# Molecular determination of *Leptospira* spp., street and shelters dogs from the Coffee Region of Colombia

*L. C. Caballero Méndez*, L. M. González Londoño, J. C. Gómez Ruíz, M. J. Escobar Herrera, M. M. Mazo and L. N. Franco-Montoya*

## Abstract

Leptospirosis is considered a zoonotic disease with a substantial impact on animal and human health. It is distributed in tropical and subtropical climates, which improves the survival of these bacteria for a long time, affecting domestic and wild animals that act as a reservoir. Canine leptospirosis has obtained great clinical relevance, due to the susceptibility of this species to infection and the frequent exposure to leptospirosis from the environment. Canines from the street and animal shelters constitute a high-risk population due to the proximity to sources of infection. This paper describes a cross-sectional study that involves sampling dogs from shelters located in the departments of Risaralda, Valle del Cauca, and Caldas, known as the Coffee Region, located in the centre-west of Colombia. Blood samples were taken from 140 canines and analysed in the laboratory of the Veterinary Medicine and Animal Science, Technological University of Pereira. DNA was extracted, and the LipL32 gene was amplified by conventional PCR. A 15% prevalence for *Leptospira* sp. was found in dogs from the Coffee region. No correlation was found between the variables such as sex, age, origin, and socioeconomic status. However, a tendency for infection was observed with several cases of diagnosis in female dogs older than six years in low strata. This study constitutes the first report of canine leptospirosis in this region of Colombia, which will allow the design of strategies aiming to mitigate the disease in this region.

**Key words:** *leptospirosis; spirochete; polymerase chain reaction; bacterial zoonosis*

## Introduction

Leptospirosis is a disease caused by the aerobic spirochete-type bacterium known as *Leptospira*, which has more than 250 pathogenic serovars and around 20 serogroups, and is widely distributed and considered an emerging zoonosis (Reagan et al., 2019). With more than one million severe cases per year in humans, it can

Lyda C. CABALLERO MÉNDEZ*, (Corresponding author, e-mail: lydaccm_27@utp.edu.co), Grupo de investigación BIOECOS, Profesora de Medicina Veterinaria y Zootecnia Facultad de Ciencias de la Salud, Universidad Tecnológica de Pereira, Pereira, Colombia; Luisa María GONZÁLEZ LONDOÑO, Julio Cesar GÓMEZ RUÍZ, María José ESCOBAR HERRERA, Médico Veterinaria y Zootecnista. Facultad de Ciencias de la Salud, Universidad Tecnológica de Pereira, Pereira, Colombia; Margarita María MAZO, Grupo de investigación BIOECOS, Profesora de Medicina Veterinaria y Zootecnia Facultad de Ciencias de la Salud, Universidad Tecnológica de Pereira, Pereira, Colombia; Luz Natalia FRANCO-MONTOYA, Grupo de investigación CIBAV, Profesora Asociada adscrita a la Escuela de Medicina Veterinaria, Facultad de Ciencias Agrarias, Universidad de Antioquia UdeA, Medellín, Colombia

affect species such as canines, cattle, porcine, equine, equine, and sheep, and species such as rats, mice, and rodents can act as reservoirs (Klaasen et al., 2015). Canine leptospirosis has gained great clinical relevance due to the increase in cases, probably due to susceptibility to infection and frequent exposure to leptospirosis from the environment (Miotto et al., 2018). This bacterium is located in the renal tubules and genital tract of domestic and wild mammals and is therefore easily excreted, becoming viable for weeks or months depending on the heat and humidity of the environment (Ahmed et al., 2012). Therefore, infection occurs through contact with contaminated urine or contaminated soil and water, and in the case of canines, it has been shown that street habits, grazing, and hunting increase the risk of infection (Major et al., 2014). A higher incidence of canine leptospirosis has been observed in tropical countries during the rainy season, and transmission between canines and humans has been documented in regions with a temperate climate (Hua et al., 2016). Canines present a variety of symptoms, which can be asymptomatic infections, febrile syndromes, renal, hepatic, and pulmonary failure, and bleeding disorders (Bertasio et al., 2020). Signs and symptoms may vary depending on the leptospira serotype involved and the host (Sripattanakul et al., 2022). Some dogs may be asymptomatic or chronic carriers, acting as maintenance hosts and spreading the disease, and becoming a problem for human and animal health (Zaidi et al, 2018). The recommended diagnostic test is the microscopic agglutination test (MAT) that detects antibodies against sp. in serum; however, these antibodies can remain in the blood even after recovery from the disease (Ahmed et al., 2012). Molecular techniques based on the detection of DNA from pathogenic leptospirosis, such as PCR in biological samples, are more sensitive and are therefore replacing techniques such as MAT, though they are not able to identify the serovar or serogroup causing the infection (Le Guyader, 2020). The objective of this study was to determine the prevalence of the infectious agent *Leptospira* sp. in dogs from animal shelters located in the departments of Caldas, Risaralda, and Valle del Cauca (Colombia), using the conventional PCR molecular technique.

