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

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2016

Institute of Veterinary Animal
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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 36,223MA 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 Siddiqqui 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

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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 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

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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.

Chapter 1. Deer farming in New Zealand and review of potential abortifacients in deer

This chapter gives a brief introduction to the New Zealand deer industry followed by a detailed literature review of reproductive performance and causes of reproductive wastage of relevance to the New Zealand deer industry, with particular emphasis on abortion and evidence of exposure of deer to potential abortifacients.

1.1 Deer farming in New Zealand

Red deer (*Cervus elaphus*) in New Zealand were first introduced from Scotland as game animals in 1871 (Giles 1975). Since then the deer numbers have flourished in the absence of predation and gained 'pest' status following considerable damage to the environment and native forest. However, the export of venison in the early 60s from deer as game animals led to the commercialisation of deer. Following this, in 1970, the first deer farming licence was given and since then deer farming developed as an industry and began to grow further throughout New Zealand. To establish deer farms, deer were captured live from the wild taking appropriate measures (Hunt 1982; Wallis and Hunn 1982) and were raised initially for production and export of venison.

The current deer population in New Zealand counts over 900,000 (Statistics New Zealand 2016b). Sixty-nine percent of deer are concentrated in the South Island whereas 31% are present in North Island of New Zealand. Deer farms in New Zealand often also run cattle and sheep stock where all the species are raised together. Deer in New Zealand mainly originated from Europe and North America. Scottish red deer (*Cervus elaphus scoticus*), Eastern European red deer (*Cervus elaphus hippelaphus),* fallow deer (*Dama dama*), North American wapiti deer (*Cervus elaphus canadensis)* are the major breeds making up the deer population in New Zealand. Other deer breeds residing in the country include white-tailed deer (*Odocoileus virginanus borealis),* rusa deer (*Cervus timorensis russa*), moose (*Alces alces andersoni*), sika deer (*Cervus nippon*) and sambar deer (*Cervus unicolor*) (Fraser *et al.* 2000), but these are not farmed.

Deer in New Zealand are primarily raised for meat (venison) production, but velvet antler is also an important product. Seventy percent of the total venison produced is exported to Europe with Germany being the largest importer followed by Netherlands, Belgium, Luxembourg. Venison is also exported to the USA and many other countries including China. With the deer industry developing rapidly, velvet production and other deer co-products have gained importance following demand in Asian markets including South Korea and China for their use in traditional medicine. Deer for prime venison production are slaughtered either at

age of 12-24 or 15-27 months of age (Barry and Wilson 1994) to meet the chilled venison demand in overseas markets. Velvet is harvested from male deer annually during the velvetting season (October to early February) under veterinary supervision and anaesthesia following the guidelines described in 'Code of Recommendations and Minimum Standards for the welfare of deer during the removal of antler' (Animal Welfare Advisory Comittee 1992).

Deer farming contributes significantly to the New Zealand economy by generating revenue over $255 million per annum. The deer industry has developed during the last 45 years, with research studies to improve deer farming, welfare, health and benefits from deer production. However, reproduction in deer has not been optimum for many years (Statistics New Zealand 2016b) and more studies are needed to reduce the reproductive wastage occurring in deer.

There has been a significant amount of research into reproductive performance in farmed deer in New Zealand, focussing mainly on quantifying reproductive outcomes and on achievement of conception (Fennessy *et al.* 1986; Beatson *et al.* 1998; Audigé *et al.* 1999a; Campbell *et al.* 2000; Lawrence 2003; Wilson *et al.* 2012) but few have focussed on abortion as a cause of reproductive wastage. This thesis focusses on abortion as a cause of reproductive wastage, and this chapter reviews the international literature on abortion in deer.

The remainder of this chapter is prepared in the style format for submission to a scientific journal.

A review of reproductive performance in farmed red deer in New Zealand and potential infectious and management-related causes of reproductive wastage

1.2 Abstract

Reproduction in farmed deer in New Zealand is sub-optimum with reproductive efficiency (calves weaned/hinds mated) averaging about 75%. This review describes reproductive wastage including abortion in deer in New Zealand but with primary focus on proven and potential causes of abortion, based on New Zealand and overseas reports. Evidence of abortifacients known for causing abortion in cattle and sheep and reported in deer were included. Causes of reproductive wastage include failure to ovulate, abortions, and peri- and post-natal losses. Abortion rates ranging from 0.8-16% have been reported in New Zealand farmed deer herds, with small numbers reported from various deer species elsewhere.

Few publications describe abortion in deer, and fewer provide robust diagnosis of causation. Serological and DNA evidence from New Zealand suggests that *Toxoplasma gondii* appears to cause abortion in deer, and several reports show that deer are readily infected by this organism. Proven or potential bacterial causes of abortion identified from deer studies internationally include *Brucella* spp., *Listeria* spp., and *Leptospira* spp. One experimental challenge study demonstrated that *Leptospira interrogans* serovar Pomona caused abortion in white-tailed deer, though this was not supported by New Zealand data. Bovine viral diarrhoea virus (BVD) and Cervid herpes virus type-1 (CvHV-1) appear to be prevalent in deer but evidence that they cause abortion is limited. Several studies report serological evidence for infection with *Neospora caninum* with some reporting the organism in fetal tissue, hence this organism may be a cause of abortion.

Among nutritional causes, nitrate has the potential to cause abortion, but this has not been recorded in deer. Under-nutrition during pregnancy has been associated with abortion. Selenium and iodine deficiency have been shown in deer, and are reported to be associated with abortion and perinatal mortality.

This review provides evidence that *T. gondii* and possibly *N. caninum* may cause abortion in deer. It confirms that while there are few reports of abortion in deer, a number of agents and other factors known to cause abortion in other ruminant species are commonly

demonstrated in a range of deer species. These should be considered during investigation of abortion on deer farms.

Key words: Deer, abortion, fetal wastage, *Brucella* spp., *Listeria* spp., *Leptospira* spp., Bovine viral diarrhoea virus, Cervid Herpes virus, *Neospora caninum*, *Toxoplasma gondii*, nitrate poisoning, Copper, Selenium, and Iodine.

1.3 Introduction

Optimum reproduction is one of many factors affecting the economic viability of a commercial deer farm. Introduced deer were able to establish and reproduce well in favourable New Zealand conditions. For a deer farm to have good reproductive efficiency, it is necessary to achieve good pregnancy rates, to maintain the pregnancy and then successfully wean live progeny. However, reproductive efficiency in red deer (*Cervus elaphus*) on New Zealand farms has long been sub-optimum (Asher and Pearse 2002; Asher 2003; Asher and Wilson 2011). For example, the reproductive efficiency (calves weaned/hinds mated) in the last 13 years has averaged 75.2% (Statistics New Zealand 2016b). In an earlier study involving 2700 hinds from 15 red deer herds, reproductive efficiency observed in rising-two-year-old deer (R2) and mixed-aged (MA) adult hinds was 70% and 85%, respectively (Audigé *et al.* 1999a). These findings are indicative of significant reproductive wastage occurring on New Zealand deer farms.

This review briefly summarises the literature on reproductive wastage in farmed deer, largely from red deer in New Zealand, and then reviews in detail the literature on abortion in deer species throughout the world, along with that demonstrating infection or exposure to known infectious and non-infectious causes of abortion in other ruminant species.

1.4 Reproductive wastage

Reproductive wastage can be due to several reasons such as failure to reach puberty, failure to ovulate or conceive, fetal losses (abortion) and peri- and postnatal mortality. There have been a limited number of reports quantifying reproductive wastage on deer farms in New Zealand or elsewhere. Available data are summarised in Table 1.1.

