Epidemiol. Infect. (2015), **143**, 2116–2125.
doi:10.1017/S0950268815000515

Prevalence and risk factors for *Leptospira* exposure in New Zealand veterinarians

J. M. SANHUEZA[1]*, C. HEUER[1], P. R. WILSON[2], J. BENSCHOP[3] AND J. M. COLLINS-EMERSON[3]

[1] *EpiCentre; Institute of Veterinary, Animal and Biological Sciences; Massey University; Palmerston North, New Zealand*
[2] *Institute of Veterinary, Animal and Biological Sciences, Massey University, Palmerston North, New Zealand*
[3] *mEpiLab; Infectious Disease Research Centre (IDReC); Hopkirk Research Institute; Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Palmerston North, New Zealand*

Received 31 July 2014; Final revision 12 January 2015; Accepted 25 February 2015; first published online 25 March 2015

SUMMARY

This study assessed seroprevalence and risk factors for *Leptospira* (serovars Hardjo, Pomona, Ballum, Copenhageni, Tarassovi) exposure in New Zealand veterinarians. Veterinarians ($n = 277$) at one of two conferences were voluntarily enrolled and blood samples taken. Microscopic agglutination test (MAT) titres ⩾48 were considered seropositive. Fourteen veterinarians (5·1%, 95% confidence interval 2·8–8·3) were seropositive to *Leptospira*. Home slaughter of cattle or pigs were significant risk factors for *Leptospira* exposure. There were no clear relationships between the animal species handled at work and serostatus. However, veterinarians spending a ‘mid to high’ proportion of their time (>50% to ⩽75%) with pets had higher odds of being seropositive than those not working with pets. A borderline positive association ($P = 0·09$) was observed between seropositivity and clinical influenza-like illness (⩾3 days off work) in the 18 months before the study. Assuming causality, this suggests that 8·3% of these cases may be attributed to *Leptospira* exposure.

Key words: Emerging infections, epidemiology, estimating, leptospirosis, prevalence of disease, zoonoses.

INTRODUCTION

The importance of leptospirosis as a re-emerging zoonotic disease in some countries has recently been emphasized [1, 2]. The disease is endemic worldwide but a higher incidence is observed in tropical and subtropical climates where the conditions of the temperature and humidity favour the survival of *Leptospira* in the environment. Moreover, most countries in tropical regions are developing countries, where there are usually higher opportunities for contact between potentially infected animals and humans [3, 4]. Leptospirosis is often associated with rodents but all mammal species, including livestock and companion animals, can be infected. Animals can shed the organism in their urine for an extended period. Humans are infected after direct or indirect contact with urine containing the bacteria. Leptospires enter the body through mucosal membranes or abrasions in the skin. Clinical signs of disease vary from mild, self-limiting influenza-like illness (ILI) lasting 3–5 days, to a severe condition that can involve renal and

* Author for correspondence: Mr J. M. Sanhueza, Private Bag 11222, Massey University, Palmerston North, New Zealand. (Email: j.m.sanhueza@massey.ac.nz)

hepatic malfunction, and if not treated can be fatal [5]. Typical symptoms of the disease include myalgia, fever, nausea, vomiting, and headache [6].

Leptospirosis is endemic in New Zealand livestock. Surveys have estimated seroprevalence in beef cattle, sheep and deer animals ranging from 48–65%, 33–48% and 26–43% for *L. borgpetersenii* serovar Hardjo type Hardjo-bovis (Hardjo) and 25–35%, 0–45% and 11–50% for *L. interrogans* serovar Pomona (Pomona), respectively [7, 8]. Other production animals, including dairy cattle and pigs, are also at risk of leptospirosis but vaccination against serovars Hardjo and Pomona is thought to have largely reduced leptospirosis incidence and the risk of transmission to humans in New Zealand [9, 10]. However, a recent pilot study in 44 conveniently selected dairy herds with a long history of vaccination against *Leptospira* (during the last 5 to >20 years), identified 13% urinary shedders by PCR and dark-field microscopy in 30% of herds [11]. Although serovars were not determined in that study, it has been reported elsewhere that transient urinary shedding of Hardjo can occur in vaccinated cattle [12]. Serovars not included in cattle vaccines and reported from notified human cases in New Zealand include *L. borgpetersenii* serovar Ballum (Ballum) and *L. borgpetersenii* serovar Tarassovi (Tarassovi) [13].

Companion animals can also be exposed to *Leptospira*, although their role in transmission of *Leptospira* to humans is unknown. A cross-sectional study in New Zealand dogs found 15·0% of serum submissions seropositive to Hardjo, Pomona, Ballum, or *L. interrogans* serovar Copenhageni (Copenhageni). The latter serovar was the most prevalent (10·3%). Hardjo (3·5%) ranked second and was predominantly found in farm dogs [14]. Similar observations were made earlier in healthy dogs from rural or urban environments [15].

In New Zealand the incidence of leptospirosis in humans is among the highest in industrialized countries being 2·6/100 000 people [3]. The most common serovars in notified human cases during 2003–2013 were Hardjo, Pomona and Ballum [13]. The disease was identified as a common occupationally acquired infectious disease in the country and a higher incidence was described for males compared to females [16]. Working in an abattoir and livestock farming were the most frequently reported occupations in *Leptospira* cases [13].

