UNIVERSIDAD SAN FRANCISCO DE QUITO USFQ

Colegio de Ciencias Biológicas y Ambientales

Detección molecular de especies patógenas de *Leptospira* en riñones de cerdos faenados en el camal municipal de Portoviejo (Manabí, Ecuador)

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DE TRABAJO DE FIN DE CARRERA

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RESUMEN

Este estudio analizó la positividad de especies patógenas de *Leptospira* en muestras de riñones de cerdos provenientes del camal municipal de Portoviejo, en la provincia de Manabí, Ecuador. Se evaluaron 385 muestras de cerdos procedentes de 10 cantones de la provincia. El ADN fue extraído de los riñones y se utilizaron primers dirigidos a un fragmento del gen 16S del ARN ribosomal (SNP111), y del gen *lipl32*, los cuales fueron amplificados mediante qPCR con ensayos TaqMan para la detección molecular de especies patógenas de *Leptospira.* Adicionalmente, se utilizaron primers específicos dirigidos para fragmentos de los genes *secY* y un fragmento del gen *lipl32* con el fin de obtener amplicones utilizados en la secuenciación mediante la tecnología Oxford Nanopore. Los resultados mostraron una positividad total del 12.99% (50/385), de las cuales 15 muestras fueron secuenciadas para identificar las especies de *Leptospira* presentes. Las especies encontradas fueron *Leptospira santarosai* (11/15) y *Leptospira interrogans* (4/15). Este estudio resalta la importancia de las condiciones sanitarias en la crianza porcina sobre el riesgo de transmisión de especies patógenas de *Leptospira*. Los resultados también evidencian el papel del cerdo como reservorio de esta bacteria en zonas rurales marginales, donde la convivencia con otros animales y humanos en sistemas de agricultura informal y no tecnificada incrementa la exposición a fluidos potencialmente infectados, como sangre y orina.

Palabras clave: *Leptospira*, Cerdos, Manabí, zonas rurales, transmisión, especies patógenas, leptospirosis

ABSTRACT

This study analyzed the positivity of pathogenic *Leptospira* species in kidney samples from pigs collected at the municipal slaughterhouse of Portoviejo, in the province of Manabí, Ecuador. A total of 385 samples from pigs originating from 10 cantons of the province were analyzed. DNA was extracted from the kidneys, and primers targeting a fragment of the 16S ribosomal RNA gene (SNP111) and *lipl32* were used and amplified by qPCR using TaqMan assays for the molecular detection of pathogenic *Leptospira* species. Additionally, specific primers targeting fragments of the *secY* gene and another fragment of the *lipl32* gene were used to obtain amplicons for sequencing via Oxford Nanopore technology. The results showed an overall positivity of 12.99% (50/385), of which 15 samples were sequenced to identify the *Leptospira* species present. The species identified were *Leptospira santarosai* (11/15) and *Leptospira interrogans* (4/15). This study highlights the importance of sanitary conditions in pig farming in relation to the risk of transmission of pathogenic *Leptospira* species. The results underscore the role of pigs as reservoirs of this bacterium in marginal rural areas, where cohabitation with other animals and humans in informal and non-technified agricultural systems increases exposure to potentially infected fluids such as blood and urine.

Key words: *Leptospira*, Pigs, Manabí, rural areas, transmission, pathogenic species, leptospirosis

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INTRODUCTION

Leptospirosis is a bacterial zoonotic disease widely distributed across various geographical regions, being found in a wide range of mammalian hosts, including humans. This disease has emerged as a significant public health issue due to the prevalence of pathogenic species of *Leptospira* worldwide, with a high prevalence in both rural and urban communities around the globe (Barragán et al., 2017). Leptospirosis is estimated to affect over 1 million people annually, causing more than 50,000 deaths (Costa et al., 2015). Despite being recognized as a high-risk disease, it is still considered neglected.

The way of infection of leptospirosis is through direct contact between mucous membranes or injured skin and pathogenic species of *Leptospira,* a spirochete primarily found in urine excreted from infected hosts, as well as in contaminated environments like soil and water, serving as the principal routes of infection (Guzmán et al., 2024). Therefore, any infected human or animal has the potential to spread the disease to others, either through direct contact or by contaminating the surrounding environment (Barragán et al., 2017). In most cases, leptospirosis causes a mild fever, often confused with other febrile diseases and being misdiagnosed. Nevertheless, severe infections of *Leptospira* spp. can cause acute kidney and liver injuries, potentially leading to death (Costa et al., 2015).

A wide range of environmental conditions has been associated with an increased risk of leptospirosis exposure. However, the disease has a higher prevalence in rural communities with tropical climates, where bacterial transmission results favorable. *Leptospira* species are known to have a higher transmission and survival rate in humid and warm conditions. Additionally, this bacterial infection has proven to prevail in underserved areas with poor sanitary conditions (Ganoza et al., 2006). These conditions lead to an increased interaction between leptospirosis hosts and the environment, contaminating water and soil, where

Leptospira spp. is known for surviving and maintaining its virulence for 20 and 40 days, respectively, which can increase considerably the infection rate (Bierque et al., 2020).

An important aspect of leptospiral transmission is the wide host range of parasitic *Leptospira*, as all animal species, including humans, are susceptible to infection. This also relates to specific associations between hosts and particular leptospiral species and serovars, which are often endemic to certain animal species or humans (Ellis, 2014). Among the most common hosts of leptospirosis, rat species such as *Rattus norvegicus* and *Rattus rattus* are well-recognized sources of infection, functioning as maintenance hosts given their capacity to harbor the pathogen as asymptomatic carriers. Due to the overpopulation of these species in both urban and rural environments, wild rats have frequent exposure to domestic, peridomestic, livestock, and wild animals, making them important sources of zoonotic pathogens (Boey et al., 2019).

