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Investigating animals and environments in contact with leptospirosis patients in Aotearoa New Zealand reveals complex exposure pathways

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ABSTRACT

Case history: Three human leptospirosis cases from a case-control study were recruited for in-contact animal and environment sampling and *Leptospira* testing between October 2020 and December 2021. These cases were selected because of regular exposure to livestock, pets, and/or wildlife, and sampling was carried out on their farms or lifestyle blocks (sites A–C), with veterinarians overseeing the process for livestock, and cases collecting environmental and wildlife samples.

Laboratory findings: Across the three sites, a total of 137 cattle, > 40 sheep, 28 possums, six dogs, six rats, three pigs and three rabbits were tested. Herd serology results on Site A, a dairy farm, showed infection with Tarassovi and Pomona; urinary shedding showed *Leptospira borgpetersenii* str. Pacifica. Animals were vaccinated against Hardjo, Pomona and Copenhageni. The farmer was diagnosed with Ballum. On Site B, a beef and sheep farm, serology showed infection with Pomona; animals were not vaccinated, and the farmer was diagnosed with Hardjo. On Site C, cattle were shedding *L. borgpetersenii*; animals were not vaccinated, and the case's serovar was indeterminate. Six wild animals associated with Sites A and C and one environmental sample from Site A were positive for pathogenic *Leptospira* by PCR.

Conclusion: These findings highlight the complexity of potential exposures and the difficulty in identifying infection sources for human cases. This reinforces the need for multiple preventive measures such as animal vaccination, the use of personal protective equipment, pest control, and general awareness of leptospirosis to reduce infection risk in agricultural settings.

Clinical relevance: Farms with unvaccinated livestock had *Leptospira* infections, highlighting the importance of animal vaccination. Infections amongst stock that were vaccinated emphasise the importance of best practice vaccination recommendations and pest control.

Abbreviations: MAT: Microscopic agglutination test; PIC: Person in charge; PPE: Personal protective equipment

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Leptospira; leptospirosis; One Health; molecular typing; New Zealand

Introduction

Leptospirosis is a neglected zoonotic disease caused by bacterial infection with *Leptospira* spp. (Goarant *et al*. 2019). It is primarily transmitted to humans through contact with infected animals' urine or their environment (Adler 2015). Symptoms can range from mild manifestations to severe forms that may lead to jaundice, kidney failure, and in rare cases, death. Diagnosis can be complex and typically involves a combination of clinical signs, laboratory tests, and epidemiological information. Compared to other high-income countries with temperate climates, Aotearoa New Zealand has one of the highest leptospirosis incidence rates in humans (Victoriano *et al*. 2009). Occupations with exposure to livestock, particularly farm and meat workers, have predominated among notifications (Nisa *et al*. 2020), suggesting infection is occurring by direct contact with farmed animals or the farming environment. This pattern is at odds with the international experience, where exposures associated with rodents (Mwachui *et al*. 2015), flooding (Lau *et al*. 2010) and poverty (Goarant

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et al. 2019) prevail. Furthermore, compared to global patterns (Munoz-Zanzi *et al*. 2020), outbreaks have been a rare occurrence in Aotearoa, and reported outbreaks are small and mostly in occupational settings (McLean *et al*. 2014; Benschop *et al*. 2017; Munoz-Zanzi *et al*. 2020). Aotearoa has held a globally unique position in leptospirosis epidemiology and prevention both in humans and animals. The development of livestock vaccines (Marshall 1987) in the late 1970s, vaccine uptake in the pig and dairy industries (Yupiana *et al*. 2021), and pharmaceutical companies' readiness to respond to newly recognised concerns (Virbac 2023), have underpinned the protection of both human and animal health in Aotearoa.

Nonetheless, in 2017, there was an increase in the incidence of human leptospirosis accompanied by changes in the demography of cases. These changes included a higher proportion of females and of individuals employed outside the traditional high-risk occupations (Institute of Environmental Science and Research 2017). Other changes include the increase of *Leptospira borgpetersenii* serovars Ballum and Tarassovi (Nisa *et al*. 2020) and the recent anecdotal association of human leptospirosis notifications with flooding events (Vallee *et al*. 2023). Our earlier work suggests that mammalian wildlife sources (Moinet *et al*. 2021, 2023) and environmental pathways (Wilkinson *et al*. 2021) may be increasingly important in disease transmission and that an updated description of potential sources of *Leptospira* infection is required.

In 2018, we commenced a nationwide human leptospirosis case-control study with four sub-studies (Nisa *et al*. 2023). Controls were frequency-matched to cases (90% male and 65% rural dwelling) to ensure, as much as possible, that cases and controls were drawn from the same source population. Here, we present the results of one of the four sub-studies, which involved cases with regular animal exposure. To update our understanding of potential sources for human leptospirosis infection, these cases had samples collected from their in-contact animals (blood and urine from livestock, and kidneys from wildlife) and the environment (soil, mud and water). This allowed us to assess the infection status of *Leptospira* spp. in the animals and environments that human leptospirosis cases had had contact with during the month prior to their onset of leptospirosis.

