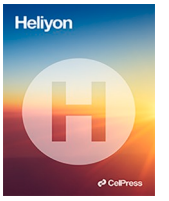
Heliyon 11 (2025) e41531

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Insights into host-pathogen interaction based on the comparison of genomes of *leptospira interrogans* isolated from dogs, humans, and a rodent in the same epidemiological context: A one health approach

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ARTICLE INFO

Keywords:
Comparative genomic analysis
Leptospira interrogans
Leptospirosis
Whole genome sequencing
One health

ABSTRACT

Leptospirosis is a zoonotic infectious disease that significantly impacts animal and public health. Comparative genomics can aid in understanding poorly understood aspects of leptospirosis pathogenesis, including infection mechanisms, antimicrobial resistance, and host interactions across different epidemiological scenarios. This study aimed to compare the genomes of *Leptospira interrogans* serogroup Icterohaemorrhagiae strains isolated from three host species in a single epidemiological scenario. Four strains of *L. interrogans* serogroup Icterohaemorrhagiae from naturally infected and clinically symptomatic dogs (C20, C29, C51, and C82) were processed for whole genome sequencing (WGS). These results were compared against WGS data from two other rodent and human strains. Phylogenetic and genomic similarity analyses demonstrated high identity and synteny between the strains isolated from humans, canines, and rodents. Small regions of divergence were observed, especially in the genome obtained from a rodent sample. The presence of 23 genes potentially associated with biofilm formation was notable, with the identification of missense mutations in eight genes. Considering the need to better understand the molecular basis involved in biofilm formation, it is of fundamental importance to elucidate the effect of mutations on the expression of the phenotype (biofilm) among different strains. The present findings highlight the necessity of One Health-based collaborative interventions to address the complex dynamics of leptospirosis transmission, involving both common hosts such as rodents and dogs, as well as less-recognized hosts.

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https://doi.org/10.1016/j.heliyon.2024.e41531
Received 8 August 2024; Received in revised form 20 December 2024; Accepted 26 December 2024
Available online 26 December 2024
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1. Introduction

Leptospirosis is an infectious zoonotic disease caused by pathogenic spirochetes belonging to the genus *Leptospira* [1]. The disease affects several mammalian species, transforming them potential sources of infection [2]. Globally, leptospirosis is recognized as a public health challenge, with estimates of approximately 1.03 million new cases yearly [3]. Brazil exhibits a high prevalence of human leptospirosis (21 %) in both urban and rural environments [4]. In Salvador, this zoonosis triggers annual epidemics that mainly affect residents living in conditions with inadequate infrastructure. Between 1996 and 2002, the average yearly number of hospitalizations due to leptospirosis in the city was 19.8 cases per 100,000 inhabitants, with a *Leptospira* infection rate of 37.8 per 1000 person-years [5].

Specific reservoirs that trigger leptospirosis infections in humans are influenced by the unique characteristics of different ecosystems [6]. In urban environments, synanthropic rodents are leading chronic carriers and vectors of leptospirosis [7]. Simultaneously, dogs emerge as potential sources of infection, given their proximity to humans, often shedding viable leptospires in their urine for long periods, even in the absence of any clinical signs of disease [8,9]. Nonetheless, the acute course of leptospirosis, triggered by incidental serovars (e.g., Copenhageni), is more common [10–13].

Sequencing and genomic comparison studies are scarce in the literature [14]. Whole genome sequencing (WGS) of autochthonous strains offers accurate identification, insights into the evolution of the infectious agent, and insight into genes related to virulence, antimicrobial resistance, and adaptability [15]. Comparing the genomes of leptospires recovered from animals and humans can contribute to the epidemiological characterization of the disease, highlighting its importance for public health. Moreno et al. [16] compared strains of serovar Canicola obtained from pigs and humans and found high similarity, indicating that pig strains may represent a significant public health risk. Another study compared *Leptospira interrogans* serogroup Icterohaemorrhagiae isolated from dogs and rodents using *secY* sequencing, variable number tandem repeat analysis, multilocus sequence typing (MLST), and multi-spacer typing analysis. The authors found that all strains were identical to the main clonal subpopulation of the Fiocruz L1-130 strain, which is responsible for human infections [17].

Bacterial zoonotic diseases, such as leptospirosis, exert a considerable impact on human morbidity and mortality; however, these infections are treatable with antibiotics and can be prevented using the One Health approach [18]. The sequencing, assembly, and annotation of the genome of *Leptospira* strains isolated from clinical samples obtained from different animals is crucial for a better understanding of the disease. Here we aimed to compare the genomes of *Leptospira interrogans* strains obtained from dogs, humans, and rodents in a single epidemiological setting. Our results will help to elucidate the circulation and transmission dynamics of pathogenic *Leptospira,* and shed light on interactions with potential hosts in a tropical epidemiological context.

2. Material and METHODS

2.1. Selection of leptospira strains

This study analyzed new sequences from four strains of *Leptospira interrogans* serogroup Icterohaemorrhagiae obtained from the inventory of the Bacterial Diseases Laboratory at the Federal University of Bahia (LABAC-UFBA). These strains, C20, C29, C51 and, C82 (accession numbers SAMN38041501, SAMN38041536, SAMN38041566, SAMN38041581, respectively.) were isolated from blood or urine samples from naturally infected dogs with well-characterized clinical symptoms and outcomes (post-mortem) [12].

In addition, we selected two sequences from two *L. interrogans* strains isolated from a human and a rodent sample in the same epidemiological context, the city of Salvador, Bahia-Brazil. The FASTA files of chromosomes 1 and 2 (Accession Numbers: NC005823 and NC005824), generated from *Leptospira interrogans* serogroup Icterohaemorrhagiae serovar Copenhageni Fiocruz reference strain L1-130 (herein referred to as L1-130), were obtained from human blood samples [19]. The raw files (Sequence Read Archive (SRA) - accession number SRX1274269) generated from *Leptospira interrogans* serogroup Icterohaemorrhagiae serovar Copenhageni Fiocruz strain R062 (herein denominated R062), were obtained from rodent kidney samples [20] in the same epidemiological setting.