Although rats are recognized as the main reservoir for leptospirosis transmission, because they shed the highest concentration of pathogenic *Leptospira,* the urine volume in which these bacterial species are found is limited. Therefore, animal density and urine volumes are essential factors in understanding leptospirosis infection in its hosts (Barragán et al., 2017). Among these potential reservoirs, livestock represent an elevated risk of bacterial exposure, given the higher urine volumes these animals shed, contributing with leptospiral distribution. Moreover, the conditions on ranches and farms where livestock are raised significantly influence the prevalence of *Leptospira*, acting as potential hotspots for infection due to the high concentration of animals and the frequent interactions between hosts and their environment. This risk is further intensified in rural communities where inadequate sanitation and poor hygiene practices contribute to increased pathogen transmission (Barragán et al., 2017).

In addition to livestock, such as cattle, pigs are increasingly recognized as significant contributors to the spread of leptospirosis. Due to the historical connotation that pigs are dirty

animals, these often contribute to the neglect of proper sanitation in farming environments. Especially in rural communities, where informal and not technified farming practices prevail, pigs are often raised under poor sanitation and inadequate conditions, frequently without any veterinary guidance (Diaz et al., 2022). These conditions can promote the spread of infectious diseases, including leptospirosis, where contaminated water and soil increase its prevalence. In pigs, leptospirosis can affect reproduction, leading to early abortions, premature births, and increased perinatal mortality. These reproductive losses carry significant economic consequences for both formal and informal pig producers, directly reducing productivity and overall herd efficiency (Pinto et al., 2016).

In Ecuador, leptospirosis is recognized as a reemerging zoonotic disease and a major public health concern. Despite its relevance, the absence of standardized control protocols and clinical management guidelines contributes to its continued classification as a neglected zoonosis (Calvopiña et al., 2023). Ecuador is considered as one of the most biodiverse countries in the world, with ecosystems and climate conditions supporting an exceptional variety of species (Bravo, 2014). Pathogenic species of *Leptospira* are particularly prevalent in the coastal regions of the country, where warm and humid environments, combined with limited sanitation infrastructure in impoverished rural communities, create ideal conditions for transmission (Diaz et al., 2022). One of the provinces with the highest prevalence of leptospirosis in Ecuador is Manabi, which is primarily rural. Of its 1,390,200 inhabitants, 617,880 live in rural communities, contributing to the growing prevalence of this bacterium over the years (Rodriguez-Paredes et al., 2021).

In this study, we analyzed the positivity rate of pathogenic species of *Leptospira* in kidney samples from slaughtered pigs in a municipal slaughterhouse in Portoviejo-Manabi called “Matadero Municipal del Cantón Portoviejo”. This establishment receives various livestock animals from across the province, many of which are peridomestic and lack formal

health controls or sanitary certification, with little regard for the animals' health status. Furthermore, every sampled pig came from informal and not technified backyard farming systems, which often lack standardized record-keeping and sanitary oversight (Sutherland & Tucker, 2011). Additionally, the slaughterhouse from which samples were obtained exhibited poor sanitary conditions, with animals often being slaughtered directly on the ground, facilitating the exposure of fluids such as urine and blood to both humans and other animals. These unhygienic practices, combined with the presence of interspecies interactions, including stray dogs and rats, created an environment highly conducive for the transmission of *Leptospira*. This study underscores the positivity rate of pathogenic *Leptospira* species in the province of Manabí, highlighting the importance of pigs as reservoirs of the infection and their role in the epidemiology of the disease.

METHODS

Sampling

The study was conducted over a two-year period from October 2018 to June 2019, and covered several rural communities in the Manabí province, located in the coastal region of Ecuador. The communities included: Chone (n = 31), El Carmen (n = 21), Flavio Alfaro (n = 36), Jama (n = 4), Jipijapa (n= 10), Manta (n = 2), Montecristi (n = 10), Pichincha (n=4), Portoviejo (n = 231) and Santa Ana (n = 36). A total of 385 slaughtered pigs were sampled from the "Matadero Municipal del Cantón Portoviejo", a municipal slaughterhouse located in Portoviejo that received animals from various locations across the province. Furthermore, each of the pigs sampled for this study retained their tails, had dark colored skin, and all males were uncastrated, these selection criteria were defined by Eduardo Díaz, followed by Patricia Zambrano, Gema Giler, Gabriela Vélez and Leonel Lazo who kindly provided the samples for analysis. Kidney samples were collected from each pig in sterile containers with 80% ethanol and transported to the Institute of Microbiology at Universidad San Francisco de Quito, which were then stored at -20 ^{o}C prior to DNA extraction.

Molecular detection

DNA extraction was performed using Chelex commercial extraction kit (Hercules, United States) following the manufacturer's protocol. The extracted DNA samples were stored at -20 ^{0}C. To assess sample quality and rule out the presence of PCR inhibitors, the *β-actin* gene was amplified from each DNA sample using conventional Polymerase Chain Reaction assays (PCR) (De Breuil, 1993). All samples successfully amplified a 300 bp fragment, proving that none of them were degraded and that the DNA quality was adequate for conducting further

tests. Additionally, this step reduced the likelihood of false negatives in subsequent PCR assays.

Two qPCR assays were implemented for the molecular detection of pathogenic *Leptospira* using TaqMan assays targeting SNP111 (Barragán et al., 2016), which belongs to the 16S rRNA gene and the *lipl32* gene (Stoddard et al., 2009). Both of these genes are specific to pathogenic *Leptospira* species; therefore, a positive result in either or both assays indicated that the sample originated from a pig infected with these bacterial species. Positive samples from Taqman assays were further analyzed via PCR, amplifying two different genes: *lipl32* and *secY.* Additionally, three fragments of the *secY* gene were amplified: two with molecular weights of 449 bp and 202 bp, using primers from Ahmed et al. (2009), and a third fragment was obtained applying a nested PCR assay with a molecular weight of 410 bp, using primers from Mosquera et al. (2024). All primers were specifically designed to target pathogenic *Leptospira* species. Amplicons obtained from both PCR assays were used for sequencing.

