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

A thesis presented in partial fulfilment of the requirements  
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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  $\leq 18$  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  $\geq 48$ . 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).

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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.

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# Table of Contents

Abstract.....	iii
Acknowledgements.....	vi
List of Publications.....	viii
List of Presentations and Posters.....	x
Table of Contents.....	xi
List of Figures.....	xvii
List of Tables.....	xx
Appendices.....	xxiii
<b>Chapter 1 General Introduction.....</b>	<b>1</b>
1    Leptospirosis Overview.....	1
1.1    Leptospira (the organism).....	1
1.2    Epidemiology of leptospirosis.....	1
1.3    Clinical features.....	3
1.4    Burden of human and animal leptospirosis.....	4
1.5    Diagnosis.....	5
2    Leptospirosis in New Zealand.....	6
3    Thesis aim and structure.....	9
4    References.....	12
<b>Chapter 2 Literature Review: Laboratory Diagnosis of Leptospirosis.....</b>	<b>19</b>
1    Introduction.....	19
2    Direct examination for leptospires and antigen detection.....	21
2.1    Dark field microscopic examination.....	21
2.2    Detection of leptospiral antigen.....	22
3    Culture of leptospires from clinical specimens.....	23
4    Serological tests.....	25
4.1    The microscopic agglutination test (MAT).....	26
4.2    Enzyme-linked immunosorbent assay (ELISA).....	31
4.3    Other serological methods.....	34
5    Molecular methods.....	35
5.1    Polymerase chain reaction (PCR).....	35
5.2    Isothermal methods.....	41
6    Conclusion.....	42
7    References.....	44

**Chapter 3 Appraisal of diagnostic assays following challenge with *Leptospira borgpetersenii* serovar Hardjobovis and *Leptospira interrogans* serovar Pomona in sheep and cattle..... 63**

1	Introduction.....	63
2	Materials and methods .....	65
2.1	Experimental Design.....	65
2.2	Challenge .....	66
2.3	Sample collection.....	67
2.4	Husbandry of animals .....	67
2.5	PCR detection of bacterial DNA.....	68
2.5.1	DNA extraction.....	68
2.5.1.1	Urine samples.....	68
2.5.1.2	Whole blood samples .....	68
2.5.1.3	Serum samples .....	68
2.5.1.4	Kidney samples .....	69
2.5.2	PCR amplification.....	69
2.6	Culture.....	70
2.7	MAT.....	70
2.8	Dark field microscopy (DFM) on urine and kidney cultures .....	71
3	Results.....	71
3.1	Sheep trials A, B and C.....	71
3.1.1	Clinical observations.....	71
3.1.2	Bacteriological findings.....	72
3.1.2.1	Leptospire in sheep urine.....	72
3.1.2.2	Leptospire in kidneys .....	75
3.1.2.3	Leptospire in whole blood and serum.....	75
3.1.3	Immune response .....	75
3.2	Cattle trial D.....	77
3.2.1	Clinical observations.....	77
3.2.2	Bacteriological findings.....	77
3.2.2.1	Leptospire in urine.....	77
3.2.2.2	Leptospire in whole bloods and serum .....	78
3.2.3	Immune response in cattle.....	78
4	Discussion.....	78
5	Acknowledgements.....	89
6	References.....	90

**Chapter 4 Shedding and seroprevalence of pathogenic leptospire in sheep and cattle at a New Zealand abattoir ..... 95**

1	Summary.....	96
---	--------------	----

2	Impacts.....	97
3	Introduction.....	97
4	Materials and Methods.....	98
4.1	Study design.....	98
4.2	Recruitment of suppliers.....	99
4.3	Sample collection and testing.....	100
4.4	Statistical analysis.....	101
5	Results.....	102
5.1	Urine qPCR.....	103
5.2	Kidney qPCR.....	103
5.3	MAT.....	104
5.4	Urinary shedding and renal colonisation in seropositive and seronegative animals.....	105
6	Discussion.....	<del>107</del> <u>107</u>
7	Acknowledgements.....	<del>114</del> <u>114</u>
8	References.....	<del>115</del> <u>115</u>