Case history

Case recruitment

The study was approved by the Massey University Animal Ethics Committee (reference number Protocol 19/11) and by the Health and Disability Ethics Committee (reference number 19/STH/80). Locality agreements and local Māori consultations were received from Aotearoa's 20 district health boards. The sampling frame consisted of a subset of 95 human cases that were enrolled in the leptospirosis case-control study between 22 July 2019 and 31 January 2022 (Nisa *et al*. 2023).

Cases were interviewed, on average, 3 months after the onset of their illness to gather information on risk factors for the case-control study. This interview was also used to identify suitable cases for participation in the current sub-study: participants were excluded from the current study if their sole exposure to animals in the month before they became ill was at abattoirs and were only eligible for inclusion if they had been otherwise exposed to livestock, pets or wildlife. Cases that met the inclusion criterion were given details of this sub-study. Cases were advised that for inclusion in this sub-study, they needed to be able to facilitate permission and subsequently sample their in-contact animals; that sampling of domestic species must occur within 6 months from the time the case was diagnosed; that sampling would be performed by the case's veterinarian; that a report would be provided to the veterinarian and the case, and that a follow-up consultation would be arranged to discuss the results.

Initially, eleven cases from the case-control study appeared suitable for inclusion; ten could be contacted but subsequently, seven of these could not facilitate animal sampling within the desired time frame, leaving three that were recruited for sampling.

For cases that consented to this study, a bespoke data collection form (see Supplementary Material 1) was used to capture number, class and species of domestic animals; animal vaccination history; details of the attending veterinarian; type and location of environments (e.g. a muddy gateway to the paddock) and pests (e.g. rats in the woodshed). The cases' veterinarians were contacted by the researchers and details of the study, animal ethics requirements and sampling protocols were explained. The cases arranged sampling dates for livestock and pets with their veterinarians. Cases were sent information by the research team on how to collect environmental samples (Supplementary Material 2) of soil, mud and water from sites around the farm that cases had contact with 1 month before they became ill with leptospirosis. Wildlife trapping and sampling were discussed with cases and if the researcher was confident in the ability of the case to arrange this, then information on how to sample killed wildlife was provided (Supplementary Material 3). Detailed methods of animal and environmental sampling, sample processing and laboratory tests are provided in Supplementary Material 4. As per the patient consents and health district ethics approvals, the cases' diagnostic test results were extracted from Aotearoa's

notifiable disease database, EpiSurv.[1] Further details of diagnostic tests used are reported elsewhere (Nisa *et al*. 2020).

Site information

All potential exposure sites were farms or lifestyle blocks. Sites A and B were landholdings belonging to their respective cases, so the case was the person in charge (PIC). For Site C, the case was not the PIC, but a city-dweller who visited the site weekly for community-organised "farm walks" to enhance their well-being by experiencing the farm lifestyle, with activities such as moving cattle, stacking firewood, clearing thistles, and setting and clearing traps.

Site A was a split-calving dairy farm, with a river traversing the farm, and approximately half of its perimeter covered by bush. Wild pigs (*Sus scrofa*), possums (*Trichosurus vulpecula*) and rats (*Rattus* spp.) were considered major pests on the farm with a recognised pig "hang-out" on a hill paddock, and hunters were frequently used to cull pigs. Rats were known to forage on garden kumara (*Ipomoea batatas)*, walnuts (*Juglans* sp.), and in animal feed (palm kernel, silage, and milk powder) and pest control was reported as "endless".

Site B was a sheep and beef farm divided into three sections, interspersed with housing and lifestyle blocks; approximately 20% of which was covered by bush, with a bush perimeter. There was a 200-hectare covenanted bush reserve containing wetlands and creeks within the farm. Hedgehogs (*Erinaceus europaeus*), rodents and possums were regularly sighted, or evidence of them seen around the chicken house and dog kennel. Poison was routinely laid for pests; hedgehogs had been inadvertently caught in drying fishing nets and mustelids were often seen as roadkill.

Site C was an 8-hectare beef lifestyle block with bush and stream boundaries, with regular pest control where rats, stoats (*Mustela erminea*) and possums were trapped at least once per day.

Laboratory findings

Human cases

Table 1 summarises the three human cases, their diagnostic results, and a description of their farm holdings. Case A tested positive for serovar Ballum and vaccinated their animals against Hardjo, Pomona and Copenhageni. Case B tested positive by PCR and serology for serovars Hardjo and Ballum, with Hardjo identified as the serovar of infection, and did not vaccinate their animals. Case C, who frequently visited a beef lifestyle block (Site C) where animals were unvaccinated, tested positive by PCR, and serology with mixed results for Hardjo and Ballum.

Table 2 summarises the sampling details from both animals and the environments across the three sites. Blood and urine samples were collected from livestock at all sites, with livestock kidney samples taken from Site C. Environmental samples were gathered from all sites, while wildlife kidney samples were collected from Sites A and C. There was willingness by the farmer on Site B to attempt trapping with support from the regional council; however, no samples were collected.

