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# Leptospirosis outbreak in Ecuador in 2023: A pilot study for surveillance from a One Health perspective

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A B S T R A C T

Leptospirosis is a neglected zoonotic disease that is endemic in tropical regions, including Ecuador. It is caused by spirochetes of the genus *Leptospira*, which can infect humans through animal reservoirs such as rats and dogs, or through contact with contaminated water or soil. In March 2023, public health authorities declared a concerning outbreak of leptospirosis in Durán Cantón, located in the Coastal region of Ecuador. For the first time in the country, a multidisciplinary approach involving physicians and veterinarians was implemented for the surveillance and management of this leptospirosis outbreak. A total of 335 samples were collected, including suspected human cases, household contacts, household dogs, synanthropic rats, and water samples within the area of human cases. Samples were processed by qPCR targeting *lipL32*, *secY,* and *rrs* fragment genes and characterized further for Sanger sequencing. Overall, 26.2 % of human samples, 43.8 % of dog samples, 38.5 % of rat samples, and 39.4 % of water samples tested positive for *Leptospira*. Further, phylogenetic analysis shows that human, dog, and rat sequences are clustered within the pathogenic subclade P1, within the branch of L. *kirschneri* and L. *interrogans*. This study is the first of its kind in Ecuador, where an ongoing outbreak of leptospirosis was managed in real-time by using molecular diagnosis and not serological tools, and where the epidemiological surveillance was done following a One Health approach. This experience should inspire public and animal health authorities in Ecuador to promote a national One Health surveillance and control program for zoonotic diseases.

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## 1. Introduction

Leptospirosis is a re-emerging and neglected zoonotic disease caused by pathogenic species of the genus *Leptospira* that pose a significant threat to both human and animal health [1,2]. The global burden of leptospirosis is estimated at 1,030,000 cases annually, which results in a total of approximately 2.90 million Disability Adjusted Life Years (DALYs), placing this disease among the leading zoonotic causes of morbidity and mortality [3,4]. Infection with pathogenic *Leptospira* presents as a life-threatening febrile syndrome that can lead to renal failure and dysfunction of other organs failure [2,5].

The diverse genus *Leptospira* has traditionally been classified into three major lineages based on pathogenicity: pathogenic, intermediate, and saprophytic [6–8]. Nevertheless, recent advancements in genomic technologies and new strain isolation have led to the genus's reclassification into two main clades: pathogenic (P) and saprophytic (S). Each of these clades is further divided into two subclades: P1 (pathogenic lineage), P2 (low-virulence intermediate clade), S1 (original saprophytic lineage), and S2 (new saprophytic clade) [6–8].

The transmission of *Leptospira* primarily happens through indirect contact with contaminated water or soil containing urine from infected animals [1,9,10]. The pathogen identification has been traditionally made using serological tests like ELISA or the Microscopic Agglutination Test (MAT). However, these methods have several limitations, especially MAT, which is time-consuming and lacks good sensitivity and specificity due to cross-reactivity between different species and serovars [11,12]. On the other hand, molecular diagnosis based on real-time or quantitative PCR (qPCR) and sequencing are becoming more used worldwide for reliable diagnosis and species identification. While the most commonly used gene target is *lipL32*, this marker is highly specific to the P1 subclade, so other gene targets, such as *SecY* or 16S rRNA (rrs), are also used for broader species identification [13–19].

Leptospirosis' characterization as a neglected tropical disease is based on facts like strong surveillance limitations and the absence of rapid and sensitive diagnostic tests that produce underreporting of cases and misdiagnosis, exacerbated by a clinical presentation similar to other endemic tropical diseases like dengue [9,12,20,21]. In South America, leptospirosis has been a significant concern, with a cumulative incidence rate of 2 cases per 100,000 inhabitants, particularly affecting Brazil, Peru, Colombia, and Ecuador [22–24]. In Ecuador, leptospirosis is a mandatory notifiable disease to the National Directorate of Epidemiological Surveillance and poses a significant public health challenge [25]. Recent years have seen an upward trend in cases, particularly linked to outdoor activities and livestock contact [11,26]. In this context, before 2023, estimates by the Ecuadorian Ministry of Health indicated a national annual incidence rate of 1.0 per 100,000 inhabitants, with 22 cases reported in 2022 [27]. However, the incidence scaled up in 2023 with 663 cases reported in Ecuador according to official data [28].