2.2. Genome sequencing and raw data quality assessment

In accordance with the manufacturer's recommendations, genomic DNA was extracted from the four novel canine strains using the QIAamp DNA Mini Kit (Qiagen, Valencia, CA, USA). The concentration of genomic DNA was determined using the Qubit dsDNA Broad Range Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA) to ensure at least 1 ng/μl for sequencing. Libraries were prepared using the Nextera XT DNA Library Preparation Kit (Illumina, San Diego, CA, USA), and sequencing was performed on an Illumina MiSeq platform using paired-end short reads, with fragments of 2 x 250 base pairs (bp). Sequencing quality was assessed using the FastQC 0.11.9 program (https://www.bioinformatics.babraham.ac.uk/projects/fastqc).

2.3. Genome assembly, annotation and analysis

For genome assembly, three different programs were used: Skesa v.2.4.0 [21], SPAdes v.3.15.3 [22], and Unicycler v.0.4.8 [23]. The resulting assembly quality was assessed using the Quast v.5.0.2 program [24]. The coverage percentage of each assembly with respect to the reference genome, the number of contigs (smallest number), the size of the largest contig, and N50, NG50, L50, and LG50 were selected as comparative parameters to evaluate the quality of the assemblies. The best-performing assembly based on these parameters was subjected to the post-assembly improvement pipeline proposed by Page et al. [25]. The scaffolding of the genome

(sorting and orienting contigs) was performed using MEDUSA v.1.6 (Multi-Draft based Scaffolder) [26], with the *L. interrogans* serogroup Icterohaemorrhagiae serovar Copenhageni strain L1-130 genome as a reference. To address the gaps generated from the previous scaffolding process, the combination of Fgap + GapBlaster was used, as suggested by De Sá et al. [27]. The pipeline was tested and validated by assembling 10 randomly chosen *Leptospira interrogans* sequences [20,28] that had been previously described and published in the Sequence Read Archive (SRA-GenBank).

All six genomes were newly annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) [29] and the Rapid Annotation using Subsystem Technology (RAST) online tools [30]. A new PGAP analysis was performed for the reference strain L1-130 to standardize annotation data. Prophages and phage-like elements were identified using the PHASTER online tool [31,32], inter-spaced short palindromic repeats (CRISPRs) and Cas regions were predicted using the CRISPRCas-finder tool [33].

The presence of sequences involved in c-di-GMP metabolism was evaluated by analyzing the genome of *L. interrogans* serogroup Icterohaemorrhagiae serovar Lai strain Lai 56601, previously described by Xiao et al. [34]. The 25 nucleotide and amino acid sequences predicted to generate proteins containing GGDEF, EAL, or HD-GYP domains were selected for comparison (13 - GGDEF domain proteins, 5 - EAL domain proteins, 3 - GGDEF-EAL domain proteins, and 4 - HD-GYP domain proteins). The sequences of the orthologous genes and proteins from each strain (C20, C29, C51, C82, L1-130, and R062) were retrieved and compared by multiple sequence alignment using ClustalW v.1.4 [35].

2.4. Variant calling

To identify single nucleotide polymorphisms (SNPs), variant calling was performed using the Snippy v.4.4.03 pipeline (https://github.com/tseemann/snippy). Briefly, the raw reads (SRA files - Supplementary Table 1) belonging to 65 strains of *L. interrogans* serogroup Icterohaemorrhagiae serovars Copenhageni and Lai obtained from dogs, humans, and rodents in different regions were mapped against the reference genome of *L. interrogans* serovar Copenhageni strain L1-130 using BWA-MEM v.0.7.12 [36]. SNPs were identified using the Freebayes v.1.1 [37] with default parameter settings. All variants were subsequently annotated using SnpEff v.4.3 [38].

2.5. Average nucleotide identity and phylogenetic analysis

Initially, the *ppk* gene, which encodes a polyphosphate kinase (720 amino acids), was used to detect relationships between the sequences of the strains isolated from dogs, humans, and rodents and the *Leptospira* species clustered in the P1 subclade (formerly the pathogenic clade). Phylogenetic analysis was performed by Mega v.6 using the Neighbor-Joining method; the phylogenetic tree was built using the Tamura-Nei model [39]. A more robust phylogenetic evaluation was performed among the sequences from dogs,

Table 1
Distribution of features of each subsystem in genomes of *L. interrogans* strains obtained from dog, human, and rodent samples in the same epidemiological scenario.

Subsystem category	Feature counts per isolate					
	C20	C29	C51	C82	L1-130	R062
Cofactors, vitamins, prosthetic groups, pigments	121	121	121	121	121	121
Cell wall and capsule	18	18	18	18	18	18
Virulence, disease, and defense	18	18	18	18	18	18
Potassium metabolism	5	5	5	5	5	5
Phages, prophages, transposable elements, plasmids	1	1	1	1	1	1
Membrane transport	20	20	20	20	20	20
Iron acquisition and metabolism	1	1	1	1	1	1
RNA metabolism	27	27	27	27	28	27
Nucleosides and nucleotides	32	32	32	32	32	32
Protein metabolism	93	93	92	92	92	92
Cell division and cell cycle	4	4	4	4	4	4
Motility and chemotaxis	71	71	71	71	71	71
Regulation and cell signaling	29	29	29	29	29	29
Secondary metabolism	4	4	4	4	4	4
DNA metabolism	57	57	57	57	57	57
Fatty acids, lipids, and isoprenoids	31	31	31	31	31	31
Nitrogen metabolism	7	7	7	7	7	7
Dormancy and sporulation	2	2	2	2	2	2
Respiration	54	54	54	54	54	54
Stress response	54	55	54	54	54	54
Metabolism of aromatic compounds	5	5	5	5	5	5
Amino acids and derivatives	139	139	139	139	140	139
Sulfur metabolism	5	5	5	5	5	5
Phosphorus metabolism	11	11	11	11	11	11
Carbohydrates	65	65	65	65	65	65
Miscellaneous	12	12	12	12	13	12
# of covered CDSs	886	887	885	885	888	885

humans and rodents, as well as the sequences of all *Leptospira* spp. reported by the Systematics of Prokaryotes Subcommittee on the Taxonomy of *Leptospiraceae* (i.e., those recognized by the International Committee). Average Nucleotide Identity (ANI) analysis was performed using pyani v.0.2.7 [40]. Subsequently, a new analysis encompassed the six genomes of the strains studied here and one genome from each species in the P1 subclade available on GenBank. Information on the genomes evaluated herein is available in Supplementary Table 2.

