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Genotyping Reveals Potential Sources of Human Leptospirosis Outbreaks in Aotearoa New Zealand

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ABSTRACT

Introduction: The introduction of PCR testing for leptospirosis in Aotearoa New Zealand has reduced the availability of serotyping data, and current diagnostic PCRs do not routinely genotype *Leptospira*. This study genotyped *Leptospira* from PCR-confirmed human cases between 2016 and 2023 and compared them with genotypes found in animals to identify potential sources of infection in a 2023 human leptospirosis outbreak.

Methods: Human samples were genotyped using *glmU* amplicon sequencing and compared to animal genotypes from previous studies. In addition, human national surveillance data were analysed to provide broader epidemiological context including regional distribution to reveal outbreak areas; diagnostic test usage to assess trends; serotyping results to evaluate consistency across methods; and demographic information to evaluate the representativeness of the genotyped dataset. Chi-squared and Poisson regression were used to assess host-genotype associations, and phylogenetics evaluated genetic relatedness.

Results: Surveillance data showed flood-associated outbreaks in several regions and a significant shift in diagnostic practice ($p \leq 0.001$), with increased use of PCR. Genotyping of PCR-confirmed cases revealed a rise in Pomona infections in 2023 across rural flood-associated regions (Gisborne, Hawke's Bay, Manawatū-Whanganui, Waikato and Wairarapa). In contrast, the Auckland region—including Aotearoa's largest city which also experienced flooding—had infections linked to Ballum, Copenhageni and Balcanica NZ. In animals, Pomona was primarily detected in sheep (*Ovis aries*), followed by cattle, while Ballum, Copenhageni and Balcanica NZ were primarily detected in mice (*Mus musculus*), Norway rats (*Rattus norvegicus*) and brushtail possums (*Trichosurus vulpecula*), respectively.

Conclusions: Flooding-driven outbreaks in rural areas with pastoral livestock were predominantly linked to livestock-associated strains, while urban cases were associated with rodents and small wildlife. These findings highlight the need for tailored mitigation strategies addressing distinct epidemiological risks in rural and urban settings. Surveillance strategies should be adapted to preserve typing capabilities to better inform public health responses in future outbreaks.

Summary

- People living in rural areas with pastoral livestock were predominantly infected with the livestock-associated strain *L. interrogans* serovar Pomona (Pomona), while those in urban regions were infected with wildlife-associated strains including *L. borgpetersenii* serovars Ballum (Ballum) and Balcanica NZ (Balcanica NZ), and *L. interrogans* serovar Copenhageni (Copenhageni).
- Sheep, followed by cattle, were identified as likely maintenance host for Pomona; rodents for Ballum and Copenhageni; and possums, followed by wild deer for Balcanica NZ.
- Flood-affected regions experienced an increase in leptospirosis cases, which appeared to be driven by the predominant maintenance host(s) present in each region.

1 | Introduction

Leptospirosis is a neglected zoonotic disease that is estimated to cause 1 million cases and 60 thousand deaths annually, though the true global burden is likely underreported due to its presentation as an undifferentiated febrile illness (Costa et al. 2015). The disease is caused by bacteria belonging to the highly diverse genus *Leptospira*, which is divided into two major clades: pathogenic (P) and saprophytic (S). Each clade is further divided into two subclades (P1, P2, and S1, S2 respectively), with P1 containing high-virulence species and P2 comprising low-virulence species (Vincent et al. 2019). Mammals are the primary hosts for pathogenic species, harboring the bacteria in their kidneys and intermittently shedding them into the environment, where they remain virulent for extended periods (Bierque et al. 2020). Transmission occurs when mucosal membranes or cuts/abrasions come into contact with infected urine or contaminated environments (Levett 2001).

Leptospirosis can be diagnosed with serology, culture, histopathology and molecular testing. The two most common methods are the microscopic agglutination test (MAT—a serological assay), and the polymerase chain reaction (PCR—a molecular method) (Levett 2001; Faine 1982). MAT relies on accurate knowledge of locally circulating serovars, requiring regular surveillance to maintain a comprehensive panel of antigens for relevant serological tests (Musso and La Scola 2013). With over 300 serovars identified worldwide (Picardeau 2017), MAT is impractical in regions with many circulating serovars and may fail to detect exotic serovars. Additionally, it requires two samples taken at least two weeks apart to detect a rise in antibody titres, leading to diagnostic delays or incomplete diagnoses. Furthermore, cross-reactivity between serovars and serogroups complicates the interpretation of results (Faine 1982; Goarant 2016). PCR overcomes these barriers by detecting bacterial DNA and providing faster results from a single sample; however, it is a presence/absence test that does not provide any lineage information (Goarant 2016). This absence of typing data can have serious implications for surveillance and mitigation strategies, as different serovars can have distinct maintenance hosts (Cordonin et al. 2020), making it difficult to accurately identify sources of infection.