The risk of *Leptospira* exposure for workers of a sheep-only abattoir was quantified as 3–54 carcasses per day. Risk depended on work position and year [17, 18]. The seroprevalence of Hardjo and Pomona in abattoir workers of another sheep abattoir was estimated as 9·5% [microscopic agglutination test (MAT) $\geqslant$24, 2008] [19]. During 2009/2010 a more comprehensive serosurvey of abattoir workers estimated that 10·9% of the workers had evidence of exposure to Hardjo or Pomona (MAT $\geqslant$48): 5·4% in beef, 12·9% in sheep and 17·5% in deer abattoir workers [20].

Veterinarians are not commonly listed in notified cases. During 2003–2013, only two cases (one veterinary technician in 2012 and one veterinarian in 2013) were notified [13]. However, veterinarians are probably at high risk of leptospirosis due to potential exposure to urine of infected animals. A previous study conducted in New Zealand found 1/86 veterinarians seropositive for Hardjo (MAT $\geqslant$100) [21]. Another serosurvey found no antibody titres for *Leptospira* in 302 veterinary students during 2010/2011 [22] but no recent estimate of the extent of exposure to *Leptospira* in veterinarians was available. This study aimed to estimate the seroprevalence of five *Leptospira* serovars and to evaluate risk factors for exposure in New Zealand veterinarians.

METHODS

Study design

A cross-sectional study was designed to estimate seroprevalence of *Leptospira* and assess risk factors associated with *Leptospira* serostatus. Veterinarians attending the Deer Special Interest group conference of the New Zealand Veterinary Association in Queenstown (May 2012) or the New Zealand Veterinary Association annual conference in Hamilton (June 2012) were eligible for inclusion. A station was set up in the exhibition hall of each conference where certified phlebotomists sampled blood from voluntary participants. Samples were kept refrigerated during the day at 4 °C. Tubes with blood were sent overnight inside bio-bottles™ (Bio-Bottle New Zealand Ltd) to the Hopkirk Institute, Massey University for serum extraction. Sera were kept frozen until testing. A modified MAT as described by Faine *et al.* [5] was used to detect antibodies against Hardjo, Pomona, Ballum, Copenhageni and Tarassovi. In summary, eight twofold dilutions of serum were made using sterile 0·9% saline solution as a diluent.

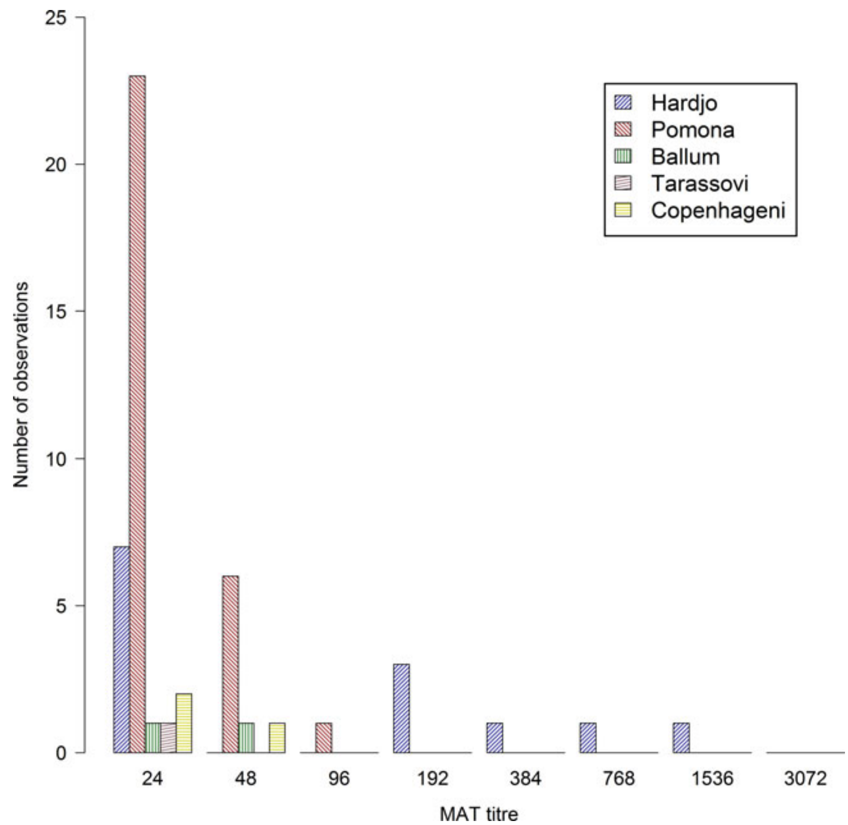

Fig. 1. Distribution of the number of samples at each microscopic agglutination test titre for Hardjo, Pomona, Ballum, Copenhageni and Tarassovi.

An equal volume of live *Leptospira* antigen was then added to each dilution to make a final serial dilution that ranged from 1:24 to 1:3072. The last dilution able to agglutinate more than 50% of leptospires was taken as the final titre for each serovar.