**Chapter 5 Inter- and between-specimen comparisons of diagnostic tests for leptospirosis in sheep and cattle.....** ~~121~~121

1	Abstract.....	<del>122</del> <u>122</u>
2	Introduction.....	<del>122</del> <u>122</u>
3	Materials and methods.....	<del>125</del> <u>125</u>
3.1	Study design.....	<del>125</del> <u>125</u>
3.2	Quantitative real-time PCR.....	<del>126</del> <u>126</u>
3.2.1	DNA extraction.....	<del>126</del> <u>126</u>
3.2.1.1	Urine samples (HLRL).....	<del>127</del> <u>127</u>
3.2.1.2	Urine samples (GV).....	<del>127</del> <u>127</u>
3.2.1.3	Kidney samples (HLRL).....	<del>127</del> <u>127</u>
3.2.2	DNA amplification.....	<del>127</del> <u>127</u>
3.2.2.1	Hopkirk Leptospirosis Research Laboratory.....	<del>127</del> <u>127</u>
3.2.2.2	Gribbles Veterinary.....	<del>128</del> <u>128</u>
3.3	Microscopic agglutination test.....	<del>128</del> <u>128</u>
3.3.1	Hopkirk Leptospirosis Research Laboratory.....	<del>128</del> <u>128</u>
3.3.2	Gribbles Veterinary.....	<del>129</del> <u>129</u>
3.4	Statistical Analysis.....	<del>129</del> <u>129</u>
4	Results.....	<del>131</del> <u>131</u>
4.1	Inter-laboratory comparisons.....	<del>131</del> <u>131</u>
4.1.1	Urine qPCR.....	<del>131</del> <u>131</u>
4.1.2	MAT.....	<del>132</del> <u>132</u>
4.2	Intra-laboratory comparisons between tests on different specimens.....	<del>135</del> <u>135</u>

4.2.1	Urine and kidney qPCR .....	<u>135</u> <del>135</del>
4.2.2	Urine and kidney qPCR and MAT .....	<u>136</u> <del>136</del>
4.3	Kidney culture .....	<u>140</u> <del>140</del>
5	Discussion .....	<u>140</u> <del>140</del>
6	Acknowledgements .....	<u>146</u> <del>146</del>
7	Sources and manufacturers .....	<u>146</u> <del>146</del>
8	Declaration of conflicting interests .....	<u>147</u> <del>147</del>
9	Funding .....	<u>147</u> <del>147</del>
10	References .....	<u>147</u> <del>147</del>

**Chapter 6 Multilocus Sequence Typing of *Leptospira* field strains isolated from sheep and cattle in New Zealand .....** ~~153~~153

1	Introduction .....	<u>153</u> <del>153</del>
2	Materials and Methods .....	<u>158</u> <del>158</del>
2.1	Cultures for <i>Leptospira</i> isolates .....	<u>158</u> <del>158</del>
2.2	Leptospire typing by MLST .....	<u>159</u> <del>159</del>
2.2.1	DNA extraction .....	<u>159</u> <del>159</del>
2.2.2	PCR amplification .....	<u>159</u> <del>159</del>
2.2.3	Purification of the amplified PCR products .....	<u>161</u> <del>161</del>
2.2.4	Sequencing .....	<u>161</u> <del>161</del>
2.2.5	Sequence analysis .....	<u>161</u> <del>161</del>
3	Results .....	<u>162</u> <del>162</del>
3.1	<i>Leptospira</i> isolates .....	<u>162</u> <del>162</del>
3.2	PCR amplification .....	<u>162</u> <del>162</del>
3.3	Sequence analysis .....	<u>162</u> <del>162</del>
3.4	Phylogenetic tree analysis .....	<u>163</u> <del>163</del>
3.5	MLST results .....	<u>165</u> <del>165</del>
4	Discussion .....	<u>166</u> <del>166</del>
5	Acknowledgements .....	<u>172</u> <del>172</del>
6	References .....	<u>173</u> <del>173</del>