Aotearoa's uncommon natural history—an island nation with only two endemic bat species as native terrestrial mammals and all others being introduced—has resulted in limited *Leptospira* diversity, enabling host–serovar associations to be reliably drawn for several decades (Hathaway and Blackmore 1981; Hathaway 1981; Moinet et al. 2021; Wilkinson et al. 2024). Only two species from the P1 clade and seven types are known to be endemic in animals (Wilkinson et al. 2024; Marshall and Manktelow 2002). These include *Leptospira borgpetersenii* serovars Ballum, Tarassovi, Balcanica, Hardjo and str. Pacifica, and *Leptospira interrogans* serovars Pomona and Copenhageni. Except for Hardjo and Balcanica, and Tarassovi and str. Pacifica, all serovars identified to date in Aotearoa belong to different serogroups. Therefore, serosurveillance has historically played a major role in the management of leptospirosis. In the 1970s, high incidence rates in humans were linked to serovars Hardjo and Pomona, with dairy cattle and pigs identified as maintenance hosts (Marshall and Manktelow 2002). By the 1980s, widespread vaccination of dairy cattle (Yupiana et al. 2019) and commercial pigs (Fairly 1997) against these serovars together with guidelines on dairy farm operations, and personal protective equipment (PPE) among agricultural workers were implemented (Ministry of Primary Industries 2017). These measures were followed by a steady decline in associated human cases over time (Figure S1), however there was an increase in cases associated with Ballum and Tarassovi (Thornley et al. 2002; El-Tras et al. 2018; Nisa et al. 2020). Since the introduction and uptake of diagnostic PCR in 2013 (Nisa et al. 2020; Hall et al. 2024), there has been a decline in the availability of typing data. Recent advances in molecular assays now enable genotyping of the seven endemic strains and all species within the P1 clade (Wilkinson et al. 2024, 2021). However, *Leptospira* genotyping is not currently used in diagnostic laboratories, leaving a gap in the identification of infecting serovars and understanding the change in the epidemiology of leptospirosis.

In early 2023, the Hawke's Bay region of Aotearoa experienced a sudden increase in leptospirosis cases following floods (Jones et al. 2023), prompting increased use of PCR-based diagnostics for rapid results. To investigate this outbreak, PCR-positive samples from human diagnostic laboratories were collected for genotyping and compared to the preceding seven-year period (2016–2022). Human-associated *Leptospira* genotypes were compared with genotypes found in animals, addressing a critical gap in understanding the potential sources and transmission pathways of the leptospirosis outbreak in 2023. Additionally, human national surveillance data including regional notifications, diagnostic test usage and serotyping results between 2016 and 2023 were analysed to provide a comprehensive picture of human infection.

2 | Material and Methods

2.1 | Sample Sources

Human DNA samples were obtained from patients tested for leptospirosis via PCR at either diagnostic laboratories or the research laboratory (Molecular Epidemiology and Public Health Laboratory, [m]EpiLab) between 4 February 2016 and 26 December 2023 as part

of various research projects (Table S1). Associated metadata included region (Table S2), and for 2023, sex and ethnicity.

In diagnostic laboratories, DNA was extracted and tested using their standard methods and stored at −20°C. DNA from PCR-confirmed cases was sent to ᵐEpiLab in batches under chilled conditions, where it was re-stored at −20°C until genotyping. In the research laboratory, DNA was extracted using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions and stored at −20°C until genotyping. A total of 96 human samples were genotyped from urine ($n=34$), plasma ($n=26$), whole blood ($n=23$), serum ($n=9$), cerebrospinal fluid ($n=2$) and culture ($n=2$).

2.2 | Data Sources

Human surveillance data from 1 January 2016 to 31 December 2023 was sourced from publicly available reports in the notifiable disease database (Institute of Environmental Science and Research, n.d.). This included notification dates, sex, ethnicity, serotypes, regional distribution and diagnostic test information.

Where available, genotype data were matched to serotype results from the same case. Serotyping was performed either at diagnostic laboratories or at ᵐEpiLab as part of other research projects (Table S1).

All animal data were sourced from previous surveys aimed at monitoring *Leptospira* prevalence in healthy animals across Aotearoa. Genotyping and serotyping data from domestic and wild mammals were generated by the authors through separate research projects conducted at ᵐEpiLab between 2007 and 2023 (Table S1) using previously published methods (Wilkinson et al. 2024). This study utilises data from 315 animals (Table S3), with samples collected from urine ($n=191$), kidney ($n=99$), culture ($n=22$) and serum ($n=3$). If available, the genotype and serotype from the same animal were matched.

2.3 | Molecular Typing and Analysis

DNA samples were subjected to PCR using *glmU* primers (Table S4) and amplicons were subjected to Sanger sequencing. Sequence data were compared to reference sequences using the Geneious software (version 10.2.6) to assign species and serovars as previously described (Wilkinson et al. 2024).

Samples that could not be amplified using the *glmU* primers but were either positive with culture or to a diagnostic PCR were amplified with 16S rRNA primers (Table S4) and Sanger sequenced as previously described (Thibeaux et al. 2018). All 16S rRNA sequences were analysed using the Basic Local Alignment Search Tool (BLAST) using the core nucleotide database provided by the National Center for Biotechnology Information to identify submitted matching or closely related sequences.

A Maximum Likelihood (ML) phylogenetic tree was constructed using *glmU* sequences from this study, alongside reference sequences from clade P1 species (Table S5), to illustrate genetic relatedness between strains. A Neighbour-Joining (NJ) tree was generated from a distance matrix calculated from nucleotide sequences, and ML estimation was applied to the NJ tree using the General Time Reversible (GTR) model of nucleotide substitution in PhyML (version 3.3.2) (Guindon et al. 2010).

2.4 | Statistical Analyses

All data were analysed using R (version 4.3.1) (R Core Team 2023) with packages presented in Table S6. Population at risk denominators for each year and region were estimated via linear interpolation of 'usually resident' census data (Stats NZ, n.d.) to calculate incidence rates. A Poisson regression model was used to assess the effect of region and period (2016–2022 vs. 2023), including population size as an offset and an interaction term between region and period. Model fit was assessed using a chi-squared test, and estimated marginal means were computed for pairwise comparisons of period within each region to evaluate differences in incidence rates.

Temporal trends in MAT and PCR use were analysed using a multinomial logistic regression with test type as the response variable and MAT + PCR as baseline.

Fisher's exact test was performed for each *Leptospira* type to assess differences in the proportions of serotypes and genotypes between 2016–2022 and 2023.