Leptospirosis cases in Ecuador are mainly distributed in the provinces of the Coastal Regions due to environmental risk factors such as climate, soil characteristics, and poor sanitation [22,25]. Seasonal floods spread *Leptospira* from overflowing sewers to clean water sources and agricultural areas, heightening the risk of infection [29]. Furthermore, the high population density of rodents in coastal areas exacerbates the transmission dynamics [19,30–32]. Under this scenario, an alarming outbreak occurred in the first half of 2023 in the Cantón Durán in the Guayas province of Ecuador. There were more than 160 recorded cases by June 2024 only in this location [27,33].

This study aimed to characterize the leptospirosis outbreak that happened in the Cantón Durán in the Coastal Region of Ecuador in 2023. In this study, new methodologies for diagnosis based on qPCR and Sanger sequencing were improved for the first time at the National Institute of Public Health Research in Ecuador. Moreover, for the first time in Ecuador, a One Health approach was implemented for the surveillance and control of an outbreak of leptospirosis, including tracking animal and environmental reservoirs to understand transmission dynamics.

## 2. Methods

### 2.1. Study setting and sample collection

The study was carried out from March to October 2023, resulting in a dataset comprising 335 samples: 164 from humans, 112 from dogs, 33 from water sources, and 26 from rats. Samples were collected from the parish within the Cantón Durán, in the province of Guayas, Coastal Region of Ecuador (southwest of Ecuador) were the leptospirosis outbreak was declared. All the samples were collected in a less than 5 km diameter area around the households were the human cases happened. The human samples collection was done by active epidemiological surveillance, involving individuals exhibiting symptoms indicative of leptospirosis. Patients were initially admitted to local healthcare facilities, where informed consent was obtained prior to the collection of blood samples. In cases where a positive diagnosis was confirmed, an extensive home and peridomicile visit protocol was implemented: 1) systematic collection of blood samples from family members and/or close contacts residing with the infected individual; 2) pet samples collection within the household; 3) water samples collection from nearby reservoirs; 4) rat capture using Tomahawk traps.

### 2.2. DNA extraction and identification of Leptospira by qPCR

Water samples (50 mL) underwent a pre-processing step before DNA extraction, involving centrifugation at 6000 rpm for 1 h to facilitate the separation of components. Subsequently, the supernatant was discarded, and 1× PBS buffer was added to the solid residue (pellet) formed after centrifugation. DNA extraction for all samples was then carried out using a column method with the Purelink Genomic DNA kit (Invitrogen, USA), following the manufacturer's instructions. For sample processing, 200 μL of both blood and urine samples were used for DNA extraction.

For the detection of *Leptospira*, real-time PCR (qPCR) was employed with specific primers and probes targeting *lipL32, secY, and rrs* genes, and internal control to monitor the presence of potential amplification inhibitors (*b-actin* gene) [34,35]. Primers and probe sequences used for the lipL32 gene, included the forward primer (F_lip32: AAG CAT TAC CGC TTG TGG TG), reverse primer (R_lip32: GAA CTC CCA TTT CAG CGA TT), and probe (taq—189P: FAM-AAA GCC AGG ACA AGC GCC G-BHQ1). For the secY gene, the forward primer (F_Lint2: CTT GAG CCT GCG CGT TAY C), reverse primer (R_Lint2: CCG ATA ATT CCA GCG AAG ATC), and probe (TaqLint2: HEX-CTC ATT TGG TTA GGA GAA CAG ATC A-BHQ1) were used. For the rrs (16S) gene, the forward primer (F_Lept: CCCGCGTCCGATTAG), reverse primer (R_Lept: TCCATTGTGGCCGRA/GACAC), and probe (P_Lept: FAM-CTCACCAAGGCGACGATCGGTAGC-BHQ1) were utilized. Finally, for the b-actin gene, the forward primer (F_actin: GGC TCY ATY CTG GCC TC), reverse primer (R_actin: GCA YTT GCG GTG SAC RAT G), and probe (P_actin: Cy5-TAC TCC TGC TTG CTG ATC CAC ATC-BHQ2) were employed.