Domestic animals

Table 3 summarises the test results for the livestock, wildlife and environments tested in the study. For

Table 1. Case details, leptospirosis test results, location and description of land holdings where cases were exposed to animals, and animal vaccination histories in a case series investigating in-contact animals and environments of three human leptospirosis cases in Aotearoa New Zealand.

	Case A	Case B	Case C
Onset of illness	April 2020	August 2020	March 2021
Interview conducted	June 2020	September 2020	April 2021
Consultations	May 2021, April 2022	June 2021	May 2022
Diagnostic results			
MAT infecting serovar(s)	Ballum	Hardjo	Indeterminate
Titres			
Indeterminate			March 2021: < 25
Ballum	April 2020: < 25 May 2020: 3,200	August 2020: < 25 September 2020: 200 October 2020: 100	April 2021: 200
Hardjo		August 2020: < 25 September 2020: 400 October 2020: 400	April 2021: 400
PCR	Negative	Positive	Positive
Holding			
Region	Northland	Canterbury	Northland
Type	Commercial split-calving dairy farm	Commercial sheep and beef farm	Beef lifestyle block
Animal vaccination			
Cattle	April 2020: Hardjo, Pomona, Copenhageni	Nil	Nil
Dogs	August 2020: Copenhageni	Nil	Nil

MAT = microscopic agglutination test

Table 2. Sampling information, timing and types of samples collected from in-contact animals and environments of three human leptospirosis cases in Aotearoa New Zealand.

Site and sample source	Sampling months	Sample types (number)	Notes
A			
Livestock	November – December 2020	Heifers: blood (20, 22), urine (20, 22)[a] Mature cows: blood (20, 24), urine (20, 24)[a] Dogs: blood (2, 2), urine (2, 2)[a]	Approximately 20 of each class of cattle[b] were sampled with 16/70 (22%) of these sampled twice with 31 days between sampling periods.
Trapping of wildlife	May–September 2021	Possum (16) kidney; pig (3) kidney; rat (3) kidney	Farmer's regular trapping
Environmental	February 2021	Soil (5); mud (3); water (4)	From bailage and palm kernel storage areas, landscaping and gardening areas, a creek, and the effluent treatment system
B			
Livestock	October – November 2020	Breeding cows: blood (20, 21), urine (20, 21)[a] Two-year old cattle: blood (20, 20), urine (20, 20)[a] Breeding ewes: blood (20, 20), urine (20, 20)[a] One-year old ewes: blood (20, 20), urine (20, 20)[a] Dogs: blood (4, 4), urine (4, 4)[a]	Approximately 20 of each class of livestock[c] were sampled with 35/46 (76%) cattle sampled twice. The shortest duration between samplings was 16 days for 2-year old cattle and breeding ewes. All other stock classes had 28 days between samplings.
Environmental	May 2021	Soil (5); mud (4); water (4)	From wetlands, house garden and paddock
C			
Livestock	July 2021	Breeding cows: blood and urine (15); calves: urine (7)	All cattle were sampled
Livestock	December 2021	Two-year old cattle: kidney (6)	All cattle were sampled
Trapping of wildlife	August 2021	Possum: kidney (12); rabbit: kidney (3); rat: kidney (3)	Traps and guidance were provided by Northland Regional Council
Environmental	August 2021	Soil (5); mud (4); water (2)	From pond, garden, and a creek collected by the person in charge. From a fishpond, garden, and a rat trail collected by Case C around their home.

[a]Sites A and B had two sampling periods for livestock (both blood and urine). The number of samples in each period is separated by a comma.
[b]Animals on-site: 200 heifers, 400 mature cattle, two dogs.
[c]Animals on-site: 90 breeding cows, 80 two-year old cattle, 400 breeding ewes, 100 one-year old ewes, four dogs.

Site A, herd-level serology results for cattle indicated recent infections with serovars Ballum, Tarassovi and Pomona and exposure to Hardjo and Copenhageni (Figure 1). Due to minimal reactivity, results for Copenhageni are not shown. For Tarassovi and Pomona, up to half of the titres were ≥ 384. When paired serology results were examined for the 16 cattle that were sampled twice (Supplementary Table 1), 10/16 (62.5%) Tarassovi and 8/16 (50%) Pomona titre changes were of two or more dilutions, suggesting current infection spreading between animals. However, for Ballum, the paired profiles were more similar with only 4/16 (25%) titre changes of two or more dilutions; suggesting that the infection was not spreading between animals and implying a different epidemiology. One dog was seropositive to Tarassovi, Ballum and Pomona (antibody titres of 48, 48 and 384, respectively), consistent with the serovar patterns

Table 3. Animal and environmental sample laboratory results in a case series investigating in-contact animals and environments of three human leptospirosis cases in Aotearoa New Zealand.

