Leptospirosis is a zoonotic illness caused by spirochaetes of the genus *Leptospira*. The organism is carried by animals with chronic renal infection, which excrete the organism in urine. Human infection is by direct or indirect contact with infected animal urine, and this can include contact with damp soil and water. In New Zealand, maintenance hosts include both domestic (eg, cattle, sheep, deer and pigs) and wild (eg, possums, hedgehogs and rodents) species. Six serovars have been isolated from New Zealand animals with *Leptospira interrogans* serovar Pomona (Pomona) and *Leptospira borgpetersenii* serovars Hardjo and Ballum responsible for most human cases. Different serovars are associated with one or more maintenance hosts that have persistent renal carriage of leptospires and generally little or no signs of infection. Humans are an accidental host for leptospires. Since domestic farmed species play an important role in transmission to humans in New Zealand, livestock vaccination is a mainstay for the control of human leptospirosis particularly in systems where there is regular and close contact between animals and people. Approximately 95% of dairy herds are vaccinated and all commercial pig producers are mandated to do so.

The clinical illness in humans is typically biphasic. The early phase (5–7 days) is characterised by flu-like symptoms of fever, muscle aches and headache. The late phase (4–30 days) is characterised by prolonged fever and a range of possible systemic complications, including jaundice, renal failure, respiratory insufficiency and confusion. Up to 30% of those with acute disease suffer long-term effects such as depression and chronic fatigue. In 2015...
there were 63 notified cases of leptospirosis in New Zealand. Of these, 36 were farmers or farm workers, nine worked in the meat processing industry and five others worked in close contact with animals. These numbers are likely a significant underestimate of true incidence as leptospirosis is under-ascertained both in New Zealand and internationally due to the non-specific clinical signs and the fact that the disease may be self-limiting. Additionally there may be a lack of awareness or suspicion by the doctor, and diagnostic tests are frequently not requested or the type of test selected is not optimal for the stage of the disease. In this report we describe a one-health investigation of three cases of leptospirosis among workers on a dairy farm with unvaccinated cattle in New Zealand.

Case history
The affected individuals were employed on a 130 hectare dairy farm in the North Island of New Zealand. This was a seasonal calving (planned start of calving 20 July) herd milking 230 adult cows through a rotary milking shed (Herd 1). One of the workers periodically raised a small number of pigs near the milking shed and there were four pigs present at the time of the disease outbreak. The farm owners also had an adjacent dairy farm covering 190 hectares and were milking 400 cows through a herring bone shed (Herd 2). The heifer replacements (approximately 160 in total each year) for both farms were reared together on nearby grazing blocks. None of the livestock on either of the farms had been vaccinated against leptospirosis and this had been the farm policy for at least 20 years. There had been no evidence of disease due to leptospirosis among livestock on the farms.

Three human cases of leptospirosis were notified to Whanganui Public Health between January and March 2015. All were employed to work only on one of the dairy farms (Herd 1) and their work involved milking and general farm duties. They had no contact with cows from the adjacent farm (Herd 2). The individual cases were followed up by phone interview using standard report forms by one of the co-authors (MT). In addition to the formal notification to health authorities, a co-author (JB) was notified in March 2015 by the local veterinarian attending the herd about two of the human cases. Serological testing of the cattle and pigs was organised through this veterinarian’s practice and the Institute of Veterinary Animal and Biomedical Sciences, Massey University (Massey University Animal Ethics Committee Approval No. 15/57). This case study has been recorded on the Low Risk Database, which is reported in the Annual Report of the Massey University Human Ethics Committee.

Clinical findings
Case A became acutely unwell while tramping on 25 January 2015 (day one), with sweats and chills, back and loin pain, and dry retching. He was transported to hospital by helicopter on day two and was treated with ceftriaxone. His condition was further complicated by hypotension, reduced renal function, pulmonary oedema and coagulopathy. On day six he was transferred to his local hospital, then discharged on day nine. Case A has continued to be unwell with severe lethargy, headaches and visual disturbances. He has not returned to full-time work.

Case B became unwell on 25 February 2015 with chills and sweats, shaking and polyuria. He was seen by a general practitioner on day two and commenced treatment with oral antimicrobials (doxycycline). After initial improvement he felt worse two days later and was admitted to hospital on day four. He responded well to IV ceftriaxone and was discharged on day seven. He was soon able to return to work with no ongoing problems.