**Chapter 7 Comparison of diagnostic tests for acute leptospirosis in humans in New Zealand .....** ~~182~~182

1	Introduction .....	<u>182</u> <del>182</del>
2	Materials and Methods .....	<u>185</u> <del>185</del>
2.1	Study design .....	<u>185</u> <del>185</del>
2.2	Ethical approval .....	<u>185</u> <del>185</del>
2.3	Recruitment .....	<u>186</u> <del>186</del>
2.3.1	GP recruitment .....	<u>186</u> <del>186</del>

2.3.2	Hospital doctors/phlebotomists recruitment .....	<u>187187</u>
2.3.3	Patient recruitment procedure .....	<u>187187</u>
2.3.4	Data and serum samples sourced from ESR .....	<u>188188</u>
2.4	Sample collection and processing .....	<u>188188</u>
2.5	Diagnostic tests .....	<u>189189</u>
2.5.1	MAT .....	<u>189189</u>
2.5.2	Culture .....	<u>190190</u>
2.5.2.1	ESR .....	<u>190190</u>
2.5.2.2	HLRL .....	<u>190190</u>
2.5.3	PCR .....	<u>190190</u>
2.5.3.1	ESR qPCR on serum and whole blood .....	<u>190190</u>
2.5.3.2	HLRL qPCR on serum and whole blood .....	<u>191191</u>
2.5.3.3	CHL conventional PCR on whole blood .....	<u>192192</u>
2.6	Database set up and data analysis .....	<u>193193</u>
3	Results .....	<u>193193</u>
3.1	Recruited patients .....	<u>193193</u>
3.2	Serum samples sourced from ESR .....	<u>198198</u>
4	Discussion .....	<u>199199</u>
5	Acknowledgements .....	<u>206206</u>
6	References .....	<u>207207</u>

**Chapter 8 Seroprevalence and exposure to risk factors for leptospirosis among veterinary students at Massey University.....213213**

1	Abstract .....	<u>214214</u>
2	Introduction .....	<u>214214</u>
3	Materials and methods .....	<u>216216</u>
3.1	Study design .....	<u>216216</u>
3.2	Student recruitment .....	<u>217217</u>
3.3	Survey development .....	<u>217217</u>
3.4	Serological test .....	<u>218218</u>
3.5	Statistical analyses .....	<u>218218</u>
4	Results .....	<u>218218</u>
4.1	Demographic characteristics .....	<u>219219</u>
4.2	Potential exposure within the veterinary curriculum .....	<u>219219</u>
4.3	Potential exposure outside the veterinary curriculum .....	<u>221221</u>
4.4	Previous leptospirosis cases and Influenza-like symptoms .....	<u>221221</u>
5	Discussion .....	<u>222222</u>
6	Acknowledgements .....	<u>226226</u>
7	References .....	<u>227227</u>



<b>Chapter 9 General Discussion.....</b>	<b><u>230230</u></b>
1 Introduction.....	<u>230230</u>
2 Research findings in context.....	<u>231231</u>
2.1 Leptospirosis diagnosis.....	<u>231231</u>
2.1.1 Choice of diagnostic tests and specimen at different stages of infection.....	<u>231231</u>
2.1.2 Evaluation of gyrB gene based qPCR with veterinary and human clinical samples.....	<u>234234</u>
2.1.3 Clinical symptoms and leptospirosis diagnosis in humans.....	<u>235235</u>
2.2 Test comparison.....	<u>238238</u>
2.2.1 Inter-laboratory comparison of urine qPCR.....	<u>238238</u>
2.2.2 Inter-laboratory comparison of MAT.....	<u>240240</u>
2.2.3 Association between serological test and testing for shedding/renal colonisation.....	<u>243243</u>
2.3 Host-serovar relationship between Hardjobovis/Pomona and sheep/cattle in NZ.....	<u>244244</u>
2.4 Reflection on occupational exposure to leptospires.....	<u>246246</u>
2.5 The value and challenge of multi-disciplinary research.....	<u>249249</u>
2.6 Implication of thesis findings for vaccination of sheep and cattle in NZ.....	<u>253253</u>
3 Reflective critique of study methodologies.....	<u>255255</u>
3.1 Design of challenge trials.....	<u>255255</u>
3.2 Utility of the microscopic agglutination test.....	<u>257257</u>
4 Suggested areas for future work.....	<u>260260</u>
4.1 Reporting the accuracy of diagnostic tests.....	<u>260260</u>
4.2 Quality control of microscopic agglutination test.....	<u>262262</u>
4.3 Assessment of occupational exposure to leptospirosis.....	<u>262262</u>
4.4 Inclusion of urine testing in animal studies.....	<u>263263</u>
4.5 The ability of qPCR to distinguish serovars using melting temperature.....	<u>264264</u>
4.6 Genotyping of <i>Leptospira</i> isolates in New Zealand.....	<u>264264</u>
5 Conclusion.....	<u>265265</u>
6 References.....	<u>267267</u>
<b>Appendix.....</b>	<b><u>280280</u></b>
Chapter 3.....	<u>280280</u>
Chapter 4.....	297
Chapter 6.....	<u>309309</u>
Chapter 7.....	<u>325325</u>
Chapter 8.....	338