Sequencing and species identification

From the 50 qPCR-positive samples, 15 amplicons were selected for sequencing using Oxford Nanopore technologies. The process began with the purification of the amplicons using the AMPure kit (Beckman Coulter, USA), followed by DNA quantification with the Qubit 1X dsDNA high sensitivity kit (Thermo Scientific, Invitrogen, USA). Consequently, the sequencing library was then prepared using Oxford Nanopore Native Barcoding Kit 96 V14 (SQK-NBD114-96), followed by ligation using the Ligation Sequencing LSK-109 kit. A total of 200 fmol of the library were loaded to a FLO-MIN114 (FBA23290) flowcell and sequenced in the GridION GXB04074 (Oxford Nanopore). The settings for the run established a 1200 bps super-accurate basecalling with a minimum Q score of 10. All the reads were processed with

MinKNOW 24.02.16, Bream 7.8.9, Configuration 5.9.18, Dorado 7.3.11 and MinKNOW Core 5.9.12.

The sequences were processed using the amplicon_sorter pipeline (Vierstraete & Braeckman, 2022) to obtain consensus sequences by classifying all reads based on similarity and length after aligning all of them without any external reference. The resulting consensus sequences were compared with sequences from the NCBI's database, GenBank using the BLASTn tool to determine species identity for each barcode. Additionally, MEGA12 was used to generate a phylogenetic analysis of the positive sequenced samples aligning these with the samples obtained from GenBank and creating multiple phylogenetic trees using the Maximum Likelihood method.

RESULTS

Positivity of pathogenic *Leptospira* species in the province of Manabi

As shown in **Figure 1**, a total of 385 pigs from ten different cantons across the province of Manabi were transported to the municipal slaughterhouse "Matadero Municipal del Cantón Portoviejo" located in the city of Portoviejo, where kidney samples were collected from each slaughtered pig. Of these, 50 tested positive for pathogenic *Leptospira* species, meaning that all of them had a positive result in at least one or both of the qPCR assays and/or amplified with at least one of the four primers used in the PCR assays. This corresponds to an overall positivity rate of 12.99% where the cantons with the highest positivity rate of pathogenic *Leptospira* species were Santa Ana (27.77%, n = 36), Chone (22.58%, n = 31) and Flavio Alfaro (19.44%, n = 36). Furthermore, Portoviejo canton had a lower positivity of 6.49% (n = 231).

Species characterization based on sequenced positive samples and phylogenetic trees construction

Out of the 15 positive samples used for sequencing, all presented a Q score higher than 10, and a molecular phylogenetic analysis was performed based on their consensus sequences, resulting in the construction of three distinct phylogenetic trees using four sets of primers. **Figure 2** presents 14 positive sequenced samples successfully grouped into clades based on a 202 bp fragment of the *secY* gene, where 3 samples (P051, P025 and P047) were assigned as *Leptospira interrogans*, and 11 samples (P035, P088, P095, P082, P042, P106, P355, P006, P001, P040 and P089) as *Leptospira santarosai*. **Figure 3** displays 9 positive sequenced samples classified into clades based on 410 bp and 449 bp fragments of the *secY* gene, where 2 samples (P051 and P025) were assigned as *Leptospira interrogans* and 7 samples (P042,

P355, P006, P106, P001, P040 and P089) as *Leptospira santarosai*. Lastly, **Figure 4** shows 2 positive sequenced samples assigned to a clade based on 474 bp fragment of the *lipl32* gene, where both (P010 and P051) were assigned as *Leptospira interrogans*.

Based on the phylogenetic analysis, samples P089, P040, P001, P006, P355, P106, P042, P035, P088, P095 and P082 were identified as belonging to the *Leptospira santarosai* species, while samples P051, P025, P047 and P010 were classified as *Leptospira interrogans*.

DISCUSSION

This study was conducted under favorable conditions for leptospirosis transmission, including frequent animal interspecies interactions, lack of sanitary conditions and warm and humid weather; factors that made the study site ideal for understanding the role of pathogenic *Leptospira* in pigs and its positivity rate in the province of Manabi (Barragán et al., 2017). Of 385 total kidney samples collected over a two-year period, 12.99% (50/385) tested positive for pathogenic *Leptospira*.

The cantons with the highest positivity rate were Santa Ana (27.77%, 10/36), Chone (22.58%, 7/31) and Flavio Alfaro (19.44%, 7/36). In a study conducted by Barragán et al. (2016), Santa Ana was found to be a canton with a high leptospirosis positivity rate with a 26.9% (25/93) of pathogenic *Leptospira* in pig urine samples, along with a positivity rate of 35.9% (42/117) in cattle urine samples. This supports that Santa Ana is a canton with a high positivity rate of leptospirosis, being found in several animal hosts related to a peridomestic and livestock context (Barragán et al., 2016). According to Correa et al. (2018), both Santa Ana and Chone were part of a cluster of five cantons with persistently high levels of extreme poverty from 1990 to 2010. Importantly, the remaining three cantons were not included in this study, leaving a gap in understanding the full extent of leptospiral prevalence across all high-poverty regions in the province (Correa et al., 2018). These elevated poverty levels are often associated with inadequate sanitation infrastructure, limited access to veterinary care, and poor health regulation of peridomestic animals. Such conditions can create an ideal environment for the persistence and transmission of zoonotic pathogens like *Leptospira*, which may explain the higher positivity rate found in these two cantons (Diaz et al., 2022).