## Materials and methods

### *Bioethical aspects*

The present study was approved by the animal bioethics committee of the Santa Rosa de Cabal University Corporation (Unisarc) (Acta No 001) and was considered minimal risk research since external secretions were collected that did not involve invasive methods in the patient or affect the integrity of the animal.

### *Type of study and population sampled*

A cross-sectional study was carried out with the canine population of shelters in municipalities such as Pereira, Cartago, Chinchina, and Santa Rosa de Cabal in the Coffee region of Colombia. A population of 140 domestic canines was sampled with a margin of error of 7% and a significance level of 95%, an expected prevalence of 25%, and an estimated population size in the region of 69,942, based on prevalence reports from other similar studies. Excluded animals correspond to those that were receiving antibiotic treatment or that had been vaccinated on the sampling date.

### *Blood sampling*

Prior to sampling, the owner or custodian responsible for each animal read and signed an informed consent form in which the procedure and scope of the study was explained and the owner accept the proce-

dures. The samples were taken by qualified personnel (veterinarians). For sample collection, the cephalic veins were located, vein puncture was performed with a number 24 needle, the sample was extracted by aspiration with a syringe and venous blood was collected in BD Vacutainer tubes containing EDTA (ethylenediaminetetraacetic acid) as an anticoagulant. In order to preserve the samples, the tubes were individually identified with the number assigned to the animal, and transported in portable refrigerators at a temperature of 4-8ºC to the Veterinary Medicine Multifunctional Laboratory of the Faculty of Health Sciences, Technological University of Pereira.

### DNA extraction

In the laboratory, DNA extraction from blood samples was carried out using Qiagen columns for blood samples from the QIAamp DNA Blood Mini Kit following the manufacturer's instructions. Quantification in µg/mL, and spectral revision or purity at 260/280 of the extracted DNA was carried out using the µDrops Multiskan GO equipment. The DNA obtained was visualised in 2% agarose gels to corroborate the integrity of the extracted DNA. Samples were aliquoted and stored at -80°C for later use.

### Diagnosis by Polymerase Chain Reaction (PCR)

To carry out the polymerase chain reaction (conventional PCR), we proceeded to amplify a specific DNA fragment directed to a region located between positions 270 and 692 of the lipL32 gene, which codes for the membrane lipoprotein LipL32. This sequence is highly conserved among pathogenic *Leptospira* sp. Their alignment was verified using the NCBI Blast resource, and the quality of primers was verified using the Oligoanalyzer tool. The sequence of the primers used were those used by Romero et al (Romero-Vivas et al., 2013), LipL32/270F5'-CGCTGAAATGGGAGTTCGTATGATT-3 and LipL32/692R5'-CCAACAGATGCAACGAAAGATCCTTT-3'. Conditions for PCR were initial denaturation at 95°C for five minutes, a 35-cycle phase at 94°C for one minute, 58°C for one minute, and 70°C for two minutes, followed by an extension phase, final at a temperature of 72°C for five minutes. In the PCR process, the following reagents were used to complete 25 µL as the final volume of the reaction: 5 µL 1X PCR buffer, 2 mM $MgCl_2$, 2.5 µL each primers (forward - reverse) 1 µM, 0.5 µL 200uM dNTPs and 0.5UI Taq polymerase, 2 µL template DNA (5 µg/mL). Amplificates were visualised on 2% agarose gel using SYBR Green as an intercalating agent. The bands were observed in a UV light transilluminator (BioRad). The presence of a 423 bp amplicon was indicative of the presence of *Leptospira* sp. DNA samples from cultures of *Leptospira copenhageni* serogroup icterohemorragica, and *Leptospira pomona* grown in EMJH liquid medium obtained from commercial isolates supplied by a reference laboratory were used as the positive control.