The qPCR reaction mixture comprised 2× Universal Master Mix [36], primers at a final concentration of 0.2 μM, probes at a final concentration of 0.13 μM, and nuclease-free water. Each well received 5 μL of DNA, added to 15 μL of Master Mix. qPCRs were executed on a LightCycler 480 real-time thermal cycler, employing the following conditions: initial denaturation at 95 °C for 2 min, followed by 45 cycles of denaturation at 95 °C for 15 s, and annealing/extension at 60 °C for 1 min. Positive (DNA extractions from *Leptospira* cultures from the National Reference Laboratory of the National Institute of Public Health Research in Ecuador) and non-template controls were included in all runs. A Ct value of 40 was set as a threshold for positivity for all the genes.

Amplicons that tested positive and exhibited Ct values less than or equal to 35 for *lipL32* (242 bp), *secY* (176 bp), and *rrs* genes were subjected to Sanger sequencing, utilizing the same set of forward and

reverse primers. However, *rrs*-positive amplicons were excluded from sequencing due to their small amplicon size (96 bp).

### 2.3. DNA sanger sequencing

PCR products were purified using *Exonuclease I* and *FastAP Thermosensitive Alkaline Phosphatase* (Thermo Fisher Scientific, USA). Sequencing reactions were performed with the *BigDye Terminator Cycle Sequencing Kit v 3.1* (Applied Biosystems, USA), and then purified by gel filtration using Sephadex G-100 (Cytiva, USA). Finally, samples were analyzed by capillary electrophoresis in an ABI3500 Genetic Analyzer (Applied Biosystems, USA). Amplicons were processed by the Sanger Sequencing Service at the Universidad de las Américas, Ecuador. Sanger sequencing reads were processed using the R package sangeranalyseR [37] for quality trimming, clipping of low-quality regions, and removal of secondary peaks. Forward and reverse reads were assembled into contigs.

### 2.4. Phylogenetic analysis

Contigs for both *lipL32* and *secY* sequences were identified using the Basic Local Alignment Tool Nucleotide (BLAST-N) searches of representative sequences against the NCBI nr/nt database [38]. The *lipL32* sequences were combined with 103 publicly available *lipL32* sequences, extracted from reference sequences from different *Leptospira* species (Supplementary Table 1). Sequences analyzed for *secY* were concatenated with the respective *lipL32* sequence (441 bp). Concatenated sequences were combined with 60 *Leptospira* species sequences extracted from publicly available genome assemblies (Supplementary Table 1). Sequences were aligned with ClustalW using the EMBL-EBI Job Dispatcher sequence analysis tools framework [39]. Phylogenetic analysis was conducted in IQ-TREE [40], using the best-fit model (SYM + I + G4) identified by ModelFinder; bootstrap values were calculated using 1000 replicates. Tree visualization was achieved through the software TreeViewer 2.2.0 [41].

## 3. Results

### 3.1. Prevalence of Leptospira in human, animal, and environmental samples assessed by qPCR in Cantón Duran (Guayas, Ecuador)

Table 1 shows the detection of *Leptospira* in different sample types collected during the leptospirosis outbreak in Cantón Durán, Ecuador. Out of 164 human samples tested, 43 (26.2 %) were positive for *Leptospira*. The average values for the Cts obtained for human samples were 35.86, 33.78 and 34.18 for the *lipL32* (Range: 34.47–38.8, $n = 19$), *rrs* (Range: 32.5–36.7, $n = 22$) and *secY* (Range: 27.61–37.97, n = 19) genes, respectively (Supplementary Table 2).

