


ORIGINAL ARTICLE

Investigation and response to an outbreak of leptospirosis among raspberry workers in Australia, 2018

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Abstract

Background: In 2018, an outbreak of leptospirosis was identified among raspberry workers from a mixed-berry farm in New South Wales, Australia. Initial testing had not revealed a cause, but eventually leptospirosis was detected via polymerase chain reaction (PCR). Further serological testing detected *Leptospira borgpetersenii* serovar Arborea, of which rodents are the predominant reservoir. Leptospirosis is rare in Australia, with outbreaks usually related to flooding. We conducted an investigation to identify risk factors for infection, to inform control measures.

Methods: Cases were detected through laboratory notifications, hospital-based syndromic surveillance, awareness-raising among farm employees and clinician alerts. Confirmed cases had a four-fold rise in antibody titre or single titre ≥ 400 on microscopic agglutination test, and a positive IgM. Probable cases had a positive *Leptospira* PCR or IgM, and possible cases had a clinically compatible illness. We conducted a case-control study among raspberry workers on the farm and compared reported exposures between cases and seronegative controls. We assessed environmental risks on-site and tested rodents for leptospirosis.

Results: We identified 84 cases over a 5-month period (50 confirmed, 19 probable and 15 possible). Compared with controls, cases were less likely to wear gloves and more recently employed. Cases also more commonly reported always having scratched hands, likely from the thorns on raspberry plants. We observed evidence of rodent activity around raspberry plants and three of thirteen trapped mice tested positive for *Leptospira* Arborea. Control measures included enhanced glove use, doxycycline prophylaxis and rodent control.

Conclusions: This is the largest known outbreak of leptospirosis in Australia. Workers were likely exposed through scratches inflicted during harvesting, which became contaminated with environmental leptospires from mice. Leptospirosis should be considered an occupational risk for raspberry workers, requiring protective measures.

Chemoprophylaxis may assist in controlling outbreaks. PCR assists in the early diagnosis and detection of leptospirosis and should be included in surveillance case definitions.

KEYWORDS

agricultural workers' diseases, disease outbreaks/prevention & control, environment and public health, leptospira/immunology, leptospirosis, zoonoses

1 | BACKGROUND

Leptospirosis is a zoonotic disease caused by pathogenic spirochaetes from the genus *Leptospira*. It is transmitted to humans through direct contact of abraded skin or mucous membranes with infected animal urine or tissue, or indirectly via contaminated water, vegetation or soil (Galloway, Guerra, & Shadomy, 2015). The incubation period is 2–30 days (usually 5–14). Clinical disease ranges from asymptomatic infection to a mild self-limiting febrile illness, or to fulminant, potentially fatal conditions.

There is a range of animal reservoirs, but rodents are the most important source of human infection (Haake & Levett, 2015) and the principal host of *Leptospira borgpeterseni* serovar Arborea (Lau, Skelly, Dohnt, & Smythe, 2015). Leptospirosis is endemic in tropical regions and considered an important re-emerging disease due to changing risks groups, increasing magnitude and frequency of outbreaks and the emergence of new predominant serovars (Hartskeerl, Collares-Pereira, & Ellis, 2011; Lau et al., 2015). However, in Australia, outbreaks are rare and usually related to flooding. Sporadic cases typically have recreational or occupational exposures, frequently sugar cane or banana farming, abattoir work or veterinarian work (Lau, Townell, Stephenson, van den Berg, & Craig, 2018).

In May 2018, local doctors reported an increased number of berry pickers presenting to hospital with a febrile illness in northern New South Wales (NSW), Australia. Although this had been recognized since April, investigations for a range of pathogens, including leptospirosis serology, had not revealed a cause. Eventually, leptospirosis was detected in a number of cases via polymerase chain reaction (PCR) on blood specimens. Initial interviews revealed that all patients were raspberry workers from a single mixed-berry farm, and many were recent migrants or backpackers on short-term work visas. The farm is in a subtropical berry-growing region and employs over 2,000 people during peak picking season. Raspberries are picked year-round, and blueberries are picked from June to January, with pickers paid by weight of fruit picked.

As outbreaks of leptospirosis are rare in NSW and specific exposure and risk factors for berry workers not previously understood, we conducted an investigation to identify the outbreak source and risk factors for infection and to help guide prevention and control measures.

Impacts

- We investigated the largest known outbreak of leptospirosis in Australia, among raspberry workers on a berry farm. We think workers were infected through scratches on their hands, which became contaminated with leptospores from mice in the picking environment. Raspberry workers were likely at increased risk of scratches due to the thorns present on raspberry plants and from inconsistent use of inadequately protective gloves. Protective gloves should be worn in the future.
- To protect workers during the outbreak, we gave them antibiotic prophylaxis with doxycycline. Antibiotic prophylaxis against leptospirosis has been used in limited settings, usually for short-term exposures in endemic areas, but its use in outbreak control in an occupational context appears novel.
- Polymerase chain reaction (PCR) can be used to diagnose leptospirosis in the early phase in the illness (which serological testing usually cannot). Therefore including PCR in surveillance definitions can enhance case detection.

