# Leptospirosis diagnostics and exposure at the human and animal interface in New Zealand

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# Abstract

The studies presented in this thesis investigate key questions about leptospirosis diagnostics in animals and humans in New Zealand (NZ): how do different diagnostic tests perform on various specimens collected at different stages of infection; how well do tests from a commercial and a research laboratory agree; how do serological test results and urine/kidney quantitative real-time PCR (qPCR) results compare; and what is the utility of PCRs on blood from acute human cases? Additional studies investigate occupational risk at the human-animal interface.

In trials where the animals were challenged with *Leptospira borgpetersenii* serovar Hardjobovis (Hardjobovis) and/or *Leptospira interrogans* serovar Pomona (Pomona), sequential samples were taken from sheep and cattle to evaluate diagnostic tests at various known times post-infection. Although no statistically significant differences were found, results suggested that during the early stage of a Pomona infection in sheep, qPCR on serum had the highest sensitivity for detecting leptospires in blood, followed by blood culture and qPCR on whole blood. In sheep infected under carefully controlled experimental conditions, culture tended to have higher sensitivity for detecting leptospires (either Hardjobovis or Pomona) in urine than qPCR; whereas in cattle with Hardjobovis infection, higher sensitivity was apparent using qPCR. Sensitivity was similar in culture and qPCR for detecting leptospires in kidney from sheep with either Hardjobovis or Pomona infections. There was low sensitivity and specificity of dark field microscopy for both urine and kidney samples, thus questioning the usefulness of this technique in veterinary settings.

A cross-sectional study was carried out at a NZ sheep and cattle abattoir to investigate the seroprevalence (by microscopic agglutination test (MAT)), shedding rate (by urine qPCR), and renal colonisation rate (by kidney qPCR) of slaughtered animals. Urine, kidney and blood samples were collected from carcasses of 399 sheep and 146 cattle. The animal-level seroprevalence found in sheep (57%, predominately lambs) and cattle (73%, predominately ≤18 months old) was substantially higher than in previous studies; these and the recorded shedding rate (27%) and renal colonisation rate (27%) raised occupational health concerns that meat workers from this abattoir may be at risk of exposure to leptospires during their daily work routine.

Samples from this abattoir study were used to investigate the inter-laboratory test agreements between a research (HLRL) and a commercial veterinary diagnostic laboratory (GV), and test agreements (HLRL) between specimens for leptospirosis diagnosis. Urine qPCR results on from the two laboratories had almost perfect agreement (kappa = 0.93). The MAT agreement between these two laboratories was higher for Hardjobovis (kappa = 0.94) than Pomona (kappa = 0.53). This serovar-dependent difference suggested that the different MAT results may be more likely due to the different source of antigen cultures (especially serovar Pomona) used in two laboratories than observer variation. These inter-laboratory comparisons can assist researchers and diagnosticians in understanding the sometimes discrepant test results received. Within HLRL, almost perfect agreement (kappa = 0.84) between qPCR results on urine and kidney suggested that the qPCR on these two specimens can be used interchangeably. The comparisons between MAT and qPCR on both kidney and urine, suggested that except from Hardjobovis-seropositivity in sheep, Pomona-seropositivity in sheep and seropositivity of both Hardjobovis and Pomona in cattle was not considered to be predictive for indicating shedding/renal colonisation at individual animal level.

A pilot panel of isolates from 18 sheep and five cattle kidney cultures demonstrated the utility of a multi-locus sequence typing scheme for genotyping *Leptospira* spp. field isolates from sheep and cattle in NZ. The sequence results provided sufficient genetic variability to assign the isolates to two distinct species, those being *L. borgpetersenii* and *L. interrogans*. Two dominant serovars (Hardjobovis and Kenniwicki) were identified. Identical sequences found in Hardjobovis isolates from sheep and cattle provided evidence for inter-species transmission of *Leptospira* spp.

Aiming to establish the best diagnostic test or combination of tests for the early diagnosis of human leptospirosis, suspect leptospirosis patients were recruited via rural general practitioners (GP), hospital doctors and phlebotomists within the Waikato District Health Board area. For each recruited patient (n = 14), blood culture, MAT (on acute and convalescent serum), and whole blood/serum PCRs (by three laboratories) were performed. Although it is difficult to make conclusions based on findings from 14 patients recruited from one region, this is the first attempt to compare different diagnostic tests for acute leptospirosis cases in NZ. The information of clinical

symptoms, demographics, and exposure to risk factors can contribute to the GPs' suspicion of future leptospirosis cases.