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.

Year	Country	Age group and breed	No. of farms	No. scanned	Mean % pregnant (range)	% abortion between scanning and calving (number aborted)	Reproductive efficiency (%)[a]	Reference
1984-85	New Zealand	R2, red deer	1	92	84.8 (75-94)	2.3% (2)	NA	Fennessy *et al.* (1986)
1992-93	New Zealand	R2, red	15	791	84.7 (50-100)	0.6 (2) from 8 farms	70.0	Audigé *et al.* (1999a)
		MA, red	15	3,466	96.8 (85-100)	0.79 (13) from 8 farms	84.1	
1997	New Zealand	R2, red	13	1059	82.3 (57-100)	2 in one herd (7)	NA	Beatson *et al.* (1998)
		MA, red	14	4746	88.5 (73-100)	-	NA	
1998	New Zealand	R2, red	16[b]	1214	85.2 (80-100)	-	75.8	Campbell *et al.* (2000)
		MA, red		3660	91.8 (79-98)	1 (NA)	83.1	
1999-2001	USA	MA, elk cows	-	-	-	Abortion:16 (4/25), stillbirth: 8 (2/25)	-	Etter and Drew (2006)
2011	New Zealand	R2, red	1	Year-1: 480, year-2: 425	91.7 both years	Year-1: 10.0 (44/440), Year-2: 8.7 (34/390)	NA	Wilson *et al.* (2012)
			1	-	-	Year-1: 16.0 (61/380), Year-2: 9.6 (27/280)	NA	
			1	1687	84.7	Early June at usual scanning: 0.01 (17/1,687), early September: 7.2 (106/1,472)	NA	
			1	903	83.8	1.7 (NA)	NA	
2011	Spain	Young, Iberian red	1	29	24.1	28.6 (2/7)	NA	Gomez-Nieto *et al.* (2011)
		Adult, Iberian red	1	60	65	10.3 (4/39)	NA	

[a] reproductive efficiency=no. of calves weaned/no. mated. [b]16 deer farms, NA = not available.

1.4.1 Failure to ovulate, conceive, or carry a conceptus

There is a limited number of studies on puberty and ovulation in R2 hinds (Fisher *et al.* 1990; Asher *et al.* 2005a; Asher *et al.* 2011). About 16-17% of yearling (R2) hinds were reported as having failed to conceive (Audigé *et al.* 1999a). However, those Authors used pregnancy scanning data early in gestation as a proxy for conception *per se*, hence may reflect both failure to conceive and embryonic and early fetal loss. Puberty failure in R2 hinds has been linked to failure to achieve pre-mating threshold live weight of 65-70 kilograms (kgs) (Kelly and Moore 1978; Hamilton and Blaxter 1980; Deer Industry Manual 2000), frequent yarding, social inhibitions, environmental stress (Asher 2003; Asher and Wilson 2011), poor nutritional environment in early life (Asher and Cox 2013), lower growth rate, lower percentage of New Zealand blood line, stress, low body condition score (BCS) and lower average daily temperatures (Audigé *et al.* 1999b). Failure to conceive in R2 hinds has also been linked to the introgression of North American wapiti (*Cervus elaphus* subsp. *nelson, manitobensis, and roosevelti*). Inter-breeding with red deer results in a higher live-weight threshold for puberty. Famers may not recognise that their deer have wapiti genes and therefore greater feed requirement to achieve puberty weight, resulting in higher non-pregnancy rates (Asher *et al.* 2005a). In another study, exogenous melatonin treatment helped achieve puberty and subsequently higher pregnancy rates in R2 hinds suggesting that photoperiod may be having an effect on puberty (Asher *et al.* 2011).

In MA hinds, non-pregnancy rates have varied from 3.1 to 12.5% in different studies (Audigé *et al.* 1999a; Campbell *et al.* 2000; Lawrence 2003). In a study by Audigé *et al.* (1999c), higher pregnancy rates were observed in MA hinds with higher body condition score (>2.5), having reared a calf in the previous reproductive cycle, from mobs with ≥1 back up sire, and when experienced sires were used, whereas pregnancy rates were lower in hinds from mobs closer to the road, and from mobs with frequent yarding and handling between paddocks, from mobs with frequent changes in mob composition, and from areas with lower temperatures.

Early embryonic and fetal losses may also be one of the reasons of R2 or MA hinds being detected non-pregnant at pregnancy scanning. However, to Author's knowledge, there are no studies reporting those losses in farmed deer. Therefore, pregnancy rate from ultrasound pregnancy scanning is a composite measure of early reproductive failure.

1.4.2 Perinatal and postnatal (pre-weaning) mortalities

Survival of a calf at birth and from birth to weaning is as important as conception and maintenance of pregnancy to calving. It has conventionally been believed that most reproductive losses occur during this period. However, not much literature is available on losses at calving and calf mortality rates or causes in deer as it is difficult to individually observe calving hinds and to monitor new-born calves for mortality. However, from few observational studies, the calving rate reported at 86% (Asher and Adam 1985) and apparent calf losses observed at 10% to 12.4%, based on recording number of pregnant hinds at calving and the number of calves weaned depicts the extent of wastage at and after birth (Audigé *et al.* 2001). Investigation into neonatal and post-natal (pre-weaning) losses have reported problems such as dystocia and stillbirth to be the major causes of perinatal loss with other contributing factors such as mismothering, dehydration/starvation, and misadventure contributing to postnatal losses (Gill 1985; Audigé *et al.* 2001). In addition to these probable causal factors, Audigé *et al.* (2001) also observed that sub-optimum calving environment and failure to access favourable conditions (sunny weather and lower temperatures in summer) by parturient hinds have also contributed to neonatal mortalities. However, only approximately half of the calves known to have been born could be recovered and made available for necropsy to determine cause, despite intensive observation of calving. In an earlier study, infectious agents such as *Corynebacterium pseudotuberculosis*, *Staphylococcus aureus*, and *Cryptosporidium* spp. have been linked to postnatal calf losses (Gill 1988) but their significance needs to be established quantitatively.

Deer farmers wean calves at a time to minimise stress levels and to prevent any subsequent physical injuries to calves. The mean proportion of hinds calving and subsequently rearing a live calf to weaning was reported at 84% in R2 hinds (range: 64 - 100% between farms) and 92% (range: 81 - 99.3% between farms) in adult (MA) hinds (Audigé *et al.* 1999a). In the same study, factors such as age, early conceptions, body condition score (BCS), presence of stag before weaning, frequent mob/paddock visit by farmer were positively related to calf survival, whereas higher temperatures and land topography (more trees, hilly areas, and gullies) were negatively associated with calf survival to weaning. Recent studies have shown that leptospirosis may be contributing to the lower weaning rates, since vaccination against *Leptospira borgpetersenii* serovar Hardjobovis *and Leptospira interrogans* serovar Pomona has been shown to reduce calf losses to weaning by an average of 5.6 percentage points in five R2 herds (Subharat *et al.* 2011b) and Ayanegui-

Alcerreca (2006) showed a 8.7 percentage points higher weaning rate in vaccinated MA hinds in one herd that in controls.