Leptospira test	Site A	Site B	Site C
Livestock			
Serology[a]	Tarassovi[b], Pomona[b], Ballum[b], Hardjo[b], Copenhageni	Tarassovi, Pomona[b], Ballum[b], Hardjo, Copenhageni	Tarassovi, Hardjo, Copenhageni
PCR (urine)[c,d]	Positive (n = 2)	Negative	Positive (n = 3)
Culture	Positive (n = 1)	Negative	Negative
Type[e]	*Leptospira* spp. (n = 1); *Leptospira borgpetersenii* str. Pacifica (n = 1)	Not applicable	*Leptospira borgpetersenii* (n = 1)
Wildlife – PCR (kidney)[c,d]	Positive	Not applicable	Positive
Environment			
Culture	Positive	Positive	Positive
16S PCR[f]	Positive	Positive	Positive
lipL32 PCR[c]	Positive	Negative	Negative
glmU PCR[d]	Negative	Negative	Negative

[a]Only serovars with titres ≥ 48 are shown in the table.
[b]Serovars with titres ≥ 384 suggesting current or recent infection.
[c]*lipL32* quantitative PCR detects *Leptospira* spp. from the pathogenic clades P1 and P2.
[d]*glmU* conventional PCR detects pathogenic *Leptospira* spp. from the P1 clade
[e]Determined by sequencing of PCR amplicons.
[f]16S PCR detects all *Leptospira* spp. from both the pathogenic and saprophytic clades.

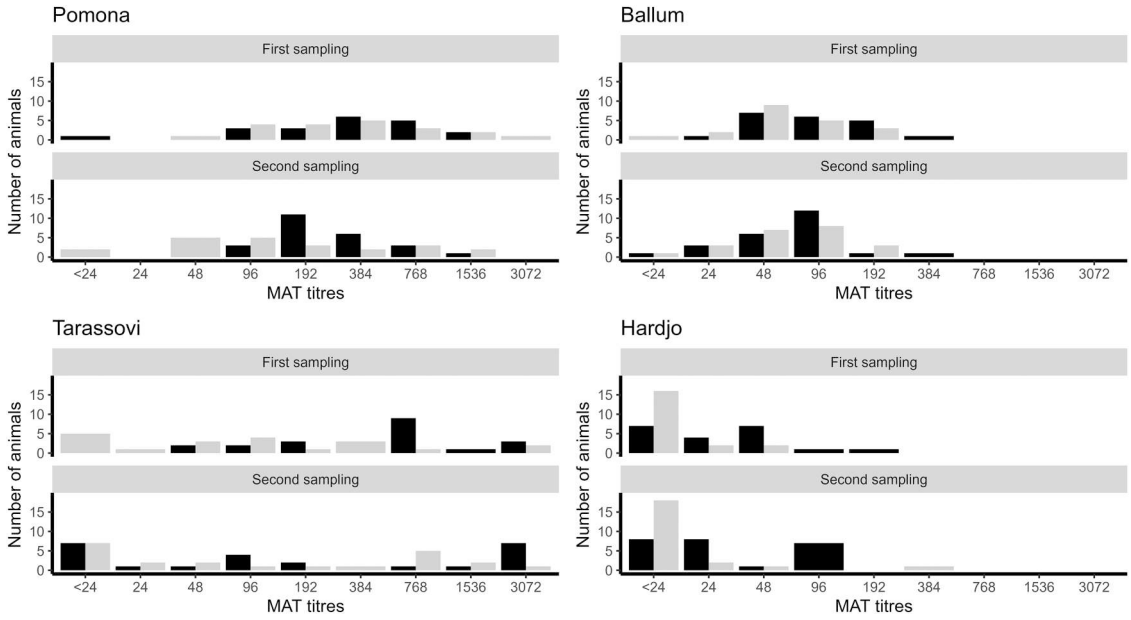

Figure 1. Microscopic agglutination test (MAT) titres of 20 heifers (grey) and 20 cows (black) on Site A for *Leptospira* serovars Pomona, Ballum, Tarrassovi and Ballum at first (3 November 2020) and second (4 December 2020) sampling for an investigation of in-contact animals in a case series of three human cases of leptospirosis in Aotearoa New Zealand. Sixteen of the animals from one location were sampled on both occasions.

seen in cattle. The second dog was seropositive only to Copenhageni (antibody titre 768). PCR results from urine indicated two cattle were shedding pathogenic *Leptospira*. One cow was PCR-positive at the first (urine) sampling. *Leptospira* classification was available at the genus level only due to the poor quality of sequence data. This cow's urine was also culture-positive as identified by dark field microscopy, however this culture could not be maintained and further characterised. A heifer was PCR-positive at the second (urine) sampling; amplicon sequencing classified this as *L. borgpetersenii* strain Pacifica (Wilkinson *et al*. 2024). All canine urine samples were PCR- and culture-negative.