Although the majority of the pigs sampled originated from Portoviejo canton, accounting for 60% (231/385) of the total samples, its positivity rate was relatively low, at only 6.49% (15/231). Portoviejo, the capital of Manabi, stands out as one of the province's most

urbanized areas and functions as a key commercial hub (Cevallos, 2018), this can explain the low positivity rate of leptospirosis, understanding that sanitary conditions may be better than rural communities in other cantons from the province. However, according to Zambrano et al. (2017), 107 human cases of leptospirosis were reported in Portoviejo between 2014 and 2017. This relatively high positivity in humans, despite the low positivity in pigs, suggests the involvement of other animal reservoirs in the transmission of *Leptospira* to humans.

Other cantons that showed notable positivity rates were El Carmen (14.29%, 3/21) and Montecristi (40%, 4/10). While this study confirmed the presence of pathogenic *Leptospira* species in pigs from these areas, there is limited information regarding the overall prevalence of leptospirosis in these two cantons, as most research has primarily focused on Portoviejo canton. This highlights the need for future studies aimed at these cantons, taking into account their positivity rate. Additionally, the remaining four cantons sampled in this study: Jama (25%, 1/4), Manta (0%, 0/2), Pichincha (50%, 2/4) and Jipijapa (10%, 1/10), had small sample sizes, which affected the total positivity rate, but limited any conclusions in a canton level. Therefore, broader and more systematic sampling in these areas is necessary to better assess the true prevalence and distribution of *Leptospira* in swine populations across these cantons.

The analysis of 15 consensus sequences derived from positive samples, confirmed the effectiveness of the protocol for ONT sequencing. The use of four primer sets, three targeting the *secY* gene and one targeting *lipl32* gene, allowed for a successful species-level identification of all 15 samples within the *Leptospira* genus. The sequencing revealed the presence of two pathogenic *Leptospira* species in the pig samples: *Leptospira santarosai* and *Leptospira interrogans*. Both species are well-documented causative agents of leptospirosis in humans and animals, underscoring their zoonotic importance (Chinchilla et al., 2023). Among the sequenced samples, *L. santarosai* was the most prevalent, present in 73.33% (11/15) of samples, while *L. interrogans* was identified in 26.66% (4/15) of the cases.

The identification of these species as primary hosts of pathogenic *Leptospira* in both livestock and peridomestic animals was reported by Chinchilla et al. (2023), where they proved that *Leptospira interrogans* is the most distributed species of pathogenic *Leptospira* in the world. In contrast, there is limited information on the reservoirs and geographical distribution of *Leptospira santarosai.* This species is considered endemic to South and Central America, with Ecuador recognized as one of the primary countries where it circulates (Chinchilla et al., 2023). A study by Barragán et al. (2016), sampled cattle, pigs and rats, in Santa Ana canton, Manabi province. In pig urine samples, they reported high positivity rates of *L. interrogans* (28%, 7/25) and *L. santarosai* (20%, 5/25), highlighting the significant role these two species play in leptospiral transmission among pigs in rural settings, as well as confirming their presence in swine populations in Manabí province (Barragán et al., 2016).

Another critical factor in the analysis of both *L. santarosai* and *L. interrogans* is their wide host range, with confirmed prevalence across multiple animal species. On one hand, *L. santarosai* has been identified in a variety of hosts, including humans, dogs, rodents, cattle, and pigs, being found mostly in South and Central America (Chinchilla et al., 2023). On the other hand, *L. interrogans* is known to be responsible for the majority of human leptospirosis cases worldwide. However, it has also been identified in a range of animals, including rodents, dogs, pigs, cattle and horses (Guglielmini et al., 2019). Furthermore, a high positivity rate of *L. santarosai* and *L. interrogans* was reported in samples from cattle, pigs and rodents in the cities of Santa Ana and Calderon, further confirming the circulation of these two species in Manabi province (Barragán et al., 2016). The repeated detection of the same two pathogenic *Leptospira* species in the same animal hosts across different studies suggests the possibility of interspecies transmission. Rural and underserved communities often provide ideal conditions for interaction among peridomestic animals, thereby increasing the likelihood of leptospiral transmission between species (Ganoza et al., 2006). In the context of this study, the poor

sanitary conditions where pigs are raised, based on informal and not-technified farming likely facilitated interspecies contact, potentially contributing to the high positivity rate observed.

After analyzing the positivity rate of pathogenic *Leptospira* species in pig samples from Manabi province, it is essential to evaluate the broader significance of these findings. This includes not only understanding the mechanisms of infection in swine and their prevalence in Ecuador, but also recognizing how in these rural areas, pigs are often raised in the backyards of people's homes, which increases their contact with other animals and with humans, including entire families. This close coexistence, typical of informal and non-technified farming, creates favorable conditions for the transmission of Leptospira and poses a significant public health risk, as poor sanitary and environmental conditions impact not only pigs but also other animals, humans, and the surrounding environment, which may act as a channel for continued transmission (Ganoza et al., 2006). Leptospirosis is a bacterial zoonotic disease characterized by its increased survival rate in warm and humid environments. It thrives particularly in settings with inadequate sanitation, where such conditions facilitate the persistence of pathogenic *Leptospira*, enabling the infection of new hosts and contributing to the continued spread of the disease (Ganoza et al., 2006).

For this reason, it is important to understand leptospirosis under the framework of the One Health concept, which promotes a collaborative and interdisciplinary strategy that integrates human, animal and environmental health. This approach allows for a full understanding of the ecological dynamics of zoonotic diseases like leptospirosis (Mackenzie & Jeggo, 2019). Furthermore, it enables a comprehensive risk assessment and supports the development of effective strategies of prevention, surveillance, and control measures for this neglected zoonotic disease (Degeling & Rock, 2020).