2 | METHODS

2.1 | Case detection

Cases were identified through several enhanced active surveillance measures, in addition to routine passive surveillance. Leptospirosis is a notifiable disease in Australia, and in NSW laboratories send positive test results for leptospirosis to public health authorities. We reviewed previous and prospective notifications across the state and followed up cases with possible links to the farm or region. We conducted real-time syndromic surveillance of berry pickers presenting to local hospitals, through a keyword alert of electronic emergency triage notes (Muscatello et al., 2005), and followed up patients with clinically compatible symptoms for testing. Additionally, we identified cases by contacting local primary care doctors, sending alerts to clinicians, raising awareness among farm employees and asking if cases had any unwell colleagues or contacts.

2.2 | Study design

As all identified cases occurred in raspberry workers on the farm (and not among blueberry pickers, other workers on the farm, or the wider community), we conducted a case-control study among raspberry workers on the farm. Raspberry workers were based in nine teams, each containing raspberry pickers, "runners," packers and supervisors who were all eligible for inclusion.

Cases were defined as people who reported symptom onset after 1 April 2018 and worked in a raspberry team for any duration in the 30 days before onset. The confirmed case definition used Australia's notifiable leptospirosis case definition, which only defines "confirmed" cases. The probable case definition drew on the national laboratory case definition, which includes detection of pathogenic *Leptospira* species by nucleic acid test as a suggestive criterion (Communicable Diseases Network Australia, 2004; Public Health Laboratory Network, 2008). Confirmed cases were people with either: (a) a four-fold or greater rise in *Leptospira* microscopic agglutination test (MAT) antibody titre between acute and convalescent sera (obtained ≥ 14 days apart); (b) a single MAT titre $\geq 1:400$ plus a positive enzyme-linked immunosorbent assay (ELISA) IgM; or (c) a positive culture for a pathogenic *Leptospira* species. Probable cases had clinically compatible symptoms plus either a positive *Leptospira* PCR or IgM (with convalescent serology unavailable). Possible cases had a clinically consistent illness, including a history of fever or rigours plus at least one other compatible symptom.

Controls were opportunistically recruited from current raspberry workers on-site or at a temporary clinic held in late July. Controls had to have worked in a raspberry team for at least two weeks since the start of the outbreak and prior to June 20, when on-site investigations commenced. If potential controls reported a history of clinically compatible symptoms, they were recruited as a possible case and offered serological testing. All controls were asked to provide a blood sample, to serologically screen for asymptomatic infection.

Study staff obtained verbal consent and interviewed participants using a structured questionnaire developed for this outbreak, using an interpreter (in-person or via telephone) if required. The questionnaire collected information on demographics, employment, clinical history, work practices and possible risk factors. Cases were asked about exposures during their incubation period, and controls were asked about exposures during the month of May, chosen as a representative period during the outbreak.

Sample size calculations were performed based on a range of risk-factor exposure prevalences among controls (20%–50%). The most conservative estimate (20% exposure) found that with 60 cases, 68 controls would be required to detect an odds ratio (OR) of ≥ 3 with 80% power and 95% confidence. To enable analysis of seronegative controls only, allowing for a 20% asymptomatic infection rate and 25% of controls declining blood collection, 112 controls were required to be recruited.

This investigation was carried out under provisions of the NSW Public Health Act 2010.

2.3 | Laboratory methods

Sera from cases and controls were screened for IgM antibodies to *Leptospira* using a commercially available ELISA (Panbio). IgM-positive sera were further tested by MAT, which detects leptospire-specific antibodies (IgM or IgG) using a panel of live organisms representing the serovars indigenous and exotic to the region (Table S1) (Faine, Adler, Bolin, & Perolat, 1999). The antigen with the highest associated titre was taken to represent the infecting serovar. Asymptomatic infection was defined as a positive IgM and MAT titre $\geq 1:400$, in a person recruited as a control (i.e. who did not report any history of illness).

Polymerase chain reaction was performed on sera collected within 7 days of onset, where available, using a TaqMan real-time PCR that targets the *rrs* (16S rRNA) gene in leptospires (Slack et al., 2007).

No patients had *Leptospira* culture performed, due to local unavailability of the required Ellinghausen-McCullough-Johnson-Harris (EMJH) media.

2.4 | Statistical analyses

Analysis was per an unmatched case-control study. Confirmed and probable symptomatic cases and asymptomatic IgM-negative controls were included in the primary analysis.

