

## Assessment of occupational exposure to leptospirosis in a sheep-only abattoir

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### SUMMARY

This study estimated the frequency of exposure of meat workers to carcasses infected with *Leptospira* serovars Hardjobovis or Pomona in a sheep-only abattoir in New Zealand. A stochastic spreadsheet model was developed to assess the daily risk of exposure of eviscerators, meat inspectors and offal handlers to live leptospires in sheep carcasses from May to November 2004 (high-risk period), and from December 2004 to June 2005 (low-risk period). The average sheep processed per day were 225 for an eviscerator, 374 for a meat inspector, and 1123 for an offal handler. The median daily exposures during high- and low-risk periods were 11 [95% distribution interval (DI) 5–19] and three (95% DI 1–8) infected carcasses/day for eviscerators, 18 (95% DI 9–29) and six (95% DI 2–12) for meat inspectors, and 54 (95% DI 32–83) and 18 (95% DI 8–31) for offal handlers, respectively. Stochastic risk modelling provided evidence that processing of sheep carcasses exposed meat workers regularly to live leptospires with substantial seasonal variation.

**Key words:** Leptospirosis, occupation-related infections, risk assessment.

### INTRODUCTION

Leptospirosis is recognized as the most important occupationally acquired zoonotic disease in New Zealand and the highest risk group has predominantly been abattoir workers and males living in rural areas. An increase in the incidence of notified cases of the disease to an average annual rate of 3·1/100 000 persons during 2001–2004 was a cause for concern because it was a significant change from the

long-term decline observed since the early 1980s to a minimum level of 2·3/100 000 persons in 1999 [1–6]. The incidence again declined in 2005 and 2006 to 2·7 and 2·1 notified cases/100 000 persons, respectively, and significantly increased in 2008 over that of 2007 [7]. An increase in the overall incidence was associated with a higher proportion of meat processing workers being affected. Meat workers constituted 32·6% (31/95), 48·9% (64/131), 46·1% (47/102), 64·7% (66/102), 47·6% (39/82) and 29·6% (24/81) of all notified leptospirosis cases among the recorded occupations in the successive years from 2001 to 2006, respectively. These rates were higher than the rates for farm workers who until then had represented the highest proportions [1–6].

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Baker & Lopez [2] reported associations between *Leptospira* serovars in notified cases and patient contact with animals prior to illness for the period 2001–2003. They found that most human infections were associated with contact with cattle, either alone or in combination with other animal species. Sheep were the second most important contact species, either alone or in combination with other species (beef cattle, deer), highlighting the importance of sheep as a source of human infection. The majority of affected patients who had contact with sheep or sheep in combination with other animals, were caused by serovars Hardjobovis and Pomona and both of these serovars occurred in similar proportions of patients.

A serological survey in New Zealand, carried out in the early 1980s from six meat processing plants, including 1215 meat inspectors and 1248 meat processing workers, showed 9.5% of meat inspectors and 4.1% of meat processing workers were seropositive with titres compatible with occupational exposure to domestic stock [8]. A recent survey of a sheep-only abattoir again resulted in an overall seropositivity of 9.5% in workers based on voluntary participation [9]. To our knowledge, no study has been conducted to assess the frequency of exposure of meat processing workers to leptospires in any abattoir, although meat processing workers have been commonly reported to be affected with leptospirosis throughout the world [2, 3, 10–15].

This study aimed to assess the frequency of exposure of meat processing workers in a sheep abattoir in New Zealand to sheep carcasses potentially shedding live leptospires, by modelling data collected in a previous study investigating the prevalence of *Leptospira* spp. in a sheep abattoir [16].