The association between *Leptospira* genotypes and sources was assessed using a chi-square test for independence and a Poisson regression model was fitted to the count data, including an interaction term between host and genotype. Estimated marginal means from the model were exponentiated to obtain predicted counts for each host–genotype combination. To assess the relative contribution of each *Leptospira* genotype within hosts, predicted counts were summed across genotypes for each host, and the proportion of each genotype was calculated as a percentage of the total predicted count per host.

2.5 | Ethical Approval

This study received ethics approval from the Massey University Human Ethics Committee, reference numbers SOA 19/51 and OM1 23/39 as well as from the Health and Disability Ethics Committee (HDEC), reference number 19/STH/80 and locality agreements, together with local Māori consultation from the 20 District Health Boards in Aotearoa at the time (Nisa et al. 2023). According to Massey University's Animal Ethics Committee guidelines, no additional ethical approval was required beyond that obtained for the original sampling as part of the previous projects (Table S1).

3 | Results

3.1 | Leptospirosis Notification Data

Analysis of human leptospirosis notification data in Aotearoa from 2016 to 2023 revealed that 2023 had the highest incidence rate during the study period (3.4 per 100,000; Figure 1A), and

the highest peak since 2002 (Figure S1). Another peak occurred in 2017 (3.1/100,000), followed by a marked decline in 2020 (1.2/100,000; Figure 1A).

When stratified by region and period (2016–2022 vs. 2023), a significant increase in incidence rates was observed in Gisborne ($p=0.002$), Hawke's Bay ($p<0.001$), Manawatū-Whanganui ($p=0.03$) and Wairarapa ($p=0.05$), with the highest incidence reported in Gisborne (23/100,000) and Hawke's Bay (21/100,000; Figure 1B). Both region and period were significant predictors of case numbers ($p<0.001$), with a significant interaction between the two ($p=0.02$), suggesting that the effect of period on case numbers varied by region. The 2023 peak was associated with a large increase in notifications in March and higher than usual notifications in January, February and April (Figure S2). The regional increase in monthly cases in 2023 (Figure S3) followed extreme weather events and flooding in the affected regions (Table S7).

3.2 | *Leptospira* Typing

There was a significant shift in diagnostic practice over time ($p\leq0.001$), with the proportion of cases diagnosed by MAT declining and PCR increasing (Figure 2A). From 2016 to 2022, 29.3% (110/376) PCR-positive samples were available for genotyping and 36.7% (40/110) were successfully genotyped. In 2023, all ($n=102$) PCR-positive samples were available and 48.3% (56/102) were genotyped. The availability of all samples in 2023, combined with serotyped cases, reduced the proportion of cases that could not be typed compared to the previous years (Figure 2B).

Genotyping identified 5 types that can be detected by serotyping (Pomona, Hardjo, Ballum, Copenhageni and Australis, Table 1). The comparison of genotyped cases between 2016–2022 and 2023 revealed a significant increase in the proportion of Pomona cases in 2023 (40%) compared to 2016–2022 (18%, $p=0.04$), while serotyping results for Pomona remained consistent (~16%) across both periods (Table 1). Conversely, the proportion of Ballum cases identified via genotyping remained stable (~33%), but the proportion via serotyping decreased significantly in 2023 (13%) compared to 2016–2022 (31% $p=0.001$, Table 1). Hardjo was the most frequently detected by serotyping in both periods (37% in 2016–2022 and 48% in 2023). In contrast, genotyping results varied, with Ballum as the most common genotype in 2016–2022 (33%), and Pomona in 2023 (40%, Table 1). No Tarassovi cases were detected via genotyping, and a low prevalence was identified through serotyping in both periods (Table 1). Low prevalence of Australis was detected by both methods and one case with Canicola was identified by serotyping over the 8-year period. Canicola and Pomona cannot be distinguished through glmU genotyping; all such sequences were classified as Pomona, the endemic serovar in New Zealand.

Genotyping also identified 5 types that cannot be detected by serotyping with MAT. These include Balcanica NZ, an endemic serovar previously associated with brushtail possums (*Trichosurus vulpecula*) and wild deer (*Cervus elaphus*) (Wilkinson et al. 2024); *L. borgpetersenii* sv. Balcanica str. Burgas, a serovar found in humans overseas and considered exotic in Aotearoa (Mateew and Manew 1975); Pacifica, a strain previously identified in Aotearoa dairy and beef cattle

