

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

Epidemiological investigation into abortion in farmed red deer in New Zealand

A thesis presented in partial fulfilment of the requirements for the degree of

Doctor of Philosophy in Veterinary Sciences

at Massey University, Palmerston North, New Zealand

Kandarp Khodidas Patel

2016

Institute of Veterinary Animal
and Biomedical Sciences
Massey University
Palmerston North
New Zealand

September 2016

Abstract

Reproductive performance in rising two-year-old (R2) and mixed-aged (MA) adult hinds is suboptimal in farmed red deer in New Zealand due to failure to conceive, fetal loss, and perinatal and postnatal mortality. Reproductive efficiency (calves weaned/hinds mated) in the last decade has averaged 75% (Statistics New Zealand 2016). Previous studies have identified risk factors for conception/pregnancy. However, while abortions are considered rare, they have been reported at low levels in a few earlier studies, but more recently a clinical investigation reported up to 10% mid-term abortion in four herds. Hence, abortion may be going unobserved on deer farms.

This epidemiological study was designed to investigate fetal wastage in farmed deer in New Zealand. The work presented in this thesis includes estimation of incidence and prevalence along with putative investigation into infectious causes based on blood, uteri and aborted fetal tissue, and analysis of farm and management risk factors based on data collected by questionnaire. It also includes the validation of an ELISA for *Toxoplasma gondii* which, based on recent clinical observations, was considered a likely contributor to abortion. Gold standard and Bayesian methodology showed this test to be 78.9% and 98.8% sensitive and 97.5% and 92.8% specific, respectively.

Eighty-five deer farms were recruited over two-years, comprising 87 R2 and 71 MA herds and 22,130 R2 and 36,223 MA hinds. The mean pregnancy rate at usual scan (Scan-1) was 82.0% (range: 7.0 - 100%) in R2 hinds and 92.6% (range: 39.8 - 100%) in MA hinds. Observations of aborting fetuses at scanning, along with a pilot study of early abortion confirms that sub-optimum pregnancy scan results are not attributable to sub-optimum conception rate alone as conventionally believed. A second pregnancy scan (Scan-2) was performed after a mean interval of 90 and 87 days from Scan-1 in a subsample of 11,005 R2 and 7,374 MA hinds, respectively, to determine fetal wastage in the 90-day between-scan (mid-term) period. Abortions were recorded in 73% and 61% of R2 and MA herds, respectively. The mean mid-term abortion rate, in herds with abortion, of 3.9% (range: 0.4 - 19.1%) in R2 was significantly higher than 2.2% (range: 0.6 - 9.1%) in MA hinds (Chisq. $p=0.009$). Repeatability of abortions investigated in 15 R2 (Student's t-test $p=0.15$) and seven MA (Student's t-test $p=0.75$) herds was poor demonstrating unpredictability between years. In a supplementary pilot study, abortions earlier than usual Scan-1 were detected in 2/3 R2 and 1/1 MA herd indicating that abortions do occur prior to mid-term. The abortion rates detected were higher than reported earlier and economically significant for many deer farmers, justifying investigation of causation.

Serology and/or PCR for *T. gondii*, *Leptospira* spp., *Neospora caninum*, Bovine Virus Diarrhoea virus (BVD), and Cervid Herpesvirus type -1 (CvHV-1) were performed on selected samples from hinds pregnant, non-pregnant and aborting at Scan-1, aborted between scans, and aborting and pregnant at Scan-2, and fetal material as appropriate.

Toxoplasma gondii sero-positive R2 hinds at Scan-2 were 1.6 times more likely to have aborted than sero-negative hinds (Chisq. $p=0.03$). *Toxoplasma gondii* sero-prevalence was positively related to herd-level abortion rates in R2 hinds (T-test $p=0.02$). In addition, *T. gondii* DNA was detected in aborting fetal tissues at Scan-1 and Scan-2 and from uteri of non-pregnant and aborting hinds at Scan-1 and aborted hinds at Scan-2. Combined, these data provide evidence that approximately 8% of abortions in R2 hinds are likely to be attributed to *T. gondii*.

There was no evidence for *Leptospira* spp., *N. caninum*, BVD, or CvHV-1 infection played a significant role in abortion. Serology for those pathogens was not associated with mid-term abortion or non-pregnancy at Scan-1 (*Leptospira* spp. only). No *Leptospira* spp. DNA was detected in aborted fetal tissue or aborted hind uteri.