1.4.3 Abortion

The third component of overall reproductive inefficiency is embryonic and fetal mortality. However, to the Author's knowledge there have been no studies of embryonic loss so this section focusses only on fetal loss. Fetal deaths and subsequent abortions have been considered of lesser importance than peri- and post-natal losses, despite lack of evidence to support this assumption, and hence have not been studied sufficiently in the past. Pregnancy testing of hinds just before calving is not a usual practice on New Zealand deer farms. Therefore, losses between conception and calving have likely remained underestimated. However, fetal losses reported from the few abortion studies in New Zealand have reported abortion rates ranging between 2% to 2.6% (Fennessy *et al.* 1986; Beatson *et al.* 1998) in R2 hinds and 0.79% for MA hinds (Audigé *et al.* 2001) (Table 1.1). In addition, abortion rates between 1.2% to 16% in four R2 herds and 4% in one MA herd were reported from a clinical investigation that involved repeat pregnancy scanning on study farms (Wilson *et al.* 2012). Outside of New Zealand, one North American study has reported abortions of 16% (4/25) in adult cows in Elk population of Idaho state of United States of America (USA) and one Spanish study reported abortions in two of seven young (1-2 years) and 4/39 (10.3%) in adult (3-9 years) red hinds.

There are several potential infectious and non-infectious causes of abortion in deer. There are few reports of diagnostic investigations that definitively demonstrate pathogens or non-infectious factors as the cause of abortion. In addition to reviewing specific diagnostic reports of abortion in deer, this section reviews evidence for the potential causes of abortion which have been reported to be present in deer in New Zealand and elsewhere.

1.5 Infectious causes of abortion

Several pathogens that are known for causing abortions in other ruminants including cattle and sheep are also reported in deer in natural or experimental conditions. They can be broadly categorised into bacterial, viral and parasitic causes. Evidence for their presence in deer is presented in Tables 1.2 and 1.3.

1.5.1 Bacterial

1.5.1.1 Brucella *species*

Abortions due to *Brucella abortus* in cattle in the early 1940s (Buddle 1950) and *Brucella ovis* in sheep in early 1950s (McFarlane *et al.* 1952; Buddle 1956) were reported in New Zealand. Subsequently, *B. abortus* was eradicated from the cattle population by applying effective control strategies (Adlam 1978; Davidson 2002). In deer, experimental *B. abortus* infections in North America have been shown to cause abortion in captive pregnant elk (*Cervus elaphus*) (Kreeger *et al.* 2000; Cook *et al.* 2002; Kreeger *et al.* 2002) (Table 1.2)

Sero-prevalence studies on free ranging elk in the USA have shown *B. abortus* antibodies. In a study from 1998 to 2002, sero-prevalence to *B. abortus* between 12% and 80% in elk from different artificial feeding areas in Idaho state, and 2.6% in hunter-killed elk deer were reported (Etter and Drew 2006) (Table 1.3). In another study carried out in Wyoming between 1993 and 2009, 21.9% of 3,327 elk in brucellosis endemic feeding grounds and 3.7% of 3,787 hunter killed elk showed antibodies to *B. abortus* (Scurlock and Edwards 2010) (Table 1.3). Both those studies included elk sampled from *Brucella* endemic feed grounds and by using hunters. These studies show a higher sero-prevalence in areas where elk were artificially fed, consistent with a greater transmission rate where population density is higher. *Brucella abortus* was isolated from sero-positive deer with abortions (4/25) and stillbirth (2/25) in a wild elk population in Ihado in USA (Etter and Drew 2006) (Table 1.3). However, no samples from sero-negative aborted deer were tested for *B. abortus* in that study to allow analysis for association between *B. abortus* and abortion. In New Zealand, no cases of *B. abortus* have been reported in farmed deer after its eradication from the cattle population (O'Neil 1996).

In New Zealand, *B. ovis* occurs in sheep flocks causing infertility and epididymitis in rams (Lawrence 1961). It was first isolated from semen of a stag in 1996 (Bailey 1997). Since then, cases in deer have remained sporadic. A sero-survey in New Zealand deer reported sero-prevalence of 11% (118/1,074) using the complement fixation test (CFT) used in sheep (Ridler *et al.* 2002b) (Table 1.3). An experimental vaginal infection study on red deer hinds in New Zealand showed venereal transmission of *B. ovis* from hinds to stags. However, no reproductive losses were reported in that study (Ridler *et al.* 2002a). Direct transmission of *B. ovis* between stags (West *et al.* 1999) and indirect transmission from rams to stags (Ridler *et al.* 2000), when co-grazing, was demonstrated in New Zealand farmed deer. These findings

suggest that infection to hinds through infected stags can spread rapidly in the mating season posing a potential risk for conception and perhaps pregnancy. However, to the Author's knowledge, no *Brucella ovis* abortions have been reported to date in red deer in New Zealand.

1.5.1.2 Listeria *species*

Abortions during the last trimester of pregnancy caused by *Listeria monocytogenes* are reported worldwide in cattle, sheep, and goats (Dennis 1974; Kirkbride 1993; Thornton 1996; Smits *et al.* 1997; Gill 1999; Moeller 2001). In Europe, listeriosis has been reported in wild red deer (*Cervus elaphus*) (Schwaiger *et al.* 2005), fallow deer (*Dama dama*) (Eriksen *et al.* 1988; Muller *et al.* 2003), reindeer (*Rangifer tarandus*), roe deer (*Capreolus capreolus*) (Nilsson and Karlsson 1959), wapiti deer (*Cervus canadensis roosevelt*) (Martyny and Botzler 1975), black buck antelope (*Antelope cervicapra*) (Webb and Rebar 1987), and llamas (*Lama lama)* (Butt *et al.* 1991). *Listeria monocytogenes* can also be isolated from forage, soil and feces, and feeding of poorly fermented silage has been reported for the transmission of listeriosis (Weiss and Seeliger 1975; Schwaiger *et al.* 2005). *Listeria* spp. has also been isolated from brains of fallow deer on a farm with high mortality in Sweden (Tham *et al.* 1999) (Table 1.3), deer brain samples (roe and red deer), plants, feeding places, and soil in Germany (Weiss and Seeliger 1975; Schwaiger *et al.* 2005), and from brain and healthy deer intestines in Denmark (Eriksen *et al.* 1988).

In New Zealand, *L. monocytogenes* has been observed in sheep, cattle, and goats (Smits *et al.* 1997; Staples 1997; Watts *et al.* 2012; Walker *et al.* 2014), and deer are subjected to the same risk factors as experienced by these species. Nervous disorders and subsequent fetal losses under pastoral grazing conditions are common in sheep with listeriosis. Moreover, reservoir sheep and cattle animals can shed *Listeria* spp. in their feces. Under these circumstances, co-grazing or alternate grazing of sheep or cattle with deer likely increases the risk of *Listeria* spp. transmission to farmed deer. In pregnant deer, silage feeding can lead to *Listeria* spp. transmission and possible subsequent abortion although no such cases have been reported in New Zealand. In the past, studies have shown fecal isolation of *L. monocytogenes* from wild deer (Lyautey *et al.* 2007) such as sika deer (*Cervus nippon)* in Japan (Sasaki *et al.* 2013), barking deer (*Muntiacus muntjak)* in India (Sarangi and Panda 2013) and farm-raised white-tailed deer (*Odocoileus virginianus)* in the USA (French *et al.* 2010), which may act as a reservoir and/or potential shedder (Table 1.3). These findings

show that deer can become infected and therefore potentially be at risk of abortion. However, there is scant evidence for listerial abortions in deer. Neonatal mortalities were observed in reindeer (Evans and Watson 1987; Nyyssönen *et al.* 2006) and Canadian farmed elk (Pople *et al.* 2001) wherein *L. monocytogenes* was isolated from several internal organs of dead fawns (Table 1.2).