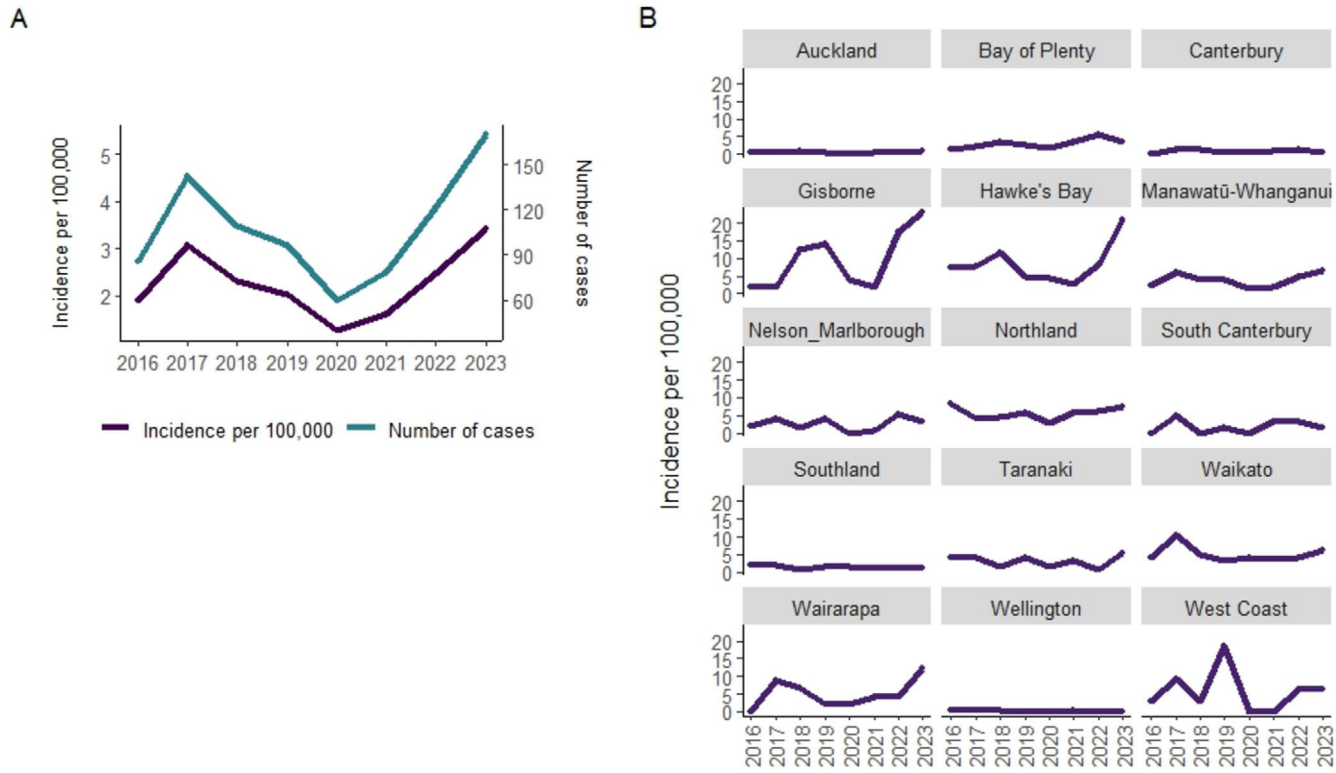

FIGURE 1 | Notified human leptospirosis cases in Aotearoa New Zealand from 2016 to 2023, showing (A) case numbers and incidence rates, and (B) incidence rates stratified by District Health Boards (DHBs). Some bordering DHBs were combined where Auckland includes Waitemata, Auckland and Counties Manukau; Waikato includes Waikato and Lakes; Wellington includes Capital and Coast and Hutt Valley. See Table S2 for geographical classifications.

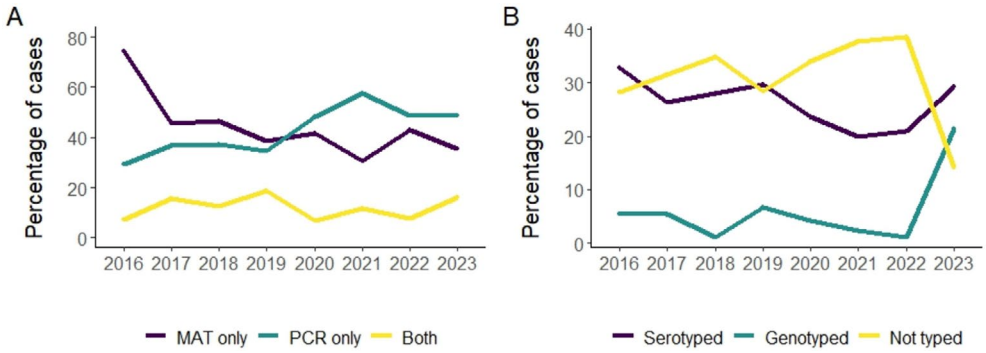

FIGURE 2 | Laboratory tests used to diagnose human leptospirosis cases in Aotearoa New Zealand from 2016 to 2023, showing (A) tests used by human diagnostic laboratories and (B) cases that were typed where serotyping data originated from human diagnostic laboratories via the microscopic agglutination tests and genotyping data from the research laboratory using *glmU* amplicon sequencing.

TABLE 1 | Comparison of genotyped and serotyped human leptospirosis cases between 2016–2022 and 2023 in Aotearoa New Zealand.

	Genotype, *n* (%)		Serotype, *n* (%)	
Genotype (serotype)[a]	**2016–2022**	**2023**	**2016–2022**	**2023**
L. interrogans sv. Pomona (Pomona)[b]	7 (17.5)	22 (39.3)*	46 (16.2)	13 (16.9)
L. borgpetersenii sv. Hardjo (Hardjo)[b]	4 (10)	5 (8.9)	105 (36.9)	37 (48)
L. borgpetersenii sv. Tarassovi (Tarassovi)[b]	0	0	31 (11)	9 (11.7)
L. borgpetersenii sv. Ballum (Ballum)[b]	13 (32.5)	19 (33.9)	89 (31.3)	10 (13)*
L. interrogans sv. Copenhageni (Copenhageni)[b]	8 (20)	6 (10.7)	10 (3.5)	7 (9.1)
L. borgpetersenii str. Pacifica (Tarassovi)[b]	3 (7.5)	2 (3.6)	NA	NA
L. borgpetersenii sv. Balcanica NZ (Hardjo)[b]	0	1 (1.8)	NA	NA
L. borgpetersenii sv. Balcanica str. Burgas (Hardjo)[c]	1 (2.5)	0	NA	NA
L. interrogans sv. Australis (Australis)[c]	1 (2.5)	0	2 (0.7)	1 (1.3)
L. interrogans sv. Canicola (Canicola)[c]	NA	NA	1 (0.4)	0
L. kirschneri[c] (Unknown)	0	1 (1.8)	NA	NA
Leptospira clade P2[c] (Unknown)	1 (2.5)	0	NA	NA
Leptospira clade P1 (Unknown)	2 (5)	0	NA	NA
Total typed	40 (100)	56 (100)	284 (100)	77 (100)

[a]Serotype each strain reacts/cross-reacts with.
[b]Endemic strains.
[c]Considered exotic.
*$p \leq 0.05$.