Recording of risk factors

A questionnaire was designed to capture information about exposure to potential risk factors during the previous 18 months. This time-frame of exposure was selected based on the duration of titres previously estimated as 10 months for Pomona and 29 months for Hardjo [23].

The questionnaire was available for participants at the conferences either online (Survey Gizmo®, www.surveygizmo.co.uk/) or paper. It covered personal information, animal exposure in the work place, animal and environmental exposure outside the work place, previous leptospirosis illness, ILI in the last 18 months, and general opinions about leptospirosis.

ILI was defined as an episode of illness accompanied by some or all of the following signs/symptoms: fever, headache, myalgia, photophobia, sweating, and severe general debility.

Statistical analysis

Antibody titres (24–3072) were described graphically using numbers (Fig. 1). Seroprevalence and 95% confidence intervals were estimated using the titre cut-off of ⩾48 for a positive sample.

Gender and age were described using percentages and summary statistics [median, minimum value (min), first quartile (Q1), third quartile (Q3) and maximum value (max)], respectively. Differences in the time spent working with different animal species between females and males were assessed using Wilcoxon's rank-sum test. The association between gender and home slaughter of pigs or cattle was explored using logistic regression.

The percentage of time spent in different activities within the veterinary practice was described and kernel density plots were used to graphically compare percentages in seropositive and seronegative veterinarians.

The number of previous leptospirosis episodes and the most common signs and symptoms associated with these episodes were recorded. The association between *Leptospira* serostatus and past episodes of leptospirosis was explored using prevalence ratios.

The number of ILIs in the last 18 months was recorded. ILI was categorized according to the time veterinarians spent off work due to this illness: no illness, off work <3 days, off work ⩾3 days. The relationship between *Leptospira* serostatus and ILI (yes/no) or time off work categories was explored. The population attributable fraction (PAF) was estimated. PAF is a measure of effect in the population. In this case it estimates the percentage of ILI in the population of veterinarians during the last 18 months that may be attributed to *Leptospira* exposure.

For inclusion in the multivariable analyses, the percentage of time spent in contact with different animal species was categorized as: none (0%), low (>0–25%), mid-low (>25–50%), mid-high (>50–75%), and high (>75%). For example, a veterinarian could be exposed to dairy cattle for 60% (mid-high) of his/her working time, to pets for 10% (low) and office work for the remaining 30% (mid-low) of work-related time.

Unconditional associations between each of the putative risk factors and *Leptospira* serostatus were evaluated using logistic regression. Variables with a P value ⩽0·35, tested by the likelihood ratio test (LRT), were selected for inclusion in the multivariable logistic model. Backward elimination of variables from a full multivariable model was based on the LRT with significance threshold of $P>0{\cdot}05$. Akaike's Information Criterion was used to select the final multivariable model [24]. Goodness of fit of the multivariable model was assessed using Pearson and deviance χ^2 tests and the Le Cessie–van Houwelingen test [25]. Observations with large Pearson residuals and delta-beta values were removed temporarily to investigate their influence on the coefficients and standard errors of the final multivariable model.

Analyses were performed using R software version 3.03 [26].

Ethical standards

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. The study was approved by the Massey University Human Ethics Committee, application number 12/09.

Table 1. *Number of participants positive and negative and seroprevalence for individual serovars at a titre of ⩾48*

Serovar	Positive (n)	Negative (n)	Seroprevalence % (95% CI)
Pomona	7	270	2·5 (1·0–5·1)
Hardjo	6	271	2·2 (0·8–4·7)
Ballum	1	276	0·4 (0·0–2·0)
Copenhageni	1	276	0·4 (0·0–2·0)
Tarassovi	0	277	0·0 (0·0–2·0)
Overall	14*	263	5·1 (2·8–8·3)

CI, Confidence interval.

* One sample positive for Hardjo and Pomona.

RESULTS

Data from 277 veterinarians, working in New Zealand that were sampled and had completed the questionnaire, were used for analysis.

Titre distribution

Antibody titres for *Leptospira* (MAT ⩾ 48) were detected in 14 veterinarians. The most frequent serovar was Pomona ($n=7$) followed by Hardjo ($n=6$). Antibodies detected for Pomona had a maximum titre of 96, while for Copenhageni and Ballum the maximum titre was 48. Comparatively higher antibody titres of up to 1536 were observed for Hardjo. Figure 1 shows the titre distribution for Hardjo, Pomona, Ballum, Copenhageni and Tarassovi serovars.

Seroprevalence

Seroprevalence of *Leptospira* (⩾48 MAT titre), all serovars included, was 5·1% [95% confidence interval (CI) 2·8–8·3]. One participant was seropositive for both Hardjo and Pomona. No veterinarian was seropositive for Tarassovi. Table 1 summarizes seroprevalence for each serovar individually and for any of the five serovars.

Age and gender

The median age of study participants was 42 years (min = 22, Q1 = 33, Q3 = 53, max = 73 years).