### Data processing and statistical analysis

To determine the prevalence of the infectious agent, all data were tabulated on the same scale of values and subjected to normality analysis. Once the premises were met, a descriptive statistical analysis was performed to determine the frequencies of positive cases of the infectious agent, as well as the frequencies of presentation evaluating the possible risk factors in variables such as sex, age (four age groups in canines: group 1 juveniles < 1 year, group 2 young (1–6 years), group 3 adults (>6 years) and group 4 without age reported), place of origin of the canine and socioeconomic strata where the shelters

were located (one (low-low), two (low) and three (medium-low)), that correspond to areas with limited economic resources that do not have easy access to veterinary services. Additionally, possible correlations were examined between the variables and the prevalence of the infectious agent using the Pearson correlation analysis with a significance level of $P \leq 0.05$. All data were analysed using GraphPad Prism 8.0 statistical and graphing software.

## Results and discussion

Blood samples from 140 domestic dogs from the municipalities of Pereira, Dosquebradas, Chinchina, and Santa Rosa were analysed. In total, 21 positive cases were for *Leptospira* sp., giving a 15% prevalence, which is similar to other reports (Felt et al., 2011; González et al., 2012). Some studies have reported a prevalence close to 30% using the same diagnostic technique (Calderón et al., 2014; Rahman et al., 2021). Due to the MAT technique, the prevalence figures are in contrast to other studies (Domínguez et al., 2013). Since this is a serological technique, PCR is the gold standard test to identify serogroups of the bacteria and can be used as a diagnostic technique since it can detect bacterial DNA in different samples such as blood, semen, and urine, due to its sensitivity and specificity (Latosinski et al., 2018). In addition, serological techniques require expertise, and the variation between laboratories is considerably high, so they must be interpreted carefully (Di Azevedo et al., 2021). Other limitations associated with the use of these techniques are the difficulties for early diagnosis since antibodies appear only two weeks after exposure, the need for subcultures of various leptospiral strains, and the lack of differentiation between vaccinations or antimicrobial antibodies from natural infection (Lau et al., 2017). On the other hand, the culture of biological samples is quite laborious since the bacterium requires a specialised growth medium and a long incubation period. In addition, its sensitivity is low since it can give false negatives due to low bacterial load or antibiotic therapy (Di Azevedo et al., 2021).

Currently, the diagnosis of Leptospirosis in canines is based on suggestive clinical or clinical pathological signs and serological tests such as ELISA and MAT. However, molecular techniques such as PCR have recently been used in veterinary medicine since these tests can reduce the challenge of interpreting antibody-based tests (Stull et al., 2022). The present study detected a region of the gene that codes for a lipoprotein known as LipL32, and dogs with 423bp amplicons were considered positive (Figure 1). This gene codes for a lipoprotein present in the external membrane of the pathogen that is highly conserved and considered the most abundant and frequent in pathogenic leptospires used in serological and molecular diagnosis (Blanchard et al., 2021). Molecular tools have proven to be sensitive and specific for detecting leptospires in the early stages of infection (Rajapakse et al., 2015). The PCR technique allows for the detection of pathogenic genes such as LipL32 of *Leptospira* sp. in blood and urine. However, the sensitivity and specificity of this test in urine samples usually ranges between 88-100%. The sensitivity in blood samples decreases to 50% in the first five days of illness. Therefore, in future studies, it is suggested that PCR be conducted on urine samples, or both blood and urine should be analysed to improve diagnostic sensitivity (Lau et al., 2017), even using PCR variations that increase the specificity of the diagnosis (Caballero et al., 2023).

Leptospirosis in canines is of great epidemiological importance since these

animals are considered primary reservoirs and sources of infection. Studies in canines in Colombia have shown different prevalences in regions such as Antioquia ranging from 7 to 22% (Perez-Garcia et al., 2022), in Córdoba, a prevalence of 70% (Ensuncho-Hoyos et al., 2017) and 21% were registered in the Department of Tolima (Romero et al., 2010), while the prevalence of this study was 15%, similar to other regions in Columbia, with the exception of the Department of Risaralda. Prevalence studies show the current situation of this agent, and therefore this study constitutes a first report for the design of control and prevention strategies.