(*Bos taurus*) as well as farmed deer (*Cervus elaphus*, Figure S4) (Wilkinson et al. 2024); and 2 exotic strains that were only identified at the species level based on partial 16S rRNA sequence: *L. kirschneri*, a species from clade P1 and a *Leptospira* species from clade 2, which aligned with several species in clade 2 including *Leptospira wolffii, Leptospira haakeii, Leptospira licerasiae* and *Leptospira neocaledonica*. Due to poor-quality sequence, 2 samples were only identified at the clade level as P1.

Stratification of genotypes by region revealed that genotyped *Leptospira* remained largely consistent between the two periods in most regions, with some exceptions (Figure 3). For instance, Manawatū-Whanganui reported cases infected with Pomona, Pacifica and Ballum, while Waikato had cases infected with Pomona, Ballum and Copenhageni in both periods (Figure 3). In contrast, the strains identified in Northland shifted from Pomona and Hardjo in 2016–2022 to Ballum and Copenhageni in 2023 (Figure 3). The 2023 increase in genotyped Pomona cases (Table 1) was primarily associated with the Gisborne, Hawke's Bay, Manawatū-Whanganui, Waikato and Wellington regions, whereas Ballum cases were ubiquitous in most regions (Figure 3).

The demographic profile of genotyped cases from 2023 was comparable to that of all notified cases in 2023, suggesting the genotyping data are representative of cases nationwide. Additionally, both the regional distribution (Figure 3) and demographic profile of 2023 cases were similar to those from 2016

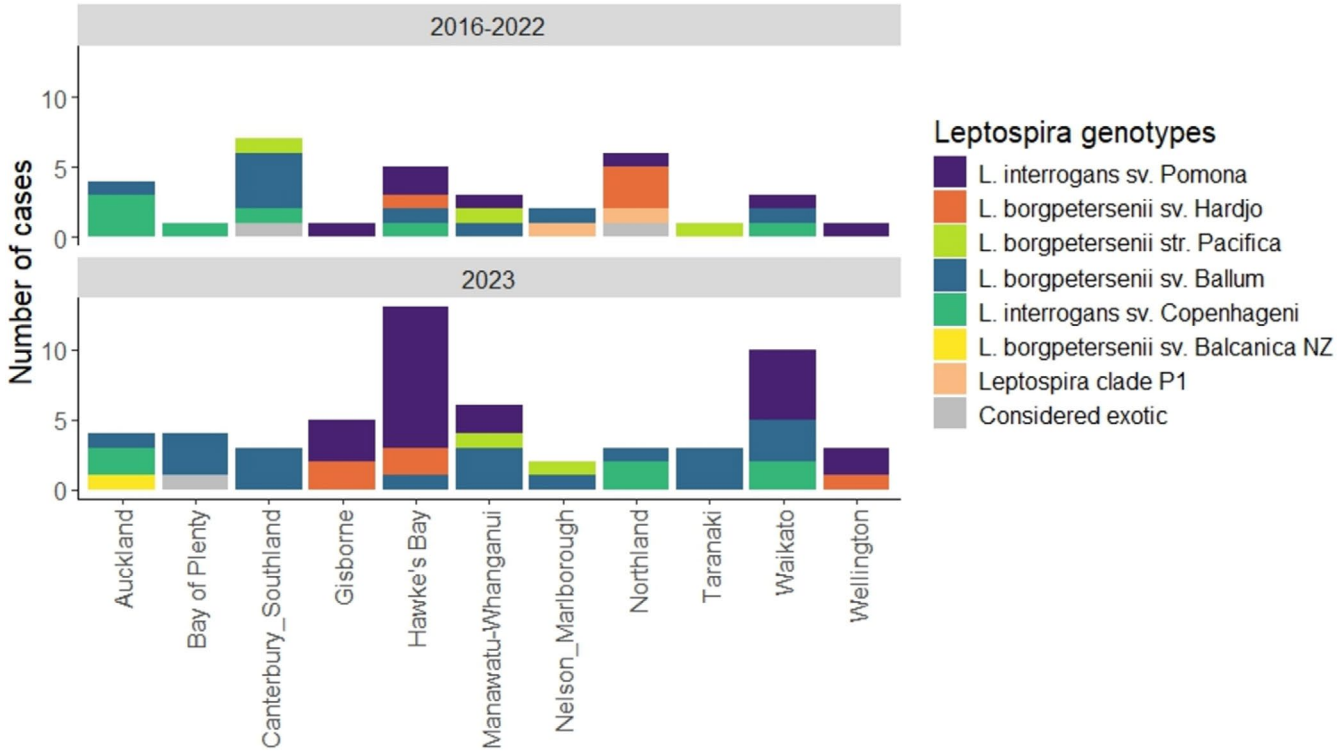

FIGURE 3 | Regional distribution of genotyped cases for 2016–2022 and 2023. Strains considered exotic include *L. interrogans* serovar Australis, *L. kirschneri* and *Leptospira* clade P2. Some bordering regions were combined where Auckland includes Waitemata, Auckland and Counties Manukau; Waikato includes Waikato and Lakes; Wellington includes Capital and Coast, Hutt Valley and Wairapapa; Nelson_Marlborough includes Tasman and West Coast; Canterbury_Southland includes South Canterbury. See Table S2 for geographical classification.

to 2022 (Table S8). Therefore, genotyped data from both periods were combined to increase the sample size for assessing potential sources.