Case C became unwell on 14 March 2015 with headache, and later that day muscle cramps and a high fever. He was seen at a local emergency clinic on day two, blood samples were collected and oral antibiotics (doxycycline) given. The headache worsened and he was seen at a hospital emergency department on day three. A CSF sample was taken and no abnormalities were detected. He was not admitted at this time but his clinical signs did not improve and he was admitted to hospital on day five. He was treated with IV fluids and oral doxycycline, and was discharged on day six. He recovered gradually over the next three months before returning to work full-time. He reports ongoing photosensitivity and alcohol intolerance.
Table 1: Summary of diagnostic tests performed on three cases of leptospirosis.

<table>
<thead>
<tr>
<th>Case</th>
<th>Leptospira IgM ELISA* screen</th>
<th>Leptospira DNA</th>
<th>Serology (microscopic agglutination test)</th>
<th>serovar</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st Sample</td>
<td>2nd Sample</td>
<td>Sample</td>
<td>Result</td>
</tr>
<tr>
<td>A</td>
<td>neg(2)</td>
<td>pos(13)</td>
<td>urine(4)</td>
<td>detected</td>
</tr>
<tr>
<td>B</td>
<td>neg(2)</td>
<td>pos(15)</td>
<td>urine(7)</td>
<td>detected</td>
</tr>
<tr>
<td>C</td>
<td>neg(2)</td>
<td>pos(18)</td>
<td>plasma(4)</td>
<td>detected</td>
</tr>
</tbody>
</table>

Numbers in parentheses and italics refer to the days post onset of symptoms.

*Bioline Leptospira IgM, Standard Diagnostics, Gyeonggi-do, Republic of Korea.

1-2PCR performed at Canterbury Health Laboratories (primers based on Merien et al)9 and at a regional hospital laboratory (primers based on Smythe et al)10 respectively. The MATs were performed at ESR using MoH funding.

Table 1 reports the diagnostic test results. In each case initial samples were taken for an ELISA screen and a microscopic agglutination test (MAT: reference antigen panel Hardjo, Pomona, Ballum, Tarassovi, Copenhageni, Canicola, Grippotyphosa and Australis) within 48 hours of the onset of symptoms. In all three cases the first Leptospira ELISA screen test and two of three initial MATs were negative. Leptospira DNA was detected as early as four days after the onset of symptoms.

Investigation of human cases

Case A was notified on 30 January 2015 and interviewed 9 February 2015 on return home from Hawkes Bay Hospital. Potential exposures included regular contact with cattle in dairy shed, being urinated on while in shed, having nicks and cuts on arms that were uncovered, contact with effluent and the recent shearing of sheep on lifestyle block.

Case B was notified on 4 March 2015 and interviewed 9 March 2015. He was the relief milker for Case A. Potential exposures included frequent contact with urine in the dairy shed, uncovered cuts on hands, splashes of urine in face, splashes of urine-contaminated water in face when washing down shed, exposed to blocked effluent pipe, which he repaired and he did not wash hands for some time afterwards, and exposure to pigs on the dairy farm.

Case C was notified on 16 March 2015 and interviewed 19 March 2015. He reported regular contact with cattle in dairy shed and that he was usually vigilant about wearing PPE in shed (apron, gumboots, gloves, eye protection). He owned cattle at his home block that were sourced from the employer’s farm.

Investigation of cattle and pigs

Eighteen to 30% of the adult milking cows from Herd 1 had blood convenience sampled on two occasions, 12 days apart, in early March 2015 to determine infection status. Blood was also collected once from each of the four pigs. At first sampling (6 March), 41/230 cattle (18%) were sampled, while at second sampling (18 March) a further 68 cattle (30%), including some of the previously sampled animals, were sampled. Serum was tested using the MAT against Leptospira borgpetersenii serovars Hardjo and Ballum and Leptospira interrogans serovars Copenhageni (Copenhageni) and Pomona. Urine was also collected from 33 cattle at the second sampling from Herd 1 to detect shedding of leptospires by real-time PCR targeting the gyrB gene. Cows from Herd 2 were also blood and urine sampled on the same dates and in similar proportions (10–22%). Testing was performed at the Molecular Epidemiology and Public Health Laboratory (EpiLab), Massey University.