For Site B, livestock serology results were notable for Pomona, particularly in sheep with four titres at 384, suggesting recent infection. Additionally, one prime (2-year-old) cattle beast had a Pomona titre of 384. Ballum was also notable with one titre of 384 in a breeding ewe (Supplementary Figures 1 and 2). There was evidence of exposure in both sheep and cattle to all other serovars except for Hardjo in sheep. Paired serology indicated very little reactivity in the beef animals at site B, with low and static titres between acute and convalescent samples. The serology results for dogs were negative. All urine samples were negative by both culture and PCR.

For Site C, there was evidence of exposure to serovars Copenhageni (n = 1), Tarassovi (n = 1) and Hardjo (n = 5), with titres of 48 or 96. Three of 15 adult cattle urine samples were positive by PCR, and amplicon sequencing identified one as *L. borgpetersenii*, while the other two had poor-quality sequence data that meant classification was only at the genus level as pathogenic *Leptospira* spp. All urine samples were negative by culture.

Wildlife

Kidney samples from 28 possums, six rats, three wild pigs and three rabbits (*Oryctolagus cuniculus*) were collected across two sites. Two of 16 possums (3%) from Site A, and 3/12 possums (25%) and 1/3 rabbits (33%) from Site C were PCR-positive for pathogenic *Leptospira* spp., but poor sequence quality meant classification was only at the genus level. All other kidneys were negative with PCR.

Environmental samples

A total of 10 water, 11 mud and 15 soil samples were collected across the three sites. All environmental samples were culture-positive by dark field microscopy and tested positive with the 16S PCR, which detects both pathogenic (P1 and P2 clades) and saprophytic (S1 and S2 clades) *Leptospira*. However, only one sample from Site A tested positive to the *lipL32* PCR, which detects all pathogenic *Leptospira*, indicating 1/36 samples contained pathogenic *Leptospira*, while the remaining 35 samples contained saprophytic *Leptospira*. This sample was not positive to the *glmU* PCR, which detects pathogenic *Leptospira*

[1]Maintained by the Institute of Environmental Science and Research on behalf of the Ministry of Health, https://episurv.esr.cri.nz/

from the P1 clade and is used for typing, suggesting the presence of *Leptospira* from the P2 clade in this sample.

Outcome

Reports of results were shared with the PIC of each site and their veterinarian and, where possible, a three-way discussion was held to provide information and support with respect to positive findings. For Site A, the discussion focused on the cattle results, challenges with vaccination timing under a split-calving pattern, the potential exposure to wildlife, and measures to ensure people's safety. Meeting outcomes included the veterinarian revisiting the vaccination programme with supporting information supplied by the research team (Heuer *et al*. 2012; Yupiana *et al*. 2021). For Site B, the discussion largely focused on Pomona results, animal vaccination, and leptospirosis prevention and awareness. Both the case and the veterinarian identified that there was little community awareness of leptospirosis in the region. The dissemination of results and raising awareness in the local community was discussed, e.g. at discussion groups which the research team offered to attend. For Site C, a discussion on treatment and vaccination was had with the veterinarian, and the cattle and wildlife results were emphasised, as was ensuring the safety of people who live on and visit the holding. This included measures such as the use of cattle vaccination, personal protective equipment (PPE), and taking care with hand hygiene and wound coverage when gardening and trapping, and when handling animal feed and firewood. The PIC shared these results with the case and with the community group that organised the "farm walks". The PIC advised that while many lifestyle block owners trap possums, they may not have awareness of leptospirosis. The PIC also advised that there was a local pet food company that used possum carcasses. The researchers contacted the regional council to raise awareness in the trapping/hunting networks, including school-based programmes on possum trapping and skinning (Northland Regional Council 2024).

Discussion

To the authors' knowledge, this case series represents the first attempt to detect pathogenic *Leptospira* spp. in both animal and non-animal putative sources using a comprehensive range of methods that revealed the complexity of potential exposure pathways associated with human leptospirosis cases in Aotearoa.

Case A was diagnosed with Ballum, the second most common serovar amongst human cases in New Zealand (Nisa *et al*. 2020) and considered highly associated with rodents (Moinet *et al*. 2021). However, it is not known whether rats (Hathaway and Blackmore 1981), mice (Moinet *et al*. 2023) or livestock (Wilson *et al*. 2021) were the source of infection in this case. The site was a dairy farm that practised leptospirosis vaccination; split calving was identified as a critical factor for consideration in the timing of an effective vaccination programme (Yupiana *et al*. 2021). The high titres to Pomona in livestock were unlikely to be vaccinal (Yupiana *et al*. 2019a), suggesting Pomona may either be endemic among cattle or associated with the wild pigs on the farm. Although a 1982[2] survey of wild pigs hunted in the North Island found no Pomona antibodies, and kidney cultures were negative, the presence of unvaccinated domestic pigs on a dairy farm has been linked to infections in both cattle (Yupiana *et al*. 2019b) and humans in 2016 (Benschop *et al*. 2017). Furthermore, cattle at this site had high titres to Tarassovi and urinary shedding, consistent with findings from a nationwide study of 200 dairy herds (Yupiana *et al*. 2019a). Genotyping confirmed that the strain shed in the urine was Pacifica, aligning with the previous study of 200 herds (Wilkinson *et al*. 2021).