# List of Figures

## Chapter 1

- Figure 1.1** Flow diagram of epidemiology of leptospirosis in animals and humans..... 2
- Figure 1.2** Proportion of laboratory-reported cases infected with serovar Hardjobovis, Pomona, and Ballum by year 2002-2012 (The Institute of Environmental Science and Research Ltd. 2003-2013)..... 7
- Figure 1.3** Leptospirosis notifications and laboratory reported cases by year 2002-2012 (lines); proportion of notified cases with occupations recorded as farmers and meat workers (bars) (The Institute of Environmental Science and Research Ltd. 2003-2013)..... 8

## Chapter 2

- Figure 2.1** Schematic representation of the biphasic nature of leptospirosis and relevant diagnostic investigations at different stages of disease. For serology specimens 1 and 2 are acute-phase specimens, 3 is a convalescent-phase specimen which may facilitate detection of a delayed immune response, 4 and 5 are follow-up specimens which can provide epidemiological information, such as the presumptive infecting serogroup. This figure was adapted with permission from (Levett 2001). ..... 21
- Figure 2.2** Response patterns of anti-leptospiral IgM and IgG class antibodies in leptospirosis infection. This figure was adapted with permission from (Picardeau 2013). ..... 26

## Chapter 3

- Figure 3.1** Number of sheep tested positive by culture, qPCR and DFM on urine on all sampling days (Day 0 to 42) after challenge among 16 sheep for Trial C..... 74
- Figure 3.2** Geometric mean of MAT titres against serovar Hardjobovis, and seroprevalence on eight sampling days (Day 0 to 37) after challenge for seven sheep (B6 were excluded from this analysis) from Trial B. Data from Day 21 onwards does not include that from sheep B4 as it died on Day 15..... 76
- Figure 3.3** Geometric mean of MAT titres against serovar Pomona, and seroprevalence on six sampling days (Day 0 to 42) after challenge among 16 sheep for Trial C..... 77

## Chapter 4

- Figure 4.1** Seroprevalence of serum samples with positive MAT titre ( $\geq 48$ ) from sheep and cattle by serovar..... 104

<b>Figure 4.2</b> Frequency histogram of positive MAT titres ( $\geq 48$ ) to serovars Pomona and Hardjobovis in sheep and cattle. ....	105
---	-----

## Chapter 5

<b>Figure 5.1</b> Flowchart showing sampling and testing procedure.....	<a href="#">126</a> <del>126</del>
<b>Figure 5.2</b> Bubble plots comparing the MAT titres for serum samples between GV and HLRL stratified by animal species: a) Hardjobovis in sheep; b) Pomona in sheep; c) Hardjobovis in cattle; d) Pomona in cattle. Bubble size is proportional to the number of samples. ....	<a href="#">134</a> <del>134</del>
<b>Figure 5.3</b> Histogram of the frequency of differences (the interval at HLRL minus the interval at GV) in serum sample storage time (between sample collection date and MAT testing date) between HLRL and GV; by serovar Hardjobovis (left) and Pomona (right). ....	<a href="#">135</a> <del>135</del>
<b>Figure 5.4</b> Percentage (and 95% CI) of urine and kidney samples that were qPCR positive in sheep and cattle that had different levels of MAT titres against serovar Hardjobovis or Pomona: a) percentage of urine samples that were qPCR positive in sheep; b) percentage of urine samples that were qPCR positive in cattle; c) percentage of kidney samples that were qPCR positive in sheep; d) percentage of kidney samples that were qPCR positive in cattle. ....	<a href="#">139</a> <del>139</del>