1.5.1.3 Leptospira *species*

Leptospira infection caused by *Leptospira borgpetersenii* serovar Hardjobovis *and Leptospira interrogans* serovar Pomona is widely prevalent in the New Zealand deer population (Flint *et al.* 1988; Wilson *et al.* 1998; Ayanegui-Alcerreca *et al.* 2007; Ayanegui-Alcérreca *et al.* 2010). Other serovars reported in deer in New Zealand include Copenhageni, Australis, Ballum, and Tarassovi (Flint *et al.* 1988; Wilson *et al.* 1998). On New Zealand deer farms, serovar Hardjobovis is highly endemic with 78% of herds sero-positive whereas serovar Pomona remains sporadic at a herd-level sero-prevalence of 20% (Ayanegui-Alcérreca *et al.* 2010). At the animal level, the same study reported a mean sero-prevalence of 60.8% for Hardjobovis and 8.4% for Pomona and 6.6% for dual positivity. In an earlier study, a sero-prevalence of 9.8% for Hardjobovis and 1.4% for Pomona was reported in randomly selected sub-sample of sera (n=417) from 575 herds sera bank based on a higher titre (≥1:100) (Reichel *et al.* 1999). However, the sero-prevalence estimates by Reichel *et al.* (1999) may not be robust as only two animals/herd were bled for the establishment of serum bank.

Leptospiral infection and subsequent decrease in live weight gain in weaner deer was demonstrated as an effect on production (Ayanegui-Alcerreca 2006; Subharat *et al.* 2011a). However, the effect of *Leptospira* spp. on reproduction including pregnancy is not yet fully characterised. Recent studies have shown that vaccination against *Leptospira* spp. resulted in improved weaning percentage by an average of 5.6 percentage points in five R2 herds (Subharat *et al.* 2011b) and 8.7 percentage points in a MA herd (Ayanegui-Alcerreca 2006) but whether the effect was on abortion or perinatal/postnatal mortality was not determined. Neither of those studies proved abortion *per se*, but it was suggested that deer carried a fetus to term, but it did not survive to weaning. Hence, the losses due to *Leptospira* spp. are more likely to be perinatal and early postnatal than abortion. However, leptospiral abortion has been demonstrated in an experimental challenge study in white-tailed deer in North America wherein leptospires from aborted fetal kidney were detected through real-time PCR assay

(Trainer *et al.* 1961) (Table 1.2). *Leptospira* spp. DNA was noted in 1 of 27 fetal tissue samples in an attempt to isolate *Leptospira* spp. from hinds and normal *in utero* fetuses from slaughtered hind in New Zealand (Subharat *et al.* 2010). Combined, these equivocal findings suggest that *Leptospira* spp. may cause abortion, yet there is little evidence that such abortions are occurring in New Zealand farmed deer.

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.

Pathogen	Deer breed	Year, country	Pregnancy fate ((No. affected/No. observed) or (no observed))	Pathogen challenge	Samples collected from (no. positive/ no. tested)	Strain and serotype isolated	Pathogen isolated/demonstrated serologically	Pathogen DNA/RNA detection	Reference
Brucella spp.	Elk (*Cervus elaphus*)	1996, USA	Abortion (13/13)	Experimental	NA	NA	NA	NA	Cook *et al.* (2002)
		1998, USA	Abortion (5/7)	Experimental	NA	NA	NA	NA	Kreeger *et al.* (2000)
		2000, USA	Abortion (15/17)	Experimental	NA	NA	NA	NA	Kreeger *et al.* (2002)
		1999-2001, USA	Abortion (4/25), Still birth (2/25)	Natural	Dam (9/25[a]) and calves (6/21)	*B. abortus* biovar 1 and 4	NA	NA	Etter and Drew (2006)
Listeria spp.	Reindeer (*Rangifer tarandus tarandus*)	1985, USA	Neonatal death (1)	Natural	Calf (1/1)	*L. monocytogenes*	Liver, lung	NA	Evans and Watson (1987)
		2006, Finland	Neonatal death (4)	Natural	Calves (4/4)	*L. monocytogenes*	Brain, lung, liver, spleen, umbilical cord, kidney	NA	Nyyssönen *et al.* (2006)
Leptospira spp.	White-tailed deer (*Odocoileus virginiasus*)	1960, USA	Abortion (4/5)	Experimental	Fetus (3/3) and dam (4/4)	*L. pomona*	Kidney, liver, blood in fetuses, and Kidney in dam	NA	Trainer *et al.* (1961)
	Red deer (*Cervus elaphus*)	2008, New Zealand	Fetuses in normal pregnancy (1/27)	Natural	Fetus (1/ 27)	*Leptospira* spp DNA	NA	DNA in Fetal kidney	Subharat *et al.* (2010)

Bovine Viral Diarrhoea virus	Mouse deer (*Tragulus javanicus*)	2002, The Netherlands	PI fawn (2)	Natural	Live fawns (2/2), and dam (1/1)	BVDV type 1f	Serum	RNA in serum	Grondahl *et al.* (2003)
	White-tailed deer (*Odocoileus virginiasus*)	2005, USA	live PI fawn (1) and mummified fetus (1)	Experimental	Live fawn (1/1)	BVDV type-2 strain PA131	serum and buffy coat. IHC in ear notch	RNA in serum and buffy coat	Passler *et al.* (2007)
		2007, USA	live born calves (3) and stillbirth (2)	Natural	Fetuses (2/2)	BVDV type-1	Nasal swab, buffy coat in live fawn, and Lungs, spleen, thymus, lymph node in dam	RNA in thymus, lungs, lymphnode and spleen	Passler *et al.* (2009)
		2008, USA	PI fawn and live fawn	Natural	Live (1/1) and PI fawn (1/1)	BVDV type-1b	NA	RNA in serum, buffy coat and IHC in skin	Passler *et al.* (2010)
Cervid Herpes virus	Reindeer (*Rangifer tarandus tarandus*)	2005, Norway	Fetuses (12/48) and dams in normal pregnancy (27/143)	Natural	Fetuses (12/48) and dams (27/143)	CvHV type-2	NA	DNA in fetal liver, spleen, lung, blood in fetuses and, trigeminal ganglia and nasal swabs in dam	das Neves *et al.* (2009b)
		2008, Norway	Abortion (1)	Experimental	Fetus (1/1) and dam (1/1)	CvHV type-2	Nasal swab, vaginal swab, in dam	DNA in lung, liver spleen, kidney, trigeminal ganglia of dam and fetus both, kidney in dam only, and mediastinal lymph node in fetus only.	das Neves *et al.* (2009a)
Neospora	Eld deer (*Cervus eldi siamensis*)	1996, France	Still birth (1)	Natural	Still born calf (1/1)	*N. caninum* tissue cyst	Brain	NA	Dubey *et al.* (1996)
	White-tailed deer	2008 and	Fetuses in normal pregnancy	Natural	Fetus (2/155)	*N. caninum*	Mice bioassay and cell culture	DNA in mouse lung cell culture	Dubey *et al.* (2013)

	(*Odocoileus virginiasus*)	2009, USA	(2/155)				from fetal brain		
	Reindeer (*Rangifer tarandus tarandus*)	2002, USA	Still birth (1)	Natural	Still born calf (1/1)	*T. gondii*	Myocardium, placenta, brain		Dubey *et al.* (2002)
Toxoplasma	White-tailed deer (*Odocoileus virginiasus*)	2007, USA	Fetuses in early (n=9/27) and mid-pregnancy (n=6/61)	Natural	Fetuses	*T. gondii*	Mice bioassay from fetal homogenate or brain	DNA in mice tissues	Dubey *et al.* (2008b)
		2008, 2009, 2010, USA	Fetuses in normal pregnancy (1/155)	Natural	Fetuses	*T. gondii*	Mice bioassay from fetal brain	DNA in mice tissues	Dubey *et al.* (2014)
	Red deer (*Cervus elaphus*)	2012, New Zealand	Abortion (16/1600)	Natural	Fetuses (8/9) and non-pregnant hinds (2/10)	*T. gondii*	NA	DNA in fetal brains and uteri	Wilson *et al.* (2012)

[a] All sero-positive dams, NA- not available

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.