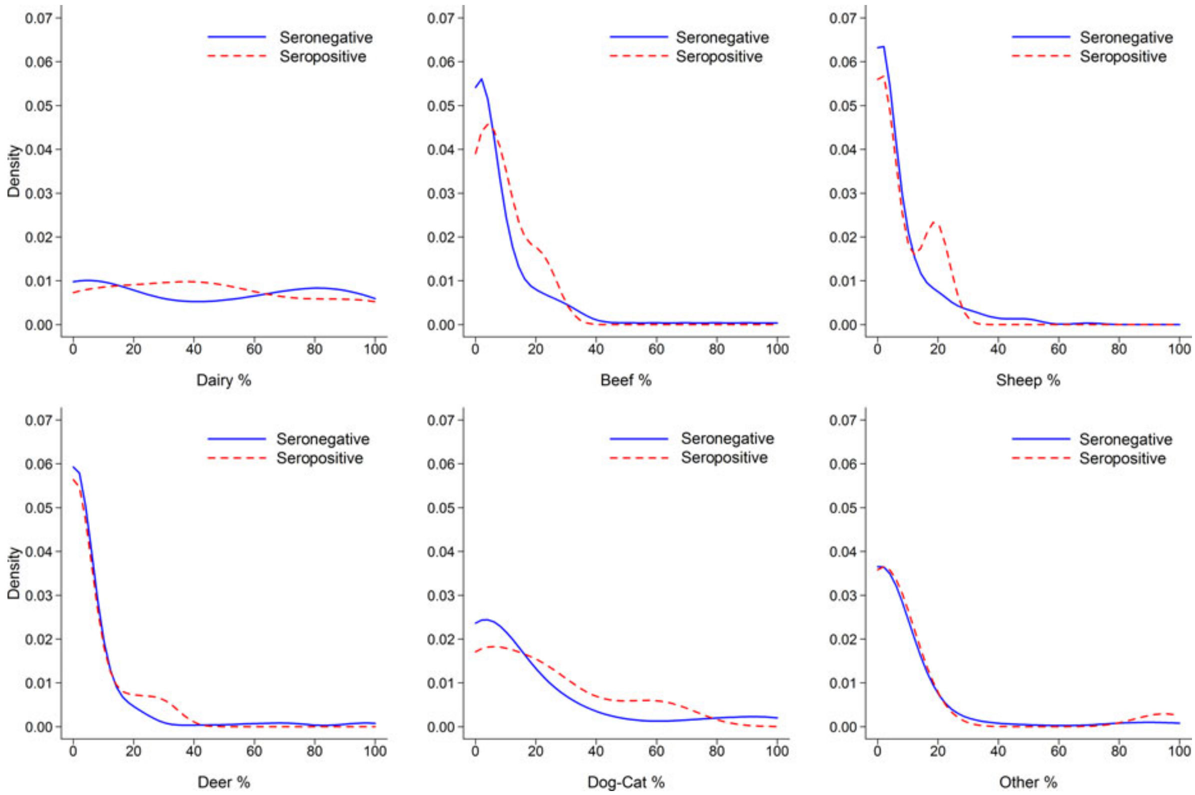

Fig. 2. Smoothed proportion of seropositive and seronegative participants (density) by percentage of time spent in contact with different animal species at work.

Thirty-nine per cent of veterinarians were females ($n = 109$). Male participants were in general older (median 47, min = 23, Q1 = 37, Q3 = 57, max = 73 years) than female participants (median = 35, min = 22, Q1 = 29, Q3 = 45, max = 59 years). There was no significant difference ($P = 0·40$) between the seroprevalence in men (10/168 positives) and women (4/109 positives). Females spent significantly ($P < 0·001$) more time on average working with dogs and cats (23·5%, 95% CI 17·5–29·6) than males (11·4%, 95% CI 8·2–14·6). On the other hand, males spent significantly ($P < 0·001$) more time working with deer (6·7%, 95% CI 4·1–9·3) than females (2·7%, 0·5–5·0%). Time spent with dairy cattle, beef cattle, and sheep was not significantly different between males and females. Home slaughter of cattle ($P = 0·54$) or pigs ($P = 0·41$) were not associated significantly with gender.

Occupational exposure to animals

Most veterinarians ($n = 155$) worked at least a quarter of their time with dairy cattle. By contrast, a small proportion of veterinarians worked more than 25% of their time with beef ($n = 18$), sheep ($n = 15$), or deer ($n = 12$). Few ($n = 24$) worked 100% of their time with a single species, while 22 had no animal contact at work.

Figure 2 shows the smoothed distribution of the proportion of time that seropositive and seronegative veterinarians spent working with different animal species. These plots suggest that seropositive veterinarians were not more involved than seronegative veterinarians in any of the extreme ends of exposure to any species, but some differences were apparent for middle portions of exposure to some species.