Dogs had the highest positivity rate, with 49 out of 112 samples testing positive (43.8 %). The average Ct values were 28.88, 32.41, and 29.59 for *lipL32* (range: 15.78–35.55, $n = 3$), *rrs* (Range: 11.95–39.47, $n = 6$), and *secY* (range: 12.33–39.37, $n = 49$) genes, respectively (Supplementary Table 2).

For rats, 10 out of 26 samples (38.46 %) tested positive. The average Ct value was 35.2 for *secY* (Range: 29.4–35.2, $n = 10$), while the only positive sample for both the *lipL32* and *secY* had Ct values of 39.9 and 39.2, respectively (Supplementary Table 2).

Lastly, for water samples, 13 out of 33 (39.39 %) were positive. The average Ct was 33.88 for *rrs* (range: 30.62–37.98, $n = 12$), while just one sample amplified for *lipL32* (Ct = 39.31) and none for *secY* (Supplementary Table 2).

**Table 1**
Detection of Leptospira by qPCR in the different sample types included in the study.

| Sample Type | Total Sample Size | Overall qPCR Positivity (%) | lipL32 Positive (%) | secY Positive (%) | rrs Positive (%) |
|---|---|---|---|---|---|
| Human | 164 | 26.2 (43/164) | 11.6 (19/164) | 11.6 (19/164) | 14.6 (24/164) |
| Dog | 112 | 43.8 (49/112) | 1.8 (2/112) | 42.9 (48/112) | 4.5 (5/112) |
| Rat | 26 | 38.5 (10/26) | 3.8 (1/26) | 3.8 (1/26) | 38.5 (10/26) |
| Water | 33 | 39.4 (13/33) | 3.0 (1/33) | N/A | 36.4 (12/33) |

### 3.2. Phylogenetic analysis

In Fig. 1, the results for the maximum likelihood phylogeny among pathogenic *Leptospira* strains based on the *lipL32* gene sequences is shown. The tree reveals a clear separation between the pathogenic subclades P1 and P2 *Leptospira* species, and the 9 sequences obtained from samples included in this study belong to the P1 subclade and clustered with L. *kirschneri* sequences. Moreover, the 6 human samples, 1 dog sample, and 1 rat sample for a cluster with L. *kirscheri* branch. Only one of the sequences obtained from a dog is not clustered with the rest of the sequences obtained in this study. The accession number for the 9 *lipL32* sequences generated in this study is provided in Supplementary Table 3.

In Fig. 2, the results for the maximum likelihood phylogeny among pathogenic *Leptospira* strains based on the analysis of concatenated *lipL32* and *secY* gene sequences are shown. There were only two samples from dogs from our study that generated sequences for both genes: samples B1C54 and C2C1 (Supplementary Table 2). Both sequences are clustered within the L. *interrogans* branch of the P1 subclade. The accession number for the 2 *secY* sequences generated in this study is provided in Supplementary Table 3.

## 4. Discussion

In the study, we described the management of an outbreak of leptospirosis happening in Cantón Durán of Ecuador in 2023 from an integrated and multidisciplinary approach involving physicians, veterinarians, and molecular biologists. This was also a multi-institutional intervention involving the Ministry of Health of Ecuador, the National Institute of Public Health Research of Ecuador, and universities. So far, to the best of our knowledge, this was the first pilot study for the implementation on epidemiological surveillance and control of leptospirosis from a One Health perspective in Ecuador involving real time tracking of an outbreak under the leadership of local public health authorities. This multidisciplinary approach was translated into collection of human samples, animal samples for suspected reservoir like rats and dogs, and environmental samples (water) within the area where the leptospirosis outbreak was declared to address potential sources of transmission. The high positivity rates either in water samples, cats and dogs within the area of the human outbreak strongly suggest an environmental risk of transmission through contaminated water sources or contact with infected pets. This information was shared with the physicians working with the affected communities to advice their patients about this infectious disease and its way of transmission.