CONCLUSIONS

This study provides essential information of the positivity rate and molecular characterization of pathogenic *Leptospira* species in pigs from the Manabí province, Ecuador, highlighting the role of *L. santarosai* and *L. interrogans* as key agents of leptospiral infection in rural and underserved areas. The higher positivity rate evidences the importance of the poor sanitary conditions presented in pigs' breeding environment, as one of the main reasons for leptospiral transmission during the study. In particular, backyard pig rearing practices, common in these communities, significantly increase the risk of exposure, as pigs are often kept in close proximity to humans and other animals under minimal sanitary control. These findings emphasize not only the presence of these pathogens in swine populations but also their potential role in interspecies transmission, particularly in environments where sanitary infrastructure is limited, and close contact between peridomestic animals, livestock, and humans is common. This underscores the urgent need to approach leptospirosis from a One Health perspective, integrating human, animal, and environmental health efforts to develop effective strategies for prevention and control. Ultimately, this work contributes to the growing understanding of leptospirosis in Latin America and calls for more focused surveillance in underrepresented regions and host species.

FIGURES

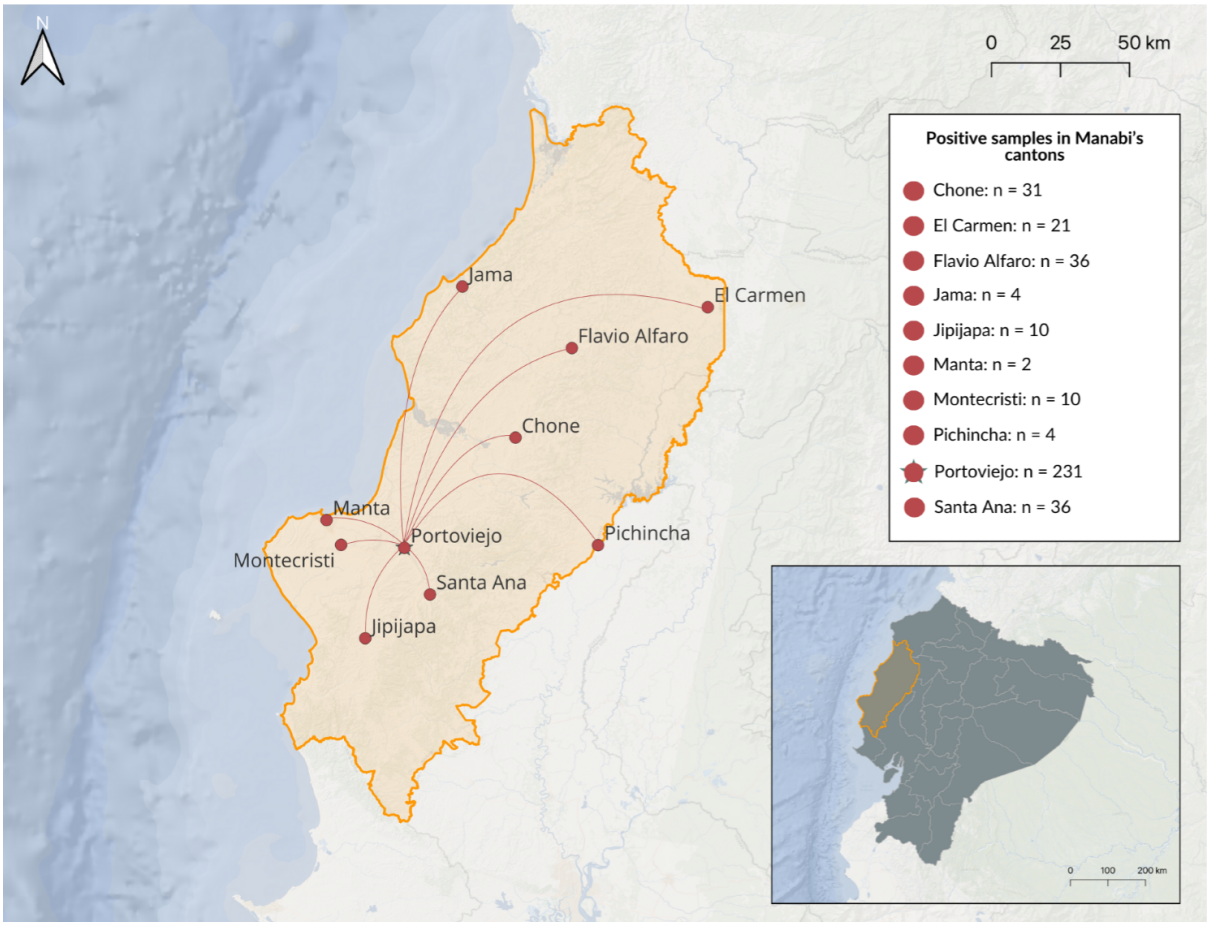

Figure 1. Geographic location of the cantons in Manabi where samples were collected. The map displays the location of the 10 cantons where kidney samples were obtained. *n* represents the number of samples collected.