The association between host animals shedding *Leptospira* and having a concurrent immune response (as measured by microscopic agglutination test; MAT) is complex. While positive association can be found at herd or flock levels, as with Tarassovi in dairy heifers (Yupiana *et al*. 2019a) and with Hardjobovis titres in prime sheep (Fang *et al*. 2015; Vallee *et al*. 2015), these associations are influenced by animal factors (age and vaccination status), pathogen factors (e.g. serovar or strain), test factors (timing and sensitivity) and environmental factors (e.g. season and endemicity). The five cattle identified as shedding pathogenic *Leptospira* spp. comprised three adult beef cows on Site C (one typed as *L. borgpetersenii*), and a dairy cow and a heifer (typed as *L. borgpetersenii* strain Pacifica) on Site A. Serology results were only available for 2/5 animals and were unremarkable (titres of 24 and 96 to Tarassovi), but at the herd level there were high titres pointing to recent infection. Thus, with a small case series, general inferences on the association between serology and shedding is not reliable, and different detection tools provide information that is not always comparable. However, this study demonstrates the value of repeated MAT investigations on groups of animals to provide information on the likely stage of infection, as was seen with Sites A and B. In general, MAT lacks specificity for the infecting serogroup

[2]JM Collins-Emerson: data on file.

(Levett 2003), limiting its use in individual cases. However, the limited number of serovars present in New Zealand (Wilkinson *et al*. 2024), which themselves mainly fall into different serogroups, allows for greater confidence in predicting the correct serovar from the MAT results compared to countries where multiple, serologically similar *Leptospira* strains may be circulating.

Environmental testing identified pathogenic *Leptospira* in1/36 samples by PCR. This is at odds with previous work where each of the 24 samples taken from a dairy farm were positive for pathogenic *Leptospira* (Wilkinson *et al*. 2021). However, only 10 of our 36 samples were taken from water (one of which was positive) while Wilkinson *et al*. exclusively tested water samples. Additionally, Wilkinson *et al*. defined positivity using targeted metabarcoding sequence data. This is a more sensitive method that can detect low-abundance species in a complex sample such as those taken from the environment and is less affected by inhibitors often found in such samples. Furthermore, environmental reservoirs of *Leptospira* are influenced by climatic drivers (Lau *et al*. 2010), as observed in the months that followed Cyclone Gabrielle (MPI 2023; Vallee *et al*. 2023). Therefore, detection of *Leptospira* in environmental samples may vary depending on rainfall, sample type (e.g. soil, water or mud), and the challenges associated with extracting DNA from the different sample types. Future research should focus on identifying the most effective DNA extraction kits and protocols for various types of environmental sample and employ methods such as metabarcoding that better assess diversity.

Lastly, this study highlighted the public health implications of leptospirosis to the wider community and provided an opportunity to raise awareness within these communities. The PIC of Sites A and C identified that, in their communities, possums were not recognised as potential sources of *Leptospira,* yet possums are frequently culled on farms by friends and community trappers, and there was a local pet food factory that processed possums. Discussions with the Northland Regional Council further identified that high school students attended programmes for National Certificate of Educational Achievement credits where they are "shown how to … skin possums and … hand pluck their fur" (Northland Regional Council 2024). Approaches to raise awareness in communities included a media release and bespoke educational materials for the Department of Conservation, community predator-free groups and hunters/trappers including the use of PPE, vaccination of dogs, and a description of symptoms. While leptospirosis is well recognised in Aotearoa's rural communities (Prinsen *et al*. 2023), it is important to realise that visitors or those new to rural settings may have no such knowledge. The fact that one of the three cases in this study was a city-dweller highlights that leptospirosis is not confined to those living rurally. Furthermore, both the veterinarian and the case from Site B said their farming community considered leptospirosis to be a dairy farm problem. They have since been informing their community of the risk through veterinary practice newsletters, meetings, and local field days.

This study had several limitations. Firstly, when this sub-study was originally proposed (Nisa *et al*. 2023), we had planned to enrol up to 20 cases, with the sampling of in-contact animals and environment planned to occur promptly after a patient became ill with leptospirosis. However, factors associated with the COVID-19 pandemic, such as travel restrictions and general caution about visitors on farms, meant it was difficult to arrange sampling. Livestock sampling by veterinarians was impacted by veterinary shortages. These factors led to delays between the onset of illness and sampling of animals and environmental sites of 7, 3 and 5 months for cases A, B, and C respectively. Furthermore, the number of PCR-positive animals from which we could identify the *Leptospira* genotype (species, serovar or strain) in both livestock and wildlife was low, limiting our ability to draw meaningful conclusions from the findings due to the restricted scope of the samples.