3.3 | Potential Sources of Human Leptospirosis

The distribution of *Leptospira* genotypes differed significantly between sources investigated (χ^2 $p \leq 0.01$), with each genotype occurring in different proportions across host species (Figure 4). Pomona were primarily found in sheep (48%, 10/21), with much lower proportions found in beef (5%, 1/21) and dairy cattle (5%, 8/164). Hardjo infections were found in farmed deer (90%, 26/29) and beef cattle (86%, 18/21), followed by sheep (52%, 11/21) and dairy cattle (10%, 17/164). Ballum showed strong associations with mice (*Mus musculus*: 84%, 31/37), ship rats (*Rattus rattus*: 67%, 4/6) and wild deer (50%, 7/14), with a much lower proportion in dairy cattle (6%, 10/164). Copenhageni was predominantly detected in wildlife, including Norway rats (*Rattus norvegicus*: 75%, 6/8), followed by possums (27%, 3/11) and dairy cattle (7%, 12/164). Pacifica was most commonly identified in dairy cattle (68%, 112/164), with lower proportions in farmed deer (7%, 2/29) and beef cattle (5%, 1/21). Balcanica NZ was primarily associated with possums (73%, 8/11) and wild deer (50%, 7/14).

Phylogenetic analysis of the *glmU* amplicon revealed that identical *Leptospira* genotypes, as defined by this region, were detected across multiple sources (Figure 5), suggesting potential interspecies transmission in shared environments.

4 | Discussion

Genotyping human leptospirosis samples revealed 10 genetically diverse *Leptospira* strains in Aotearoa over the 8-year study period. This included 6 genotypes previously identified in animals and considered endemic (Pomona, Hardjo, Ballum, Copenhageni, Pacifica and Balcanica NZ) (Wilkinson et al. 2024); and at least 4 strains that are considered exotic (*L. interrogans* sv. Australis str. Ballico, *L. borgpetersenii* sv. Balcanica str. Burgas, a *L. kirschneri* species and a *Leptospira* species from clade P2). The endemicity of the latter 3 exotic strains cannot be ruled out as they are not routinely monitored like Australis, and genotyping was only performed on a subset of cases (Figure 2). Serotyping identified 5 endemic serovars (Tarassovi, Pomona, Hardjo, Ballum and Copenhageni) and two exotic serovars (Australis and Canicola). Although some discrepancies exist between genotyping and serotyping results, Pacifica serologically cross-reacts with Tarassovi (Figure S4), and Balcanica NZ and Balcanica Burgas cross-react with Hardjo (Wilkinson et al. 2024). Thus, the five endemic-serovar panel remains relevant for leptospirosis surveillance in Aotearoa. However, this panel may not differentiate between serovars that cross-react or detect many new or exotic types, as the exotic-serovar panel includes only three additional serovars (Australis, Canicola and Grippotyphosa).

Genotyping identified Pomona as the predominant type during the 2023 outbreak; however the proportion identified via serotyping remained similar for both time periods (Table 1). This

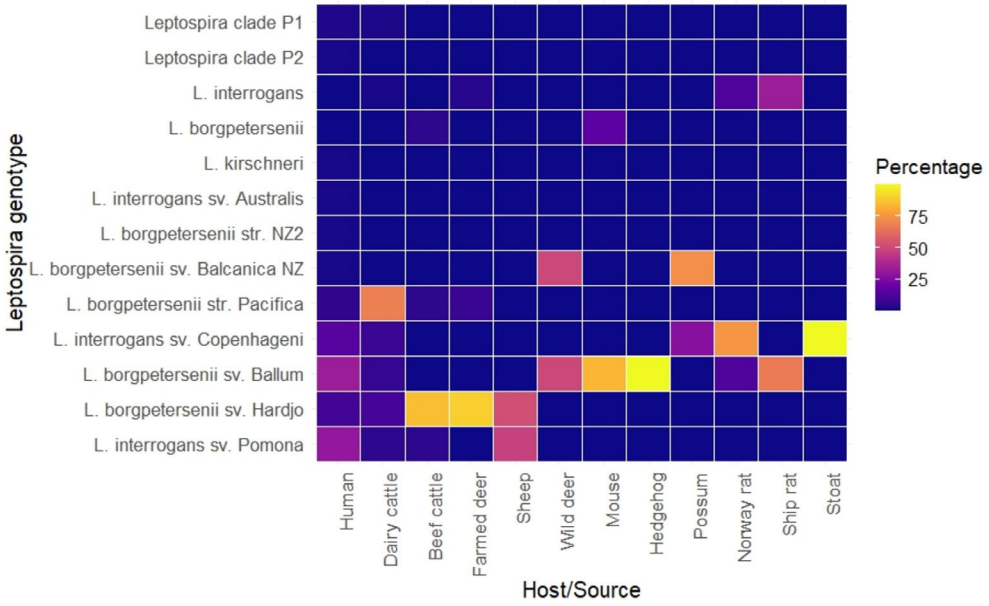

FIGURE 4 | Percentage of *Leptospira* genotypes detected across different host and/or sources in New Zealand. Due to low sample sizes ($n = 2$), genotype proportions from stoats and hedgehogs may be uncertain and are therefore not interpreted as evidence of primary maintenance hosts for the genotypes identified in this study.

discrepancy may reflect regional differences in diagnostic practices (PCR vs. MAT), which can influence strain detection and local epidemiology, which can be further amplified by environmental factors. For instance, in Hawke's Bay, a region with historically high Pomona incidence (Nisa et al. 2020), the increased use of PCR in 2023 following severe flooding (Table S7) likely contributed to the higher detection rates via genotyping (Figure 3). Increases in Pomona-associated cases in 2023 were observed in four other regions (Gisborne, Manawatū-Whanganui, Waikato and Wellington, Figure 3), with Wellington cases likely originating from Wairarapa, a rural DHB within the Wellington region (Table S2) as Wellington did not report any cases in 2023 while Wairarapa had a significant increase (Figure 1B). These patterns align with strains typically circulating in their respective regions (Nisa et al. 2020) and appeared to be exacerbated by extreme rainfall events (Table S7). A significant interaction between region and period was observed, with case numbers peaking approximately one month after flooding in affected regions (Figure S3); although some variation was noted, as each serovar follows a distinct seasonal pattern in Aotearoa (Nisa et al. 2020). The time lag aligns with the incubation period of 2–20 days for leptospirosis (Haake and Levett 2015), and a previous study demonstrated a strong association between heavy rainfall and flooding and human leptospirosis (Tana et al. 2025).