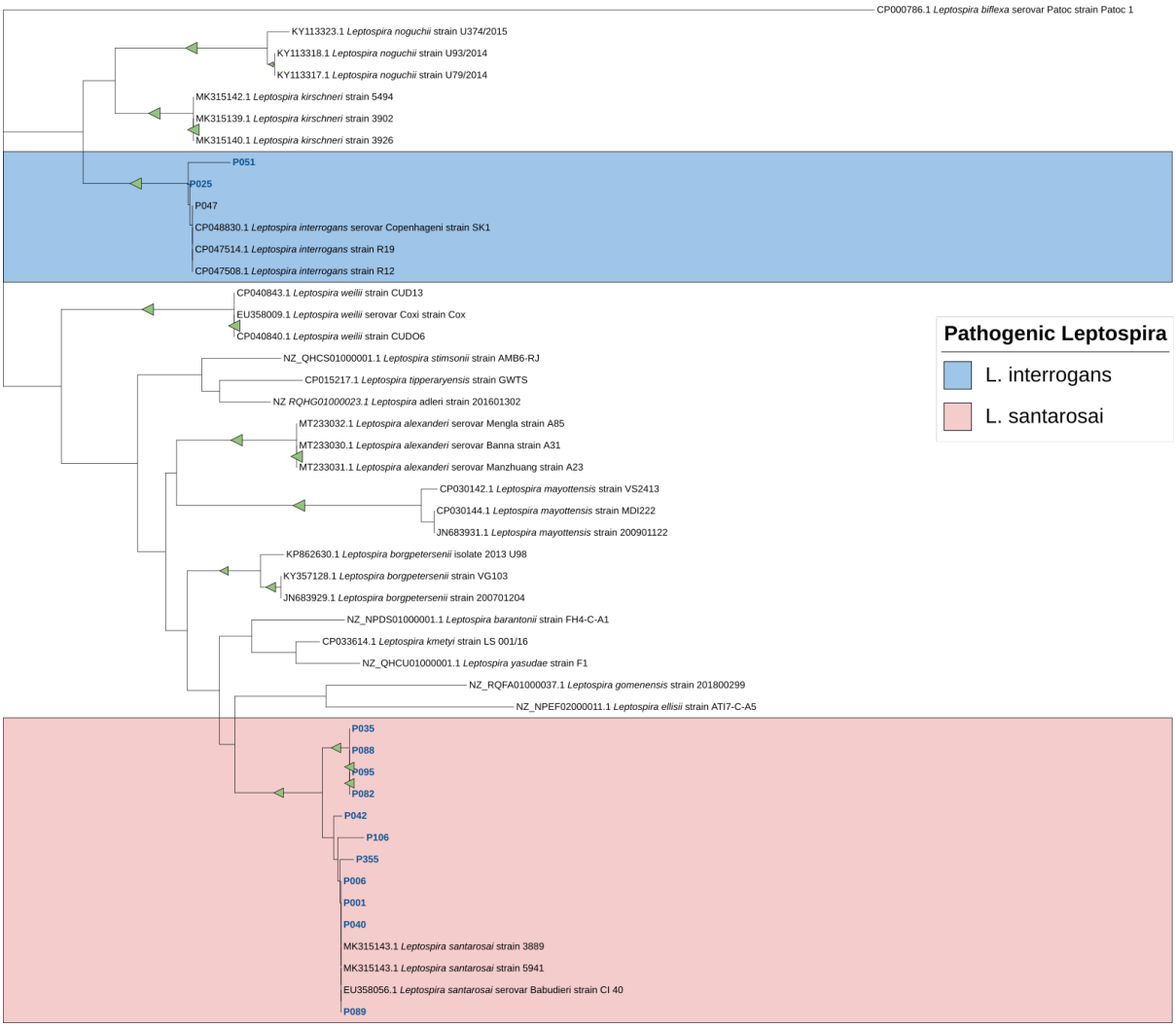

Figure 2. Phylogenetic tree of the pathogenic *Leptospira* spp. genus based on a 202 bp fragment of the *secY* gene. The molecular phylogenetic analysis separates pathogenic *Leptospira* species into clades, represented in different colors. Bootstrap values (500 replicates) > 85 are labeled and indicated with a green triangle. Sequenced positive samples are grouped in clades assigning them into species level.