We compared the distribution of teams in which cases and controls worked, using cross-tabulation, unadjusted ORs and chi-squared tests.

For each questionnaire item, distribution of responses among cases and controls was examined using contingency tables, and associations tested using unadjusted ORs and Wald tests, calculated from univariable logistic regression. Covariates associated with illness on crude analysis ($p < .2$) were included in an unconditional multivariable logistic regression model, with age and sex included a priori. Stepwise backwards elimination was used to determine the final model of factors independently associated with illness, using $p \geq .05$ for removal of variables. Where multiple variables described the same exposure (e.g. "any glove use" and "frequency of glove use"), both were examined for a univariable association with illness. If the ordinal variable showed an association stronger than or equal to that of the binary variable, and there was evidence of a dose-response relationship, the ordinal variable was considered for inclusion in the final model, otherwise the binary variable was used. Variables were checked for correlation and where two variables were highly correlated, only one was considered for inclusion in the model (or to tease out correlations with having a picking role, a sensitivity analysis was performed). As glove use and having scratched hands were associated, and scratches were considered to lie on the causal pathway between glove use and infection, we included glove use in the multivariable model, because self-assessment and recall of this variable was considered likely to be more accurate.

Three sensitivity analyses were conducted: firstly, including controls who were not screened for asymptomatic infection; secondly, on a subgroup of only people who worked in a picking role (including all controls); and thirdly, using a forward selection process for model selection. Additionally, to help explain some observed associations between illness and certain variables that were not deemed to be causal, we performed exploratory bivariable analysis of these variables with other factors.

All analyses were on a complete-case basis, using Stata v14.

2.5 | Environmental investigations

We conducted three farm inspections, to understand operations and assess potential sources and risk factors for infection, including work practices, handwashing, protective equipment and drinking water supply. The farm and surroundings were explored for possible contributing factors, such as rodent habitats, surface water and land use. The location of fields in which cases and raspberry teams had worked over the outbreak period was mapped. We reviewed rainfall data from the nearest weather station (Australian Bureau of Meteorology, 2018).

Additional rodent traps were placed around the farm, and samples of caught mice were sent to a reference laboratory for leptospirosis testing. Mouse serum was tested via MAT as per human testing. Frozen kidney specimens were tested via PCR and culture. Kidney specimens for culture were transported in semi-solid EMJH media and subsequently subcultured into EMJH broth.

3 | RESULTS

3.1 | Outbreak description

We identified 84 cases during the outbreak, including 50 confirmed, 19 probable and 15 possible cases. The outbreak occurred over a 140-day period, from April 14 to August 31, 2018 (Figure 1).

The median age of cases was 30 years, and 50 (60%) of 84 cases were male. All cases reported raspberry work during their incubation period. No cases were identified among other farm employees, including blueberry workers, or in the wider region, including other farms.

Employment records showed 642 people had worked in a raspberry team during the outbreak period, yielding a crude attack rate of 11% (69/642) for confirmed and probable cases, and 13% (84/642) for all cases.

In most cases, clinical disease was mild; many cases did not seek medical care during their illness and were diagnosed retrospectively. There were no deaths, and we did not identify any severe complications. However, 22 (32%) of 69 confirmed or probable cases reported at least one night's hospital stay.

3.2 | Laboratory results

All 69 confirmed and probable cases were anti-leptospiral IgM-positive, of which 58 (84%) had a reactive MAT. Of these, 57 had highest (or equal highest) titres against *Leptospira* Arborea, including 47 cases with titres $\geq 1:400$ and 28 with titres $\geq 1:800$. One case had a higher titre against *Leptospira interrogans* serovar Zanoni (1:1,600) than *Leptospira* Arborea (1:800). This case had also been diagnosed with leptospirosis 1 year earlier, with mixed MAT results at that time.

Forty-one (59%) of 69 confirmed and probable cases had *Leptospira* PCR performed on acute sera (the remaining cases did not have acute sera collected or available for PCR testing). Thirty-seven (90%) were positive, comprising 54% of the 69 confirmed and probable cases.

We recruited 115 potential controls and 82 (71%) consented to serological screening. Of these, 13 (16%) were anti-leptospiral IgM-positive. Only one participant had a MAT titre $\geq 1:400$ (1:800 against *Leptospira* Arborea), giving an asymptomatic infection rate of 1%.