Moreover, new methodologies based on diagnosis by qPCR followed by Sanger sequencing were implemented for a more sensitive and specific diagnosis and a more accurate approach to zoonotic transmission to humans. Consequently, our findings revealed a high *Leptospira* infection rate in humans, household dogs, and synanthropic rats within the area of study, as well as a high *Leptospira* contamination of water sources. Considering the demographics of the study site, our finding could be extrapolated to an incidence rate of 150 per 100,000 inhabitants, a high incidence rate that has been reported in other endemic countries worldwide like Malaysia, India, Tanzania, or Australia [42–47].

We call attention to the fact that this study was started in the middle

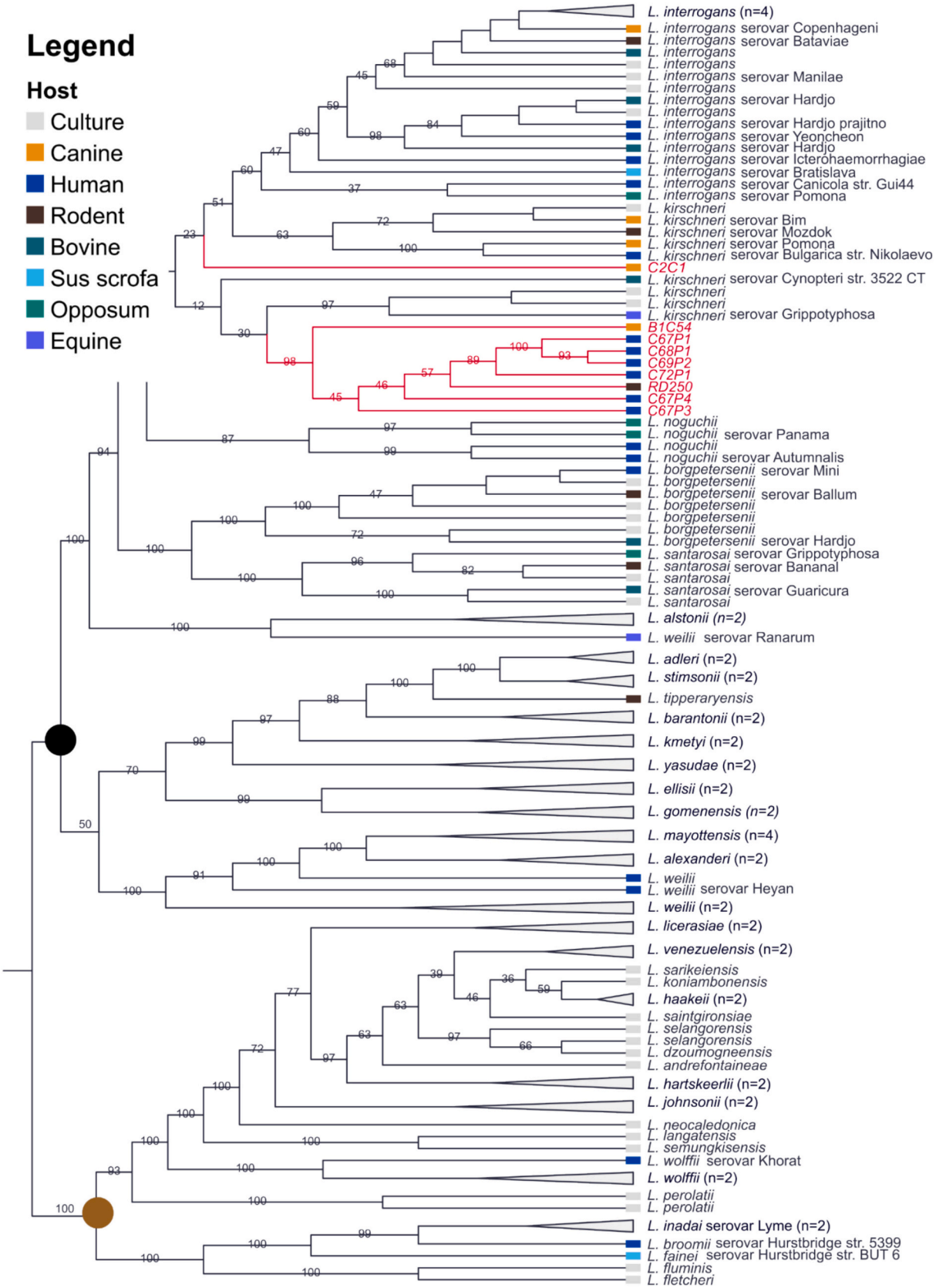


**Fig. 1.** Maximum likelihood phylogeny constructed using the amplicon sequenced of the *lipL32* gene, including sequences obtained from human, canine, and rat samples collected in this study (all highlighted in red) and those available in public databases (black text). Black dot: P1 lineage of pathogenic *Leptospira spp.;* Light brown dot: P2 clade of low-virulence intermediate *Leptospira spp.* (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