History of previous leptospirosis episodes

Leptospirosis was previously diagnosed by general practitioners and laboratory test (serology) 11 times in 10 participants. Nine were able to recall symptoms of disease, which were: headache ($n = 9$), sweating ($n = 9$), fever ($n = 8$), myalgia ($n = 7$), sore eyes ($n = 6$), photophobia ($n = 6$), severe debility ($n = 5$) and meningitis ($n = 1$). Eight recalled being off work a median time of 7 days (min = 2, Q1 = 3, Q3 = 21, max = 105 days) and one recalled being hospitalized because of

Table 2. *Association between* Leptospira *serostatus and time off work due to ILI in the last 18 months*

Serostatus	⩾3 days ill	<3 days ill	Not ill	PR* (95% CI)	PR† (95% CI)
Positive	3	5	6	1·4 (0·8 to 2·2)	2·8 (0·9 to 8·2)
				PAF* (95% CI)	PAF† (95% CI)
Negative	20‡	86‡	151	1·8 (−1·6 to 5·0)	8·3 (−6·3 to 20·9)
Total	23	91	157		

ILI, Influenza-like illness; CI, confidence interval; PR, prevalence ratio; PAF, population attributable fraction.
* PR and PAF were estimated using values for ILI and not ill in the last 18 months.
† PR and PAF were estimated using values for ⩾3 days ill and <3 days or not ill in the last 18 months.
‡ Four veterinarians, seronegative to *Leptospira*, had ILI but did not state number of days off work.

the disease. Eight of these episodes of leptospirosis occurred from 43 years to 16 years prior to participation in this study, while one occurred 3 months before sampling. Having clinical leptospirosis in the past was significantly associated ($P < 0·001$) with seropositivity [prevalence ratio (PR) = 10·7%, 95% CI 4·0–28·3]. This strong association was also observed when excluding the recent episode of leptospirosis (PR 8·9, 95% CI 2·9–26·9). Previous leptospirosis was excluded from the multivariable model since it could mask the effect of other risk factors associated with serostatus.

Leptospira exposure and illness

A total of 118/275 veterinarians experienced ILI in the previous 18 months. Of *Leptospira* seropositives, 8/14 had ILI in the last 18 months compared to 110/261 in seronegative veterinarians. ILI cases in seropositive veterinarians was therefore 1·4 times greater than in seronegative veterinarians ($P = 0·28$). However, seropositive veterinarians were more likely to spend ⩾3 days off work due to ILI than seronegative veterinarians (Table 2) ($P = 0·09$). Assuming a causal relationship between *Leptospira* exposure and ILI, the percentage of ILI in the last 18 months associated with ⩾3 days off work that was attributable to *Leptospira* exposure in the population of veterinarians, was 8·3% (PAF).

The ILI variable was excluded from the multivariable model as it was likely to be a consequence of leptospirosis rather than a cause, hence not a confounder for other risk factors.

Unadjusted associations

Unadjusted odds ratios (ORs) (Table 3) suggested that home slaughtering of cattle, sheep or deer in the previous 18 months significantly increased the odds of seropositivity compared to veterinarians who were not involved in such activities. Categories of the time spent with beef cattle, sheep, deer or other animals; hunting wild pigs, wild goats, rabbits, or birds; owning cattle, sheep, horses, dogs or cats; home slaughter of deer; age and gender were largely not associated (LRT $P > 0·35$) with *Leptospira* serostatus (results not shown in Table 3).

Multivariable analysis

In the final multivariable model, home slaughter of cattle or pigs was positively associated with *Leptospira* seropositivity. For example, the odds of being seropositive for veterinarians involved in home slaughter of cattle in the previous 18 months were 4·6 times the odds of being seropositive for those not involved in this activity, after controlling for the effects of home slaughter of pigs and occupational exposure to dogs and cats. Moreover, veterinarians spending from 50% to 75% of their time working with dogs or cats had significantly higher odds of being seropositive than those not working with dogs or cats (Table 4).

DISCUSSION

In this sample of veterinarians, 14/277 were seropositive (5·1%, 95% CI 2·8–8·3). A previous study investigated the seroprevalence of veterinarians in New Zealand for Hardjo, Pomona, Ballum, Copenhageni, Tarassovi, plus serovars Canicola, Autumnalis, Andaman and Medanensis not tested in our study, observing 1/86 (1·2%) veterinarians seropositive for Hardjo [21]. The MAT titre cut-off used in that investigation was one dilution higher (⩾100) than the one used in the present study. At an equivalent MAT titre of 96, 7/277 veterinarians were seropositive (2·5%,

Table 3. *Unadjusted associations between potential risk factors and* Leptospira *serostatus* ($P \leqslant 0{\cdot}35$)

Variable	Levels	*n*	OR (95% CI)	LRT *P*
Dairy exposure	High	77	2·6 (0·3–30·0)	0·3
	Mid-high	40	3·4 (0·3–39·0)	
	Mid-low	38	7·7 (0·8–71·1)	
	Low	56	5·0 (0·5–46·1)	
	None	66	Reference	
Dog-cat exposure	High	19	n.a.	0·22
	Mid-high	9	6·3 (1·0–38·7)	
	Mid-low	24	2·0 (0·4–11·1)	
	Low	109	1·1 (0·3–3·8)	
	None	116	Reference	
Home slaughter of cattle	Yes	32	4·8 (1·5–15·2)	0·02
	No	240	Reference	
Home slaughter of pigs	Yes	11	8·5 (2·0–36·6)	0·01
	No	261	Reference	
Home slaughter of sheep	Yes	65	3·5 (1·2–10·2)	0·03
	No	207	Reference	
Hunting wild deer	Yes	55	2·4 (0·8–7·3)	0·16
	No	221	Reference	
Camping lakes/rivers	Yes	120	2·5 (0·8–7·6)	0·11
	No	157	Reference	
Fishing	Yes	59	3·0 (1·0–8·9)	0·06
	No	218	Reference	
Own deer	Yes	15	3·2 (0·7–15·8)	0·20
	No	262	Reference	
Own pigs	Yes	16	3·0 (0·6–14·6)	0·23
	No	261	Reference	

LRT, Likelihood ratio test; OR, Odds ratio; CI, confidence interval; n.a., not identifiable as no seropositives were observed.