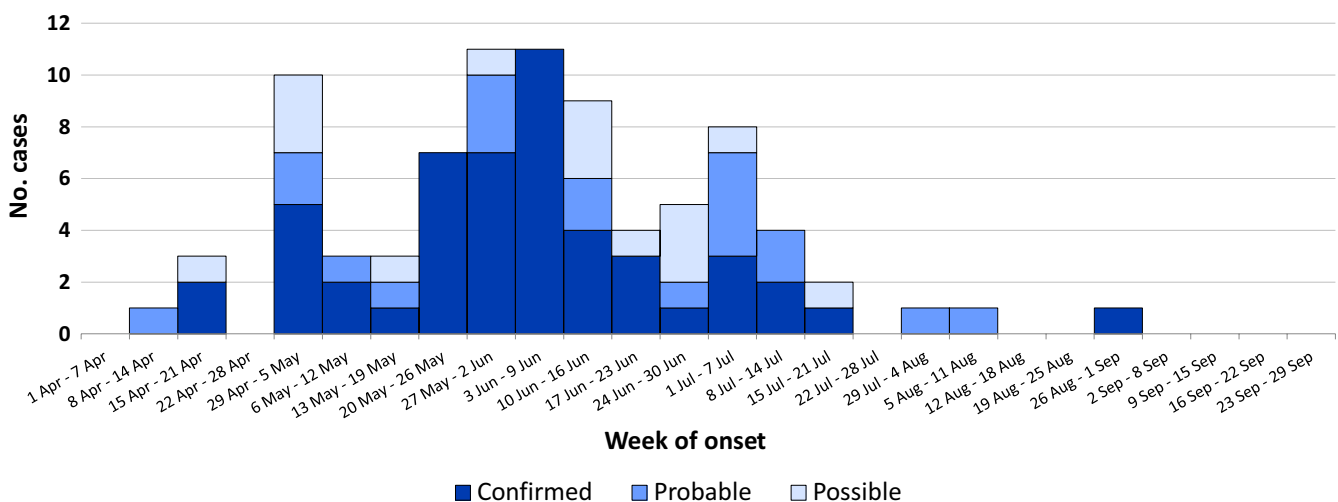


FIGURE 1 Distribution of leptospirosis cases, by case status and week of onset, during an outbreak among raspberry workers, New South Wales, Australia, April–August 2018 [Colour figure can be viewed at wileyonlinelibrary.com]

3.3 | Case-control study findings

We interviewed 67 of 69 confirmed and probable cases, and these cases were included in the primary analysis, with the 69 IgM-negative controls.

Similar proportions of cases and controls worked in each of the nine raspberry teams (Table S2). In crude analysis, there was no association between case status and age, sex, packing work, hand-washing, skin contact with irrigation water, working in the rain, eating berries at work, drinking from disposable cups, using a personal water bottle or receiving a scratch that bled (Table 1). Cases were more likely than controls to have required an interpreter for interview and to report working in a picking role, having skin contact with mud, seeing rodents, drinking water from a trailer in the field, and “always” having scratches on their hands. There was a protective association with being employed for longer, working in a supervisor role and wearing gloves, with the greatest association seen in those reporting “always” wearing gloves at work.

In multivariable analysis (Table 1), compared to controls, cases were found to have been employed for shorter (for each additional year worked, OR: 0.76, 95% confidence interval [CI] 0.61–0.95), were more likely to require an interpreter (OR: 4.00, 95% CI: 1.63–9.86) and report seeing rodents (OR: 7.09, 95% CI: 1.29–38.93), and less likely to wear gloves (OR: 0.30, 95% CI: 0.12–0.75).

To explore why employment duration and requiring an interpreter may be associated with disease, we assessed the associations of these variables. Participants who reported having scratches on hands had been employed for less time (median 0.46 vs. 3.00 years, Mann-Whitney $p < .0001$) as had pickers (median 0.55 years for pickers, vs. 3.50 years for non-pickers, $p = .017$). Participants who required an interpreter were more likely to work in a picking role (64/65 [98%] vs. 125/140 [89%], chi-squared $p = .023$) and less likely to be a supervisor (0/65 [0%] vs. 18/140 [13%], Fisher's exact test $p = .002$).

Sensitivity analyses produced largely similar results, with the same variables retained in the multivariable models, with comparable effects (Table S3).

3.4 | Environmental investigations findings

The farm covered 930 ha, including 280 ha of crops, of which approximately one-third comprised raspberry fields. Raspberry teams rotated through different fields each day. No clustering of cases in specific areas was identified. Raspberry plants were grown on trellises in covered, open-sided tunnels.

Workers were provided with cotton gloves which workers cut the tips off to assist picking (Figure 2). Potable water was transported by tanker from the local government's treated piped water supply and stored in sealed water tanks on-site, before being transferred to containers on a trailer in each field. No clear opportunities for contamination were identified.

Farm management reported several changes on the farm in the preceding year, including expansion of one of the five on-site

irrigation dams, removal of scrap metal from some areas and expansion of new fields for crops. The farm borders other farms and areas of native woodland vegetation.