## Chapter 6

<b>Figure 6.1</b> Neighbour-joining tree built on Tamura-Nei model of 23 isolates for <i>adk</i> gene. The bar indicates 0.02 estimated substitution per sequence position.....	<a href="#">164</a> <del>164</del>
<b>Figure 6.2</b> Neighbour-joining tree built on Tamura-Nei model based on concatenated sequences of six loci from 21 isolates. The bar indicates 0.02 estimated substitution per sequence position. ....	<a href="#">164</a> <del>164</del>

## Chapter 7

<b>Figure 7.1</b> Map showing the seven towns in Waikato region of North Island, New Zealand, from which the 11 medical centres were recruited for this study. Insert: map of New Zealand showing the location covering the study area (Waikato region). ....	<a href="#">186</a> <del>186</del>
<b>Figure 7.2</b> Flow chart for blood sample collection and dispatch to laboratories (WHL= Waikato Hospital laboratory; Pathlab= PathLab Waikato laboratories; ESR = Institute of Environmental Science and Research Ltd, Wellington; HLRL = Hopkirk Leptospirosis Research Laboratory, Massey University; CHL = Canterbury Health Laboratory, Christchurch), and tests performed. ....	<a href="#">189</a> <del>189</del>
<b>Figure 7.3</b> Number of patients that reported each symptom stratified by cases (n=4) and non-cases (n=9); 13 of 14 patients had recorded symptom information. ....	<a href="#">195</a> <del>195</del>

## Chapter 9

**Figure 9.1** Predicted synopsis with 95% confidence interval (represented by dashed lines) of clinical Pomona infection in sheep, from Day 0 to Day 42 after infection; a) predicted probability of leptospire being detected in blood; b) predicted probability of leptospire being detected in urine; c) predicted log MAT titre against serovar Pomona; based on data from 16 sheep that were inoculated with serovar Pomona in Trial C; raw data are represented by ‘+’..

.....[233233](#) |

**Figure 9.2** Framework of the multidisciplinary collaborative approach for the Health Research Council study; “T” hospital laboratory<sup>a</sup> = Taumarunui, Te Kuiti, Tokoroa and Thames hospital laboratory; PathLab blood collection centres<sup>b</sup> = Path Lab Te Awamutu, Leamington, Cambridge, Morrinsville-Dallas, and Matamata blood collection centres; ESR = Institute of Environmental Science and Research Ltd, Wellington; Massey = Hopkirk Leptospirosis Research Laboratory, Massey University; CHL = Canterbury Health Laboratory, Christchurch; WDHB = Waikato District Health Board. ....