Table 4. *Results of a multivariable logistic regression model between* Leptospira *serostatus and potential risk factors*

Risk factor	Levels	OR (95% CI)	LRT *P*
Dog-cat exposure	High	n.a.	0·17
	Mid-high	9·2 (1·4–62·8)	
	Mid-low	2·5 (0·4–14·9)	
	Low	1·1 (0·3–4·3)	
	None	Reference	
Home slaughter of cattle	Yes	4·6 (1·3–16·1)	0·03
	No	Reference	
Home slaughter of pigs	Yes	7·9 (1·7–37·5)	0·02
	No	Reference	

LRT, Likelihood ratio test; OR, Odds ratio; CI, confidence interval; n.a., not identifiable as no seropositives were observed.

95% CI 1·0–5·1); which is similar to that of the previous study.

The selection of the MAT titre cut-off depends on the purpose for testing. For a clinical case of leptospirosis based on MAT, the World Health Organization (WHO) considered a MAT titre of ⩾400 in a single or paired serum sample, or a fourfold increase in MAT titre in acute and convalescent phase in subjects experiencing clinical signs compatible with leptospirosis [27]. There is not a defined cut-off for antibody MAT titres when assessing *Leptospira* exposure in apparently healthy individuals working in high-risk occupations, hence the cut point must be considered when comparing seroprevalence studies.

For example, exposure to Hardjo and Pomona in apparently healthy abattoir workers was estimated at 10·9% (95% CI 8·5–13·9) using the MAT titre of ⩾48 [20], which is 2·5 times higher than the exposure observed to these two serovars in veterinarians (4·3%, 95% CI 2·3–7·5). Exposure to Hardjo, Pomona and Ballum has also been assessed in apparently healthy veterinary students where none of the 302 participants was positive for these serovars at a titre of ⩾48 [22]. This finding suggests that exposure to *Leptospira* most likely occurs during the years of active work.

Leptospirosis is likely under-reported in New Zealand, especially for mild infections. The disease in people is characterized by non-specific symptoms

[6] that may be confused with flu. We observed a marginally significant association between *Leptospira* exposure and ILI ($\geqslant$3 days off work) in the previous 18 months. Although, the cross-sectional design used does not allow assessment of when exposure and outcome occurred; we believe that some of the ILIs recorded are a consequence of *Leptospira* exposure. This is consistent with recent findings in abattoir workers where new Hardjo or Pomona infections increased the risk of ILI by 1·9 (95% CI 1·3–2·7) in a year and the proportion of ILI cases attributed to Hardjo or Pomona exposure (PAF) was 10·0% [23]. If we assume a causal relationship between *Leptospira* exposure and being off work for $\geqslant$3 days with flu-like signs of illness in the last 18 months, it can be said that 8·3% of these cases could be prevented if *Leptospira* exposure of veterinarians was controlled (Table 2).

A significant risk factor for *Leptospira* exposure was home slaughtering of cattle ($n = 32$) or pigs ($n = 11$). The average predicted seroprevalence increased from 3·2% (95% CI 1·0–16·9) in veterinarians not performing these activities to 16·4% (95% CI 4·4–46·0) in veterinarians performing any of these activities. This result contrasts with findings from the serosurvey in veterinary students of New Zealand, where no student was seropositive and yet 20·9% stated that they performed home slaughter in the 18 months prior to sampling [22]. A plausible explanation for the difference observed between students and veterinarians is the presumably shorter length of time that students were exposed to home slaughter due to their young age. A recent study of abattoir workers did not find a significant association between home slaughter and *Leptospira* seropositivity [20] but no information was available about the species slaughtered at home or the frequency of this practice. Possibly, the additional risk of *Leptospira* exposure from home slaughter in abattoir workers was relatively small compared to veterinarians.

Home slaughter of sheep was not a significant risk factor in the multivariable model, most likely due to its significant association with slaughtering cattle (OR 2·5, $P = 0{\cdot}02$) and pigs (OR 9·5, $P = 0{\cdot}001$). Hence, those involved in home slaughter appear to process more than one species. It is therefore impossible to make inferences about which home-slaughtered species was the likely source of infection for veterinarians.

A positive correlation between *Leptospira* seroprevalence and the time spent with a specific species was expected, especially since earlier studies demonstrated *Leptospira* exposure in livestock [7, 8] and that between 21% and 41% of seropositive animals potentially shed *Leptospira* in their urine [17, 28]. Instead, seroprevalence tended to be higher when veterinarians worked with multiple species (Fig. 2). If any species, it was the contact with pets and dairy cattle that tended to be positively associated with seropositivity. However, the data are too sparse to draw clear conclusions about the comparative risk of infection from any particular animal species that veterinarians are handling during their clinical work.