Evidence of rodent activity (droppings, rodent holes and dead mice) was seen around the raspberry plants and storage sheds. Mice (*Mus musculus*) were caught in the rodent traps. Farm pest-control staff reported that mice numbers were not above usual levels, but this was unquantified. Thirteen mice were referred for laboratory testing (caught at two time-points, in June and July). Three (25%) of 12 mice tested via MAT were positive for *Leptospira* Arborea antibodies (titres 1:200–1:400; serum not collected from one mouse). Three (23%) of 13 kidney specimens tested via PCR were positive, from the same mice that were MAT-positive. Culture could not be performed due to bacterial overgrowth of the kidney specimens during transportation.

Meteorological records showed above usual rainfall during March (162 mm vs. a 12-year median of 98 mm), but rainfall in all other months in 2018 before and during the outbreak (range: 11–62 mm per month) was below median.

4 | DISCUSSION

We identified 69 confirmed or probable cases of leptospirosis, and 15 possible cases, making this the largest known site-specific outbreak in Australia. All cases were raspberry workers from a single farm. While cultivated varieties of thornless raspberry bushes are available, this farm used the standard thorny variety. Not wearing gloves, seeing rodents, requiring an interpreter and being employed more recently were risk factors for disease, and there was suggestive evidence that “always” having scratches on hands was a risk factor. Laboratory investigation indicated the putative serovar was *Leptospira* Arborea, the presence of which was also demonstrated in trapped mice.

Workers were likely infected through exposed scratches on their hands, which came into contact with leptospire from mouse urine in the picking environment. Raspberry workers were likely at increased risk of scratches due to the thorns present on raspberry plants (not present on blueberry plants) and from inconsistent use of inadequately protective gloves. While we considered other potential transmission routes, particularly drinking water which was associated with illness on crude analysis, these were not supported by environmental investigations and did not fit the distribution of disease being among raspberry workers only. The lack of strong association with reporting scratches on hands may be due to recall error or underreporting of small scratches deemed insignificant.

The association with shorter employment duration may be partially explained by longer-term employees working in different roles, such as supervisory roles. However, this relationship remained even in the subgroup analysis of pickers. Selection bias may have contributed, since only current employees were recruited as controls, and longer-term employees may have been more likely to participate, due to more personal interest in the outbreak. The association is

TABLE 1 Crude and adjusted analysis of the distribution of potential risk factors between leptospirosis cases and controls

| Risk factor | Group | Cases (%) | Controls (%) | Crude OR | 95% CI | <i>p</i> value ^a | Adjusted OR ^b | 95% CI | <i>p</i> value ^a |
|---|---------------------|-------------|--------------|--------------|------------|-----------------------------|--------------------------|------------|-----------------------------|
| Male sex | | 42/67 (63) | 32/69 (46) | 1.94 | 0.98–3.85 | .057 | | | |
| Median age (years) | | 29 | 32 | 0.99 | 0.95–1.02 | .43 | | | |
| Median years employed | | 0.54 | 1 | 0.84 | 0.71–0.96 | .045 | 0.76 | 0.61–0.95 | .015 |
| Interpreter required | | 32/66 (48) | 18/65 (28) | 2.46 | 1.19–5.08 | .015 | 4.00 | 1.63–9.86 | .003 |
| Raspberry picking work | | 65/67 (97) | 60/69 (87) | 4.87 | 1.01–23.48 | .048 | | | |
| Raspberry packing work | | 23/67 (34) | 29/69 (42) | 0.72 | 0.36–1.44 | .36 | | | |
| Raspberry supervisor work | | 0/67 (0) | 8/69 (12) | ^c | | | | | |
| Any handwashing | | 58/58 (100) | 69/69 (100) | undefined | | | | | |
| Skin contact with irrigation water | | 23/64 (36) | 17/69 (25) | 1.72 | 0.81–3.63 | .16 | | | |
| Skin contact with mud | | 25/55 (45) | 17/68 (25) | 2.50 | 1.17–5.36 | .019 | | | |
| Worked in the rain | | 49/58 (84) | 57/62 (92) | 0.48 | 0.15–1.52 | .21 | | | |
| Saw rodents | | 10/67 (15) | 2/69 (3) | 5.88 | 1.24–29.93 | .026 | 7.09 | 1.29–38.93 | .024 |
| Ate berries at work | | 29/67 (43) | 31/69 (45) | 0.94 | 0.48–1.84 | .85 | | | |
| Drank water from trailer | | 61/67 (91) | 54/69 (78) | 2.84 | 1.02–7.79 | .045 | | | |
| Drank trailer water using cup | | 42/67 (63) | 48/69 (70) | 0.74 | 0.36–1.50 | .40 | | | |
| Drank from own water bottle | | 31/67 (46) | 37/69 (54) | 0.74 | 0.38–1.46 | .39 | | | |
| Had any scratches on hands | | 52/67 (78) | 43/69 (62) | 2.10 | 0.99–4.45 | .054 | | | |
| Frequency of scratches on hands (vs. "never") | Rarely | 11/67 (16) | 11/69 (16) | 1.73 | 0.61–4.95 | .31 | | | |
| | Often | 13/67 (19) | 14/69 (20) | 1.61 | 0.60–4.32 | .34 | | | |
| | Always | 28/67 (42) | 18/69 (26) | 2.70 | 1.13–6.43 | .025 | | | |
| Received a scratch that bled | | 17/48 (35) | 15/42 (36) | 0.99 | 0.42–2.34 | .98 | | | |
| Any glove use (vs. "never") | | 39/67 (58) | 53/69 (77) | 0.42 | 0.20–0.88 | .022 | 0.30 | 0.12–0.75 | .010 |
| Frequency of glove use (vs. "never") | Mostly did not wear | 3/62 (5) | 2/69 (3) | 0.91 | 0.14–6.02 | .92 | | | |
| | Mostly wore | 9/62 (15) | 6/69 (9) | 0.91 | 0.28–3.01 | .88 | | | |
| | Always wore | 22/62 (35) | 44/69 (64) | 0.30 | 0.14–0.67 | .003 | | | |