The maximum likelihood (ML) tree was built using concatenated core-SNPs from the *L. interrogans* serovar Copenhageni strains C20, C29, C51, C82 isolated from dogs, and 61 other strains obtained from humans and rodents from various locations (Supplementary Table 1). The sequences were aligned and filtered using Gubbins software in the phylogenetic analysis based on concatenated core-SNPs [41]. The phylogenetic tree was generated using the ML method in the RAxML program [42]. The GTR model with a gamma distribution of nucleotide substitutions was applied using a bootstrap analysis with 1000 replicates to calculate the statistical support of the tree branches. The tree was visualized and edited using the web version of FigTree v.4 (http://tree.bio.ed.ac.uk/software/figtree).

2.6. Comparative genome analysis

The BRIG (BLAST Ring Image Generator) program was utilized for genome comparisons using the BLAST algorithm to generate circular comparison images of the genomes of chromosomes I and II [43]. In addition, we also performed comparative genome analysis by aligning and ordering the sequences obtained using Mauve [44], and by constructing synteny graphs using the ACT (Artemis Comparison Tool) program [45]. The genomes of the five strains utilized for comparison purposes in this study were compared with the reference genome of *L. interrogans* strain L1-130, which also belongs to the same serogroup and serovar.

3. Results and DISCUSSION

3.1. General genome analysis

Obtaining high-quality genomes remains a challenging task. The combined use of diverse tools designed to improve the assembly process, scaffolding, and gap closure resulted in the acquisition of two chromosomes and a significant reduction in the number of gaps: C20 (n = 37), C29 (n = 38), C51 (n = 36) and C82 (n = 35) (Supplementary Table 3). Previous studies have described the obtainment of genomes resulting in the presence of multiple contigs as well as substantial gaps in some regions [16,46,47]. According to Nieves et al. [48], the lack of complete or high-quality draft genomes makes it difficult to reliably reconstruct relevant gene clusters due to information loss, which directly influences subsequent comparative analysis. Therefore, implementing specific techniques to mitigate these deficiencies is recommended to increase the final quality of the assembly [25].

Compared to the L1-130 strain, all five non-human strains demonstrated similar numbers of genes and coding sequences (CDSs) (total, coding, and "with defined function" - Supplementary Table 4). The annotation performed by the RAST server functionally classified the CDSs of each strain into 26 subsystems, with the number of predicted proteins ranging between 885 and 888. Metabolism of amino acids and derivatives was the most abundant subsystem: 140 (L1-130) to 139 for the other genomes. CDSs related to co-factors, vitamins, prosthetic groups, and pigments totaled 121 in every strain evaluated. The distribution of the predicted CDS subsystems in each strain is shown in Table 1.

The annotation of the genomes using the subsystems methodology (RAST) confirms the high conservation of CDSs among *L. interrogans* strains, as evidenced by similar numbers of CDSs and subsystem categories. Most genes were found to encode proteins responsible for fundamental biological processes such as transcription, translation, cell wall synthesis, and motility. Studies carried out on strains belonging to other species and/or serovars have described higher numbers of CDSs [46,47,49]. It is our belief that this may be due to the higher quality of our assembly, which was achieved through the pipeline employed to enhance genome assembly.

All the five strains evaluated exhibited nearly the same number of coding regions and the same products found in the annotation carried out for the L1-130 reference strain. The differences that stood out were the presence of an enoyl-coA hydratase family protein (present in L1-130, R062, C20, C29, and C82, and absent in C51), a potassium-transporting ATPase (present in L1-130, R062, C20, C29, and C82), insertion sequences (IS3, IS1500A), beta-barrel-shaped outer membrane proteins (absent in the sequences of C20, C29,

Table 2
– Analysis of variants in the genome of leptospires isolated from samples of dogs and rodents from the city of Salvador, Bahia-Brazil.

Variant type	Strain ID				
	C20	C29	C51	C82	R062
Variant complex	20	19	20	20	13
Deletion	23	18	17	21	14
Insertion	41	42	37	40	34
MNP	1	1	0	1	1
SNP	62	61	52	61	50
Variant Total	**147**	**141**	**126**	**143**	**112**
Missense	38	34	36	36	33
Synonymous	4	10	4	5	7

MNP = Multiple Nucleotide Polymorphism; SNP=Single Nucleotide Polymorphism.

C82, R062), and lipoproteins with adhesin function (ligA and ligB), which were present in the reference strain L1-130, but absent in the rodent strain R062 and in all four strains isolated from dogs. It is known that copy number variation can occur between serovars and strains of a given serovar [50]. Additionally, the absence of sequences in strains may also lead to unassembled regions (i.e., gaps) [48].

Most genes in the *Leptospira* genome are annotated as hypothetical proteins, or show less similarity to characterized proteins [20]. We observed similar numbers of hypothetical proteins in the genomes of the strains obtained from canines and rodents compared to the reference strain L1-130 genome (Supplementary Table 4). Previous studies have highlighted the presence of hypothetical proteins related to fitness, adaptation, and virulence [16,51,52]. The presence of these proteins in our study suggests their potential role in *Leptospira* spp., underscoring the need for further research to understand their influence and relationships across different species of *Leptospira*.