## MATERIALS AND METHODS

### Data collection

Data collection, diagnostic procedures and prevalence of seropositive and culture-positive carcasses were reported earlier [16]. Briefly, serum and kidney samples were collected from sheep by systematic random sampling from randomly selected sheep-lines (30 per line) in one sheep-only abattoir in New Zealand from 18 May to 30 November 2004 (study period 1), a period immediately following a major flood in the area of the abattoir and surrounding districts (February 2004), and again from 1 December 2004 to 15 June 2005 (study period 2), a period of average

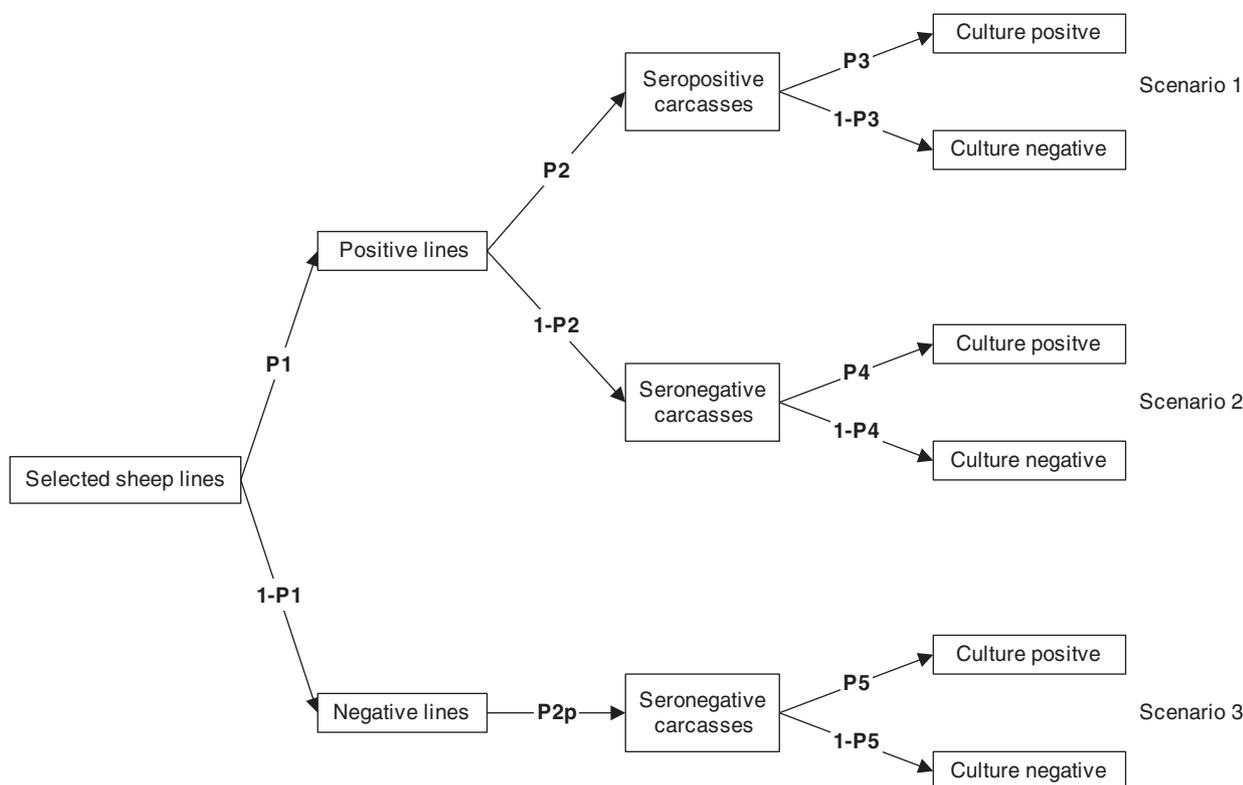
rainfall and temperature. Due to the extremely seasonal production cycle of New Zealand sheep farming, sheep sampled in first study period were born during August–September 2003 and slaughtered from May to November 2004. Sheep sampled in study period 2 were born during August–September 2004 and slaughtered from December 2004 to June 2005. Except for 6/89 farms that were sampled twice, a slaughter line represented a single farm.

The standard microscopic agglutination test (MAT) described by Faine [17] was used for serology against serovars Hardjobovis and Pomona with a positive cut-off titre of  $\geq 1:48$ . Lines were considered positive if one or more carcasses were seropositive to either one or both serovars. From December 2004 onwards (period 2), all kidneys from seropositive carcasses were cultured in Ellinghausen–McCullough–Johnson–Harris medium containing 5-fluorouracil [18] and examined every 1–3 weeks for the presence of leptospires, using dark-field microscopy. In addition, kidneys from the first 15 carcasses from each of 34 randomly selected lines were cultured, irrespective of their serostatus ( $n=509$ ; including one line with 14 carcasses of which all were sampled). Kidney culture results from the carcasses that tested seronegative ( $n=499$ ) were used for an estimate of the culture isolation rate from seronegative carcasses used in the risk model.