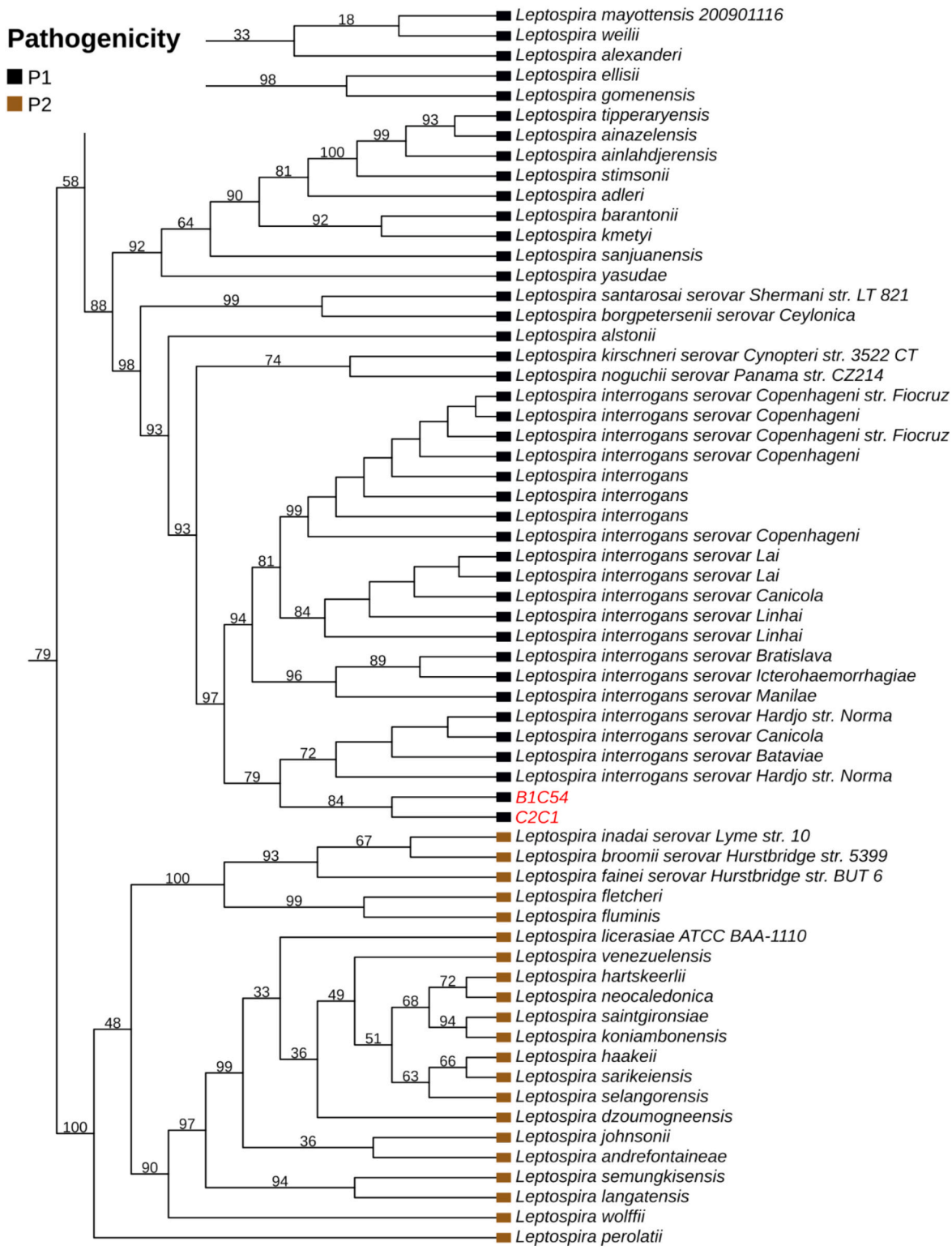


**Fig. 2.** Maximum likelihood phylogeny constructed using the concatenated amplicon of the *lipL32* and *secY* gene, including sequences obtained from canines (highlighted in red) collected in this study and those available in public databases (black text). P1 and P2 sequences are represented by black and brown squares, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

of the rainfall season of 2023 in the Coastal Region of Ecuador. In fact, the rainfall frequency in the rain season in 2023 was higher than usual as reported by the National Institute of Meteorology of Ecuador [48]. The correlation of leptospirosis outbreaks with rain season has been previously shown in other studies in the region of South America, including Ecuador [49], showing the importance of climate variables in the epidemiology of leptospirosis, underscoring the impact that climate change could have on enhancing outbreaks of leptospirosis [50]. This scenario is particularly worrisome for underserved urban and rural parishes like the ones included in this study, where limited urban planning governance has led to the strain on stormwater and drainage systems, resulting in chronic flooding as a prevalent issue [51].

Our molecular and phylogenetic analysis confirmed the widespread environmental contamination and identified animal reservoirs contributing to the zoonotic transmission of leptospirosis during the outbreak in Cantón Durán in 2023. The high positivity rates observed in household dogs related to human cases, synanthropic rats, and water samples within those locations underscore the significance of considering animal and environmental