Organism	Deer breed	Study type	Year, country	Serology test[a]	Sample	Strain/serovar from culture	Prevalence (%) (no. tested) from sera/culture	Detection in tissues % prevalence (no. tested)	Reference
Brucella abortus	Elk (*Cervus elaphus*)	Experimental	1998, USA	Dot blot assay	Blood	Strain 2308	Vaccinated 71.4 (5), control- 100 (7)	-	Kreeger *et al.* (2000)
		Clinical report	1998-2002, USA	Screening- card test, SPT, BAPA Confirmatory- CFT	Blood	Biovar 1 and biovar 4 from seropositive calves	Trapped elk- 32 (366), hunter killed elk- 2.6 (227), calves from sero-positive dams- 72.2 (18)	-	Etter and Drew (2006)
		Serological survey	1991-2008, USA	Screening- Card test, SPT, RIV, Confirmatory- CFT, FPA, cELISA	Blood	-	Trapped elk- 22 (3,327), hunter killed elk- 3.7 (3787)	-	Scurlock and Edwards (2010)
Brucella suis	Red deer (*Cervus elaphus*)	Serological survey	2000, New Zealand	CFT	Blood	-	Farmed- 11 (1074)	-	Ridler *et al.* (2002b)
Listeria spp.	NA	Survey	1972-74, Germany	Culture	Feces	1/2a, a/2b, 4b, 4c, 4d, 4f, 4g, 5	15.7 (102)	-	Weiss and Seeliger (1975)
	Reindeer (*Rangigfer tarandus tarandus)*	Clinical report	1987, USA	Culture	Liver, lung, kidney	-	100 (1)	-	Evans and Watson (1987)
	Fallow deer (*Dama dama*)	Survey	1985-86, Denmark	Culture	Brain	1/2a, a/2b, 4b	83.3 (42)	-	Eriksen *et al.* (1988)
	Fenced fallow deer	Clinical report	1990-91, Sewden	Culture	Brain	1/2a, 4b	35.7 (14)	-	Tham *et al.* (1999)
	Reindeer	Clinical report	2005, Finland	Culture	Lung, liver, spleen, brain,	-	100 (4)	-	Nyyssönen *et al.* (2006)

				umbilical cord				
NA	Survey	2003-05, Canada	Culture	Feces	-	5.9 (34)	-	Lyautey *et al.* (2007)
white-tailed deer (*Odocoileus virginianus*)	Survey	2006-07, USA	Culture	Feces	-	3.3 (30 farms)	-	French *et al.* (2010)
Sika deer (*Cervus nippon*)	Survey	2010, Japan	Culture	Feces	1/2a, 1/2b, and 4b	22.8 (128)	-	Sasaki *et al.* (2013)
Wapiti (*Cervus Canadensis roosevelti*)	Survey	1972, USA	Culture	Feces	1,4	19 (72)	-	Martyny and Botzler (1975)
Roe, red deer	Survey	2005, Germany	Culture	Brain	-	Roe deer 6.6 (654), red deer 2.6 (189)	-	Schwaiger *et al.* (2005)
Barking Deer (*Muntiacus muntjak)*, Chital (*Axis axis)* Sambar (*Rusa unicolor)*, Chousingha (*Tetraceros quadricornis*),	Survey in zoo animals	2013, India	Culture	Feces	-	27.8 (18)	-	Sarangi and Panda (2013)

	Hog Deer (*Hyelaphus porcinus)*, Barasinga *(Rucervus duvaucelii)*								
Leptospira spp	Wild red deer (*Cervus elaphus*)	Serological survey	1966, New Zealand	MAT	Blood	-	PM 0.9 (109)	-	Daniel (1966)
	Farmed red (mixed-age)	Clinical report	1984, New Zealand	MAT, culture	Urine	-	HJ 8.3 (12), PM 75 (12)	HJ 20 (5), PM 80 (5)	Fairley (1984)
	Farmed red calves	Clinical report	1984, New Zealand	MAT, culture	Urine	-	PM 62.5 (16)	HJ 16.7 (6), PM 83.3 (6)	Fairley *et al.* (1984)
	Wild deer	Serological survey	NA, New Zealand	MAT, culture	Urine	-	PM 16.6 (24), PM+IC 16.6 (24), IC 8.3 (24)	-	Inglis (1984)
	Farmed red (mixed-age)	Vaccination trial	1984, New Zealand	MAT	Blood	-	Hardjobovis- 33.3 (6)	-	Wilson and Schollum (1984)
	Farmed ND	Serological survey	NA, New Zealand	MAT	Blood	-	HJ 23 (26)	-	Flint (1985)
	Farmed ND	Serological survey	NA, New Zealand	MAT	Blood	-	HJ+CO 32.5 (120)	-	Williams (1985)
	Farmed red (mixed-age)	Serological survey	1986, New Zealand	MAT	Blood, urine	-	Hardjobovis-22.2 (27)	HJ 29.6 (27), CP 11.1 (27)	Flint *et al.* (1986)
	Farmed red, fallow (mixed-age)	Serological survey (5 farms)	1988, New Zealand	MAT	Blood	-	HJ 39 (360), PM 13.9 (360), CO 43 (155/360), Australis 13.1 (360), Ballum 53.1 (360), Tarassovi 26.1 (360)	-	Flint *et al.* (1988)
	Farmed red, wapiti	Serological survey	1998, New	MAT	Blood, kidney	-	HJ 73.6 (675), PM 41.5 (675), CO 11.3 (675),	HJ 30(10), PM 10(10), TS 60 (10)	Wilson *et al.* (1998)