N. caninum sero-prevalence was 0.6% in 348 samples analysed. Hence, further investigation was not justified. Sero-prevalence to BVD was 12.5%, and while not related to abortion, suggests a possibility of a persistently infected (PI) deer. The sero-prevalence of CvHV-1 was higher in MA than R2 hinds but unrelated to abortion (Chisq. $p<0.001$). The significance of Cervid Rhadinovirus type-2 (CRhV-2) DNA detected in maternal tissues is unknown.

Farm, management, health, and environment autumn and winter risk factors, analysed for pregnancy (Scan-1) and having aborted by Scan-2 showed that winter hay feeding, presence of dairy cattle on farm and co-grazing of hinds with beef cattle were associated with abortion. This risk factor analysis suggests that attention to good nutrition and health, and effective grazing management reduces the risk of abortion.

The observed abortion rates were higher than estimates used for power analysis at the study design stage. Therefore, despite that the number of farms able to be recruited was slightly below target, the abortion rates reported are robust. A potential limitation of this study was that the recruitment of farms could not be achieved by random selection, hence results may have been affected by volunteer bias. Further, it was necessary to adopt a cross-sectional blood sampling methodology since a longitudinal study design involving repeat sampling, while preferable, was not possible for logistical reasons due to the scale of this study on commercial farms.

Overall, while a major proportion of abortions remained unexplained, this study showed that abortions, sometimes in high numbers, are occurring on deer farms. The mid-term abortion rate observed, if consistent across the industry, would result in losses of \$2.10 million. If that rate was consistent throughout gestation, the loss could be up to \$5.58 million. Given the magnitude of abortion rates on many properties, further research into causation is justified. However, the poor repeatability or predictability of abortion will make such research using the epidemiological approach adopted here difficult. Due to *T. gondii* being implicated as a cause of abortion in R2 hinds, research into developing an effective vaccine may be warranted.

The research undertaken in this study effectively contributes to knowledge on reproductive inefficiency in farmed deer, providing data on the prevalence, incidence, and causation of abortion, and helping explain sub-optimum pregnancy scan results. These data contribute to understanding of BVD, *N. caninum* and CvHV-1 which have been little studied in farmed deer and will guide further studies to help the deer industry plan and implement measures to enhance reproductive efficiency.

Acknowledgements

First of all, I am grateful to the Lord of the world because of whom I was able to undertake and complete the PhD study.

I would like to give sincere thanks to my main supervisor, Prof. Emeritus Peter Wilson to consider me for this project and, for all the encouragement, supervision, inspiration, and help throughout my PhD study. I would also like to thank my co-supervisors; Dr. Laryssa Howe, Prof. Cord Heuer and Dr. Geoffery Asher for their enthusiasm, patience, supervision, and guidance through my PhD. I also acknowledge the epidemiological advice and help from Prof. Ian Dohoo, Prof. Wesley Johnson, and A/Prof. Geoff Jones. I am also thankful to Dr. Wlodek Stainslawek (MPI, Wallaceville) for undertaking virus neutralisation tests.

Special thanks to Deer Reproductive Efficiency Group (Southland), Southland Deer Farmers Association, and individual farmers, particularly Landcorp Farming Ltd to make this study possible. I gratefully acknowledge the in-kind contribution of all participating farmers and the assistance of a large number of veterinary practices, deer slaughter premises veterinarians, and scanners for scanning and, blood and tissue sample collection.

This study was funded by AgResearch, Agmardt, DEEResearch, MSD Animal Health, IVABS Postgraduate Research Fund, and Massey University. I also acknowledge IVABS Postgraduate Travel Fund for supporting my conference travels.

I also thank the technical team at IVABS and Hopkirk for their invaluable assistance. Liz Burrows for the laboratory work and Neville Haack for laboratory and field work. Special thanks to Dr. Laryssa Howe for providing training on all the serology and PCR techniques and for the moral support throughout the PhD. I would also like to thank several other helping hands in the laboratory; Gayathri Gopakumar, Harneet Bajwa, Ameesha Salaria, Sameer Siddiqui and Komal Arora for their help in tissue processing and laboratory work. I would also like to thank the visiting students at IVABS, Mariam Nouvel Bagayako (France), and Inge Janssen and Nannet Fabri (The Netherlands) for assistance in blood and tissue collection and processing.