[251251](#) |

## List of Tables

### Chapter 3

<b>Table 3.1</b> The providers and the source of challenge cultures used for each trial.....	66
<b>Table 3.2</b> Detection of leptospire in urine on each sampling day after challenge, and kidney on Day 42, by culture (C), qPCR (P) and Dark Field Microscopy (D) and antibody titres against serovars Hardjobovis and Pomona by MAT for sheep Trial A. ....	72
<b>Table 3.3</b> Detection of leptospire in urine on each sampling day after challenge, and kidney on Day 42, by culture (C), qPCR (P) and Dark Field Microscopy (D) and antibody titres against serovar Hardjobovis by MAT for sheep Trial B. ....	73
<b>Table 3.4</b> Detection of leptospire in blood, serum, urine and kidney by culture (C), qPCR (P) and Dark Field Microscopy (D) and antibody titres against serovar Pomona by MAT from each sampling day after challenge for sheep Trial C. ....	74
<b>Table 3.5</b> Detection of leptospire in urine on each sampling day after challenge by culture (C), qPCR (P) and Dark Field Microscopy (D) and antibody titres against serovars Hardjobovis and Pomona by MAT for cattle Trial D.....	78
<b>Table 3.6</b> Summary of number of sheep tested positive by culture, qPCR, and DFM on each sampling day and proportion of animals tested positive during the observation period for 16 sero-converted (Pomona) sheep in Trial C, two sero-converted (Hardjobovis) sheep in Trial A, and three sero-converted (Hardjobovis) cattle in Trial D. ....	82
<b>Table 3.7</b> Summary of number of sheep tested positive by culture, serum qPCR, and whole blood qPCR on each sampling day and proportion of sheep tested positive during the observation period for 16 sero-converted (Pomona) sheep in Trial C. ....	84

### Chapter 4

<b>Table 4.1</b> Number of sheep and cattle sampled from each supplier (sample size), number of samples tested (N) by urine, kidney qPCR and MAT, number of samples tested positive (N pos), and percentage positive with 95% confidence intervals (CI) adjusted for the effect of correlation of animals within slaughter line.....	103
<b>Table 4.2</b> Number of sheep and cattle which had positive MAT titres ( $\geq 48$ ) (seropositive) and negative MAT titres ( $< 48$ ) against serovar Hardjobovis and/or Pomona, Hardjobovis-only and Pomona-only and had urine or kidney samples tested by qPCR (N), number of samples tested positive by urine and kidney qPCR (N pos) and percentage positive with 95% confidence intervals (CI) adjusted for the effect of correlation of animals within slaughter line. ....	<del>106</del>

## Chapter 5

**Table 5.1** Number of urine samples tested positive (Pos) or negative (Neg) by HLRL and GV qPCR when the GV suspect results ( $36 < Ct \leq 40$ ) were and were not considered as positive, and agreement (presented by kappa) between these two qPCRs, stratified by animal species.....[132-132](#)

**Table 5.2** Number of serum samples that were MAT positive (Pos) or negative (Neg) by HLRL and GV, and agreement (presented by kappa) between these two MATs, stratified by serovar and animal species. ....[133-133](#)

**Table 5.3** Number of animals tested urine and kidney qPCR positive (Pos) or negative (Neg), and agreement (presented by kappa) between urine and kidney qPCR results, stratified by animal species. ....[136-136](#)

**Table 5.4** Number of urine and kidney samples tested qPCR positive (Pos) or negative (Neg) by MAT results (titre  $\geq 48$ , against Hardjobovis and/or Pomona), and agreement (presented by kappa) between urine, kidney qPCR results and MAT results, stratified by animal species. ....[136-136](#)

**Table 5.5** Percentage of urine and kidney samples that were qPCR positive in seropositive (MAT titre  $\geq 48$ ) and seronegative (MAT titre  $< 48$ ) animals, prevalence ratio of urine and kidney qPCR positivity in seropositive versus seronegative animals, with 95% confidence intervals (CI) adjusted for the effect of correlation of animals within slaughter line .....[138-138](#)

## Chapter 6

**Table 6.1** *Leptospira* species endemic in New Zealand and animal reservoirs to which the serovars are adapted (Hathaway 1981; Marshall and Manktelow 2002; Ayanegui-Alcerreca *et al.* 2007; Dorjee *et al.* 2008). ....[157-157](#)

**Table 6.2** Strain type of each of the six serovars of *Leptospira spp.* reference cultures maintained by HLRL .....[158-158](#)

**Table 6.3** The six MLST genes and primers used in this study .....[160-160](#)

**Table 6.4** Supplier and animal species, MLST allelic groupings for the 23 isolates, and microscopic agglutination test (MAT) titres against serovar Hardjobovis and Pomona for the animals from which the 23 isolates were obtained in this study .....[165-165](#)