Flooding has also been linked to Pomona outbreaks in sheep (Vermunt et al. 1994). Given the farming patterns in Aotearoa (Stats NZ 2023), Pomona infections in Hawke's Bay, Gisborne, Manawatū-Whanganui and Wairarapa are likely associated with sheep and beef cattle, while infections in Waikato may be linked to beef and dairy cattle. This is supported by a recent study showing that extreme rainfall, surface runoff and other climate variables were associated with increased leptospirosis in sheep, with weaker associations in cattle (Sadler et al. 2025). The same study observed increases in sheep leptospirosis in the same regions as humans after the 2023 floods. Although, leptospirosis vaccination coverage is high in dairy cattle (~99%), it remains much lower in beef (18%–25%) and sheep (<1%) (Yupiana et al. 2019; Dreyfus et al. 2018; Sanhueza et al. 2017). Bivalent (Pomona and Hardjo) leptospirosis vaccinations are licensed for cattle, deer and sheep and trivalent (bivalent + Copenhageni) and tetravalent (bivalent + Copenhageni + Tarassovi) are licensed for cattle. As all ruminant stock classes are pastorally grazed, unvaccinated animals can contribute substantially to environmental contamination, particularly in high stocking density systems. This poses a heightened risk during flooding events, when contaminated water can facilitate the spread of *Leptospira*.

In contrast, cases in Auckland, a large city that was also affected by flooding (Table S7), were due to infections with Ballum, Copenhageni and Balcanica NZ (Figure 3), which were largely associated with wildlife (Figure 4). Small wild mammals are globally recognised as the primary sources of infection in urban areas (de Faria et al. 2008; Garcia-Lopez et al. 2024; Sayanthi and Susanna 2024) and extreme weather can increase small mammal populations (Polgreen and Polgreen 2017). A study in Brazil found that rat abundance, along with environmental and hydrological factors, plays a significant role in *Leptospira* spillover (Soni et al. 2024). However, each host species can harbour multiple strains (Figure 5). For example, while Ballum infections in an urban area may be typically linked to mice or rats, this may not be the case in rural regions, where people can be exposed to any Ballum hosts (dairy cattle, wild deer, mice, hedgehogs or rats) (Moinet et al. 2021). Notably, Ballum was the most widespread serovar present across multiple regions (Figure 3).

Leptospira can persist in soil and water for extended periods and is resuspended following rainfall (Thibeaux et al. 2017, 2024). A meta-analysis found flood water contact significantly increases leptospirosis risk (Naing et al. 2019). We postulate that the 2023 flooding led to resuspension and mobilisation of *Leptospira* from