I would also like to thank my colleagues at IVABS and EpiCentre for all the help and time during my PhD; Emilie Vallee, Daniela Tapia Escerate Felipe Lembeye, Jose Solis-Ramirez, Juan Sanhueza, Arata Hidano, Nelly Narquetoux, Alicia Coupe, Rima Shreshta,

Masasko Wada, Sara Azarpeykan, Doris Adeyanka, Rebecca, Kate Littlewood, Asmad Kari, Alfredo Lepori, Shirli Notcovich, Shashwati Mathurkar, Deepa Patel, Tessy George, Gauri More, Sharini Somasiri, and many more.

I also thank all my friends in India and the Massey Hindu Society for all the social gatherings, and activities during the PhD that helped me get relaxed. I deeply thank my fiancée, Bhumi Savaliya, who provided an immense support during my PhD study. Without her, this journey would have been difficult. Last but not least, I am thankful to my family, especially my mother who always stood by me in all stages of my life, and was a huge inspiration. Her loss has left a huge void in my family.

List of publications

Patel KK, Wilson PR, Asher G, Howe L, Heuer C. Study of risk factors for abortions in New Zealand farmed deer. *Proceedings of the Annual conference of the Deer Branch of New Zealand Veterinary Association (Cervetec 2016)*, 2016 in press

Patel KK, Wilson PR, Asher G, Howe L, Heuer C. Fetal wastage in New Zealand farmed red deer. *Proceedings of the 8th International Deer Biology Congress*, 2014

Patel KK, Wilson PR, Asher G, Howe L, Heuer C. Update of a study of fetal wastage in red deer. *Proceedings of the Annual conference of the Deer Branch of New Zealand Veterinary Association (Cervetec 2014)*, 2014

Patel KK, Howe L, Wilson PR, Heuer C, Asher G. Does *Toxoplasma* play a role in deer abortions in New Zealand? *Proceedings of the New Zealand Society for Parasitology Conference and Annual Meeting No. 41*, 2014

Patel KK, Wilson PR, Asher G, Howe L, Heuer C. Preliminary results from a study of reproductive wastage in deer. *Proceedings of the Annual conference of the Deer Branch of New Zealand Veterinary association (Cervetec 2013)*, 241-245, 2013

Wilson PR, Patel KK, Asher G, Howe L, Heuer C, Sinclair G. Clinical investigations of foetal loss in farmed deer. *Proceedings of the Annual conference of the Deer Branch of New Zealand Veterinary Association (Cervetec 2012)*, 107-110, 2012

Patel KK, Wilson PR, Howe L, Heuer C, Asher G. Potential infectious causes of abortion in deer. *Proceedings of the Annual conference of the Deer Branch of New Zealand Veterinary association (Cervetec 2012)*, 101-105, 2012

Patel KK, Howe L, Asher G, Wilson PR. Possible role of *Toxoplasma gondii* in deer reproductive failure and route for human infection. *Proceedings of the New Zealand Society for Parasitology Conference and Annual Meeting No. 39*, 51, 2011

List of presentations and poster

Patel KK, Wilson PR*, Asher G, Howe L, Heuer C. Study of risk factors for abortions in New Zealand farmed deer. *Annual conference of the Deer Branch of New Zealand Veterinary Association (Cervetec 2016), Dunedin, New Zealand, 2016*

Patel KK*, Wilson PR, Asher G, Howe L, Heuer C. Fetal wastage in New Zealand farmed red deer. *8th International Deer Biology Congress, Harbin, China, 2014*

Patel KK*, Wilson PR, Asher G, Howe L, Heuer C. Update of a study of fetal wastage in red deer. *Annual conference of the Deer Branch of New Zealand Veterinary Association (Cervetec 2014) and Food safety, Animal Welfare & Biosecurity, Epidemiology & Animal Health Management branch of the New Zealand Veterinary Association, Queenstown and Hamilton, New Zealand, 2014*

Patel KK*, Howe L, Wilson PR, Heuer C, Asher G. Does *Toxoplasma* play a role in deer abortions in New Zealand? *New Zealand Society for Parasitology Conference and Annual Meeting No. 41, Palmerston North, New Zealand, 2013*

Patel KK*, Wilson PR*, Asher G, Howe L, Heuer C. Preliminary results from a study of reproductive wastage in deer. *Annual conference of the Deer Branch of New Zealand Veterinary association (Cervetec 2013), and Food safety, Animal Welfare & Biosecurity, Epidemiology & Animal Health Management branch of the New Zealand Veterinary Association, Patagonia, Argentina and Palmerston North, New Zealand, 2013*