Data on monthly numbers of slaughter of sheep during the study period, numbers of eviscerators (handling kidneys and bladders), meat inspectors and offal handlers were retrieved from the abattoir database. Since live *Leptospira* are primarily found in kidneys and bladder [19, 20], the assessment of exposure risk to live leptospires focused on groups of meat workers handling those organs. It was assumed that these workers represented the group with the highest risk. The abattoir processed sheep in two work shifts covering 24 h, each with different sets of workers.

### Analysis

Estimates of serological and culture prevalence obtained from the aforementioned, previous observational study of leptospirosis at a sheep-only abattoir [16] were used for exposure risk assessments. Seroprevalence data for serovars Hardjobovis and Pomona were combined as both serovars can cause severe disease in humans. In addition, the exposure risk for each occupational group of meat workers was



**Fig. 1.** Scenario tree outlining the probability for exposure of meat-processing workers to infection from leptospirosis from infected kidneys potentially shedding live leptospires in seropositive and seronegative lines of sheep.

assessed separately for each of the two study periods as there was a strong seasonal difference in prevalence [16]. A scenario tree outlining pathways leading to sheep kidneys potentially shedding live leptospires and subsequent exposure of meat workers to leptospirosis is illustrated in Figure 1. The total probability of carcasses that were potentially shedding live leptospires was calculated as the sum of conditional probabilities of kidney culture-positives from seropositive (scenario 1) and seronegative (scenario 2) carcasses within seropositive sheep lines, and kidney culture-positives from seronegative carcasses within seronegative sheep lines (scenario 3) as follows (Fig. 1):

$$\begin{aligned} \text{Scenario 1 (S1)} &= P1 * P2 * P3, \\ \text{Scenario 2 (S2)} &= P1 * (1 - P2) * P4, \\ \text{Scenario 3 (S3)} &= (1 - P) * P2p * P5, \\ P_{\text{total}} &= S1 + S2 + S3. \end{aligned}$$

Where, P1 is the probability of a line being seropositive, P2 is the probability of a carcass from a seropositive line being seropositive, P3 is the probability of a kidney sample from a seropositive carcass of a seropositive line being positive, P4 is the probability of a kidney sample from a seronegative carcass of a

seropositive line being positive, P5 is the probability of a kidney sample from a seronegative line and seronegative carcass being positive, and  $P_{\text{total}}$  is the probability of a carcass being kidney culture-positive.

Because all culture-positive kidneys from seronegative carcasses were from seronegative lines, P4 could not be measured and was therefore considered to be at least as high as P5, thus it was assumed that  $P4 = P5$ . Moreover,  $P2p = 1$  as all seronegative carcasses in scenario 3 were, by definition, from seronegative lines. Estimates of culture isolation rates from seropositive and seronegative carcasses obtained in the second sampling period were also applied for analyses of the first sampling period. MAT and culture sensitivity were not considered in the model, as both were regarded as high and because tissue culture was regarded as being highly comparable to PCR [21].

The final risk of daily exposure of meat workers was performed using a stochastic model with a binomial distribution:

$$\chi = \text{Binomial}(n, P_{\text{total}}),$$

where  $\chi$  = the number of sheep carcasses potentially shedding leptospires that were processed by one worker (eviscerator, meat inspector, or offal handler)

Table 1. Spreadsheet cell definitions for input data and assumed distributions for input parameters and output variable (cell B37) for the @Risk model by example of an eviscerator in period 1