In New Zealand dogs, Copenhageni was the most commonly observed serovar, followed by Hardjo which was commonly present in dogs from rural locations or farm working dogs [14, 15]. However, only one veterinarian (who did not work with dogs or cats) was seropositive for Copenhageni. This contrasts with six veterinarians seropositive for Hardjo; three of whom had no or only minor exposure to dogs or cats (0–3%), and the other three had moderate exposures (20–65%). Furthermore, none of the 19 veterinarians who worked >75% of the time with dogs or cats were seropositive.

A strong and highly significant positive association between previous leptospirosis episodes and seropositivity to *Leptospira* observed in this survey was also reported from abattoir workers [20]. Antibodies are usually detectable from 5 to 10 days after infection but they may persist for months or even years after infection at low antibody titres [29]. Re-testing of 69 meat inspectors with previous positive titres, irrespective of their clinical history, showed that MAT titres of 384–192, 96, and 48–24 persisted for at least 30, 43 and 52 months, respectively, in some individuals [30]. Nevertheless, the persistence of titres is difficult to assess because often individuals at high risk of infection are studied and it is unknown whether persistent titres are the product of a slow decay, or continued re-exposure. The latter hypothesis could be questionable since seroconversion in seronegative meat inspectors was estimated in 4·4% a year [30]. Previous episodes of clinical *Leptospirosis* in veterinarians of this study occurred between 16 and 43 years before sampling; it is therefore unlikely that the strong association between previous leptospirosis and seropositivity was due to a slow decay of antibodies, thus they may rather reflect continuous re-exposure to *Leptospira* resulting in mild or subclinical infections.

Gender was not significantly associated with *Leptospira* exposure ($P = 0{\cdot}9$) in this group of veterinarians. National data show males have a higher incidence of leptospirosis than females which may be due to the predominance of males in high-risk occupations [16]. For example, male abattoir workers were more likely to be seropositive and seroconverted more often to *Leptospira* than female workers [20, 23]. In our data, females represented 39·4% of participants and they spent on average more time with dogs or cats and less time with deer than males. Other occupational exposures and the proportion of veterinarians involved in home slaughter of cattle or pigs were not different between females and males.

The 277 participant veterinarians represented 11% of the veterinarians registered in New Zealand [31] ($n = 2521$) in the year of the study. The median age of veterinarians in New Zealand was 43 years, similar to our study (42 years) but a slightly higher proportion of females (62%) and males (31%) under 40 years was observed in comparison to population demographics of veterinarians (59% and 23%, respectively). Potential selection bias may have been introduced by design as only veterinarians attending the conferences had the opportunity to participate in the study. The effect this might have had on seroprevalence and ORs is difficult to predict and subject to speculation. Nonetheless, we can see no reason to believe that selection bias affected the overall conclusions presented in this study.

CONCLUSION

Leptospira seroprevalence in veterinarians is about half as high as that of abattoir workers. High-risk activities for *Leptospira* exposure in veterinarians included home slaughter of cattle or pigs. No clear associations were found with the animal species that veterinarians were in contact with during professional activities. The borderline association between *Leptospira* exposure and ILI resulting in $\geqslant 3$ days off work suggested that 8·3% of these ILI cases were attributable to *Leptospira* infection during the 18 months prior to sampling. However, caution should be exercised when interpreting this result since the cross-sectional design does not warrant a causal relationship.

ACKNOWLEDGEMENTS

We express our gratitude to all the veterinarians that voluntarily participated in the study and made it possible, to Neville Hack for all the technical assistance and support provided, and to MSD Animal Health, Zoetis and Vibac for computers and online connection for participants to access the questionnaire.

This study was funded by MSD Animal Health, Zoetis and Vibac, New Zealand Government through an International Doctoral Research Scholarship and by Massey University.

DECLARATION OF INTEREST

None.