Wilson PR*, Patel KK, Asher G, Howe L, Heuer C, Sinclair G. Clinical investigations of foetal loss in farmed deer. *Annual conference of the Deer Branch of New Zealand Veterinary Association (Cervetec 2012), Queenstown, New Zealand, 2012*

Patel KK*, Wilson PR, Howe L, Heuer C, Asher G. Potential infectious causes of abortion in deer. *Annual conference of the Deer Branch of New Zealand Veterinary association (Cervetec 2012), Queenstown, New Zealand, 2012*

Patel KK*, Howe L, Asher G, Wilson PR. Possible role of *Toxoplasma gondii* in deer reproductive failure and route for human infection. *New Zealand Society for*

Parasitology Conference and Annual Meeting No. 39, Palmerston North, New Zealand,
2011

(*Speaker)

Poster presentation

Patel KK, Wilson PR, Asher G, Howe L, Heuer C. Fetal wastage in New Zealand deer: estimating misclassification bias. *Proceedings of the 14th International Symposia on Veterinary Epidemiology and Economics (ISVEE14), Merida, Mexico,* 2015

Table of Contents

Abstract.....	iii
Acknowledgements	vii
List of publications.....	ix
List of presentations and poster	x
Table of Contents	xii
List of Tables	xviii
List of Figures.....	xxiii
Chapter 1. Deer farming in New Zealand and review of potential abortifacients in deer.....	1
1.1 Deer farming in New Zealand	2
1.2 Abstract	4
1.3 Introduction	5
1.4 Reproductive wastage	5
1.4.1 Failure to ovulate, conceive, or carry a conceptus	7
1.4.2 Perinatal and postnatal (pre-weaning) mortalities.....	8
1.4.3 Abortion.....	9
1.5 Infectious causes of abortion	9
1.5.1 Bacterial.....	10
1.5.2 Viral.....	25
1.5.3 Protozoa.....	28
1.6 Non-infectious causes of abortion	31
1.6.1 Nitrate poisoning	31
1.6.2 Nutrition	31
1.6.3 Trace element deficiency.....	32
1.7 Conclusion.....	33
1.8 References	34
Chapter 2. Mating management, pregnancy and mid-term abortion rates in farmed red deer in New Zealand.....	53
2.1 Abstract	54
2.2 Introduction	55
2.3 Materials and methods.....	56
2.3.1 Study design	56
2.3.2 Animals and pregnancy determination	57

2.3.3 Abortion repeatability.....	58
2.3.4 Early fetal loss	58
2.3.5 Farm data collection	58
2.3.6 Statistical analysis	59
2.4 Results	60
2.4.1 Farm recruitment	60
2.4.2 Scan-1 pregnancy results.....	63
2.4.3 Abortion at Scan-1	67
2.4.4 Scan-2 data and abortion rates.....	67
2.4.5 Abortion repeatability.....	71
2.4.6 Early fetal losses.....	73
2.5 Discussion.....	76
2.6 Conclusion.....	81
2.7 Acknowledgements.....	82
2.8 References	83
Chapter 3. Sensitivity and specificity of ELISA, latex agglutination test, and Western blot to detect Toxoplasma gondii antibodies in farmed red deer sera	85
3.1 Abstract	86
3.2 Introduction	87
3.3 Materials and methods.....	88
3.3.1 Samples.....	88
3.3.2 Serological assays.....	88
3.3.3 Statistical analysis	90
3.4 Results	92
3.4.1 Western blot.....	92
3.4.2 LAT compared with Western blot.....	93
3.4.3 ELISA compared with Western blot	94
3.4.4 Bayesian latent class analysis	95
3.5 Discussion.....	97
3.6 Conclusion.....	99
3.7 Acknowledgements.....	99
3.8 Sources and manufacturers.....	100
3.9 References	100
Chapter 4. Investigation of association between Toxoplasma gondii and early pregnancy and abortion rates in New Zealand farmed red deer	103