	A	B	C
1	Item	Number	Cell-B Formula/Description
2	Total lines	21	Input parameter
3	Seropositive lines	19	Input parameter
4	betadist alpha 1	20	=B3 + 1
5	Betadist alpha 2	3	=B2-B3 + 1
6	Prevalence of positive lines	0.86957	= RiskBeta(B4, B5)
7			
8	Total sera tested within positive lines	565	Input parameter
9	Seropositive sera	115	Input parameter
10	Betadist alpha 1	116	=B9 + 1
11	Betadist alpha 2	451	=B8-B9 + 1
12	Seroprevalence	0.20459	= RiskBeta(B10, B11)
13			
14	Total seropositive kidneys	43	Input parameter
15	Total culture-positive kidneys	9	Input parameter
16	Betadist alpha 1	10	=B15 + 1
17	Betadist alpha 2	35	=B14-B15 + 1
18	Culture-positive rate of serpositive kidneys	0.22222	= RiskBeta(B16, B17)
19			
20	Total seroneg. kidneys	499	Input parameter
21	Total culture-pos. kidneys	5	Input parameter
22	Betadist alpha 1	6	=B21 + 1
23	Betadist alpha 2	495	=B20-B21 + 1
24	Culture-pos. rate of seropos. kidneys	0.01198	= RiskBeta(B22, B23)
25			
26	Total probability of culture-positive carcasses	0.04938	= B6*(B12*B18 + (1-B12)*B24) + (1-B6)*B24
27			
28	Shifts/day	2	Input parameter
29	Eviscerators/shift	5	Input parameter
30	Total 14 months kill	9 43 413	Input parameter
31	Total daily kill	2246	=B30/(14*30)
32	Per shift daily kill	1123	=B31/B28
33	Per shift daily kills/person	225	=ROUND(B32/B29,0)
34	Standard deviation [of B33]	50	Input parameter
35	Distribution daily kills/person	225	= RiskNormal(B33,B34)
36			
37	Daily exposure rate per worker	11	= RiskOutput() + (RiskBinomial(B35,B26))

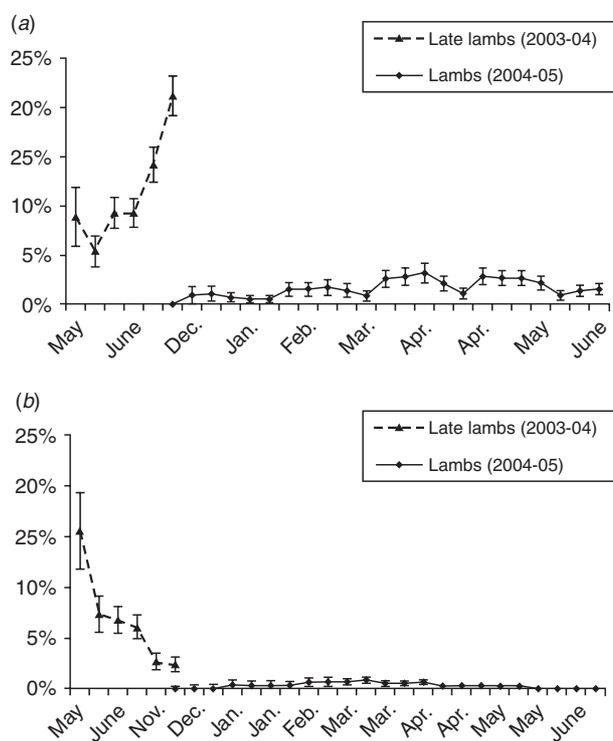
on an average working day, and  $n$  represented the number of sheep carcasses processed per worker per day. The number of carcasses per worker per day was assumed to follow a normal distribution with a standard deviation of  $\pm 22\%$  of the mean. The standard deviation was calculated from abattoir data.

Uncertainties about the prevalence ( $p$ ) of seropositive sheep lines, seropositive carcasses and culture-test positives were modelled using a beta distribution:

$$p = \text{beta}(x + 1, n - x + 1),$$

where  $x$  = number of positive carcasses and  $n$  = number of carcasses sampled.

All model parameters and uncertainties were combined to produce the output distribution for  $\chi$ , the number of kidney culture-positive carcasses processed per person per day. Inputs, cell formulas and the output variable are defined in Table 1 by example of an eviscerator in the first study period. Simulations were performed by using @RISK software version 5.5.0, 2009 (Palisade Corporation, USA). Each simulation consisted of 10 000 iterations using the Latin Hypercube sampling method which achieved sufficient convergence of the results as monitored by change in percentiles, means, and standard deviations (all changes  $< 1.5\%$ ). Estimates of daily exposure risks are presented as medians and their 95%



**Fig. 2.** Monthly carcass prevalence of MAT antibody to (a) *Leptospira borgpetersenii* serovar Hardjobovis and (b) *Leptospira interrogans* serovar Pomona of 9–14 months old, late-season lambs (study period 1, May–November 2004, and 4–10 months old lambs (study period 2, December 2004–June 2005) in a sheep-only abattoir in New Zealand. Error bars are 95% confidence intervals of point estimates.

distribution intervals (DI). A sensitivity analysis of the model was performed by calculating Spearman rank correlation coefficients between the model response (the number of shedding carcasses per worker per day) and each model input parameter.