3.2. Phage, crispr-cas system, and putative virulence genes

The use of molecular techniques for genomic analysis of leptospires remains limited [14]. PHASTER analysis demonstrated no intact prophages were present in any of the genomes evaluated; however, prophages characterized as "incomplete" ranged from 6.2 kb to 11.6 kb (Supplementary Table 5). To date, only 3 phages [53] and 128 plasmids have been described (NCBI - accessed April 28, 2024 - https://www.ncbi.nlm.nih.gov/genome/browse#!/plasmids/leptospira). Putative prophages have been detected in the genomes of several pathogenic and intermediate leptospiral strains, suggesting a possible role in the evolution of these species [15]. The incomplete prophages described in this study have previously been reported in different pathogenic *Leptospira* species [46,47,49], including in *Leptospira interrogans* serogroup Pyrogenes related to severe leptospirosis in humans [54].

The number of CRISPR arrays varied among the strains; L1-130 had 11 arrays, while C20 and R062 had 10, and C29, C51, and C82 presented 9. In addition, it is possible to note that pairs of identical sequences were observed across all the strains evaluated. Based on the presence of Cas signature proteins, only one CRISPR subtype (CAS-TypeIC) was identified among the analyzed genomes (Supplementary Table 6). While we observed no divergence in the type of CRISPR-Cas systems among the genomes evaluated in this study, different types of systems have previously been detected in *L. interrogans* and other species [15,46,47]. Knowledge about the

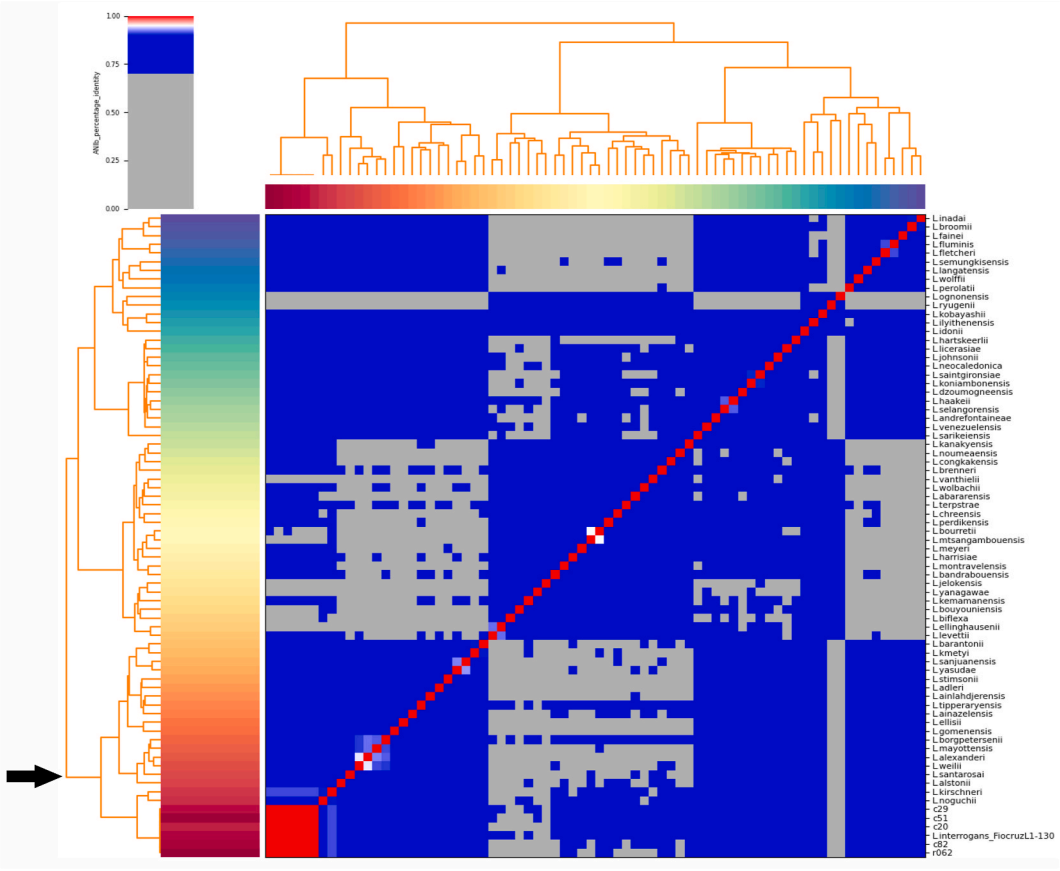

Fig. 1. Graphical representation of the average nucleotide identity matrix (ANIb) between the genomes of the strains studied herein and sequences from 69 species of the *Leptospira* genus. Separation of clades is observed where gray tones represent values > 70 %, blue tones represent values < 95 %, white represent 95 %, and red tones >95 %. The arrow indicates the node in which pathogenic species (Subclade P1) most frequently involved in human and animal diseases cluster.