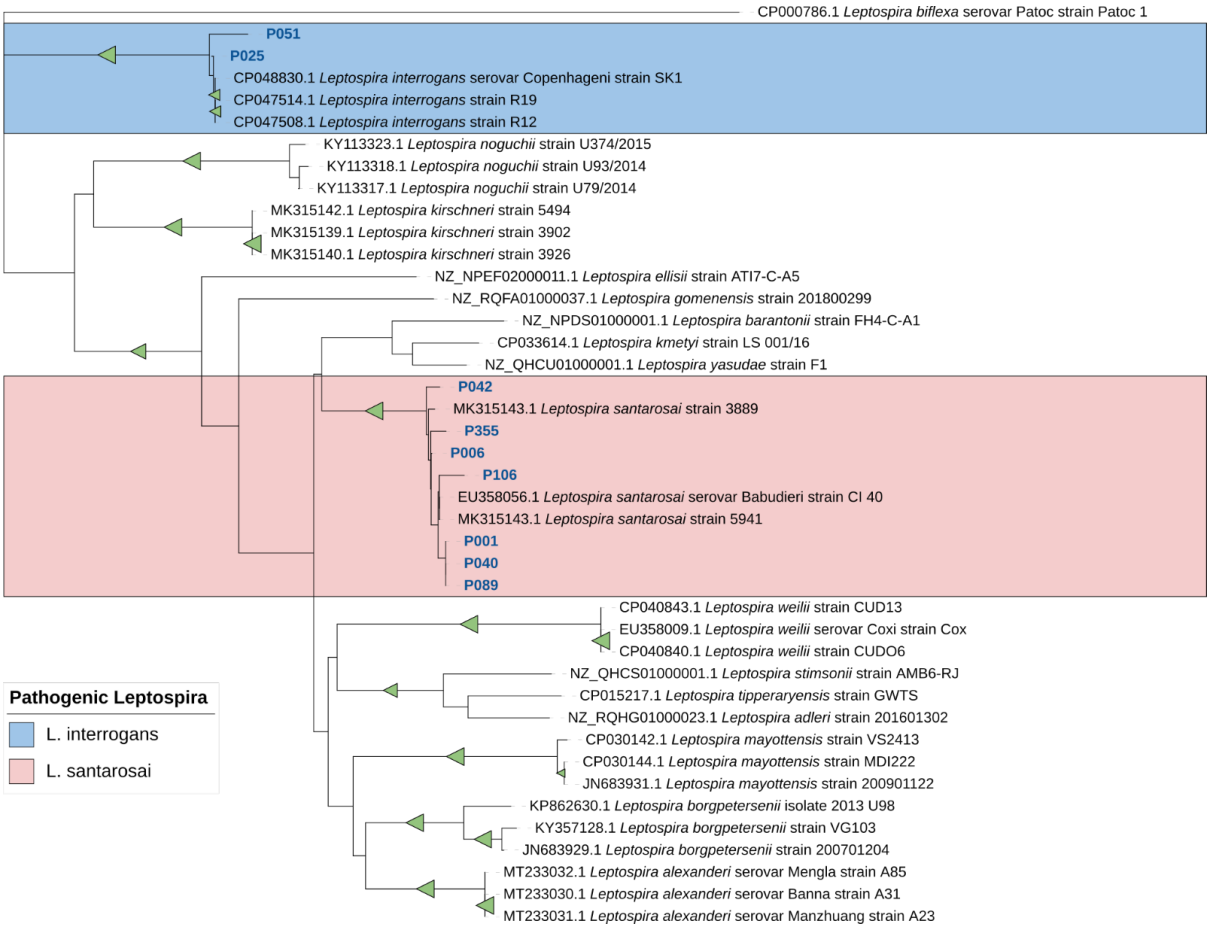

Figure 3. Phylogenetic tree of the pathogenic *Leptospira* spp. genus based on 410 bp and 449 bp fragments of the *secY* gene. The molecular phylogenetic analysis separates pathogenic *Leptospira* species into clades, represented in different colors. Bootstrap values (500 replicates) > 85 are labeled and indicated with a green triangle. Sequenced positive samples are grouped in clades assigning them into species level.

Figure 4. Phylogenetic tree of the pathogenic *Leptospira* spp. genus based on 474 bp fragment of the *lipl32* gene. The molecular phylogenetic analysis separates pathogenic *Leptospira* species into clades, represented in different colors. Bootstrap values (500 replicates) > 85 are labeled and indicated with a green triangle. Sequenced positive samples are grouped in clades assigning them into species level.

REFERENCES

Ahmed, A., Engelberts, M. F. M., Boer, K. R., Ahmed, N., & Hartskeerl, R. A. (2009). Development and Validation of a Real-Time PCR for Detection of Pathogenic Leptospira Species in Clinical Materials. *PLoS ONE*, *4*(9), e7093. https://doi.org/10.1371/journal.pone.0007093

Barragan, V., Chiriboga, J., Miller, E., Olivas, S., Birdsell, D., Hepp, C., Hornstra, H., Schupp, J. M., Morales, M., Gonzalez, M., Reyes, S., De la Cruz, C., Keim, P., Hartskeerl, R., Trueba, G., & Pearson, T. (2016). High Leptospira Diversity in Animals and Humans Complicates the Search for Common Reservoirs of Human Disease in Rural Ecuador. *PLoS Neglected Tropical Diseases*, *10*(9), e0004990. https://doi.org/10.1371/journal.pntd.0004990

Barragan, V., Nieto, N., Keim, P., & Pearson, T. (2017). Meta-analysis to estimate the load of Leptospira excreted in urine: beyond rats as important sources of transmission in low-income rural communities. *BMC Research Notes*, *10*(1). https://doi.org/10.1186/s13104-017-2384-4

Bierque, E., Thibeaux, R., Girault, D., Soupé-Gilbert, M., & Goarant, C. (2020). A systematic review of Leptospira in water and soil environments. *PLoS ONE*, *15*(1), e0227055. https://doi.org/10.1371/journal.pone.0227055

Bravo, B. V. (2014). La Biodiversidad en el Ecuador. *Universidad Politécnica Salesiana*. https://dspace.ups.edu.ec/handle/123456789/6788

Boey, K., Shiokawa, K., & Rajeev, S. (2019). Leptospira infection in rats: A literature review of global prevalence and distribution. *PLoS Neglected Tropical Diseases*, *13*(8), e0007499. https://doi.org/10.1371/journal.pntd.0007499

Calvopiña, M., Romero-Alvarez, D., Vasconez, E., Valverde-Muñoz, G., Trueba, G., Garcia-Bereguiain, M. A., & Orlando, S. A. (2023). Leptospirosis in Ecuador: Current Status and Future Prospects. *Tropical Medicine And Infectious Disease*, *8*(4), 202. https://doi.org/10.3390/tropicalmed8040202

Cevallos, M. (2018). La innovación como estrategia para el desarrollo económico del cantón Portoviejo. *Journal Of Science And Research: Revista Ciencia E Investigación*, *3(4)*, 12-16. https://revistas.utb.edu.ec/index.php/sr/article/view/566/431

Chinchilla, D., Nieves, C., Gutiérrez, R., Sordoillet, V., Veyrier, F. J., & Picardeau, M. (2023). Phylogenomics of Leptospira santarosai, a prevalent pathogenic species in the Americas. *PLoS Neglected Tropical Diseases*, *17*(11), e0011733. https://doi.org/10.1371/journal.pntd.0011733

Correa-Quezada, R., García-Vélez, D. F., De la Cruz del Río-Rama, M., & Álvarez-García, J. (2018). Poverty Traps in the Municipalities of Ecuador: Empirical Evidence. *Sustainability*, *10*(11), 4316. https://doi.org/10.3390/su10114316