## RESULTS

### Serological and culture prevalence

During the study period, the abattoir processed on average 25–40 lines (9416–21 728 sheep) per week. The monthly numbers of sheep slaughtered during the study period were more or less uniform but were highest during the peak of the processing period from January to March 2005, and lowest during the off-season period from August to October 2004. The average daily number of sheep carcasses processed by an eviscerator was 225, and by a meat inspector and an offal handler were 374 and 1123, respectively, over the entire study period.

The number of slaughter lines sampled per month ranged from three in May 2004 to 15 in April and

May 2005 with an average of nine lines per month. The number of sheep carcasses sampled ranged from 90 in May 2004 to 432 in May 2005 with an average of 276 per month. In total, 2758 carcasses from 15 855 sheep in 95 lines originating from 89 farms in 11 districts of New Zealand (this included subset of samples wherein kidneys from first 15 randomly selected carcasses were cultured irrespective of serological status) were tested for MAT antibodies against serovars Hardjobovis and Pomona.

The monthly prevalence of seropositive lines ranged from 2/10 in January 2005 to 3/3 in May 2004. Overall, 42/95 lines (44.2%, 95% confidence interval (CI) 34.6–54.2) equivalent to 40/89 farms (44.9%, 95% CI 35.0–55.3) had one or more seropositive sheep. The line prevalence was significantly ( $P < 0.001$ ) and substantially higher during the first study period (19/21 lines, 90.5%, 95% CI 71.1–97.4) than during the second study period (23/74 lines, 31.1%, 95% CI 21.7–42.3).

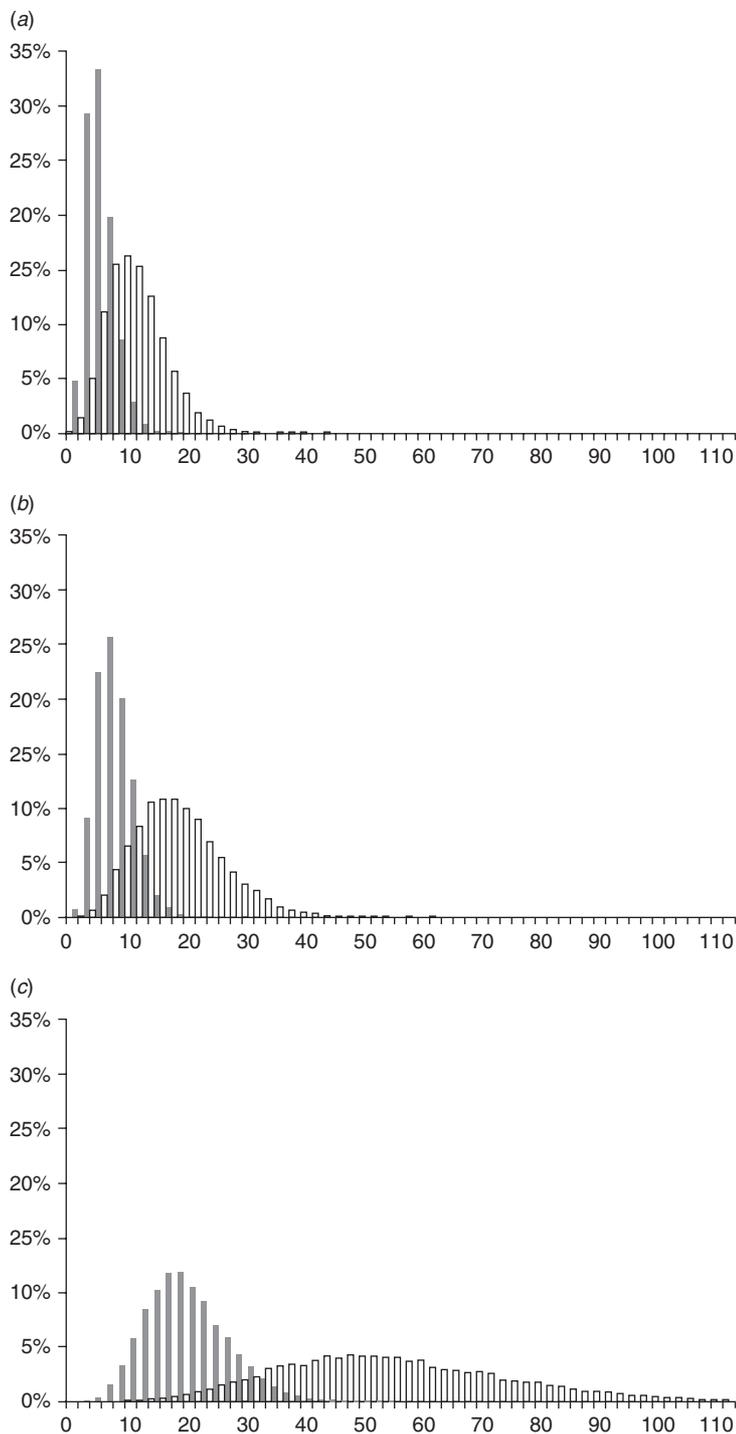
The prevalence of seropositive sheep ranged from 2/300 (0.7%, 95% CI 0.1–2.7) in January 2005 to 61/240 (25.4%, 95% CI 20.1–31.5) in November 2004. Overall, a total of 158/2758 sheep (5.7%, 95% CI 4.2–6.7) were seropositive to either one or both serovars, again with substantial differences between study periods (Fig. 2). The prevalence within seropositive lines was 115/565 (20.4%, 95% CI 17.2–23.9) during the first and 43/633 (6.8%, 95% CI 5.1–9.0) during the second study period.