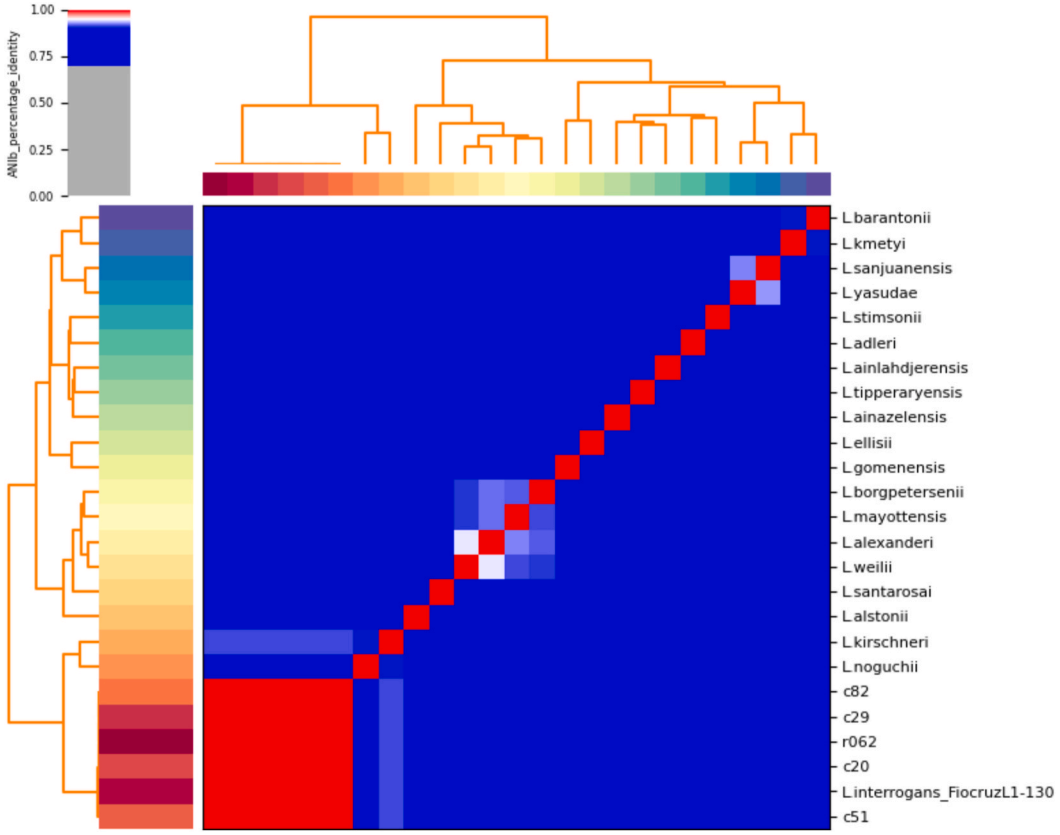

Fig. 2. Graphical representation of the average nucleotide identity matrix (ANIb) between the genomes of the strains studied herein and the sequences of species that make up the P1 subclade. Separation of clades is observed where gray tones represent values > 70 %, blue tones represent values < 95 %, white represent 95 % and red tones >95 %. All strains of *L. interrogans* serogroup Icterohaemorrhagiae demonstrated similarity above 99.88 % and grouped together with the genome sequence of the human reference strain L1-130.

actual role of the CRISPR-Cas system in *Leptospira* is limited [46]. Studies have suggested that CRISPR-Cas systems may play a role in *Leptospira* pathogenesis, since these are only found in the genomes of pathogenic and intermediate species [15,55]. We emphasize the need for studies to elucidate the importance of these divergences between CRISPR-Cas systems among various species of the *Leptospira* genus.

The number of elements in a genome can vary among different serogroups and serovars of *Leptospira* [50]. Sequencing well-characterized indigenous strains is essential to understanding factors related to the evolution of the genus *Leptospira* and the epidemiology of leptospirosis across different species. Here, we compared the genomes of well-characterized strains. Our results indicate differences in the number of CDSs, hypothetical proteins, phages and prophages, as well as CRISPR arrays in comparison to strains belonging to other serovars. Therefore, we highlight the importance of conducting further studies focused on obtaining, sequencing, and analyzing local isolates.

3.3. Analysis of genes related to biofilm formation

Different signaling molecules regulate biofilm formation. We observed the presence of 12 proteins with a GGDEF domain, five proteins with an EAL domain, two with a GGDEF-EAL domain, and four with an HD-GYP domain across the six genomes studied herein. It has been suggested that these domains may be involved in c-di-GMP metabolism. The second messenger bis-(3′-5′)-cyclic dimeric guanosine monophosphate (c-di-GMP) has been described as a molecule associated with biofilm formation in several species of bacteria, including *L. interrogans* [56]. Biofilm formation confers relevant properties to bacteria, notably resistance to antimicrobials, toxins, ultraviolet radiation, salinity, dehydration, oxidation, environmental stresses, and host immune responses [57].

Mutations were observed in 20 of the 23 nucleotide sequences found in the genomes compared here. However, in only eight sequences, such mutations cause changes in amino acid chains and, consequently, in the final protein sequence. The identification of these sequences and the number and site of each mutation is detailed in Supplementary Table 7. Despite the presence of these mutations, a recent study by Carvalho et al. [58] showed that the same dogs strains evaluated herein can form biofilms *in vitro*. Additionally, the expression of this phenotype was found to lead to greater resistance to antimicrobial drugs. The molecular basis of biofilm formation remains poorly understood, especially in the *Leptospira* genus. Given the mutations found and the persistence of biofilm

(caption on next page)

Fig. 3. The phylogenetic relationship between strains of *Leptospira interrogans* serovar Copenhageni was meticulously constructed using the ML method with 1000 bootstrap repetitions. This method, renowned for its robustness and accuracy, serves to validate the credibility of our analysis. The constructed tree clearly describes the phylogenetic relationships between strains belonging to the Copenhageni serovar isolated in various geographic locations. This analysis is based on concatenated core-SNPs and the number of different SNPs among 53 strains of *L. interrogans* serovar Copenhageni. The sequences are color-coded according to country in which each strain was isolated, as indicated in the legend. *L. interrogans* serovar Lai was used as an outgroup for this analysis.

formation capacity *in vitro*, we call attention to the need to study the expression of genes responsible for or linked to biofilm formation to prevent and control the spread of *Leptospira* strains.