Companion animals such as canines are an indicator of the distribution of *Leptospira* serovars in nature since a resurgence of canine leptospirosis has been reported in some regions of the world, though the use of bivalent vaccines has been successful in decreasing its frequency (Lau et al., 2017). Canines living on the streets constitute a group at risk of contracting leptospirosis due to their preference to hunt street rats and mice, while dogs living at home are regularly fed by their owners (Altheimer et al., 2020). This study sampled street dogs in animal shelters considered to be primary reservoirs and sources of infection by pathogenic *Leptospira*. In addition, in the canine population, some dogs can become asymptomatic, chronic carriers, excreting pathogenic leptospires and increasing the possibility of horizontal transmission between canines in the same shelter (Miotto et al., 2018).

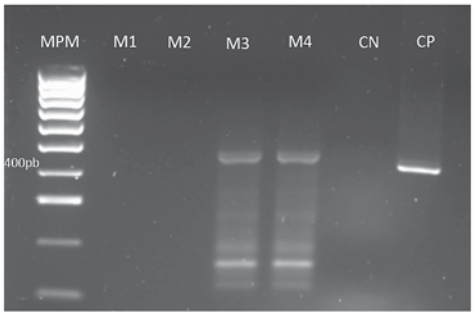


**Figure 1.** Electrophoresis agarose gels 2% for *Leptospira* sp. MP: 100 bp molecular weight marker, M1, M2, M3, M4: samples from domestic canines, CN: negative control, CP: positive control.

In this study, more females were positive for *Leptospira* sp. than males (Table 1), which agrees with other reports (Romero et al., 2010). However, Pearson's correlation analysis did not show sex to be highly correlated as a risk factor for this agent. Other authors also reported that factors such as sex and breed are not considered risk factors for acute leptospirosis (Schuller et al., 2015).

Concerning the ages of the sampled population, half of the sampled canine population were adults (> 6 years), where the highest number of positive cases occurred, despite the lack of a significant

**Table 1.** Prevalence of *Leptospira* sp. by sex in 140 dogs sampled from the Coffee Region, Colombia

| Infection | Female | | | Male | | | Total Sampling dogs/ Total Prevalence |
|---|---|---|---|---|---|---|---|
| | Positive | Negative | Total Females | Positive | Negative | Total Males | |
| **Leptospirosis** | 15 | 70 | 86 | 6 | 48 | 54 | 140 |
| **Prevalence by sex** | | 10.71% | | | 4.28% | | 15.00% |

correlation between age and the number of positive cases. In the presence of the disease, studies have shown that dogs with a median age between 6 and 7 years are more vulnerable to infection in comparison with other population ages (Fraune et al., 2013). However, studies have found a greater probability of testing positive in young populations between 0-4 years of age, and in canines under 1 year of age or over 8 years of age compared to other ages, and therefore the findings associated with age vary between studies (Smith et al., 2019). It has been suggested that middle-aged male canines (4-10 years) are more active outdoors and this increases the risk of exposure to contract Leptospirosis (Lau et al., 2017).

Several cases were registered in shelters from the Municipalities of Pereira and Cartago, determining a positive correlation for the frequency of infection by *Lepstopira* sp, in these shelters (r=0.2853; *P*=0.0006). These regions register average temperatures between 25° and 30° and humidity between 86% and 92% (IDEAM, 2023). The zones located in the tropics have a higher leptospirosis incidence due to their optimal environmental conditions that favour the development of reservoir animals, survival of the bacteria in the soil and water, and the risk of human infection due to the ease of access to sources of infection (Costa et al., 2015). This suggests the need for prompt intervention in this sampled area to prevent the spread of the infectious agent to other animals and even humans.