Leptospire were isolated from 9/43 (20.9%, 95% CI 10.6–36.5) kidneys of seropositive, and from 5/499 (1.0%, 95% CI 0.4–2.5) kidneys of seronegative carcasses.

### Risk assessment

The median daily exposure risk for eviscerators was 11 (95% DI 5–19) culture-positive carcasses during the first and three (95% DI 1–8) during the second study period. The corresponding risk for meat inspectors, was 18 (95% DI 9–29) and six (95% DI 2–12) culture-positive carcasses, respectively, and for offal handlers 54 (95% DI 32–83) and 18 (95% DI 8–31) culture-positive carcasses, respectively (Fig. 3).

The sensitivity analyses showed that the culture isolation rate from seropositive carcasses had the greatest impact on the risk of exposure when the seroprevalence was high (study period 1). Similarly, the culture isolation rate from seronegative carcasses



**Fig. 3.** Frequency distributions of 10 000 simulation runs of the number carcasses potentially shedding live leptospirae processed per day by (a) an eviscerator, (b) a meat inspector and (c) an offal handler during a high-risk period (May–November 2004, period 1; □) and a period of average risk (December 2004–June 2005, period 2; ■) in a sheep-only abattoir in New Zealand.

was highly influential when seroprevalence was low (study period 2). Another important determinant was the number of processed carcasses per day. All other input parameters had relatively little impact on the estimate of exposure risk (Table 2).

**DISCUSSION**

This study estimated the daily frequency (i.e. ‘risk’) at which workers at one sheep-only abattoir were exposed to kidney culture-positive carcasses. The

Table 2. Spearman rank correlation coefficients between model input parameters and the daily exposure risk of abattoir workers to sheep carcasses with kidney cultures of *Leptospira* serovars *Hardjo* or *Pomona* during May–November 2004 (study period 1) and December 2004–June 2005 (study period 2) at a sheep-only abattoir in New Zealand