	cross (mixed-age)	(53 farms)	Zealand				TS 15.1 (675)		
	Farmed red deer	Serological survey	1999, New Zealand	MAT	Blood	-	HJ 19.8 (417), PM 2.2 (417), CO 0.2 (417)	-	Reichel *et al.* (1999)
	Farmed red deer (mixed-age) plus weaners	Clinical report	2005, New Zealand	MAT	Blood	-	HJ 1.3 (75), PM 26.7 (75)	-	Dean *et al.* (2005)
	Farmed red deer (mixed-age + yearling)	Serological survey (111 farms)	2005, New Zealand	MAT	Blood	-	HJ 54.2 (2016), PM 1.8 (2016), CO 1.2% (2016)	-	Ayanegui-Alcérreca *et al.* (2010)
	Farmed red deer (mixed-age)	Serological and tissue survey	2008, New Zealand	MAT, culture, PCR	Blood, kidney, uterus	-	HJ 29.3 (116), PM 11.2 (116)	Culture: kidney -HJ 5.8 (120), uteri 0, fetal organs 0. PCR: kidney 4.2 (120), uteri 0, fetal organs 3.7 (27)	Subharat *et al.* (2010)
Bovine virus diarrhoea	Red, roe and fallow deer	Serological survey	1991-94, Germany	NPLA	Blood	-	Roe deer 4.7 (577), red deer 4.7 (443), fallow deer 3.5 (283)	-	Muller *et al.* (1997)
	Reindeer, roe deer, red deer	Serological survey	1993-2000, Norway	VNT	Blood	-	Reindeer 4.2 (810), Roe deer 12.3 (635), Red deer 1 (658)	-	Lillehaug *et al.* (2003)
	Farmed red deer	Serological survey	1997, New Zealand	VNT	Blood	-	9.5 (400)	-	Motha *et al.* (2000)
	Wild roe, fallow, red deer, and ND	Serological survey	1995-99, Denmark	Blocking ELISA	Blood	-	Red 3.9 (57), fallow 0 (0/29), roe 0 (0/149), ND 0 (0/8)	-	Nielsen *et al.* (2000)
	Huemul deer	Serological survey	2000, Chile	VNT	Blood	-	0 (0/7)	-	Montt *et al.* (2003)

	(*Hippocamelus bisulcus*)								
	Red deer	Serological survey	2003-10, Spain	Screening- indirect ELISA, confirmatory- blocking ELISA, RT-PCR	Blood. RNA from serum, for PCR	-	19.5 (267)	22.5 (267)	Rodriguez-Prieto *et al.* (2016)
	Red and roe deer	Serological survey	2010-14, Spain	Screening -blocking ELISA, confirmatory test- VNT, RT-PCR	Blood, RNA from serum	-	Red deer 10.8 (65), roe deer 0 (0/24)	Red deer 0 (65), roe deer 0 (24)	Fernandez-Aguilar *et al.* (2016)
Cervid Herpes virus	Red deer	Serological survey	NA, Czech Republic	VNT	Blood	CvHV-1	71.0 (165)	-	Pospisil *et al.* (1996)
	Reindeer,	Serological survey	1993-2000, Norway	VNT	Blood	CvHV-2	Reindeer 28.5 (831),	-	Lillehaug *et al.* (2003)
	Farmed red deer	Clinical report	2004, New Zealand	Viral culture, consensus PCR, sequencing	Buffy coat, conjunctival swab	CvHV-1, Cervid Rhadino virus type-2 (CRhV-2)	Buffy coat consensus PCR- 55.6 (9)	Culture 20 (10), consensus PCR 62.5 (16), CvHV-1 PCR 26.7 (15),	Squires *et al.* (2012)
	Farmed red deer	Clinical report	2009, New Zealand	PCR, virus culture, direct fluroscent antibody test	Formal in fixed penis and prepuce samples	CvHV-1		30% of stags presented for slaughter	Morgan *et al.* (2010)
Toxoplasma gondii	Farmed red deer	Serological survey	1972-77, Scotland	SFDT, HA	Blood	-	SFDT 32.5 (NA), HA-9.8 (NA)	-	Williamson *et al.* (1980)

	White-tailed deer	Serological survey	1991, USA (Pennsylvania)	MAT	Blood	-	60 (583)	-	Humphreys *et al.* (1995)
	White-tailed deer	Serological survey	1990-93, USA (Minnesota)	MAT	Blood	-	30 (1,367)	-	Vanek *et al.* (1996)
	White-tailed deer	Serological survey	2002-03, USA (Mississippi)	MAT	Blood	-	46.6 (73)	-	Dubey *et al.* (2004)
	Roe, red, reindeer	Serological survey	1992-2000, Norway	DAT	Blood	-	Roe 33.9 (760), Red 7.7 (571), reindeer 1 (866)	-	Vikoren *et al.* (2004)
	Roe deer	Serological survey	1998-2001, Italy	LAT	Blood	-	13 (207)	-	Gaffuri *et al.* (2006)
	Red, roe, fallow deer	Serological survey	1993-2005, Spain	MAT	Blood	-	Red 15.6 (441), roe 22.8 (79), fallow 21.2 (33)	-	Gauss *et al.* (2006)
	Black-tailed deer (*Odocoileus hemionus columbianus*)	Serological survey	1998-2002, USA	MAT	Blood	-	32.6 (43)	-	Dubey *et al.* (2008a)
	Roe, sika, red, fallow	Serological survey	1998-2006, Czech Republic	IFAT	Blood	-	Roe 24.1 (79), sika 50 (14), red 44.8 (377), fallow 16.8 (143)	-	Bartova *et al.* (2007)
	White-tailed deer	Clinical report	2007, USA (Iowa)	MAT	Blood	-	64.2 (84)	-	Dubey *et al.* (2008b)
	Roe deer	Serological survey	NA, Spain	MAT	Blood	-	39.2 (278)	-	Gamarra *et al.* (2008)
	White-	Serologica	2007,	MAT	Blood	-	32.3 (62)	-	Dubey *et*

	tailed deer	l survey	USA (Minnesota and Iowa)						*al.* (2009)
	Roe deer	Serological survey	2007, Spain	DAT	Blood	-	13.8 (160)	-	Panadero *et al.* (2010)
	Roe deer	Serological survey	1999-2007. Sweden	DAT	Blood	-	34.2 (199)	-	Malmsten *et al.* (2011)
	ND	Molecular and serological survey	2007-08, Ireland	LAT, nested PCR	Blood, diaphragm	-	6.6 (348)	4.2 (71)	Halová *et al.* (2013)
	Roe, red, fallow deer	Molecular and serological survey	2004-09, Belgium	SAG1-ELISA, real-time PCR	Blood, brains	-	Roe 52.1 (73), red 0 (0/7), fallow (0/7)	Roe 2 (20), red 0 (0/13)	De Craeye *et al.* (2011)
	White-tailed deer	Serological survey	2010, USA (Ohio)	MAT	Blood	-	58.8 (444)	-	Ballash *et al.* (2014)
	White-tailed deer	Serological survey	2008-10, USA (Minnesota)	MAT	Blood	-	22.6 (487)	-	Dubey *et al.* (2014)
	White-tailed deer	Serological survey	2010, USA (New York)	ELISA	Blood	-	42.2 (299)	-	Schaefer *et al.* (2013)
Neospora spp.	Roe, red, fallow deer (wild and farmed)	Serological survey	2004-09, Belgium	Indirect ELISA	Blood	-	Roe 2.7 (73), red 0 (0/7), fallow (0/7)	-	De Craeye *et al.* (2011)
	Roe, sika, red, fallow	Serological survey	1998-2006, Czech	Screening - competitive inhibition ELISA,	Blood	-	Roe 13.9 (79), sika 14.3 (14), red 6.4 (377), fallow 1.4 (143)	-	Bartova *et al.* (2007)

		Republic	confirmatory- IFAT					
Mule deer (*Odocoileus hemionus hemionus*), Black-tailed deer	Serological survey	1998-2002, USA	Screening -NAT, confirmatory- IFAT	Blood	-	White-tailed deer16.7 (42), Black tailed deer-18.6 (43)	-	Dubey *et al.* (2008a)
White-tailed deer	Serological survey	2007, USA (Minnesota and Iowa)	IFAT, NAT, ELISA and/or WB	Blood	-	Minnesota 71.0 (62), Iowa 88.2 (170)	-	Dubey *et al.* (2009)
Red deer	Serological survey	2006-07, Poland	Screening -iscom-ELISA, confirmatory- WB	Blood	-	Farmed 12.8 (47), Feral 11.3 (106)	-	Gozdzik *et al.* (2010)
Roe deer	Serological survey	2007, Spain	cELISA	Blood	-	6.9 (160)	-	Panadero *et al.* (2010)
Roe deer	Serological survey	1999-2007. Sweden	Screening- iscom ELISA, confirmatory- IB	Blood	-	1 (199)	-	Malmsten *et al.* (2011)
Farmed fallow deer	Serological survey	NA, Poland	Screening- ELISA, confirmatory- WB	Blood	-	3 (335)	-	Bień *et al.* (2012)