Regarding the socioeconomic stratum, the shelters testing positive for *Leptospira* sp. belong to stratum one (low-low), and two (low); however, it was not possible to establish a correlation with the presence of the infectious agent. Studies suggest that environmental and socioeconomic factors are related to leptospirosis outbreaks. In turn, the disordered growth of human communities and the invasion of wild animals facilitates the transmission (Raghavan et al., 2012; White et al., 2017). Leptospires excreted by animals can remain viable and infectious for up to 6 months in fresh temperate waters such as lakes or rivers and for around 20 months in mineral bottled water (Bierque et al., 2020). Lower strata have been shown to be a risk factor for the presentation and spread of the disease, due to limited access to drinking water sources, presence of rodents, overcrowding, coexistence with other animals, inadequate waste disposal, and poor food storage, among other reasons (Hernández-Rodríguez et al., 2022). Studies have shown that living in urban areas and poverty are significant predictive factors for canine leptospirosis (Smith et al., 2019). Therefore, it is necessary to design preventive strategies that help to mitigate infection between animals and humans.

## Conclusions

A 15% prevalence for the *Leptospira* sp. bacterium was observed, without showing any correlations with the study variables. The current prevalence obtained for this infectious agent constitutes an overview of the current situation of this pathogen in the domestic canine population in areas of the Coffee region (Colombia) and a cause for concern due to the zoonotic potential caused by this pathogen. It is necessary to establish sanitary and educational measures that allow timely detection, control, prevention, and vaccination to mitigate the impact on the public health of companion animals.

## Acknowledgment

The authors express their gratitude to the LMV Laboratory (Bogotá), the dog shelters, and the Vice-Rectory for Research, Innovation, and

Extension (VIIE) of the Technological University of Pereira for the financial support received during the execution of this project through the call to finance research seedbed projects. This study was performed with fundings from "Convocatoria para financiar proyectos de los semilleros de investigación de la Universidad Tecnológica de Pereira año 2021".