Period ...	Spearman rank-order correlation coefficient					
	Eviscerator		Meat inspector		Offal handler	
	1	2	1	2	1	2
Model input parameter						
Culture isolation rate from seropositive carcasses	0.47	0.14	0.59	0.14	0.76	0.20
Culture isolation rate from seronegative carcasses	0.18	0.42	0.22	0.51	0.25	0.72
Number of carcasses processed by a meat worker/day	0.47	0.33	0.34	0.26	0.16	0.11
Prevalence of seropositive carcasses within seropositive lines	0.12	0.08	0.18	0.12	0.22	0.11
Prevalence of seropositive lines	0.11	0.09	0.15	0.08	0.21	0.12

calculated risk depended entirely on the prevalence of culture isolation and on the number of carcasses processed. However, the extent to which the different intensity of handling carcasses contributed to risk differences between workers at different positions was not evaluated. Thus, assuming that all workers were equally exposed was somewhat crude but inevitable based on the data. Measuring position-specific exposure by sampling carcasses would have been almost impossible given practical circumstances at commercial slaughter plants. However, a feasible design to associate the exposure risk with type of work would be a longitudinal study of seroconversion in meat workers at different positions along the slaughter chain. Initiated by the second author of this paper, such a study is currently underway in eight processing plants in New Zealand.

Estimates of exposure risks to leptospirosis varied from moderate for individual eviscerators and meat inspectors to high for offal handlers, and were highly dependent on the slaughter season (period prevalence). Data collection coincided by chance with extensive rainfall in the summer of 2004 which resulted in surface flooding of most of the areas from which sheep were sourced for slaughter (study period 1). Such floods have occurred every 10–15 years since weather was recorded in the affected regions. The subsequent season was a typical dry summer with only occasional and moderate rainfall (study period 2). The leptospirosis seroprevalence was distinctly different and this led to a substantial difference in exposure of

abattoir workers to infected sheep carcasses. Exposure estimates during the first study period must therefore be regarded as exceptionally high while those of the second study period rather represented an ‘average’ season. The seasonal difference in prevalence is most likely due to ambient temperature and surface water, because other conditions did apparently not vary between seasons. For example, the age of lambs follows very similar seasonal patterns due to the strictly seasonal lambing that is typical for sheep breeding in New Zealand. Moreover, there were no apparent changes in breed or in locations and source farms between the two study periods.

In addition to prevalence, the other factor determining the exposure risk was the number of sheep processed per day. Each shift in a full day’s processing work at the abattoir involves one offal handler, five eviscerators and three meat inspectors, thus the relatively high exposure risk for offal handlers was due to the greater number of carcasses processed per day. A 1982 cross-sectional study conducted at six meat processing plants (three processing sheep and cattle, two sheep, cattle and pig and one pig-only) found no significant difference between the seroprevalence in slaughter floor-workers and meat inspectors [8]. This finding may not be valid now, as work practices and the use of protective clothing and equipment have changed in modern processing plants, including specialization to only processing a single species.

This study supports earlier findings of positive leptospirosis titres in meat workers and meat inspectors

compatible with occupational exposure [8, 9] and strengthens the case for a role of sheep as an important source of human leptospirosis [2]. The exposure risk was not constant over time and varied both between and within seasons depending on the prevalence of infection in sheep being processed at particular times. A reasonable assumption is that at least some of the exposures would result in successful infection given the daily frequency and the degree and nature of exposures. Eviscerators incise the kidney capsules and enucleate the kidneys as part of routine processing and to facilitate examination by the meat inspectors. Face masks may not always be worn for guarding against exposure from splashing urine, and despite suggestions that infection may be mediated through intact but dampened skin after prolonged immersion in water [19, 20], the authors know from personal observation during sampling that compliance with wearing gloves is lacking. The risk assessments were only done for those personnel, namely eviscerators, inspectors and offal handlers, who handled kidneys. Other workers who did not handle kidneys, but were in some way exposed to blood or urine would potentially be exposed to infection, too. Thus, here might be additional risks arising from those sources for those workers. However, no cultures were performed on urine and blood, hence no assessments could be made about the direct exposure risk from these infection sources.