In conclusion, this study underscores the complexity of leptospirosis exposures in farm settings, revealing that both animal- (domestic and wildlife) and non-animal sources can contribute to infection risk. Given the multiple sources, definitive identification of the infection origin is unlikely, and effective prevention requires a combination of strategies, addressing various exposure pathways. The findings also emphasise the importance of timely vaccination and informed management practices (including pest control) within livestock populations to mitigate risks. Veterinarians need to be aware that there are multiple exposure pathways and be prepared to develop bespoke approaches for leptospirosis prevention and control. This study is an important "proof of concept" for a One Health approach, demonstrating the value and limitations of using diverse source investigation methods.

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References

***Adler B.** *Leptospira and Leptospirosis*. Springer, Heidelberg, Germany, 2015

Benschop J, Collins-Emerson J, Maskill A, O'Connor P, Tunbridge M, Yupiana Y, Weston J. Leptospirosis in three workers on a dairy farm with unvaccinated cattle. *New Zealand Medical Journal* 130 (1462), 102–8, 2017

Fang F, Collins-Emerson JM, Cullum A, Heuer C, Wilson PR, Benschop J. Shedding and seroprevalence of pathogenic *Leptospira* spp. in sheep and cattle at a New Zealand abattoir. *Zoonoses and Public Health* 62, 258–68, 2015. https://doi.org/10.1111/zph.12146

Goarant C, Picardeau M, Morand S, McIntyre KM. Leptospirosis under the bibliometrics radar: evidence for a vicious circle of neglect. *Journal of Global Health* 9, 010302, 2019. https://doi.org/10.7189/jogh.09.010302

Hathaway SC, Blackmore DK. Ecological aspects of the epidemiology of infection with leptospires of the Ballum serogroup in the black rat (*Rattus rattus*) and the brown rat (*Rattus norvegicus*) in New Zealand. *Journal of Hygiene* 87, 427–36, 1981. https://doi.org/10.1017/S0022172400069679

***Heuer C, Benschop J, Stringer L, Collins-Emerson JM, Sanhueza JM, Wilson PR.** *Leptospirosis in New Zealand – Best Practice Recommendations for the Use of Vaccines to Prevent Human Exposure*. https://nzva.org.nz/assets/Policies-Guidelines-Resources/Leptospirosis_NZ_Best_Practice_Massey.pdf (accessed 13 November 2024). Massey University, Palmerston North, NZ, 2012

***Institute of Environmental Science and Research**. *New Zealand Public Health Surveillance Report: July to September 2017*. https://www.sacnzs.org.nz/assets/1Reports/Surveillance-reports-and-dashboards/NZPHSR/2014-2018/NZPHSR2017Dec.pdf (accessed 24 January 2025). Institute of Environmental Science and Research Ltd., Porirua, NZ, 2017

Lau CL, Smythe LD, Craig SB, Weinstein P. Climate change, flooding, urbanisation and leptospirosis: fuelling the fire? *Transactions of the Royal Society of Tropical Medicine and Hygiene* 104, 631–8, 2010. https://doi.org/10.1016/j.trstmh.2010.07.002

Levett PN. Usefulness of serologic analysis as a predictor of the infecting serovar in patients with severe leptospirosis. *Clinical Infectious Diseases* 36, 447–52, 2003. https://doi.org/10.1086/346208

Marshall RB. A nationwide experiment to control human leptospirosis by vaccinating dairy cattle. *Israeli Journal of Veterinary Medicine* 43, 271–6, 1987

McLean M, Ruscoe Q, Kline T, King C, Nesdale A. A cluster of three cases of leptospirosis in dairy farm workers in New Zealand. *New Zealand Medical Journal* 127 (1388), 13–20, 2014

Moinet M, Wilkinson DA, Aberdein D, Russell JC, Vallee E, Collins-Emerson JM, Heuer C, Benschop J. Of mice, cattle, and men: a review of the eco-epidemiology of *Leptospira borgpetersenii* serovar Ballum. *Tropical Medicine and Infectious Disease* 6, 189, 2021. https://doi.org/10.3390/tropicalmed6040189

Moinet M, Oosterhof H, Nisa S, Haack N, Wilkinson DA, Aberdein D, Russell JC, Vallee E, Collins-Emerson J, Heuer C, *et al*. A cross-sectional investigation of *Leptospira* at the wildlife-livestock interface in New Zealand. *PLoS Neglected Tropical Diseases* 17, e0011624, 2023. https://doi.org/10.1371/journal.pntd.0011624

***MPI**. *Notification: Increased Risk of Leptospirosis Infection in Animals*. https://www.deernz.org/home/our-stories/notification-increased-risk-of-leptospirosis-infections-in-animals/ (accessed 15 February 2024). Ministry for Primary Industries, Wellington, NZ, 2023

Munoz-Zanzi C, Groene E, Morawski BM, Bonner K, Costa F, Bertherat E, Schneider MC. A systematic literature review of leptospirosis outbreaks worldwide, 1970–2012. *Revista Panamericana de Salud Publica* 44, e78, 2020. https://doi.org/10.26633/RPSP.2020.78