## References

1. AHMED, A., H. L. KLAASEN, M. VAN DER VEEN, H. VAN DER LINDEN, M. G. GORIS and R. A. HARTSKEERL (2012): Evaluation of real-time PCR and culturing for the detection of leptospires in canine samples. Adv. Microbiol. 2, 162-170. 10.4236/aim.2012.22021
2. ALTHEIMER, K., P. JONGWATTANAPISAN, S. LUENGYOSLUECHAKUL, R. PUSOONTHORNTHUM, N. PRAPASARAKUL, A. KURILUNG, et al. (2020): Leptospira infection and shedding in dogs in Thailand. BMC Vet. Res. 16, 1-13. 10.1186/s12917-020-2230-0.
3. BERTASIO, C., M. B. BONIOTTI, L. LUCCHESE, L. CEGLIE, L. BELLINATI, M. MAZZUCATO, et al. (2020): Detection of New Leptospira Genotypes Infecting Symptomatic Dogs: Is a New Vaccine Formulation Needed? Pathogens 9, 484. 10.3390/pathogens9060484
4. BIERQUE, E. R., D. THIBEAUX, M. E. GIRAULT, C. SOUPÉ-GILBERT and A. GOARANT (2020): Systematic review of Leptospira in water and soil environments. PLOS one 15: e0227055. 10.1371/journal.pone.0227055
5. BLANCHARD, S., C. CARIOU, J. BOUVET, W. VALFORT, F. OBERLI, S. VILLARD, et al. (2021): Quantitative real-time PCR assays for the detection of pathogenic Leptospira species in urine and blood samples in canine vaccine clinical studies: a rapid alternative to classical culture methods. J. Clin. Microbiol. 59: e03006-20. 10.1128/JCM.03006-20.
6. CABALLERO MÉNDEZ, L. C., L. N. FRANCO MONTOYA, M. M. MAZO, J. C. SEPÚLVEDA-ARIAS, A. CARDONA TABARES and J. J. CARO (2023): Detección molecular de Brucella sp, Leptospira spp y Toxoplasma gondii en felinos domésticos y silvestres y el impacto zoonótico en el personal encargado de su manejo en Pereira, Colombia. Rev. de Investig. Vet. del Peru 34. 10.15381/rivep.v34i1.23066
7. CALDERÓN, A., V. RODRÍGUEZ, S. MÁTTAR and G. ARRIETA (2014) Leptospirosis in pigs, dogs, rodents, humans, and water in an area of the Colombian tropics. Trop. Anim. Health Prod. 46, 427-432. 10.1007/s11250-013-0508-y
8. COSTA, F., J. E. HAGAN, J. CALCAGNO, M. KANE, P. TORGERSON, M. S. MARTINEZ-SILVEIRA, et al. (2015): Global morbidity and mortality of leptospirosis: a systematic review. PLOS Negl. Trop. Dis. 9: e0003898. 10.1371/journal.pntd.0003898
9. DI AZEVEDO, M. I. N. and W. LILENBAUM (2021): An overview on the molecular diagnosis of animal leptospirosis. Lett. Appl. Microbiol. 72, 496-508. 10.1111/lam.13442.
10. DOMÍNGUEZ, S. D. C. B., M. Y. C DZIB, M. G. M. VELÁZQUEZ, L. A. N OREZA, M. I. G SOLANO, R. I. C. POOT, et al. (2013): Detection of reactive canines to Leptospira in Campeche City, Mexico. Rev. Argent. Microbiol. 45, 34-38.
11. ENSUNCHO-HOYOS, C., V. RODRÍGUEZ-RODRÍGUEZ, A. PÉREZ-DORIA, O. VERGARA and A. CALDERÓN-RANGEL (2017): Epidemiology behavior of leptospirosis in Ciénaga de Oro, Córdoba (Colombia). Trop. Anim. Health Prod. 49, 1345-1351. 10.1007/s11250-017-1332-6.
12. FELT, S. A., M. O. WASFY, F. WAEL, A. SAMIR, B. A. RAHAMAN, M. BOSHRA, et al. (2011): Cross-species surveillance of Leptospira in domestic and peri-domestic animals in Mahalla City, Gharbeya Governorate, Egypt. Am. J. Trop. Med. Hyg. 84, 420-425. 10.4269/ajtmh.2011.10-0393
13. FRAUNE, C. K., A. SCHWEIGHAUSER and T. FRANCEY (2013): Evaluation of the diagnostic value of serologic microagglutination testing and a polymerase chain reaction assay for diagnosis of acute leptospirosis in dogs in a referral center. JAVMA 242, 1373-1380. 10.2460/javma.242.10.1373.
14. GONZÁLEZ, F. H. D., S. T. OLIVEIRA, J. B. MESSICK, A. W. BIONDO, A. P. DOS SANTOS, R. STEDILE, et al. (2012): Exposure to Leptospira spp. in sick dogs, shelter dogs and dogs from an endemic area: points to consider. Acta Sci. Vet. 40, 1-7.
15. HERNÁNDEZ-RODRÍGUEZ, P. and B. TRUJILLO-ROJAS (2022): Saúde única: abordagem abrangente para melhorar as estratégias de prevenção e controle da Leptospirose. Rev. de Cienc. Agrovet. 21, 71-78. 10.5965/223811712112022071
16. HUA, K. K., T. W. XIAN, L .S. FONG, M. A. ROSLAN, R. RADZI, S. K. BEJO, et al. (2016): Seroprevalence and molecular detection of leptospirosis from a dog shelter. Trop. Biomed. 33, 276-284.
17. IDEAM (2023): Instituto de Hidrología, Meteorología y Estudios Ambientales. [Internet, June 2023). Available from: http://atlas.ideam.gov.co/visorAtlasClimatologico.html
18. KLAASEN, H. L. and B. ADLER (2015): Recent advances in canine leptospirosis: focus on vaccine development. Veterinary Medicine: Research and Reports 6, 245-260. 10.2147/VMRR.S59521
19. LATOSINSKI, G. S., F FORNAZARI, S. D BABBONI, K CAFFARO, A. C. PAES and H. LANGONI (2018): Serological and molecular detection of Leptospira spp. in dogs. Rev. Soc. Bras. Med. Trop. 51, 364-367. 10.1590/0037-8682-0276-2017
20. LAU, S. F., J. Y WONG, K. H KHOR, M. A. ROSLAN, M. A. RAHMAN, S. K. BEJO and A. R. BAHAMAN (2017): Seroprevalence of leptospirosis in working dogs. Top. Companion Anim. Med. 32, 121-125. 10.1053/j.tcam.2017.12.001.