[a] SPT=Standard plate agglutination test, BAPA= Buffered acidified plate agglutination test, CFT= complement fixation test, RIV= rivanol precipitation–plate agglutination, FPA= fluorescence-polarization assay, cELISA= competitive enzyme-linked immunosorbent assay, LAT= latex agglutination test, MAT= microscopic agglutination test, HJ= Hardjobovis, PM= Pomona, CO= Copenhagoni, IC= Icterohaemorrhagia, ND=not described, VNT= virus neutralisation test, RT-PCR= Reverse transcription polymerase chain reaction, CvHV-1= Cervid herpes virus type-1, CRhV-2= Cervid Rhadino virus type-2, NPLA= Neutralizing Peroxidase-linked assay, DAT= Direct agglutination test, HA= haemagglutination test, SFDT= Sabin-Feldman dye test, NAT= Neospora agglutination test, WB= western blot, IB= immunoblotting

1.5.2 Viral

1.5.2.1 Bovine viral diarrhoea virus (BVDV)

Bovine viral diarrhoea caused by a *pestivirus* is an important disease in cattle worldwide known for causing abortions and subsequent economic losses (Horner 1996; Houe 1999). Genetically, two species of BVDV have been identified: BVDV-1 and BVDV-2. Both species exist as either cytopathogenic or non-cytopathogenic biotypes. BVDV-1 has five distinct serotypes (1a, 1b, 1c, 1d, and 1e) having a worldwide distribution whereas BVDV-2 viruses are mainly restricted to North America including United States of America (USA) and Canada (Donis 1995; Brownlie *et al.* 2000). Consequences of BVDV infection in dairy cattle include early embryonic death, fetal resorption, mummification, conception failure, abortions and congenital malformations (McGowan *et al.* 1993; Fray *et al.* 2000; Lindberg *et al.* 2001). In New Zealand, BVDV-1 is known to cause reproductive losses including early embryonic losses leading to a high proportion of non-pregnant cows and abortions (Thobokwe and Heuer 2004). An important outcome of this disease is birth of a persistently infected (PI) calf. A PI animal is an important source of BVD infection that regularly excretes high amount of viruses in saliva, semen, nasal discharge, urine, feces, milk and tears (Fray *et al.* 2000).

In a recent New Zealand study, approximately 44% of cattle from 50 herds and 28% of calves from 32 herds were found antibody positive to BVDV (Thobokwe *et al.* 2004). Also, past studies have reported an estimated sero-prevalence of 60% in the cattle population, with an estimated one to five per cent of PI calves born within herds (Littlejohns and Horner 1990). Production of PI fawns from BVDV infected pregnant white-tailed deer through transplacental transmission of virus is possible (Passler *et al.* 2009; Passler *et al.* 2010). Experimental studies have shown that infected cattle co-habitating with pregnant white-tailed deer can transmit virus to deer resulting in either PI fawns or stillbirth (Passler *et al.* 2009). The infection pattern for BVDV in white-tailed deer was reported to be similar to that in cows under experimental conditions in the USA wherein abortions, stillbirth and birth of PI fawns were observed (Ridpath *et al.* 2008). In that study, the advancement of clinical signs of bovine viral diarrhoea was consistent with that observed in infected pregnant cattle.

Losses due to BVDV infection and subsequent fetal infections have been reported in the Sika deer industry in China where the BVDV sero-prevalence ranged from 60-87% (Rui *et al.* 2000). Antibodies to BVDV has been observed in other overseas deer populations

(Nielsen *et al.* 2000; Fernandez-Aguilar *et al.* 2016; Rodriguez-Prieto *et al.* 2016) (see Table 1.3). Sero-prevalence of 3.9% of 77 wild red deer was reported by Nielsen *et al.* (2000) in Denmark, and 10.8% of 65 wild red deer was reported in Spain (Fernandez-Aguilar *et al.* 2016) (Table 1.3). The Danish and Spanish studies used blocking ELISA as a screening test and VNT as a confirmatory test, and the Spanish study also used VNT at a cut-off neutralising antibody titre of ≥1:10. In another Spanish study, a sero-prevalence of 19.5% of 267 wild red deer, using indirect ELISA as screening test and blocking ELISA as confirmatory test, was reported (Rodriguez-Prieto *et al.* 2016) (Table 1.3). The overseas studies reporting sero-prevalence in deer used different tests and therefore may not be directly comparable.

In New Zealand, sero-prevalence of 9.5% in a sub-sample of 400 from a serum bank has been reported (Motha *et al.* 2000). However, the sero-prevalence data reported in that study are likely not fully representative as the sera collection was based on two deer per herd. Also, the test used in that study, a blocking antibody ELISA, was not validated for deer. In New Zealand conditions, the majority of deer farmers also rear cattle and sheep along with deer population. Given the considerable sero-prevalence of BVDV in the New Zealand cattle population and its potential transmission to deer, the pregnant deer population may be at risk of BVDV infections and subsequent losses. However, there are no reports of abortions due to BVDV in deer.

1.5.2.2 Cervid Herpesvirus (CvHV)

Herpesvirus infections are observed in several species of production animals including cattle, sheep, and goats. Bovine herpesvirus type-1 (BoHV-1) in cattle causes infectious bovine rhinotracheitis (IBR), pustular vulvovaginitis and abortion in females, balanoposthitis and penile lesions in males, and ocular lesions in both males and females (Engels and Ackermann 1996; Smith 1997; Muylkens *et al.* 2007). Cervid herpesvirus belonging to the alpha herpes viridae family is also reported in deer (Table 1.2 and Table 1.3). Two types of cervid herpesvirus (CvHV) have been isolated from deer, namely CvHV type I (Inglis *et al.* 1983) from red deer and CvHV type II from reindeer (Ek-Kommonen *et al.* 1986; Rockborn *et al.* 1990). In New Zealand, CvHV-1 was reported and isolated from semen collected from a red deer stag in 2001 (Tisdall and Rowe 2001). An earlier case of keratoconjunctivitis in red deer may have been due to herpes virus infection but no diagnostic tool for CvHV-1 was available at that time (Wilson *et al.* 1981), but it has subsequently been

identified from eye lesions (Squires *et al.* 2012). Also, CvHV-1 was detected in a deer farm in New Zealand that experienced an overall decrease in pregnancy rates (Squires *et al.* 2012). In that study, CvHV-1 was isolated from vaginal lesions including mucosal erosions and petechial haemorrhages from an immunosuppressed hind (administered dexamethasone) with a history of keratoconjunctivitis suggesting a possible role in the poor reproductive performance in the herd. The lesions grossly and histologically were similar to infectious pustular vulvovaginitis observed in cattle infected with BoHV-1 but these observations were not sufficient to establish a causative association between CvHV-1 infection and low reproductive performance problems on the farm (Squires *et al.* 2012).