4.1 Abstract	105
4.2 Introduction	107
4.3 Materials and methods	108
4.3.1 Sample collection	109
4.3.2 Sample selection for serology	109
4.3.3 ELISA	110
4.3.4 PCR.....	111
4.3.5 Statistical analysis	111
4.4 Results	113
4.4.1 Serology.....	113
4.4.2 PCR and relationship with serology in aborting and pregnant hinds	117
4.4.3 Pathology	119
4.5 Discussion	121
4.6 Conclusion	126
4.7 Acknowledgements	126
4.8 References	128
Chapter 5. Investigation of association between <i>Leptospira</i> spp. serovars Hardjobovis and Pomona and <i>Neospora caninum</i>, and pregnancy and abortion in New Zealand farmed deer	133
5.1 Abstract	134
5.2 Introduction	136
5.3 Materials and methods	138
5.3.1 Sample collection and handling.....	139
5.3.2 Serology.....	140
5.3.3 Molecular diagnostics.....	143
5.3.4 Statistical analysis	145
5.4 Results	147
5.4.1 <i>Leptospira</i> spp. serology.....	147
5.4.2 <i>Neospora caninum</i> serology	154
5.4.3 Molecular diagnostics.....	154
5.5 Discussion	155
5.6 Conclusion	160
5.7 Acknowledgements	161
5.8 References	162

Chapter 6. Investigation of association between bovine viral diarrhoea virus and cervid herpesvirus type-1, and abortion in New Zealand farmed deer	167
6.1 Abstract	168
6.2 Introduction	170
6.3 Materials and methods.....	172
6.3.1 Sample collection and handling.....	172
6.3.2 Virus neutralisation assay (VNT) for bovine viral diarrhoea virus (BVD) and cervid herpesvirus type-1 (CvHV-1)	173
6.3.3 Sample selection for serology	174
6.3.4 Herpesvirus consensus PCR.....	175
6.3.5 Statistical analysis	176
6.4 Results	177
6.4.1 Bovine viral diarrhoea virus (BVD) serology	177
6.4.2 Cervid herpesvirus type-1 (CvHV-1) serology	179
6.4.3 Herpesvirus consensus PCR.....	180
6.5 Discussion.....	180
6.6 Conclusion.....	183
6.7 Acknowledgements.....	183
6.8 References	185
Chapter 7. Analysis of farm, environment, health and management risk factors for pregnancy and abortion in New Zealand farmed red deer	205
7.1 Abstract	206
7.2 Introduction	208
7.3 Materials and methods.....	209
7.3.1 Statistical analysis	211
7.4 Results	212
7.4.1 Univariate autumn risk factors analysis for pregnancy rate at Scan-1 and daily abortion rate (DAR) at Scan-2.....	213
7.4.2 Multivariate autumn risk factor analysis for pregnancy rate at Scan-1	224
7.4.3 Multivariate autumn risk factor analysis for daily abortion rate (DAR) at Scan-2	224
7.4.4 Univariate analysis of winter risk factors for daily abortion rate (DAR) at Scan-2	225
7.4.5 Multivariate winter risk factor analysis for daily abortion rate (DAR) at Scan-2	230
7.5 Discussion.....	230

7.5.1 Autumn risk factors for pregnancy rate at Scan-1 or daily abortion rate at Scan-2	230
7.5.2 Winter risk factors for daily abortion rate at Scan-2	234
7.5.3 Possible bias	235
7.6 Conclusion.....	235
7.7 Acknowledgements.....	236
7.8 References	237
Chapter 8. General discussion	239
8.1 Introduction	240
8.2 History of fetal wastage.....	240
8.3 Selection of farms	241
8.4 Timing of scanning	242
8.5 Timing of abortions	243
8.6 Prevalence and incidence of abortion.....	244
8.7 Economic impact of abortion	245
8.8 Limitations on sampling and questionnaire data collection.....	246
8.9 Toxoplasma gondii ELISA validation for use in deer.....	247
8.10 Investigation of infectious causes of abortion	249
8.11 Toxoplasma gondii sero-status and pregnancy	250
8.12 Toxoplasma gondii vaccination cost-benefit.....	251
8.13 Other pathogens and sero-prevalence estimates	252
8.14 Farm, environment, health and management risk factors for pregnancy at Scan-1 and abortion by Scan-2	253
8.15 Limitations of the present study	256
8.16 Implications of this research for the Deer Industry	257
8.17 Proposed future research.....	258
8.18 Conclusion.....	260
8.19 References	262
Appendices.....	283
Appendix 1: Description of farms	284
Appendix 2: Toxoplasma gondii Western blot protocol	294
Appendix 3: Toxoplasma gondii (FOOD) nested PCR	301
Appendix 4: Neospora spp. real-time PCR	304
Appendix 5: Deer herpes consensus PCR.....	306
Appendix 6: Questionnaire 1 (autumn risk factors).....	309