surveillance within the control efforts to manage leptospirosis, that is, a One Health approach [52]. Furthermore, the detection of pathogenic subclade P1 *L. kirscheri* sequences clustering across humans and animals endorses the zoonotic transmission of leptospirosis from animal reservoirs as it has been shown elsewhere [6,53]. Moreover, the closer clustering of sequences from human origin with rats than with dogs suggest that rats could have a more direct role as a reservoir for the human outbreak in Cantón Durán in 2023. Additionally, the concatenated analysis including *secY* sequences was only possible for sequences of two dogs, showing that they form a cluster within the branch of P1 *L. interrogans*.

Despite the valuable insights garnered from our study, several limitations should be acknowledged. Firstly, the lack of *secY* sequences from human and environmental samples could be attributed to the low carriage of *Leptospira* DNA in those samples as revealed the high Ct values above 39, hampered our ability to obtain good-quality sequences for phylogenetic analysis. Secondly, the absence of whole genome sequence data due to high Ct values and the lack of capacity to conduct culture isolation for this study constrained our ability to fully elucidate the genetic diversity and evolutionary relationships of the circulating *Leptospira* strains, as well as get insights into specific virulence factors that may be present within the identified strains. These limitations underscore the necessity for improved molecular and microbiological techniques in future research endeavors for state-of-the-art molecular and genomic surveillance of *Leptospira* in Ecuador.