A serious concern of high risk of exposure of meat workers to leptospires would be in instances where sheep are sent to slaughter from farms with recent clinical or unrecognized outbreaks of leptospirosis. In our earlier study [16], we found that in 30 lambs sampled from 500 lambs in a line from a suspected outbreak, 13 carcasses were seropositive to Pomona, and all were kidney culture-positive. Furthermore, 3/5 kidneys from seronegative carcasses with a high score of white-spot lesions in their kidneys were culture positive, too. Thus, the risk of exposure from these lines must be regarded as extremely high. However, it is unknown how often such lines are passing through an abattoir in an average season.

No critical evaluation of the performance of MAT and culture tests has been reported in the scientific literature and for all analyses it was assumed that the sensitivity and specificity of these tests were close to 100%. The MAT is generally considered as the reference test and its sensitivity and specificity are thought to be high as it apparently does not cross-react with any other bacteria, other than leptospires

belonging to the same serogroup [22]. Conversely, the sensitivity of culture test is intuitively considered to be moderate in the absence of a gold standard [19], while being similar to PCR [21]. Our perfect test assumptions would have biased the results towards more conservative estimates if the sensitivity of culture is indeed low. The culture isolation rates in seropositive and seronegative carcasses of the second December 2004–June 2005 study period were applied for assessment of exposure risks during the first processing period. This may have introduced some bias because no cultures were done in the first May–November 2004 study period when the seroprevalence at line and individual animal levels was significantly higher compared to the second period. The first period (May–November 2004) was preceded by extensive surface floods occurring during moderate to high mean daily temperatures in February 2004. As sheep are farmed on pasture throughout the year, the flood in the source area of the sampled carcasses during the period preceding the first study period was the most plausible and strongest cause of the higher than average seroprevalence. Culture isolation rates in kidneys could have been substantially higher in the first, rather than the second, low-risk period (December 2004–June 2005). If flood and temperature were strong determinants of prevalence in the first period, the time of infection would have been relatively close to the time of slaughter giving rise to a relatively high number of acute infections than occur under typical dry conditions of an average summer. Consequently, the exposure rates for the high-risk season (May–November 2004; Fig. 2) would have been underestimated.

Input variables with the greatest influence on daily exposure risks were culture isolation rates from seropositive carcasses during a period of high prevalence and from seronegative carcasses during low prevalence. This was due to relatively low numbers of carcasses from which kidneys were cultured causing low precision of culture rate estimates. Another important input parameter was the daily number of sheep processed by each worker. This, in conjunction with the probably quite variable quality and quantity of exposure of different positions/activities at the processing chain, indicates that these are areas of uncertainty about which more information is required. Rather than sampling carcasses, a feasible research method to answer such questions is a study of infection rates in humans relating human infection status to processing activities controlling for age, time at work and lifestyle factors.

Although the exposure risk was evaluated at one abattoir only, we believe that relatively similar conditions exist in processing sheep carcasses within New Zealand because the general food hygiene regulations apply to all abattoirs. Differences between abattoirs processing sheep, e.g. the physical set up for processing, the speed of processing, the number of workers employed for each of the various processing functions, or compliance with protective clothing and equipment may be relatively small. Our findings may therefore be fairly representative for sheep-only abattoirs in New Zealand. Leptospirosis is emerging as a zoonosis worldwide [23], thus similar data about the serological or culture prevalence may exist in other countries where it is a recognized occupational disease [13, 20, 24–26]. Stochastic risk modelling as demonstrated in this paper may be useful for quantifying exposure in such situations.

Although this study has provided an insight into the daily frequencies of exposure risks at a sheep-only abattoir, the extent to which exposure risks of the observed magnitude result in successful human infection and clinical disease has yet to be determined. This latter task is a necessary step for obtaining a reasonably accurate and comprehensive assessment of risk of occupational leptospirosis in meat workers and for the formulation of effective risk reduction programmes.

In conclusion, this study demonstrates the usefulness of data from seroprevalence and culture prevalence studies and the value of stochastic risk modelling for obtaining quantifiable occupational exposure risk estimates of meat workers to leptospirosis. This study appears to be the first where data from seroprevalence and culture prevalence studies were used for an exposure risk assessment. The findings alerted public health authorities and occupational safety and health personnel about the potential size of a zoonotic risk and triggered subsequent, ongoing studies of the incidence of human infection in several abattoirs. The methodology is robust and reproducible and may have bearing on future investigations about occupational risks.

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## DECLARATION OF INTEREST

None.

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