Appendix 7: Questionnaire 2 (winter risk factors)328

List of Tables

Chapter 1

Table 1.1: Summary of studies reporting reproductive wastage including pregnancy, abortions and stillbirth, and reproductive efficiency (number of calves weaned/number of hinds at mating) in farmed red deer in New Zealand, United States of America (USA) and Spain.	6
Table 1.2: Summary of published reports of reproduction losses attributed to natural or experimental demonstrated or potential infectious causes of abortion in deer in New Zealand and worldwide.	14
Table 1.3: Summary of published reports of serological and tissue surveys, and clinical reports for demonstrated and potential infectious causes of abortion in deer in New Zealand and worldwide.	17
Table 2.1: Summary of total number of herds and animals available for mating management, Scan-1, and Scan-2 data.	62
Table 2.2: Mating management data including stag joining and stag removal dates, joining interval, and stag removal to first scan interval, by age groups and year from R2 and MA herds.	62
Table 2.3: Scan-1 and Scan-2 dates for each age group each year.	64
Table 2.4: Animal-level pregnancy rate and number (and %) of hinds observed aborting at Scan-1, number undergoing Scan-2, number aborted by Scan-2, number aborting at Scan-2, between-scan abortion prevalence for R2 and MA hinds in years 1 and 2.	64
Table 2.5: Summary of herd-level pregnancy rates at Scan-1 and daily abortion incidence rates at Scan-2.	65
Table 2.6: Number (and %) of herds with no, low, medium, and high daily abortion rates.	71
Table 2.7: Daily abortion rates for R2 herds with nil, low, medium and high abortion rates in year-1 on farms that were also scanned in year-2.	72
Table 2.8: Daily abortion rates for MA herds with nil, low, medium and high abortion rates in year-1 on farms that were also scanned in year-2.	73
Table 2.9: Mating management, scanning, and early fetal losses recorded in the R2 and MA herds scanned for detection of early abortion.	75

Table 3.1. Informative beta priors used for estimation of western blot and latex agglutination test (LAT) sensitivities (Se) and specificities (Sp) in a Bayesian latent class model.....	92
Table 3.2: Summary of sera with observation of bands with different molecular weights (kD) on western blot immunoblot image.....	93
Table 3.3. Classification of results from 252 sera tested on WB, LAT (cut-off titre: 1:32), and ELISA (cut-off S/P(%): 30) according to their sero-status for each test.....	94
Table 3.4. Test characteristics (Se, Sp, apparent prevalence, PPV, NPV, positive likelihood ratio (LR+), negative likelihood ratio (LR-), Kappa statistic, McNemar’s Chi-square statistic) values with confidence interval (95%) for latex agglutination test (LAT) and ELISA at manufacturer’s cut off.....	95
Table 3.5. <i>Toxoplasma gondii</i> sero-prevalence, and test sensitivity (Se), specificity (Sp), positive predictive value (PPV), negative predictive value (NPV) with their 95% confidence (gold standard analysis) and credible (Bayesian latent class analyses) intervals for LAT and WB as obtained from Bayesian latent class analysis with non-informative and informative priors and their comparison with estimates from gold standard analyses assuming WB as a gold standard test.....	96
Table 3.6. Comparison of sensitivity (Se), specificity (Sp), positive predictive value (PPV), and negative predictive value (NPV) with their 95% confidence (gold standard analysis) and credible (Bayesian latent class analyses) intervals for ELISA test obtained from Bayesian latent class analysis and gold standard analyses at manufacturer’s and optimised cut-off SP% after anti-log conversion.....	97
Table 4.1: Summary of total number (and range per herd) of sera tested from pregnant and non-pregnant hinds at Scan-1 and from aborted and non-aborted hinds at Scan-2.	110
Table 4.2: Sero-prevalence of <i>Toxoplasma gondii</i> in pregnant and non-pregnant hinds at Scan-1 and aborted and non-aborted hinds at Scan-2 in R2 and MA herds.	114
Table 4.3: Odds ratios and p-value for logistic models based on <i>Toxoplasma gondii</i> sero-status per se (unadjusted), or sero-status controlled for year and island (adjusted) for association between individual hind-level sero-positivity and non-pregnancy at Scan-1, and having aborted by Scan-2.....	114
Table 4.4: Mean, SE and range of within-herd <i>Toxoplasma gondii</i> sero-prevalence (%) at Scan-1 and Scan-2 in R2 and MA herds with and without aborted hinds.	115