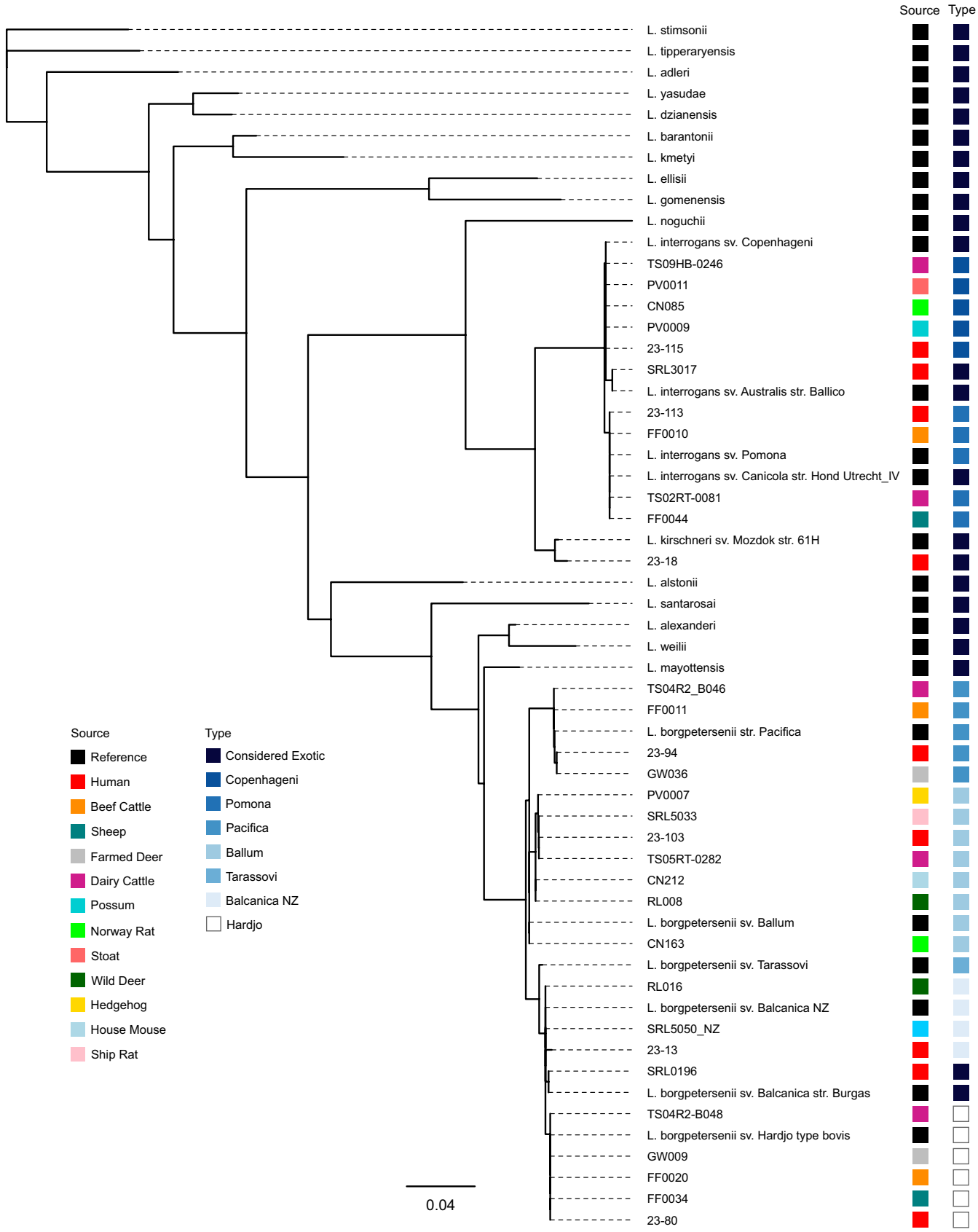

FIGURE 5 | Maximum likelihood phylogenetic tree based on *glmU* sequences of *Leptospira* species from clade P1, illustrating their associated hosts in Aotearoa New Zealand. To simplify visualisation, only one representative genotype per host is shown.

soil, as well as increased shedding from unvaccinated stressed animals, contaminating floodwaters with strains circulating in affected regions. Given the predicted increase in frequency and intensity of extreme weather events due to climate change (Robinson et al. 2021), the threat of flood-associated leptospirosis outbreaks is likely to rise, highlighting the need for effective disease mitigation strategies.

Although this study provides valuable insights, identifying the exact source is challenging as leptospirosis transmission is complex (Benschop et al. 2025). Furthermore, incomplete genotyping from 2016 to 2022 may have underestimated the genotypes, and the small sample sizes for some wildlife species (Table S3) may have resulted in imprecise association estimates. In addition, farmed deer and sheep samples were from 2007 to 2010, respectively, and may not reflect current infection status, though recent diagnostic data indicate that leptospirosis remains prevalent in sheep, with increased incidence reported following the 2023 floods (Sadler et al. 2025).

This study provides evidence supporting region-specific disease mitigation strategies to reduce transmission risks and enhance climate resilience, particularly during floods when personal protective equipment may be unavailable in Aotearoa. These strategies include better awareness between rural and urban leptospirosis, improved livestock vaccination programs and wildlife management, and considering doxycycline prophylaxis for individuals exposed to floodwaters (Chusri et al. 2014). Furthermore, integrating molecular typing—with expanded marker sets capable of distinguishing between many serovars—into routine surveillance is essential, as reliance on PCR without complementary typing limits source tracking and outbreak response. Building effective, cross-sectoral surveillance and diagnostic capacity will support leptospirosis control through a One Health approach and enable more targeted environmental interventions (Thibeaux et al. 2024).

In conclusion, these findings highlight the growing risk of leptospirosis amid climate change in Aotearoa, with livestock-associated strains affecting rural agricultural areas and mammalian wildlife-associated strains occurring in urban settings.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Appendix S1:** zph70022-sup-0001-AppendixS1.docx. **Figure S1:** Incidence of notified leptospirosis cases in Aotearoa New Zealand from 1979 to 2023. The red dashed line crosses the incidence notified in 2023 (3.4/100,000). Case numbers were sourced from EpiSurv [1] and population at risk denominators for each year were estimated via linear interpolation of 'usually resident' census data from 1981 to 2023 [2]. **Figure S2:** Incidence of notified leptospirosis cases per month in Aotearoa New Zealand from 2016 to 2023. Monthly notifications contain all cases, including suspected cases that were not confirmed with a diagnostic test. **Figure S3:** Incidence of notified leptospirosis cases stratified by month and region in Aotearoa New Zealand from 2016 to 2023. Monthly notifications contain all cases, including suspected cases that were not confirmed with a diagnostic test. **Figure S4:** Comparison of genotyping and serotyping data from the same (A) human ($n = 23$) and (B) animal ($n = 147$) in Aotearoa New Zealand, highlighting cross-reactivity in serology. Notably, animals infected with *L. borgpetersenii* str. Pacifica exhibited cross-reactivity against serogroup Tarassovi. Serotype classification was based on the microscopic agglutination test (MAT) [3], where a serotype was classified if it reached a reciprocal titre of ≥ 192 as higher titres are generally associated with shedding [4], though this may not be true all the time and may be dependent on the host species [5]. If more than one serovar reached a titre 192, the case was classified as 'Mixed'. Titres not meeting these criteria were classified as 'Unknown'. **Table S1:** Research projects that genotyped and/or serotyped *Leptospira* from human and animal samples in Aotearoa New Zealand. **Table S2:** Different geographical

classifications in Aotearoa New Zealand. **Table S3:** Species and sample types used to generate genotyping data. **Table S4:** Primers used in this study. **Table S5:** zph70022-sup-0001-AppendixS1.docx. *Leptospira* species and strain names and corresponding NCBI accession numbers for the glmU sequences used to construction of the phylogenetic tree. **Table S6:** R packages used for analysis. **Table S7:** Regions affected by extreme weather events and flooding in Aotearoa New Zealand in 2023 [25]. **Table S8:** Demography of notified and genotyped cases.