In both herds on first sampling there were many high titres (≥384) to Pomona and Hardjo. On second sampling there was a rise in the proportion of seropositive animals with higher titres, suggesting recent infections with Pomona and Hardjo. This was more pronounced in Herd 1 where 35 (51%) of 68 had titres to Pomona at the second sampling with 21 of these 35 (60%) ranging from 384 to 3,072; the pattern was similar for Hardjo. In both herds there were cattle with titres to Ballum and Copenhageni but the number of positives and titre values were much lower and mostly seen in cows with high positive Hardjo and or Pomona titres, suggesting cross-reaction. For example in Herd 1, 19 (28%) of 68 had
Ballum titres ranging from 24 to 96, and 18 (95%) of these 19 also had high titres to either Hardjo or Pomona. Seven of 33 urine samples (21%) taken from cows in Herd 1 were real-time PCR positive.

All four pigs had titres to Pomona (1 at 3,072 and 3 at 1,536) and Copenhageni (1 at each of 192, 384, 1,536 and two at 1,536).

Follow-up

Farm staff and other visitors to the farm who were likely to come into contact with urine from the cows (eg, veterinarians) were advised to use personal protective equipment (aprons, gloves, visors and boots), pay attention to personal hygiene (eg, wash hands, no eating in the shed), be cautious around effluent and stop drinking raw milk. In addition, all farm staff in contact with the cows took oral doxycycline 200mg weekly and this continued until the end of the milking season (mid-May 2015).

All cattle (milking and non-milking) received a sensitising vaccine dose for Pomona and Hardjo on 17 March 2015 with a bivalent bovine leptospirosis vaccine (Leptoshield, Pfizer Animal Health, West Ryde, NSW, Australia). They received a booster one month later and at dry off, all milking cows were treated with long-acting amoxicillin (15mg/kg, IM, Betamox LA, Noorbook, VIC, Australia). The whole herd is now on annual vaccination with calves receiving sensitising and booster doses at approximately three and four months of age.

The pigs were immediately slaughtered and were not processed for consumption. A rodent control programme was implemented as there were reports of mice infesting the cattle feed (palm kernel expeller). An investigation into rodent Leptospira carriage on the farm is in progress.

Discussion

Leptospirosis is usually sporadic in New Zealand but outbreaks have previously occurred. There were four leptospirosis outbreaks reported in 2008 and two in 2010 involving 20 and five cases, respectively. The outbreaks were all from farm or abattoir settings with exposure to infected animals or carcasses either confirmed or suspected as the source of infection. Not all outbreaks are identified as such, for example in the 2008/2009 season, a small upper North Island abattoir experienced three human leptospirosis cases among 20 staff, of which two were hospitalised. Symptoms included fever, headache, diarrhoea and meningitis. Two of the cases were associated with serovar Pomona. No stock slaughtered at the small upper North Island abattoir had been vaccinated. In August and September 2010 three cases were reported by clinicians to the local public health service in Wairarapa. All three individuals had worked on the same dairy farm during their incubation period, where there was an inadequate herd vaccination programme. Two cases were hospitalised with serovar Hardjo infection. In October 2015, four meat workers from an eastern North Island meat plant that slaughters multiple livestock species were hospitalised with leptospirosis. Two are still suffering from post-leptospirosis symptoms—at the time of writing one is on shortened hours and the other unable to return to work. The outbreak at the eastern North Island meat plant was brought to the author’s (JB) attention by health and safety personnel at the plant. The vaccination status of livestock at this plant was unknown.

For the outbreak described in this case series, all three individuals reported direct contact with cattle urine in the milking shed. A further risk factor was the spray irrigation of dairy shed effluent, and all reported contact with effluent while moving the irrigation equipment. Case A reported being “drenched” with effluent. It was also noted that there were pigs on the farm, and at the rural residence of one of the cases.

There is debate about antibiotic treatment for leptospirosis, but there is a consensus that early treatment is better than delayed as it improves the prognosis. Although the cases here presented early in the clinical course (day 2) and were given antibiotics, all were hospitalised. There is also debate about the use of post-exposure prophylaxis, which was given to staff in-contact with the milking herd until the herd had antibiotic treatment at drying-off. This was also combined with ensuring that all people on the farm were aware of the risk factors for infection and understood the measures that should be taken to avoid infection such as good hygiene and use of personal protective equipment.
equipment to cover mouth, eyes, nose and skin-breaks, when in contact with the cattle or their effluent. Prophylactic doxycycline has been used after leptospirosis outbreaks following severe flooding. The cases presented here illustrate the complexities when using laboratory test results for timely diagnosis and the advantages of using a suite of tests rather than a single diagnostic test. In all cases, an initial screen for *Leptospira* IgM was negative—it is estimated that the median time from onset of symptoms to a positive test result is 7–14 days. PCR testing for *Leptospira* DNA is both an early (positive from four days post-onset) and rapid (the current turn-around time for this test when sent from a regional centre to a reference laboratory is about two days) test for leptospirosis. Most advice on management stresses the importance of a high level of suspicion for leptospirosis leading to clinical diagnosis and empirical treatment. Given the large proportion of cases in New Zealand among workers on farms and in the meat industry, occupational enquiry is an essential aspect of the clinical assessment.