Mwachui MA, Crump L, Hartskeerl R, Zinsstag J, Hattendorf J. Environmental and behavioural determinants of leptospirosis transmission: a systematic review. *PLoS Neglected Tropical Diseases* 9, e0003843, 2015. https://doi.org/10.1371/journal.pntd.0003843

Nisa S, Wilkinson DA, Angelin-Bonnet O, Paine S, Cullen K, Wright J, Baker MG, Benschop J. Diverse epidemiology of *Leptospira* serovars notified in New Zealand, 1999–2017. *Pathogens* 9, 841, 2020. https://doi.org/10.3390/pathogens9100841

Nisa S, Vallee E, Marshall J, Collins-Emerson J, Yeung P, Prinsen G, Douwes J, Baker MG, Wright J, Quin T, *et al*. Leptospirosis in Aotearoa New Zealand: protocol for a nationwide case-control study. *JMIR Research Protocols* 12, e47900, 2023. https://doi.org/10.2196/47900

***Northland Regional Council**. *Project Pest Control*. https://www.nrc.govt.nz/projectpestcontrol (accessed 28 March 2024). Northland Regional Council, Whangārei, NZ, 2024

Prinsen G, Baker M, Benschop J, Collins-Emerson J, Douwes J, Fayaz A, Littlejohn S, Nisa S, Quin T, Yeung P. "We don't really do doctors." Messages from people diagnosed with occupational leptospirosis for medical professionals on infection, hospitalisation, and long-term effects. *Heliyon* 9, e19303, 2023. https://doi.org/10.1016/j.heliyon.2023.e19303

Vallee E, Heuer C, Benschop J, Collins-Emerson JM, Wilson PR. Serological patterns, antibody half-life and shedding in urine of *Leptospira* spp. in naturally exposed sheep. *New Zealand Veterinary Journal* 63, 301–12, 2015. https://doi.org/10.1080/00480169.2015.1049668

Vallee E, Borman B, Read D, Wada M. *First the Floods, Then the Diseases – Why NZ Should Brace for Outbreaks of Spillover Infections From Animals*. https://theconversation.com/first-the-floods-then-the-diseases-why-nz-should-brace-for-outbreaks-of-spillover-infections-from-animals-201162 (accessed 25 September 2024). The Conversation, Wellington, NZ, 2023

Victoriano AF, Smythe LD, Gloriani-Barzaga N, Cavinta LL, Kasai T, Limpakarnjanarat K, Ong BL, Gongal G, Hall J, Coulombe CA, *et al*. Leptospirosis in the Asia Pacific region. *BMC Infectious Diseases* 9, 147, 2009. https://doi.org/10.1186/1471-2334-9-147

***Virbac**. *Lepto 4-Way*. https://nz.virbac.com/lepto-4-way (accessed 1 November 2023). Virbac, Hamilton, NZ, 2023

Wilkinson DA, Edwards M, Benschop J, Nisa S. Identification of pathogenic *Leptospira* species and serovars in New Zealand using metabarcoding. *PLoS One* 16, e0257971, 2021. https://doi.org/10.1371/journal.pone.0257971

Wilkinson DA, Edwards M, Shum C, Moinet M, Anderson NE, Benschop J, Nisa S. Molecular typing of *Leptospira* spp. in farmed and wild mammals reveals new host-serovar associations in New Zealand. *New Zealand Veterinary Journal* 72, 1–9, 2024. https://doi.org/10.1080/00480169.2023.2248930

Wilson PR, Mannewald A, Collins-Emerson JM, Dreyfus A, Sanhueza JM, Benschop J, Verdugo C, Emanuelson U, Boqvist S, Heuer C. Serological study of *Leptospira interrogans* serovar Copenhageni and *L. borgpetersenii* serovars Tarassovi and Ballum in beef cattle, sheep and deer in New Zealand. *New Zealand Veterinary Journal* 69, 83–92, 2021. https://doi.org/10.1080/00480169.2020.1830867

Yupiana Y, Vallee E, Wilson P, Collins-Emerson J, Weston J, Benschop J, Heuer C. Emerging *Leptospira* strain poses public health risk for dairy farmers in New Zealand. *Preventive Veterinary Medicine* 170, 104727, 2019a. https://doi.org/10.1016/j.prevetmed.2019.104727

Yupiana Y, Wilson PR, Weston JF, Vallee E, Collins-Emerson JM, Benschop J, Scotland T, Heuer C. Epidemiological investigation of *Leptospira* spp. in a dairy farming enterprise after the occurrence of three human leptospirosis cases. *Zoonoses and Public Health* 66, 470–9, 2019b. https://doi.org/10.1111/zph.12578

Yupiana Y, Wilson PR, Collins-Emerson JM, Weston JF, Benschop J, Vallee E, Heuer C. Vaccination practices for *Leptospira* spp. on New Zealand dairy farms. *New Zealand Veterinary Journal* 69, 299–307, 2021. https://doi.org/10.1080/00480169.2021.1928563

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