Costa, F., Hagan, J. E., Calcagno, J., Kane, M., Torgerson, P., Martinez-Silveira, M. S., Stein, C., Abela-Ridder, B., & Ko, A. I. (2015). Global Morbidity and Mortality of Leptospirosis: A Systematic Review. *PLoS Neglected Tropical Diseases*, *9*(9), e0003898. https://doi.org/10.1371/journal.pntd.0003898

Degeling, C., & Rock, M. (2020). Qualitative Research for One Health: From Methodological Principles to Impactful Applications. *Frontiers In Veterinary Science*, *7*. https://doi.org/10.3389/fvets.2020.00070

Diaz, E. A., Luna, L., Burgos-Mayorga, A., Donoso, G., Guzman, D. A., Baquero, M. I., Pearson, T., & Barragan, V. A. (2022). First detection of Leptospira santarosai in the reproductive track of a boar: A potential threat to swine production and public health. *PLoS ONE*, *17*(9), e0274362. https://doi.org/10.1371/journal.pone.0274362

Du Breuil, R. M., Patel, J. M., & Mendelow, B. V. (1993). *Quantitation of beta-actin-specific mRNA transcripts using xeno-competitive PCR.* https://genome.cshlp.org/content/3/1/57

Ellis, W. A. (2014). Animal leptospirosis. *Current Topics In Microbiology And Immunology*, 99-137. https://doi.org/10.1007/978-3-662-45059-8_6

Ganoza, C. A., Matthias, M. A., Collins-Richards, D., Brouwer, K. C., Cunningham, C. B., Segura, E. R., Gilman, R. H., Gotuzzo, E., & Vinetz, J. M. (2006). Determining Risk for Severe Leptospirosis by Molecular Analysis of Environmental Surface Waters for Pathogenic Leptospira. *PLoS Medicine*, *3*(8), e308. https://doi.org/10.1371/journal.pmed.0030308

Guglielmini, J., Bourhy, P., Schiettekatte, O., Zinini, F., Brisse, S., & Picardeau, M. (2019). Genus-wide Leptospira core genome multilocus sequence typing for strain taxonomy and global surveillance. *PLoS Neglected Tropical Diseases*, *13*(4), e0007374. https://doi.org/10.1371/journal.pntd.0007374

Guzmán, D. A., Diaz, E., Sáenz, C., Álvarez, H., Cueva, R., Zapata-Ríos, G., Prado-Vivar, B., Falconí, M., Pearson, T., & Barragan, V. (2024). Domestic dogs in indigenous Amazonian communities: Key players in Leptospira cycling and transmission? *PLoS Neglected Tropical Diseases*, *18*(4), e0011671. https://doi.org/10.1371/journal.pntd.0011671

Maciel, E. A. P., De Carvalho, A. L. F., Nascimento, S. F., De Matos, R. B., Gouveia, E. L., Reis, M. G., & Ko, A. I. (2008). Household Transmission of Leptospira Infection in Urban Slum Communities. *PLoS Neglected Tropical Diseases*, *2*(1), e154. https://doi.org/10.1371/journal.pntd.0000154

Mackenzie, J. S., & Jeggo, M. (2019). The One Health Approach—Why is it so important? *Tropical Medicine And Infectious Disease*, *4*(2), 88. https://doi.org/10.3390/tropicalmed4020088

Mosquera, P., Mejia, L., Ortiz, G., Pazmino, G., Pearson, T., Barragán, V., & Trueba, G. (2024). Mixed Leptospira infections in domestic animals from a rural community with

high leptospirosis endemicity. *PLoS ONE*, *19*(10), e0312556. https://doi.org/10.1371/journal.pone.0312556

Pinto, P. S., Libonati, H., & Lilenbaum, W. (2016). A systematic review of leptospirosis on dogs, pigs, and horses in Latin America. *Tropical Animal Health And Production*, *49*(2), 231-238. https://doi.org/10.1007/s11250-016-1201-8

Rodriguez-Paredes, M. B., Vallejo-Janeta, P. A., Morales-Jadan, D., Freire-Paspuel, B., Ortiz-Prado, E., Henriquez-Trujillo, A. R., Rivera-Olivero, I. A., Jaramillo, T., Lozada, T., & Garcia-Bereguiain, M. A. (2021). COVID-19 Community Transmission and Super Spreaders in Rural Villages from Manabi Province in the Coastal Region of Ecuador Assessed by Massive Testing of Community-Dwelling Population. *American Journal Of Tropical Medicine And Hygiene*, *106*(1), 121-126. https://doi.org/10.4269/ajtmh.21-0582

Stoddard, R. A., Gee, J. E., Wilkins, P. P., McCaustland, K., & Hoffmaster, A. R. (2009). Detection of pathogenic Leptospira spp. through TaqMan polymerase chain reaction targeting the LipL32 gene. *Diagnostic Microbiology And Infectious Disease*, *64*(3), 247-255. https://doi.org/10.1016/j.diagmicrobio.2009.03.014

Sutherland, M. A., & Tucker, C. B. (2011). The long and short of it: A review of tail docking in farm animals. *Applied Animal Behaviour Science*, *135*(3), 179-191. https://doi.org/10.1016/j.applanim.2011.10.015

Vierstraete, A. R., & Braeckman, B. P. (2022). Amplicon_sorter: A tool for reference-free amplicon sorting based on sequence similarity and for building consensus sequences. *Ecology And Evolution*, *12*(3). https://doi.org/10.1002/ece3.8603

Zambrano, P., Lazo, L., Barragán, V., Morales, M., Bulnes, C., Fimia, R., & Iannacone, J. (2017). Estado actual y estrategias futuras en la epidemiología de la leptospirosis en el cantón Portoviejo, provincia de Manabí, Ecuador. *Biotempo*, *14*(2), 197-209.