21. LE GUYADER, M., C. FONTANA, N. SIMON-DUFAY, H. J. BALZER, N. PANTCHEV, J. C. THIBAULT, et al. (2020): Successful Leptospira genotyping strategy on DNA extracted from canine biological samples. J. Microbiol. Methods 176:106007. 10.1016/j.mimet.2020.106007.
22. MAJOR, A., A. SCHWEIGHAUSER and T. FRANCEY (2014): Increasing incidence of canine leptospirosis in Switzerland. IJERPH 11, 7242-7260. 10.3390/ijerph110707242
23. MIOTTO, B. A., A. G. A. GUILLOUX, B. F. TOZZI, L. Z. MORENO, A. S. DA HORA, R. A. DIAS, et al. (2018): Prospective study of canine leptospirosis in shelter and stray dog populations: Identification of chronic carriers and different Leptospira species infecting dogs. PloS one 13(7): e0200384. 10.1371/journal.pone.0200384
24. MIOTTO, B. A, B. F. TOZZI, M. D. S. PENTEADO, A. G. A. GUILLOUX, L. Z. MORENO, M. B. HEINEMANN, et al. (2018): Diagnosis of acute canine leptospirosis using multiple laboratory tests and characterization of the isolated strains. BMC Vet. Res. 14, 1-9. 10.1186/s12917-018-1547-4.
25. PEREZ-GARCIA, J., F. P. MONROY and P. AGUDELO-FLÓREZ (2022): Canine Leptospirosis in a Northwestern Region of Colombia: Serological, Molecular and Epidemiological Factors. Pathogens 11, 1040. 10.3390/pathogens11091040.
26. RAGHAVAN, R. K., K. M. BRENNER, J. J. HIGGINS, J. S. HUTCHINSON and K. R. HARKIN (2012): Neighborhood-level socioeconomic and urban land use risk factors of canine leptospirosis: 94 cases (2002-2009). Prev. Vet. Med. 106, 324-331. 10.1016/j.prevetmed.2012.04.003
27. RAHMAN, S. A., K. H. KHOR, S. KHAIRANI-BEJO, S. F. LAU, M. MAZLAN, A. ROSLAN, et al. (2021): Detection and characterization of Leptospira spp. in dogs diagnosed with kidney and/or liver disease in Selangor, Malaysia. J. Vet. Diagn. Invest. 33, 834-843. 10.1177/10406387211024575
28. RAJAPAKSE, S., C. RODRIGO, S. M. HANDUNNETTI, et al. (2015): Current immunological and molecular tools for leptospirosis: diagnostics, vaccine design, and biomarkers for predicting severity. Ann. Clin. Microbiol. Antimicrob. 14. 10.1186/s12941-014-0060-2
29. REAGAN, K. L. and J. E. SYKES (2019): Diagnosis of canine leptospirosis. Vet. Clin. North Am. Small Anim. 49, 719-731. 10.1016/j.cvsm.2019.02.008
30. ROMERO, M. H., J. A. SÁNCHEZ and L. C. HAYEK (2010): Prevalencia de anticuerpos contra Leptospira en población urbana humana y canina del departamento del Tolima. Rev. Salud. Publica 12, 268-275.
31. ROMERO-VIVAS, C. M., M. CUELLO-PÉREZ, P. AGUDELO-FLÓREZ, D. THIRY, P. N. LEVETT and A. K. FALCONAR (2013): Cross-sectional study of Leptospira seroprevalence in humans, rats, mice, and dogs in a main tropical sea-port city. Am. J. Trop. Med. Hyg. 88, 178-183. 10.4269/ajtmh.2012.12-0232
32. SCHULLER, S., T. FRANCEY, K. HARTMANN, M. HUGONNARD, B. KOHN, J. E. NALLY and J. SYKES (2015): European consensus statement on leptospirosis in dogs and cats. JSAP 56, 159-179. 10.1111/jsap.12328
33. SMITH, A. M., A. G. ARRUDA, M. D. EVASON, J. S. WEESE, T. E. WITTUM, D. SZLOSEK, et al. (2019): A cross-sectional study of environmental, dog, and human-related risk factors for positive canine leptospirosis PCR test results in the United States, 2009 to 2016. BMC Vet. Res. 15, 1-12. 10.1186/s12917-019-2148-6.
34. SRIPATTANAKUL, S., T. PRAPONG, A. KAMLANGDEE, G. KATZENMEIER, D. HALTRICH, R. HONGPRAYOON, et al. (2022): Leptospira borgpetersenii Leucine-Rich Repeat Proteins and Derived Peptides in an Indirect ELISA Development for the Diagnosis of Canine Leptospiral Infections. Trop. Med. Infect. Dis. 7, 311. 10.3390/tropicalmed7100311.
35. STULL, J. W., M. EVASON, J. S. WEESE J. YU, D. SZLOSEK and A. M SMITH (2022): Canine leptospirosis in Canada, test-positive proportion and risk factors (2009 to 2018): A cross-sectional study. Plos one 17(6): e0270313. 10.1371/journal.pone.0270313.
36. WHITE, A. M., C. ZAMBRANA-TORRELIO, T. ALLEN, M. K. ROSTAL, A. K. WRIGHT, E. C. BALL, et al. (2017): Hotspots of canine leptospirosis in the United States of America. Vet. J. 222, 29-35. 10.1016/j.tvjl.2017.02.009
37. ZAIDI, S., A. BOUAM, A. BESSAS, D. HEZIL, H. GHAOUI, K. AIT-OUDHIA, et al. (2018): Urinary shedding of pathogenic Leptospira in stray dogs and cats, Algiers: A prospective study. PLoS One 13(5): e0197068. 10.1371/journal.pone.0197068