REFERENCES

1. **Bharti AR, *et al.*** Leptospirosis: a zoonotic disease of global importance. *Lancet Infectious Diseases* 2003; **3**: 757–771.
2. **Adler B, De la Peña Moctezuma A.** *Leptospira* and leptospirosis. *Veterinary microbiology* 2010; **140**: 287–296.
3. **Pappas G, *et al.*** The globalization of leptospirosis: worldwide incidence trends. *International Journal of Infectious Diseases* 2008; **12**: 351–357.
4. **Levett PN.** Leptospirosis: a forgotten zoonosis? *Clinical and Applied Immunology Reviews* 2004; **4**: 435–448.
5. **Faine S, *et al.*** *Leptospira and Leptospirosis*, 2nd edn. Melbourne, Australia: MediSci, 1999.
6. **Vickery B, *et al.*** Leptospirosis presenting to an intensive care unit in provincial New Zealand: a case series and review. *Critical Care and Resuscitation* 2006; **8**: 192–199.
7. **Dreyfus A, *et al.*** Leptospirosis seroprevalence and associated economic loss in New Zealand livestock. In: *Proceedings of the Food Safety, Animal Welfare & Biosecurity, Epidemiology & Animal Health Management, and Industry branches of the NZVA*. VetLearn Foundation, 2011, pp. 3·12·11–13·12·10.
8. **Subharat S, *et al.*** Longitudinal serological survey and herd-level risk factors for *Leptospira* spp. serovars Hardjo-bovis and Pomona on deer farms with sheep and/or beef cattle. *New Zealand Veterinary Journal* 2012; **60**: 215–222.
9. **Marshall R.** A nationwide experiment to control human leptospirosis by vaccinating dairy cattle. *Israel Journal of Veterinary Medicine* 1987; **43**: 271–276.
10. **Marshall RB, Manktelow BW.** Fifty years of leptospirosis research in New Zealand: a perspective. *New Zealand Veterinary Journal* 2002; **50**: 61–63.
11. **Wilson PR, *et al.*** Does long term vaccination eliminate leptospiral shedding? A study of New Zealand dairy herds. *8th Scientific Meeting of International Leptospirosis Society*. Fukuoka, Japan, 2013, pp. 104.
12. **Zuerner RL, *et al.*** A *Leptospira borgpetersenii* serovar Hardjo vaccine induces a Th1 response, activates NK cells, and reduces renal colonization. *Clinical and Vaccine Immunology* 2011; **18**: 684–691.
13. **The Institute of Environmental Science and Research Ltd.** Notifiable and Other Diseases in New Zealand:

Annual Report 2003–2013 (https://surv.esr.cri.nz/). Porirua, New Zealand.
14. **Harland AL, *et al.*** A serological survey of leptospiral antibodies in dogs in New Zealand. *New Zealand Veterinary Journal* 2013; **61**: 98–106.
15. **O'Keefe JS, *et al.*** A serosurvey for antibodies to *Leptospira* in dogs in the lower North Island of New Zealand. *New Zealand Veterinary Journal* 2002; **50**: 23–25.
16. **Thornley CN, *et al.*** Changing epidemiology of human leptospirosis in New Zealand. *Epidemiology and Infection* 2002; **128**: 29–36.
17. **Dorjee S, *et al.*** Prevalence of pathogenic *Leptospira* spp. in sheep in a sheep-only abattoir in New Zealand. *New Zealand Veterinary Journal* 2008; **56**: 164–170.
18. **Dorjee S, *et al.*** Assessment of occupational exposure to leptospirosis in a sheep-only abattoir. *Epidemiology and Infection* 2011; **139**: 797–806.
19. **Benschop J, *et al.*** Seroprevalence of leptospirosis in workers at a New Zealand slaughterhouse. *The New Zealand medical journal* 2009; **122**: 39–47.
20. **Dreyfus A, *et al.*** Seroprevalence and risk factors for leptospirosis in abattoir workers in New Zealand. *International Journal of Environmental Research and Public Health* 2014; **11**: 1756–1775.
21. **Robinson RA, Metcalfe RV.** Zoonotic infections in veterinarians. *New Zealand Veterinary Journal* 1976; **24**: 201–210.
22. **Fang F, *et al.*** Seroprevalence and exposure to risk factors for leptospirosis among veterinary students at Massey University. *New Zealand Veterinary Journal* 2013; **62**: 130–135.
23. **Dreyfus A, *et al.*** Risk of infection and associated influenza-like disease among abattoir workers due to two *Leptospira* species. *Epidemiology and Infection*. Published online: 30 September 2014. doi:10.1017/S0950268814002477.
24. **Akaike H.** New look at statistical-model identification. *IEEE Transactions on Automatic Control* 1974; **AC19**: 716–723.
25. **le Cessie S, van Houwelingen JC.** A goodness-of-fit test for binary regression models, based on smoothing methods. *Biometrics* 1991; **47**: 1267–1282.
26. **R Foundation for Statistical Computing.** *R: A Language and Environment for Statistical Computing*. Vienna, Austria, 2014.
27. **World Health Organisation (WHO).** Report of the Second Meeting of the Leptospirosis Burden Epidemiology Reference Group (http://whqlibdoc.who.int/publications/2011/9789241501521_eng.pdf), 2011.
28. **Fang F, *et al.*** Shedding and seroprevalence of pathogenic *Leptospira* spp. in sheep and cattle at a New Zealand abattoir. *Zoonoses and Public Health*. Published online: 8 July 2014: doi:10.1111/zph.12146.
29. **Cumberland P, *et al.*** Persistence of anti-leptospiral IgM, IgG and agglutinating antibodies in patients presenting with acute febrile illness in Barbados 1979–1989. *European Journal of Epidemiology* 2001; **17**: 601–608.
30. **Blackmore DK, Schollum LM, Moriarty KM.** The magnitude and duration of titres of leptospiral agglutinins in human sera. *New Zealand Medical Journal* 1984; **97**: 83–86.
31. **New Zealand Veterinary Council.** Annual report 2011–2012 (http://www.vetcouncil.org.nz/documentation/VCNZ_VeterinaryWorkforce2011-12.pdf).