A cross-sectional study was conducted to determine the seroprevalence and quantify putative risk factors for both intra- and extra-curricular exposure to leptospirosis among undergraduate veterinary students at Massey University, NZ. All participating students (n = 302) were MAT negative for each serovar (Hardjobovis, Pomona, and Ballum), using a cut-point of ≥ 48. This study demonstrated that these veterinary students were at low risk of contracting leptospirosis, despite frequent exposure to potential sources of infection (e.g. animal urine within and outside veterinary curriculum, home slaughtering, hunting, and outdoor activities involving fresh water). The similar frequency of exposure to the non-work putative risky activities (hunting and home slaughtering) reported in veterinary students as previously reported in meat workers, added strength to the finding that non-work activities are less important risk factors compared to within-work activities.

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# List of Publications

**Fang F, Benschop J, Wilson PR, Collins-Emerson JM, Heuer C and Prattley D.** Seroprevalence and exposure to risk factors for leptospirosis among veterinary students at Massey University. *New Zealand Veterinary Journal* 62, 130-5, 2014.

**Fang F, Collins-Emerson JM, Cullum A, Heuer C, Wilson PR, Benschop J.** Shedding and Seroprevalence of Pathogenic *Leptospira* spp. in Sheep and Cattle at a New Zealand Abattoir. *Zoonoses and Public Health*, 2014. doi: 10.1111/zph.12146.

**Fang F, Collins-Emerson JM, Heuer C, Hill FI, Tisdall DJ, Wilson PR, Benschop J.** Inter-laboratory and between-specimen comparisons of diagnostic tests for leptospirosis in sheep and cattle. *Journal of Veterinary Diagnostic Investigation* (accepted for publication July 2014).

**Benschop J, Fang F, Heuer C, Wilson PR, Collins-Emerson J**. Fit for purpose: testing for leptospirosis in humans and animals. *Proceedings of the 8th Scientific Meeting of International Leptospirosis Society,* OR3-49, 2013.

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**Heuer C, Wilson P, Collins-Emerson J, Dreyfus A, Subharat S, Fang F and Midwinter A.** Massey researchers contribute to international leptospirosis conference. *Vetscript* 22, 26-8, 2009.

# List of Presentations and Posters

**Fang F, Collins-Emerson JM, Wilson PR, Heuer C, Tisdall D and Benschop J**. Leptospirosis infection in sheep and cattle in a New Zealand Abattoir: sero-prevalence, shedding and diagnostic test comparison. *Poster session presented at the meeting of 13th International Symposium on Veterinary Epidemiology and Economics (ISVEE)*. Maastricht, Belgium/Netherlands, 2012.

**Fang F, Benschop J, Collins-Emerson JM, Wilson PR, Heuer C and Tisdall D**. Leptospirosis infection in sheep and cattle in a New Zealand Abattoir: sero-prevalence, shedding and diagnostic test comparison. *Poster session presented at the 1st Infectious Disease Research Centre (IDReC) Symposium*. Convention Centre, Palmerston North, New Zealand, 2012.

**Fang F, Benschop J, Collins-Emerson JM, Wilson PR and Heuer C.** Leptospirosis in New Zealand: diagnostics. *The NCBID epidemiological skills development programme: Module 2.3 Laboratory Investigation Course (Session 10)*. NCBID, Upper Hutt, New Zealand, 2012.

**Fang F, Benschop J, Wilson PR, Collins-Emerson JM and Heuer C.** Sero-prevalence and shedding of pathogenic Leptospira spp. in a local mixed-species abattoir. *The VII Reunion de la International Leptospirosis Society (ILS)*. Merida, Yucatan, Mexico, 2011.

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**Fang F, Benschop J, Collins-Emerson JM, Wilson PR and Heuer C**. Effect of antibody titer cut points on inferences from a cross-sectional sero-prevalence study of leptospirosis in meat work. *Poster session presented at the 6th Annual Scientific Meeting of International Leptospirosis Society (ILS).* Gokulam Convention Centre, Cochin, India, 2009.

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