Abbreviations: CI, confidence interval; OR, odds ratio.

^aWald test *p* value.

^bAdjusted for all other variables in the multivariable model. *n* = 120.

^cOR = 0 due to a zero cell value. Fisher's exact *p* value = .006.

less likely due to prior infection, and therefore immunity, because controls had all screened IgM-negative (which can persist for years (Cumberland, Everard, Wheeler, & Levett, 2001)) and there have only been three notified cases of leptospirosis linked to the farm since 2011. However, we cannot exclude immunity as a cause, as we did not screen for leptospiral IgG and prior cases may not have been diagnosed.

The association with requiring an interpreter may partially relate to non-English speakers being more likely to work in picking roles. It may also reflect selection bias in over-recruitment of English-speaking controls, despite significant efforts to use interpreters and translate study material into commonly spoken languages.

Our investigations had a number of strengths. The multimodal case detection, thorough case follow-up and efforts made to collect



FIGURE 2 The cotton gloves worn by raspberry workers, with two finger tips cut-off [Colour figure can be viewed at wileyonlinelibrary.com]

convalescent serology and test stored sera allowed us to identify many cases. We serologically screened controls to avoid bias towards the null from including asymptomatic cases as controls. Another strength was the comprehensive and iterative environmental investigations.

Nonetheless, there are some limitations to our investigations. Outbreak detection was delayed, partially as cases went undiagnosed. This is likely due to over-reliance on serological testing for acute diagnosis, which is typically negative in the acute phase of illness (Katz, 2012; Lau et al., 2015). PCR may be underutilized because it is not included in Australia's leptospirosis surveillance case definition (Communicable Diseases Network Australia, 2004). We note that the case definition has not been reviewed since 2004. While leptospirosis PCR assays are not yet standardized in Australia, and some have the potential to cross-react with other organisms (Villumsen et al., 2012), development of a "probable" case definition for people with a positive PCR test would allow increased and earlier detection of cases of leptospirosis, and encourage confirmatory testing with convalescent serology.

The delayed detection meant some cases were interviewed well after their illness. Therefore recall bias and social desirability bias are likely factors in some associations, particularly as exposures were self-reported and some related to work rules (e.g. handwashing). The higher reporting of seeing rodents by cases may be due to awareness that leptospirosis is transmitted via rodents and is not causal in itself. Raspberry consumption may have been under-reported, as workers were not allowed to eat berries during shifts. This underreporting was likely non-differential with respect to outcome, potentially biasing this effect-estimate towards the null. However, we considered raspberry consumption an unlikely transmission route, given the absence of cases in other people who would have consumed raspberries (on-site and in the community). Finally, we asked controls about exposures during a representative period during the outbreak, as they were unable to be recruited

concurrently throughout the outbreak. This may have led to differential recall bias among controls. However, recall bias was also a potential issue for cases, and all participants likely provided answers based on their usual practice which is unlikely to have varied significantly during this time.

Environmental investigations, while comprehensive, could not definitively demonstrate the presence of rodent urine around the raspberry plants, and molecular testing for leptospires in the environment is not routinely available due to the lack of validated protocols (Wynwood, Graham, et al., 2014). Furthermore, we did not test other animals reported on-site for leptospirosis, for example kangaroos, which have been found to carry *Leptospira* Topaz (Roberts, Smythe, Dohnt, Symonds, & Slack, 2010).