Table 4.5: Beta coefficient estimate and p-value based on unadjusted <i>Toxoplasma gondii</i> sero-prevalence, or sero-prevalence controlled by year and island (adjusted), for association between within-herd <i>T. gondii</i> sero-prevalence and proportion of hinds not pregnant at Scan-1 and daily abortion rate between Scan-1 and Scan-2.	115
Table 4.6: <i>Toxoplasma gondii</i> Scan-1 sero-status of hinds scanned pregnant at Scan-1 and their Scan-2 aborted or non-aborted status.....	116
Table 4.7: Number positive/number PCR tested for <i>Toxoplasma gondii</i> in uteri, cotyledon, placenta and fetal tissue and proportion sero-positive at Scan-1 and Scan-2 from normal pregnant, non-pregnant, aborting and aborted hinds.	119
Table 5.1. Summary of total number (and range per herd) of sera tested for <i>Leptospira borgpetersenii</i> serovar Hardjobovis and <i>Leptospira interrogans</i> serovar Pomona from pregnant and non-pregnant hinds at Scan-1 and from aborted and non-aborted hinds at Scan-2.....	141
Table 5.2: Number of maternal and fetal tissue samples from pregnant and aborting hinds at Scan-1 and aborted animals by Scan-2 tested for leptospiral DNA using real-time polymerase chain reaction (qPCR) assay.....	144
Table 5.3: Number (and % of total) of sera and reciprocal titres for <i>Leptospira borgpetersenii</i> serovar Hardjobovis and <i>Leptospira interrogans</i> serovar Pomona from R2 and MA hinds at Scan-1 and Scan-2.....	148
Table 5.4: Sero-prevalence for <i>Leptospira borgpetersenii</i> serovar Hardjobovis and <i>Leptospira interrogans</i> serovar Pomona in pregnant and non-pregnant hinds at Scan-1 and aborted and non-aborted hinds at Scan-2 in R2 and MA herds.	149
Table 5.5: The odds ratio and p-value for animal-level <i>Leptospira borgpetersenii</i> serovar Hardjobovis and <i>Leptospira interrogans</i> serovar Pomona sero-status association with non-pregnancy at Scan-1 and having aborted by Scan-2 in herds with aborted hinds.	149
Table 5.6: Mean of <i>Leptospira borgpetersenii</i> serovar Hardjobovis and <i>Leptospira interrogans</i> serovar Pomona reciprocal antibody titres ($\geq 1:48$) for non-pregnant and pregnant R2 and MA hinds at Scan-1 and Scan-2 in hinds that had aborted or not aborted as determined at Scan-2.....	150
Table 5.7: Mean, SE and range of within-herd sero-prevalence for <i>Leptospira borgpetersenii</i> serovar Hardjobovis and <i>Leptospira interrogans</i> serovar Pomona at Scan-1 and Scan-2 in R2 and MA sero-positive herds.	151

Table 5.8: Model coefficient logarithmic estimate and p-value for association between Hardjjobovis and Pomona within-herd sero-prevalence and daily abortion rate at Scan-2....	153
Table 5.9: Paired Neospora caninum and Toxoplasma gondii serology and uterus PCR results for hinds sero-positive on Neospora caninum ELISA.....	154
Table 5.10: Number positive/number PCR tested for Neospora caninum in uteri, cotyledon, placenta and fetal tissue and proportion sero-positive at Scan-1 and Scan-2 from normal pregnant, non-pregnant, aborting and aborted hinds.	155
Table 6.1: Summary of total number (and range per herd) of sera tested for bovine viral diarrhoea virus (BVD) and cervid herpesvirus type-1 (CvHV-1) from aborted and non-aborted hinds at Scan-2.	174
Table 6.2: Summary of maternal samples from aborted hinds at Scan-2 and hinds with no live calf at weaning tested for herpesvirus DNA using consensus polymerase chain reaction (PCR) assay.....	176
Table 6.3: Number (and % of total) of sera with reciprocal titres for bovine viral diarrhoea (BVD) from aborted and non-aborted R2 and MA hinds. (Note: samples with reciprocal titres ≥ 8 are considered positive).	178
Table 6.4: Per cent sero-positive (and number of sera tested) for bovine viral diarrhoea virus (BVD) and cervid herpesvirus type-1 (CvHV-1) in aborted and non-aborted hinds in R2 and MA herds.	178
Table 6.5: Odds ratios and p-value for logistic regression models based on bovine viral diarrhoea virus (BVD) and cervid herpesvirus type-1 (CvHV-1) sero-status per se (unadjusted), or sero-status controlled for year and island (adjusted) for association between individual hind-level sero-positivity and having aborted.	179
Table 6.6: Number of sera (and % of total) with reciprocal titres for cervid herpesvirus type-1 (CvHV-1) from R2 and MA hinds. (Note, reciprocal titres of ≥ 1 are considered positive).	179
Table 7.1: Herd-level mean and range for within-herd pregnancy rate at Scan-1 and daily abortion rate at Scan-2 from herds with questionnaire-1 (Q1) and questionnaire-2 (Q2) data, and all herds.	213
Table 7.2: Univariate associations between pasture type fed in autumn and deer farming experience and the pregnancy rate at Scan-1.	215
Table 7.3: Univariate associations between pasture type fed in autumn and deer farming experience and daily abortion rate by Scan-2.	215