# Molekularno određivanje *Leptospira* spp. u uličnim psima i psima iz skloništa u kolumbijskoj Regiji kave

Lyda C. CABALLERO MÉNDEZ, Grupo de investigación BIOECOS, Profesora de Medicina Veterinaria y Zootecnia Facultad de Ciencias de la Salud, Universidad Tecnológica de Pereira, Pereira, Colombia; Luisa María GONZÁLEZ LONDOÑO, Julio Cesar GÓMEZ RUÍZ, María José ESCOBAR HERRERA, Médico Veterinaria y Zootecnista. Facultad de Ciencias de la Salud, Universidad Tecnológica de Pereira, Pereira, Colombia; Margarita María MAZO, Grupo de investigación BIOECOS, Profesora de Medicina Veterinaria y Zootecnia Facultad de Ciencias de la Salud, Universidad Tecnológica de Pereira, Pereira, Colombia, Luz Natalia FRANCO-MONTOYA, Grupo de investigación CIBAV, Profesora Asociada adscrita a la Escuela de Medicina Veterinaria, Facultad de Ciencias Agrarias, Universidad de Antioquia UdeA, Medellín, Colombia

Leptospiroza se smatra zoonotskom bolešću i od značajnog je utjecaja na zdravlje ljudi i životinja. Prisutna je u tropskim i suptropskim klimama što potpomaže dugoročno preživljavanje ovih bakterija, utječući na domaće i divlje životinje koje su njihovi rezervoari. Leptospiroza pasa dobila je na velikom kliničkom značenju, između ostalog i zbog prijemčivosti ove vrste bakterija na infekciju i često izlaganje leptospirozi iz okoliša. Zbog blizine izvorima infekcije psi s ulice i iz skloništa za životinje predstavljaju visokorizičnu opasnost. U ovom radu opisana je unakrsnu studija koja uključuje uzorkovanje pasa iz skloništa koja se nalaze u departmanima Risaralda, Valle del Cauca i Caldas, poznatima kao Regija kave, u srednjem zapadu Kolumbije. Uzeti su uzorci krvi 140 pasa, analizirani su u laboratoriju za Veterinarsku medicinu i znanost o životinjama Tehnološkog Sveučilišta u Pereiri, ekstrahirana je DNK te je amplificiran LipL32 gen konvencionalnim PCR-om. Dokazana je prevalencija od 15 % za *Leptospira* sp. u pasa iz Regije kave; nije otkrivena korelacija između varijabli poput spola, dobi, podrijetla i socioekonomskog statusa. Međutim, zamijećena je tendencija za infekciju s nekoliko slučajeva dijagnoze u kuja starijih od šest godina u nižim slojevima. Ova studija predstavlja prvo izviješće o leptospirozi pasa u ovoj regiji u Kolumbiji, što će omogućiti osmišljavanje strategija usmjerenih na ublažavanje ove bolesti.

**Ključne riječi:** *leptospiroza, spiroheta, lančana reakcija polimerazom, bakterijska zoonoza*