## Chapter 7

**Table 7.1** PCR (q- and conventional), culture and MAT results for the 14 patients recruited. ....[194-194](#)

**Table 7.2** Demographic, clinical course, and treatment information for the 14 patients. ...[196-196](#)

<b>Table 7.3</b> Information of occupation and exposure to risk factors of leptospirosis infection for the 14 recruited patients.....	<a href="#">197</a> <a href="#">197</a>
<b>Table 7.4</b> Information of exposure to other risk factors of leptospirosis infection for nine patients who had the Waikato Leptospirosis Questionnaire filled in by the MOoH.....	<a href="#">198</a> <a href="#">198</a>
<b>Table 7.5</b> Demographic and sampling information of the 17 cases sourced from ESR, MAT from ESR, ELISA screening from local laboratories, and qPCR (ESR and HLRL) results for the 23 serum samples from these 17 cases.....	<a href="#">199</a> <a href="#">199</a>

## Chapter 8

<b>Table 8.1</b> Demographic characteristics for the 302 veterinary students who completed the questionnaire and provided blood samples .....	<a href="#">219</a> <a href="#">219</a>
<b>Table 8.2</b> The number (and %) of students reporting exposure to animal urine within the veterinary curriculum (n=245) and outside the veterinary curriculum (n=143) by species within the previous 18 months .....	<a href="#">220</a> <a href="#">220</a>
<b>Table 8.3</b> The number (and %) of students (n=302) reporting exposure to potential sources of leptospirosis outside the veterinary curriculum in the past 18 months .....	<a href="#">221</a> <a href="#">221</a>

## Chapter 9

<b>Table 9.1</b> Clinical symptoms and diagnostic results from the 13 patients recruited for HRC study (Chapter 7) who had clinical symptoms recorded; first serum sample was collected on recruitment date; second serum sample was collected 3 weeks apart from the recruitment date; information for patients confirmed as leptospirosis cases are shaded in grey. ....	<a href="#">237</a> <a href="#">237</a>
<b>Table 9.2</b> GV (Ct value included) and HLRL urine qPCR results before and after DNAs were exchanged for the 16 samples that had different urine qPCR results originally. ....	<a href="#">240</a> <a href="#">240</a>
<b>Table 9.3</b> Reading of MAT titres from Observer 1 and Observer 2 (experienced leptospirosis researchers, HLRL) on ten serum samples of unknown serological status, positive control (serovar Pomona (pom +ve)) and negative control (saline), stratified by different methods of diluting the serum samples in the MAT process (Micro Diluter/multichannel pipettes). ....	<a href="#">242</a> <a href="#">242</a>

# Appendices

## Chapter 3

Appendix 3a Challenge and sampling schedule for all four trials .....	<del>280</del> 280
Appendix 3b Count of challenge cultures for all four challenge trials .....	282
Appendix 3c Test results for all four challenge trials .....	<del>283</del> 283

## Chapter 4

Appendix 4 Test results for the abattoir study .....	296
--	-----

## Chapter 6

Appendix 6a Template and Primer Concentration Requirements for Full Sequencing Service ...	<del>309</del> 309
Appendix 6b Details of the 292 <i>Leptospira</i> strains in the MLST database provided by Carlos Alfredo Carmona-Gasca (Carmona-Gasca <i>et al.</i> 2011) .....	<del>310</del> 310
Appendix 6c Sequence from isolate E48, Hardjobovis isolate (D9), and Pomona isolate (A6) at loci <i>lipL41</i> .....	<del>324</del> 324

## Chapter 7

Appendix 7a Cover letter for GP .....	<del>325</del> 325
Appendix 7b GP information sheet.....	<del>326</del> 326
Appendix 7c Study protocol for GPs/hospital doctors about patient recruitment.....	<del>328</del> 328
Appendix 7d Study protocol for phlebotomists about patient recruitment .....	<del>329</del> 329
Appendix 7e Information Sheet for Study Participants .....	<del>330</del> 330
Appendix 7f Leptospirosis study participant identification form .....	<del>333</del> 333
Appendix 7g Waikato Leptospirosis questionnaire .....	<del>334</del> 334

## Chapter 8

Appendix 8 Questionnaire for Veterinary Student Study .....	338
---	-----