Agricultural work is a known risk factor for leptospirosis in Australia (Lau et al., 2018); however, raspberry workers have not previously been recognized as specifically at risk. There has been one other reported leptospirosis outbreak on a berry farm, of *L. interrogans* serovar grippityphosa among strawberry harvesters in Germany (Desai et al., 2009). Similar to our findings, harvesting with open hand wounds in the rain and accidental rodent contact were the main risk factors identified.

We recommended a number of control measures throughout the outbreak. Early in the response, we promoted hand hygiene practices, protective clothing use including gloves and avoiding contact with mud. Rodent control measures were also implemented. Following analysis of the case-control study, we focused on promoting impervious glove use among raspberry workers, to prevent scratches from coming into contact with any rodent urine.

Over 11 days in late July, we provided 114 raspberry workers, of 230 employed at the time, with four weeks of doxycycline prophylaxis (200 mg weekly). Chemoprophylaxis against leptospirosis has been used in settings such as short-term exposures in endemic areas or following high-risk events such as flooding (Schneider et al., 2017; Sehgal, Sugunan, Murhekar, Sharma, & Vijayachari, 2000; Takafuji et al., 1984). However, its use in outbreak control in an occupational context appears novel. It is unclear which, if any, control measures contributed to the outbreak subsiding, though a combination of measures and conditions may have been involved.

It is unclear why this outbreak occurred at this time and location, and whether any environmental factors drove transmission. Land-use changes may have displaced local rodent populations, and rainfall around the outbreak period would have been conducive to leptospire survival in the environment, though unlikely entirely explanatory. The first case of leptospirosis due to *Leptospira* Arborea in Australia was diagnosed in 1998, in northern NSW (Slack, Symonds, Dohnt, & Smythe, 2006). In Queensland, to the north of NSW, the range, number and proportion of cases due to *Leptospira* Arborea have increased since 2001 (Lau et al., 2015). Environmental factors hypothesized to have contributed to this emergence of *Leptospira* Arborea in Queensland include population growth, land-use changes including agricultural intensification and deforestation, increased flooding and climatic changes (Lau et al., 2015; Wynwood, Craig, et al., 2014).

Leptospirosis should be considered an occupational risk for raspberry workers, especially considering changing drivers of transmission in Australia and globally. This outbreak highlights the role of PCR in the early diagnosis of leptospirosis, and for this reason, PCR should be used clinically and in surveillance case definitions. A range of control measures was implemented, including use of protective gloves, chemoprophylaxis and rodent control. The use of chemoprophylaxis in controlling outbreaks of leptospirosis is uncommon and warrants further evaluation.

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CONFLICT OF INTEREST

All authors have completed an ICMJE conflict of interest form and declare no conflicts of interest.

