in dogs is challenging due to the limitations in diagnosing the disease. Therefore, control strategies must be aimed at early diagnosis, vaccination, and education about the importance of control in preventing the disease.

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Imunološko utvrđivanje *Toxoplasma gondii* i *Brucella Canis* u pasa iz skloništa u Risaraldi i Caldasu, Regija kave – Kolumbija

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Povećanje populacije pasa u Kolumbiji povećalo je i učestalost bolesti koje predstavljaju rizike za zdravlje ljudi i životinja. *Brucella canis* je patogen koji predstavlja primarni uzrok neplodnosti u pasa. Lako se prenosi između pasa tjelesnim tekućinama, uzrokujući pobačaje u ženki, perinatalnu smrt štenaca, epididimitis i neplodnost mužjaka. S druge strane, *Toxoplasma gondii* lako je prenosivi parazit; njegov prijenos najčešće se odvija između pasa i mačaka, uglavnom konzumacijom kontaminirane hrane i vode, što prouzroči bolest koja se obično manifestira subklinički u pasa. Međutim, ovaj patogen u imunosuprimiranih pojedinaca može prouzročiti neuromuskularne, gastrointe-

stinalne i respiratorne znakove. Trenutno, zbog povećanja i geografske rasprostranjenosti populacije pasa, važno je prikupiti informacije o prevalenciji i mogućim faktorima rizika povezanim s manifestacijom ovih bolesti u okruzima Risaralda i Caldas u Kolumbiji. U tom smislu, provedena je opisna presječna studija, a uzorkovana je populacija domaćih pasa ($n=93$), ženke $n=63$ (67,74 %) i mužjaci $n=30$ (32,26 %). Kao rezultat, utvrđena je učestalost od 72 % životinja pozitivnih na *Toxoplasma gondii*, dok je za *Brucella canis* procijenjena prevalencija od 0 %.

Ključne riječi: antitijela, bruceloza, prevalencija, javno zdravlje, toksoplazmoza