Table 7.4: Univariate associations between nutritional management, environment, health, year and island risk factors in autumn and pregnancy rate at Scan-1.	216
Table 7.5: Univariate association between nutritional management, environment, health, year and island risk factors in autumn and daily abortion rate at Scan-2.	218
Table 7.6: Data from farms reporting abortion during the previous three years and their pregnancy rate and daily abortion rate in research participation year.	221
Table 7.7: Summary of source of information as provided by deer farmers to complete the questionnaires.....	223
Table 7.8: Significant autumn risk factors in the multivariate analysis for association with pregnancy rate at Scan-1, and LS mean pregnancy rate and p-values.....	224
Table 7.9: Significant autumn risk factors in the multivariate analysis for association with daily abortion rate at Scan 2, and LS mean daily abortion rate, and p-values.....	225
Table 7.10: Univariate associations between pasture types grazed in winter and the daily abortion rate at Scan-2.	226
Table 7.11: Univariate associations between risk factors in winter and daily abortion rate at Scan-2.....	228
Table 7.12: Significant winter risk factors in the multivariate analysis for association with having aborted by Scan 2, and model coefficient estimates, odds ratio and p-values...	230
Table 8.1: The cost analysis of vaccination against <i>Toxoplasma gondii</i> in approximated red R2 hinds in New Zealand.....	252

List of Figures

Chapter 2

Figure 2-1: Geographical distribution of participating deer farms in year-1 and year-2.	60
Figure 2-2: Individual R2 herd Scan-1 pregnancy rates in year-1 (1A) and year-2 (1B). Individual MA herd Scan-1 pregnancy rates in year-1 (1C) and year-2 (1D) (each bar represents one herd).	66
Figure 2-3: Individual herd between-scan daily abortion rates for R2 herds in year-1 (2A) and year-2 (2B). Individual herd between-scan daily abortion rates for MA herds in year-1 (2C) and year-2 (2D) (each bar represents one herd).	69
Figure 2-4: Individual herd between-scan daily abortion rates for R2 herds on farms scanned both in year-1 and year-2.	72
Figure 2-5: Individual herd between-scan daily abortion rates for MA herds on farms scanned both in year-1 and year-2.	73
Figure 3-1. Western blot patterns of a sub-sample of sera (b to j). Positive sera samples b,c,d,g and j show bands to <i>Toxoplasma gondii</i> antigens from 24 to 40kD. Sera labelled e, f, h, and I represent negative samples. Sera labelled 'a' and 'k' represent negative and positive control sera, respectively.	93
Figure 3-2. Receiver operating characteristic (ROC) curve plotted for ELISA S/P(%) from Bayesian latent class (BLC) analysis.	96
Figure 4-1: Uterus with haemorrhagic caruncles from an aborted <i>Toxoplasma gondii</i> DNA negative R2 hind at Scan-2.	120
Figure 4-2: Uterus from an aborted <i>Toxoplasma gondii</i> DNA positive and sero-positive R2 hind at Scan-2 with presence of pinpoint and petechial haemorrhages on caruncles and uterine floor.	121

Declaration

Chapters 2 to 7 in this thesis are set out as a paper in the style and format required of the journal. Therefore, there are some repetitions, particularly in the methods. The co-Authors listed in those chapters have made their contributions, however, my input was the greatest as I designed and executed this study including the laboratory work, data analysis, and preparation of manuscripts.