3.4. Variant calling

Compared to the human reference sequence (L1-130), variant call analysis identified 147, 141, 126, 143, and 112 variations in strains C20, C29, C51, C82, and R062, respectively. Missense mutations ranged from 33 in R062 to 38 in C20. Meanwhile, the number of synonymous mutations detected varied between 4 and 10 (Table 2). Most of the mutations were found to be present in non-coding regions or regions of hypothetical proteins: C20 (64.62 %), C29 (63.82 %), C51 (63.49 %), C82 (64.33 %), R062 (60.71 %). Among the mutations present in known regions, we highlight those observed in the sequences responsible for the synthesis of a protein containing an adenylate/guanylate cyclase domain, present in all of the sequences, and a synonymous mutation in the sequence responsible for the synthesis of the *secY* gene, present in strains C20, C29, C82, and R062.

The *secY* gene is part of the MLST panel extensively used to identify species in the genus *Leptospira* [59]. Although it does not change the final structure of the protein, it is possible that this polymorphism can lead to poor primer pairing and, consequently, a reduction in the efficiency of the PCR reaction [60]. We also observed missense mutations as SNPs, indels (insertion/deletion), and complex variations as genes with more than one alteration. The mutations observed in the sequences are responsible for synthesizing proteins containing adenylate/guanylate cyclase domain, which were previously associated with biofilm formation [61]. We emphasize the need for experimental studies to elucidate the role of these mutations in protein expression and, consequently, in biofilm formation.

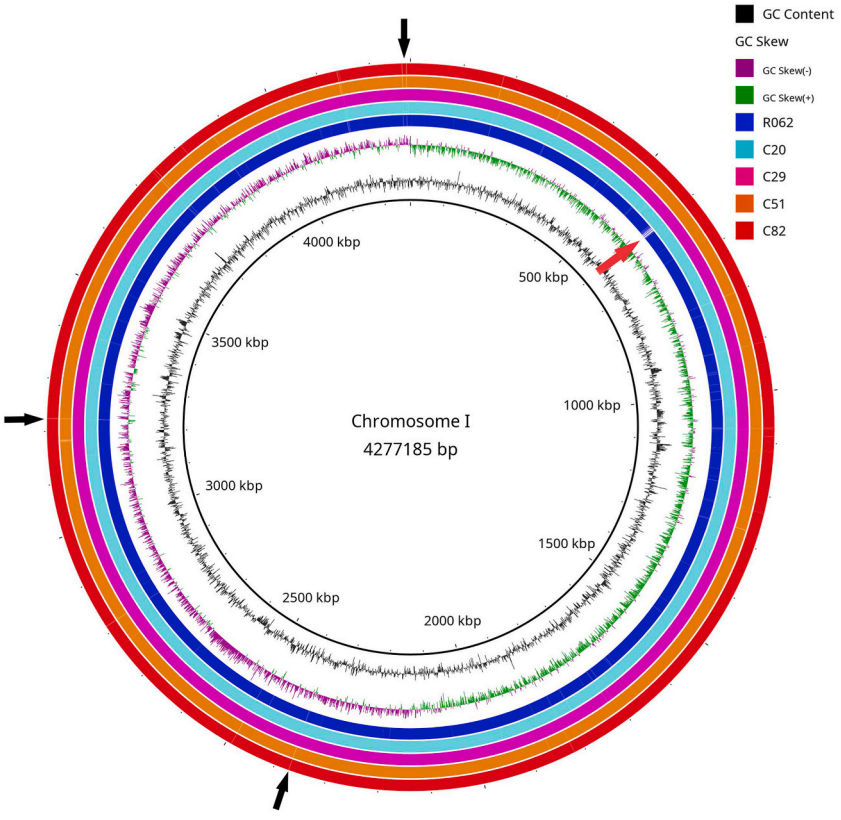

Fig. 4. Circular genome map of **chromosome 1** in five clinical isolates of *L. interrogans*, serogroup Icterohaemorrhagiae, obtained from samples of dogs, rodents, and humans. The reference strain L1-130 is represented by the black ring in the inner part of the figure. All sequences are highly related and show high synteny. Unrelated regions of the genome are represented by white spaces. The red arrow indicates a divergent area unique to the rodent-isolated strain. Black arrows indicate areas of differences between the reference strain and various sequences from dogs and rodents.

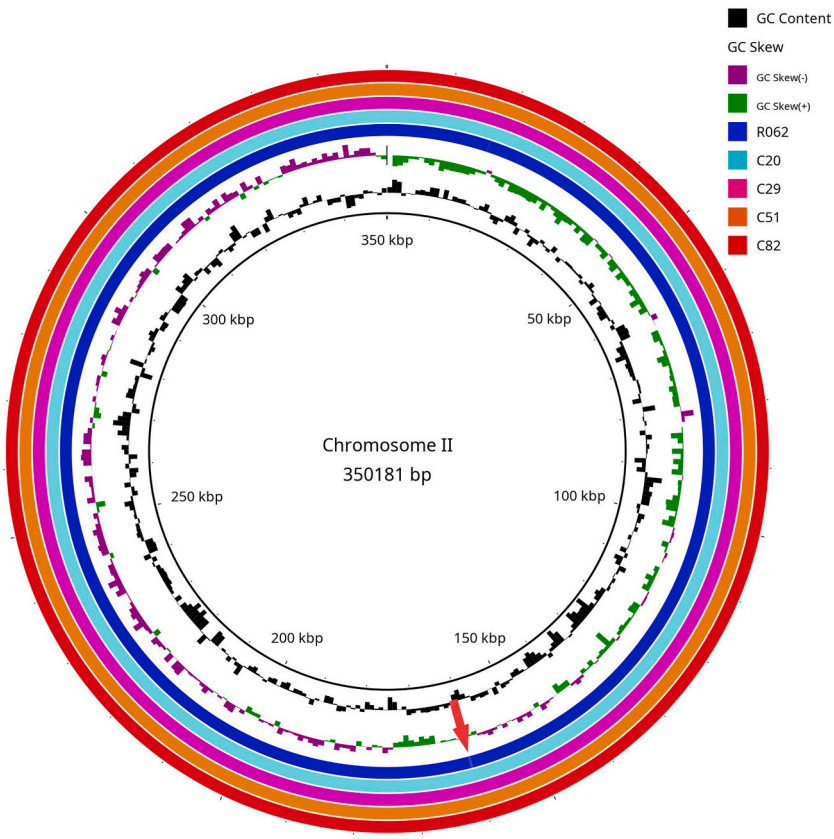

Fig. 5. – Circular genome map of **chromosome 2** in five clinical isolates of *L. interrogans*, serogroup Icterohaemorrhagiae, obtained from samples of dogs, rodents, and humans. The reference strain L1-130 is represented by the black ring in the inner part of the figure. All sequences are highly related and show high synteny. The red arrow indicates a divergent area unique to the rodent-isolated strain.