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REFERENCES

- Australian Bureau of Meteorology (2018). *Climate Data Online*. Retrieved from <http://www.bom.gov.au/climate/data/>
- Communicable Diseases Network Australia (2004). *Leptospirosis case definition*. Retrieved from http://www.health.gov.au/internet/main/publishing.nsf/content/cda-surveil-nndss-casedefs-cd_lepto.htm
- Cumberland, P., Everard, C. O., Wheeler, J. G., & Levett, P. N. (2001). Persistence of anti-leptospirosis IgM, IgG and agglutinating antibodies in patients presenting with acute febrile illness in Barbados 1979–1989. *European Journal of Epidemiology*, *17*, 601–608. <https://doi.org/10.1023/A:1015509105668>
- Desai, S., van Treeck, U., Lierz, M., Espelage, W., Zota, L., Sarbu, A., ... Jansen, A. (2009). Resurgence of field fever in a temperate country: An epidemic of leptospirosis among seasonal strawberry harvesters in Germany in 2007. *Clinical Infectious Diseases*, *48*, 691–697. <https://doi.org/10.1086/597036>
- Faine, S., Adler, B., Bolin, C., & Perolat, P. (1999). Methods. In S. Faine, B. Adler, C. Bolin & P. Perolat (Eds.), *Leptospira and Leptospirosis* (pp. 169–184). Melbourne, Australia: MediSci Press.
- Galloway, R., Guerra, M., & Shadomy, S. (2015). Leptospirosis. In D. L. Heymann (Ed.), *Control of communicable diseases manual: An official report of the American public health association* (pp. 348–353). Washington, DC: American Public Health Association Press.
- Haake, D. A., & Levett, P. N. (2015). Leptospirosis in humans. *Current Topics in Microbiology and Immunology*, *387*, 65–97. https://doi.org/10.1007/978-3-662-45059-8_5
- Hartskeerl, R. A., Collares-Pereira, M., & Ellis, W. A. (2011). Emergence, control and re-emerging leptospirosis: Dynamics of infection in the changing world. *Clinical Microbiology and Infection*, *17*, 494–501. <https://doi.org/10.1111/j.1469-0691.2011.03474.x>
- Katz, A. R. (2012). Quantitative polymerase chain reaction: Filling the gap for early leptospirosis diagnosis. *Clinical Infectious Diseases*, *54*, 1256–1258. <https://doi.org/10.1093/cid/cis037>
- Lau, C. L., Skelly, C., Dohnt, M., & Smythe, L. D. (2015). The emergence of *Leptospira borgpetersenii* serovar Arborea in Queensland, Australia, 2001 to 2013. *BMC Infectious Disease*, *15*, 1–11. <https://doi.org/10.1186/s12879-015-0982-0>
- Lau, C. L., Townell, N., Stephenson, E., van den Berg, D., & Craig, S. B. (2018). Leptospirosis. *Australian Journal of General Practice*, *47*, 105–110.
- Muscattello, D. J., Churches, T., Kaldor, J., Zheng, W., Chiu, C., Correll, P., & Jorm, L. (2005). An automated, broad-based, near real-time public health surveillance system using presentations to hospital Emergency Departments in New South Wales, Australia. *BMC Public Health*, *5*, 141. <https://doi.org/10.1186/1471-2458-5-141>
- Public Health Laboratory Network (2008). *Leptospirosis laboratory case definition (LCD)*. Retrieved from <http://www.health.gov.au/internet/main/publishing.nsf/content/cda-phlncd-leptospirosis.htm>
- Roberts, M. W., Smythe, L., Dohnt, M., Symonds, M., & Slack, A. (2010). Serologic-based investigation of Leptospirosis in a population of free-ranging Eastern Grey Kangaroos (*Macropus giganteus*) indicating the presence of *Leptospira weilii* serovar Topaz. *Journal of Wildlife Diseases*, *46*, 564–569. <https://doi.org/10.7589/0090-3558-46.2.564>
- Schneider, M., Velasco-Hernandez, J., Min, K.-D., Leonel, D., Baca-Carrasco, D., Gompper, M., ... Munoz-Zanzi, C. (2017). The use of chemoprophylaxis after floods to reduce the occurrence and impact of Leptospirosis outbreaks. *International Journal of Environmental Research and Public Health*, *14*(6), 594. <https://doi.org/10.3390/ijerph14060594>
- Sehgal, S. C., Sugunan, A. P., Murhekar, M. V., Sharma, S., & Vijayachari, P. (2000). Randomized controlled trial of doxycycline prophylaxis against leptospirosis in an endemic area. *International Journal of Antimicrobial Agents*, *13*, 249–255. [https://doi.org/10.1016/S0924-8579\(99\)00134-X](https://doi.org/10.1016/S0924-8579(99)00134-X)
- Slack, A., Symonds, M., Dohnt, M., Harris, C., Brookes, D., & Smythe, L. (2007). Evaluation of a modified Taqman assay detecting pathogenic *Leptospira* spp. against culture and *Leptospira*-specific IgM enzyme-linked immunosorbent assay in a clinical environment. *Diagnostic Microbiology and Infectious Disease*, *57*, 361–366. <https://doi.org/10.1016/j.diagmicrobio.2006.10.004>
- Slack, A., Symonds, M., Dohnt, M., & Smythe, L. (2006). The epidemiology of leptospirosis and the emergence of *Leptospira borgpetersenii* serovar Arborea in Queensland, Australia, 1998–2004. *Epidemiology and Infection*, *134*, 1217–1225. <https://doi.org/10.1017/s0950268806006352>
- Takafuji, E. T., Kirkpatrick, J. W., Miller, R. N., Karwacki, J. J., Kelley, P. W., Gray, M. R., ... Sanchez, J. L. (1984). An efficacy trial of doxycycline chemoprophylaxis against leptospirosis. *The New England Journal of Medicine*, *310*, 497–500. <https://doi.org/10.1056/nejm198402233100805>

- Villumsen, S., Pedersen, R., Borre, M. B., Ahrens, P., Jensen, J. S., & Krogfelt, K. A. (2012). Novel TaqMan® PCR for detection of *Leptospira* species in urine and blood: Pit-falls of in silico validation. *Journal of Microbiological Methods*, *91*, 184–190. <https://doi.org/10.1016/j.mimet.2012.06.009>
- Wynwood, S. J., Craig, S. B., Graham, G. C., Blair, B. R., Burns, M. A., Weier, S. L., ... McKay, D. B. (2014). The emergence of *Leptospira borgpetersenii* serovar Arborea as the dominant infecting serovar following the summer of natural disasters in Queensland, Australia 2011. *Tropical Biomedicine*, *31*, 281–285.
- Wynwood, S. J., Graham, G. C., Weier, S. L., Collet, T. A., McKay, D. B., & Craig, S. B. (2014). Leptospirosis from water sources. *Pathogens and Global Health*, *108*, 334–338. <https://doi.org/10.1179/2047773214y.0000000156>

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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