In the current outbreak, two of three cases reported persistence of symptoms at least six months after the acute episode. Overseas work suggests up to 30% of those initially affected with leptospirosis suffer with symptoms long after they were first unwell. A pilot case series conducted by some of the co-authors (summer 2015/16) studied post-leptospirosis symptoms in six men, who had independently contacted researchers at Massey University due to the persistence of symptoms after their acute episode. Persistence was defined as six months after diagnosis of the acute episode. These men reported loss of employment, disruption of community involvement and high emotional and financial demand on their immediate families. Of the six interviewed, only two had returned to full-time employment. A common theme was the delay to diagnosis of the acute disease episode and reluctance of support workers to consider whether any persistent symptoms might be linked to the previous acute episode. The focus of leptospirosis research has been almost exclusively on the acute episode, a critical unanswered question is to describe and quantify post-leptospirosis symptoms so as to fully understand the burden leptospirosis places on rural New Zealanders, their immediate families and the community as a whole.

One unexpected finding with this outbreak of disease among workers on a dairy farm was the lack of evidence of clinical disease among the cattle. The majority of the cows on both farms would have been pregnant (1–6 months gestation at the time the farm workers became ill) and abortion is usually a common sequelae to infection, particularly with Pomona. Other common signs of leptospirosis in cattle (reduced milk production, inappetance, fever, mastitis, haemolytic anaemia, haemoglobinuria, jaundice, death) were not detected in either of the herds or among the younger cattle from these herds which were grazing nearby. Although we do not have definitive evidence, this adds weight to current discussions on the role of cattle in the epidemiology of Pomona infection; cattle may well be becoming a maintenance host for Pomona in New Zealand. Speciation of the *Leptospira* by sequencing the PCR positives would add support to this discussion but this was not attempted as in the outbreak situation the interest was in shedding per se rather than source attribution.

The New Zealand Veterinary Association developed a risk management programme (Leptosure™) in 2002 that was aimed specifically at dairy farms to protect farm staff and other farm visitors from leptospirosis. While vaccination of domestic animals is considered a mainstay of disease control, other factors should also be taken into account in case of a vaccine breakdown. Waterways should be fenced off to prevent livestock contaminating them as well as these being a source of infection. Rodents and other wildlife need to be controlled particularly around stored feed, as they can be a source of *Leptospira* serovars not included in the usual livestock vaccines, eg, Ballum. Pigs should not be kept on cattle farms as they are the main source of Pomona infection, which can cause serious disease in cattle. Good personal hygiene when working around livestock and use of personal protective equipment are recommended. People should not be allowed to eat, drink or smoke when working around cattle or in a potentially contaminated environment. All staff should wear heavy-duty plastic aprons, rubber boots
and gloves when milking cows to deflect urine splash. Gloves should be used for handling aborted material and people should cover cuts if they are going to be milking cows. Effluent requires careful disposal and should be sprayed onto paddocks well in advance of the next planned grazing. All cattle on the farm should be vaccinated from as early an age as possible; this includes beef cattle and breeding bulls, which are usually brought in for just a few months. Similarly, other species including deer, sheep, and dogs should be vaccinated against leptospirosis.

In summary, this outbreak of leptospirosis is likely to have occurred because dairy farm workers were exposed to unvaccinated dairy cattle and pigs. Combining PCR testing for *Leptospira* DNA with MAT serology provided an early test result and epidemiological information. During an outbreak, having an early definitive diagnosis will raise suspicion of leptospirosis in subsequent outbreak cases. Follow-up investigations on this farm as part of a project on leptospirosis at the wildlife-domestic species interface has shown Ballum infection, PCR-positive kidneys in 22 (96%) of 23 field mice captured in October 2016, and sero-positivity in 26 (12.8%) of 203 mixed-aged cattle sampled in April 2017. Thus, an integrated approach to control must continue to include vaccination of stock, use of personal protective equipment, personal hygiene, rodent control and shared awareness of risks. To date there have been no further human cases of leptospirosis associated with this herd.

1. Marshall RB, Manktelow BW. Fifty years

**Competing interests:**
Nil.

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