The level of divergence between strains of the same species can be inferred based on identifying SNPs among bacterial genome sequences. According to Lata et al. [46], such divergence may suggest which evolutionary forces have been expressed in bacterial species over time. Strains isolated from dogs and rodents showed fewer SNPs (between 76 and 95 SNPs) than L1-130, reinforcing the similarity between the sequences isolated in this same setting. More comprehensive evaluations of genes related to virulence and strain adaptability, especially those classified as hypothetical, could help shed light on specific pathophysiology and clinical aspects of the disease in each host species.

3.5. Phylogenetic classification

Analysis of the *ppk* gene revealed that all the genomes in the present study group within the P1 subclade form a monophyletic branch together with the sequence of the pathogenic species *Leptospira interrogans* (Supplementary Fig. 1). To determine the species of the four newly sequenced genomes, we used the ANI values compared to the genomes of all 69 described *Leptospira* species (Fig. 1). Accordingly, the strains analyzed in this study were observed to belong to the P1 subclade. Subsequently, we performed another analysis, comparing these six strains only to other genomes belonging to the P1 subclade (Fig. 2). The identity values compared to strain L1-130 were 99.95 % (C20), 99.94 % (C29 and R062), 99.92 % (C51), and 99.91 % (C82) (Supplementary Table 8). These results confirm that the present isolates indeed belong to *Leptospira interrogans,* with very high similarity to the reference strain genome, L1-130.

Phylogenetic analyses have indicated that the strains of *L. interrogans* serovar Copenhageni and Icterohaemorrhagiae are highly conserved over time, with a tendency to form distinct geographical groupings (e.g., Brazil, Hawaii, French Polynesia, Egypt, Denmark, the Netherlands, Japan, Guadeloupe, Slovenia, and Colombia), suggesting close relationships in the transmission dynamics of this disease between humans, dogs, and rodents [20].

Phylogenetic analyses of core-SNPs based on ML reveal a significant genetic relationship between the strains of *L. interrogans* belonging to the Copenhageni serovar. This tree exhibits a topology wherein all the strains cluster closely with statistical support (Fig. 3). This finding is particularly noteworthy, as it demonstrates that even strains isolated at different times and from different geographical origins are grouped closely on the same branch of the phylogenetic tree, emphasizing the high conservation of leptospiral genomes [20]. It is worth highlighting that all samples analyzed here were isolated in the same epidemiological scenario. This