## 5. Conclusion

Our study is a prove of concept of the fundamental role of an integrated One Health approach in addressing the complexities of leptospirosis outbreaks in tropical settings within low- and middle-income countries like Ecuador. By combining molecular diagnostics, genetic analysis a classical epidemiological analysis, and collaboration across disciplines, we were able to get some insights in the transmission dynamics of leptospirosis in Cantón Durán of Ecuador during the 2023 outbreak, pointing out the environmental risk associated to contaminated water sources from animal carriers like rats, or due to direct interaction with infected pets like dogs. Hopefully, this successful experience would inspire to the stake holders in Ecuador to promote a national program for surveillance, prevention and control of zoonotic diseases from a One Health vision.

## Author's contribution

All the authors were involved in the study design, sample collection, data analysis, and critical review of the manuscript. SOA, NMJ, and MAGB wrote the first draft and final version of the manuscript.

## Ethical approval

The Institutional Review Board of "Universidad Técnica de Manabí" approved this study with the code of CEISH-UTM-EXT_2023-04-18_JCZL.

## CRediT authorship contribution statement

**Solón Alberto Orlando:** Writing – review & editing, Methodology, Investigation. **Naomi Mora-Jaramillo:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Conceptualization. **Darwin Paredes-Núñez:** Writing – review & editing, Supervision, Resources, Funding acquisition. **Angel Sebastian Rodriguez-Pazmiño:** Writing – review & editing, Methodology, Investigation. **Elsy Carvajal:** Writing – review & editing, Methodology, Investigation. **Ariana León Sosa:** Writing – review & editing, Methodology, Investigation. **Ariana Rivera:** Writing – review & editing, Methodology, Investigation. **Jocelyn Calderon:** Writing – review & editing, Methodology, Investigation. **David Guizado Herrera:** Writing – review & editing, Supervision, Resources, Funding acquisition. **Fabrizio Arcos:** Writing – review & editing, Supervision, Resources, Funding acquisition. **Leila Estefanía Vera Loor:** Writing – review & editing, Supervision, Resources, Funding acquisition. **Emma Viviana Pérez Oyarvide:** Writing – review & editing, Supervision, Resources, Funding acquisition. **Dennis Ignacio Quimí López:** Writing – review & editing, Supervision, Resources, Funding acquisition. **Betti Guailla Ríos:** Writing – review & editing, Supervision, Resources, Funding acquisition. **Bertha Benavides Yánez:** Writing – review & editing, Supervision, Resources, Funding acquisition. **Pablo Torres-Lasso:** Writing – review & editing, Supervision, Resources, Funding acquisition. **Patricia Zambrano Gavilanes:** Writing – review & editing, Supervision, Resources, Funding acquisition. **Mirna C. Oviedo:** Writing – review & editing, Supervision, Resources, Funding acquisition. **Manuel González:** Writing – review & editing, Supervision, Resources, Funding acquisition. **Fabiola Jiménez-Valenzuela:** Writing – review & editing, Supervision, Resources, Funding acquisition. **Melva Esperanza Morales García:** Writing – review & editing, Supervision, Resources, Funding acquisition. **Esther Guadalupe Intriago Alcivar:** Writing – review & editing, Supervision, Resources, Funding acquisition. **Jessedel Lilibeth Saltos Montes:** Writing – review & editing, Supervision, Resources, Funding acquisition. **Alfredo Medina:** Writing – review & editing, Supervision, Resources, Funding acquisition. **María Karolina López Rauschemberg:** Writing – review & editing, Supervision, Resources, Funding acquisition. **Juan Carlos Zevallos:** Writing – review & editing, Supervision, Resources, Funding acquisition. **Miguel Angel García-Bereguiain:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Funding acquisition, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.onehlt.2024.100948.

## Data availability

Data will be made available on request.

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