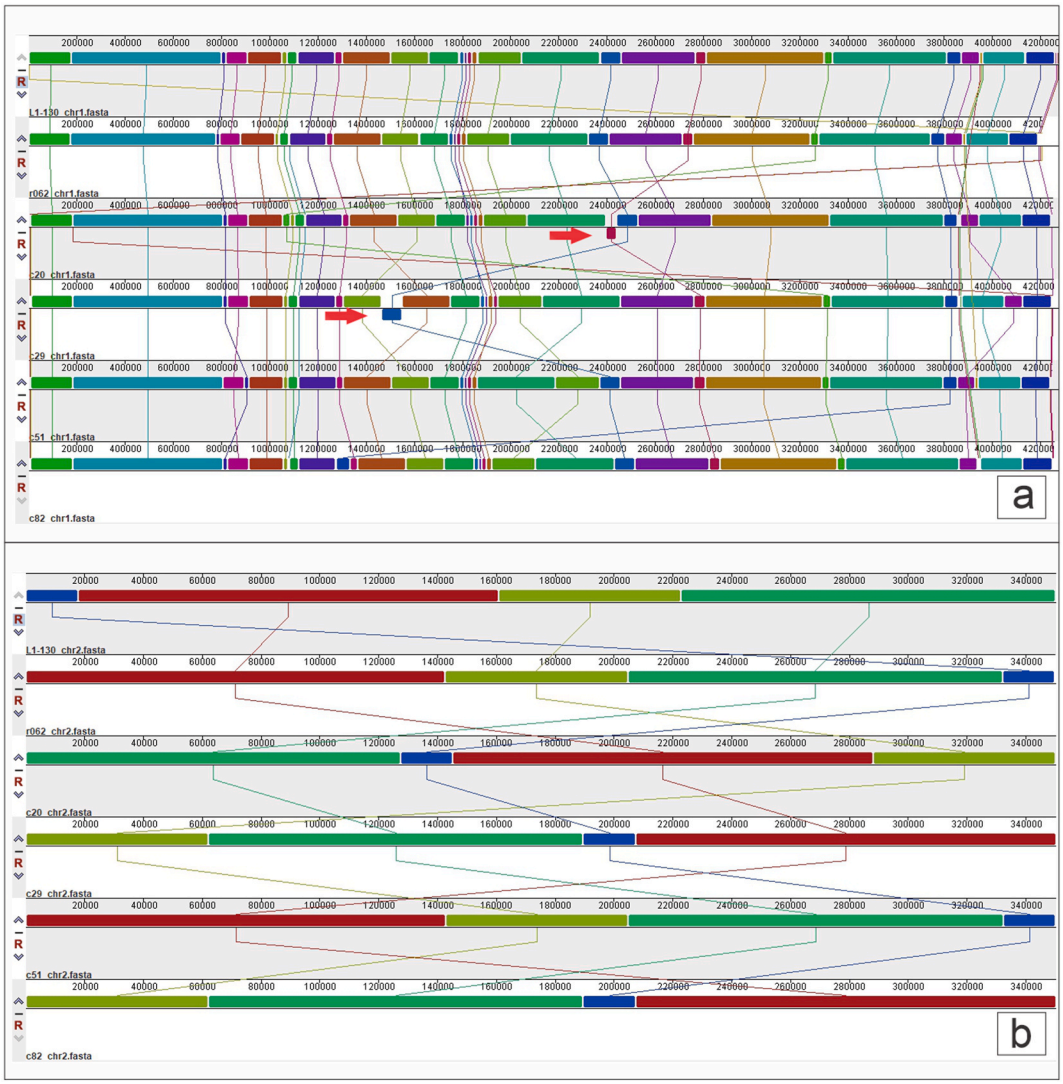

Fig. 6. Comparative analysis between genomes of *L. interrogans*, serogroup Icterohaemorrhagiae, obtained from samples of naturally infected dogs, humans, and rodents using the Mauve tool. (a) chromosome 1, (b) chromosome 2. In this comparison, horizontal lines represent the strains, with each block in a specific color representing a syntenic segment compared to the others, while vertical lines between the blocks indicate arrangements. Red arrows indicate inversions.

observation underscores the fact that, despite the temporal distance between L1-130 (1996) and C51 (2018), no gene composition changes were observed. All six strains isolated in Salvador exhibit a mixed clustering pattern together with those isolated from other countries [20]. No clustering of these six strains was observed based on the animal species from which they were obtained. This clustering pattern, associated with the high similarity between *Leptospira* strains from humans and animals, provides genetic evidence that reaffirms the maintenance of the disease in animals (rodents and dogs) and transmission between species [47].

3.6. Comparative analysis of genomes

The comparative analyses conducted in our study demonstrate a high level of similarity among the genomes of the strains isolated from dogs, rodents, and humans. BRIG, a powerful tool in our analysis, enabled the observation of a high level of synteny in the genomic regions when compared to the L1-130 strain. The observation of synteny enhances our understanding of genomic similarity. However, chromosome 1 presented small areas with deleted regions (black arrows), mainly in the sequences of strains isolated from canines (Fig. 4). Regarding the comparison data between the rodent strain (R062) and L1-130 (Figs. 4 and 5), areas of divergence were observed near the 500 and 150 kb positions on chromosomes I and II, respectively. Small variations may be related to sequencing and assembly issues, since repeat areas cannot be assembled in sequencing with short reads [25]. Previous studies have linked these divergences to areas containing transposon genes, hypothetical proteins, or insertion elements [16,62].

The entire genome of the strains was mapped on both chromosomes of the reference strain, with no extrachromosomal elements, such as phages or plasmids, identified. Structural analysis revealed arrangement variations on both chromosomes (chromosome 1 (Fig. 6a) and chromosome 2 (Fig. 6b)), including inversions (blocks of inverted sequences between the genomes) and translocations. Analysis using ACT confirmed the high degree of synteny, corroborating the rearrangements identified by Mauve, including regions of inversion (Supplementary Fig. 2). Despite some observed translocations and inversions, the sequences maintained similarity in gene content, ordering, and block size. Moreno et al. [63] pointed out that structural changes may be related to the different assembly and sorting methodologies. Despite the small differences observed herein, the presently conducted genome comparisons using the ACT and BRING tools showed higher similarity than other previous comparisons [16,47,62]. We attribute the observed difference to the combination of tools used herein, which allowed us to obtain very high-quality genomes.

Leptospira interrogans exhibits the broadest global distribution of the genus, and is frequently found in tropical regions [28]. Synanthropic rodents are widely described as chronic carriers and the major reservoirs of this species in the area under study. In this same epidemiological scenario, several studies have described the circulation of pathogenic *Leptospira* in the environment [64,65], in rodents [66], and in other wild and domestic animals, such as dogs, deer, wild cats and reptiles [12,67–70]. The high circulation of *Leptospira* spp. in this region underscores the need for surveillance based on a One Health approach to achieve a better understanding of the transmission dynamics among species, the degree and distribution of environmental contamination, and the potential use of the canine population as sentinels for predicting infections in humans [11,71].

4. Conclusions

The presently analyzed genomes of *Leptospira interrogans* serogroup Icterohaemorrhagiae, isolated from dogs, humans, and rodents in a single epidemiological setting, exhibit a high degree of conservation and similarity, demonstrating the interaction between animals and public health. Importantly, some genes related to virulence and adaptability, including genes potentially associated with biofilm formation, were found to exhibit mutations. The present findings emphasize the importance of collaborative intervention strategies based on the One Health approach to target the complexity of leptospirosis transmission dynamics in known hosts of *Leptospira*, such as rodents, as well as those not commonly considered hosts, in diverse communities worldwide.

CRediT authorship contribution statement

Lucas Nogueira Paz: Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **Laise de Moraes:** Writing – review & editing, Investigation, Formal analysis. **Luciane Amorim Santos:** Investigation, Formal analysis. **Camila Hamond:** Writing – review & editing, Investigation, Formal analysis, Conceptualization. **Melissa Hanzen Pinna:** Writing – review & editing, Supervision, Investigation, Formal analysis, Conceptualization.

Ethics Committee Approval

The study was approved by the Local Institutional Review Board on the Use of Animals at the School of Veterinary Medicine of the Federal University of Bahia (EMEVZ-UFBA) (06/2021–June 05, 2021).

Data availability statement

Data associated with this study (BioSample and SRA) has been deposited at NCBI GenBank under the BioProject accession number PRJNA1032694.

Funding Statement

Lucas N. Paz received funding via a fellowship from CAPES [88882.453699/2019-1].

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.

Acknowledgements

We thank the Superintendência de Tecnologia da Informação (STI-UFBA) for informatics support. We thank Professor Dr. Vasco Azevedo for his critical comments on the draft manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e41531.

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