Furthermore, at a New Zealand deer slaughter premises, CvHV-1 was also isolated from two stags presented for slaughter with extensive ulcerative lesions, and fibrinous and necrosuppurative balenoposthitis in 30% of stags in the same line (Morgan *et al.* 2010). These findings suggest that CvHV-1 may play a role in reduced deer reproductive performance given its isolation from both males and females.

An estimated 38.2% sero-prevalence to CvHV-1 was reported based on a randomly sampled farmed red deer population in New Zealand (Motha and Jenner 2001) (Table 1.3). However, the sero-prevalence data reported in that study was based on collection of blood samples from two deer per farm and therefore are unlikely to be fully representative. Sero-prevalence of 32% in deer was reported for bovine herpesvirus (BoHV) in New Zealand by Motha *et al.* (2000) using virus neutralisation test (VNT). Hence there may be cross-reaction between CvHV-1 and BoHV-1, which are antigenically similar (Motha and Jenner 2001; Thiry *et al.* 2006). Therefore, the BoHV-1 sero-prevalence reported in that study may be an over- estimate of the actual prevalence due to presence of cross-reaction.

A sero-prevalence for CvHV-1 of 71% was reported in a red deer population in Scotland (Pospisil *et al.* 1996). As BoHV-1 and CvHV-1 are closely related, several studies reporting a crude BoHV-1 sero-prevalence in deer have suggested cross reactivity between BoHV-1 and CvHV-1. Antibodies to BoHV-1 have been reported in a wild deer population with 0.2% of 914 roe deer, 5.4% of 499 red deer and 0.6% of 312 fallow deer in Germany (Mueller *et al.* 1997), 68% of 165 red deer in Scotland (Pospisil *et al.* 1996), 28.5% reindeer, 3% of 602 roe deer, and 0.5% of 589 red deer in Norway (Lillehaug *et al.* 2003) (Table 1.3). CvHV-1 has also been isolated from a nasal swab of healthy wild red deer fawn in Belgium (Thiry *et al.* 2007) and from the genital tract of captive red deer in France (Thiry *et al.* 2011)

suggesting that CvHV-1 is not only restricted to ocular lesions but may also reach genital organs which may be a risk for impaired reproduction.

The second type of CvHV virus, CvHV type 2 (CvHV-2), is also reported in deer. CvHV-2 vertical transmission to fetuses (das Neves *et al.* 2009b) and abortion has been demonstrated in a semi-domesticated reindeer (*Rangifer tarandus tarandus*) population where in CvHV-2 DNA was detected both from dam and fetal tissues (das Neves *et al.* 2009a) (Table 1.2). That study suggests that the presence of CvHV-2 in fetuses may have a role in impaired reproduction as observed with other ruminant herpes viruses.

Cervid Rhadinovirus type-2 (CRhV-2) was reported by Squires *et al.* (2012) in conjunctival swabs and buffy coat from normal hinds on two farms with known cases of keratoconjunctivitis and presence of vestibulo-vaginal lesions showing inflammatory changes, petechial haemorrhages and some mucosal erosions, associated with CvHV-1 infection. CRhV-2 has also been reported in Elk in North America (Li *et al.* 2005). These findings suggest that the CRhV-2 should be included in investigation of reproductive failure in deer.

1.5.3 Protozoa

1.5.3.1 Neospora caninum

Since its discovery from dog feces in Norway, *Neospora caninum*, causative agent for neosporosis, has been found worldwide in cattle and dog populations (Bjerkås *et al.* 1984). In New Zealand, *N. caninum* infections have led to abortions and reproductive disorders in cattle (Thobokwe and Heuer 2004; Weston *et al.* 2012) and sheep (West 2002; Howe *et al.* 2012) populations but its effects in deer are not known. Reservoir status of dogs with *Neospora caninum* poses a risk for horizontal transmission on New Zealand farms as oocysts shed by dogs could be picked up by grazing deer (Anderson *et al.* 1995; Dijkstra *et al.* 2001). In spite of reports of neosporosis in cattle and sheep, clinical neosporosis in deer is not reported in New Zealand. Antibodies to *N. caninum* have been reported in wild deer populations of white-tailed deer, mule deer (*Odocoileus hemionus hemionus)*, black-tailed deer (*Odocoileus hemionus columbianus)*, Vietnam sika deer (*Cervus nippon pseudaxis*), roe deer, red deer, fallow deer, and caribou ranging from 17-88% sero-prevalence in North America and up to 14% in Europe (Dubey and Schares 2011) (Table 1.3).

Sero-prevalence of 11% in 106 farmed and 13% in 46 free-ranging red deer has been reported for *N. caninum* in a Polish study from an area with documented evidence of neosporosis in cattle (Gozdzik *et al.* 2010). In addition, *N. caninum* has been isolated, confirmed by bioassay in mice, from brains (3/110) of naturally infected white-tailed deer suggesting that deer may act as intermediate hosts for *N. caninum* (Wilson 2002a; Gondim *et al.* 2004).

Neospora caninum oocysts were isolated from brain of a stillborn deer in France (Dubey *et al.* 1996) and two of 155 fetal brains obtained from hunt-killed pregnant white-tailed deer in USA suggesting congenital transmission of *N. caninum* (Dubey *et al.* 2013) (Table 1.2). Both cases showed that *N caninum* has potential to infect the fetus in pregnant deer which may possibly lead to abortion. *Neospora caninum* has also been linked to peri- and neo-natal mortalities. *Neospora caninum* DNA along with histological evidence of protozoal cysts was demonstrated in a case of clinical Neosporosis in a 3-week-old fallow deer fawn in Switzerland (Soldati *et al.* 2004). Association of *N. caninum* with neonatal mortality was further demonstrated in an Argentinian study wherein *N. caninum* was isolated from brain samples coupled with histological lesions and serological evidence in fatal cases of one axis deer (*Axis axis)* calf and four other neonates in a zoo (Basso *et al.* 2014). In New Zealand, 74% of urban and farm dogs have been shown to have antibodies to *N. caninum* (Antony and Williamson 2003). Therefore, despite no reports of clinical disease or abortion, findings suggest that exposure of *N. caninum* shed by farm dogs could infect pregnant hinds on New Zealand deer farms.

1.5.3.2 Toxoplasma gondii

Toxoplasma gondii is found worldwide and can infect all mammals including humans. An estimated 16% of the human population around the world show antibodies to *T. gondii* (Dubey 2008). *Toxoplasma gondii* has accounted for abortions in sheep around the world. In New Zealand, *T. gondii* was reported in sheep abortion storms in the late 1950s during which *T. gondii* was isolated from placental and fetal tissues from aborting sheep (Hartley *et al.* 1954). Cats act as a definitive host for this disease and regularly shed oocysts, contaminating the environment. *T. gondii* transmission occurs through ingestion of tachyzoites/bradyzoites from contaminated tissues from infected animals, or by fetal transmission from dam to offspring. The deer population, both wild or farmed, can ingest *T. gondii* oocysts from

environment and can get infected. In pregnant deer, *T. gondii* infection can lead to congenital transmission and possibly subsequent abortion (Wilson *et al.* 2012).

Sero-studies have been done in wild and/or farmed deer around the world. However, only few studies have used deer specific and validated serology assays. In wild deer, the antibody sero-prevalence reported in different studies in different years for *T. gondii* ranged between 14-76% % in white-tailed deer in different states of USA (Dubey and Jones 2008; Schaefer *et al.* 2013; Ballash *et al.* 2014; Dubey *et al.* 2014) (Table 1.3). In European wild deer populations, sero-prevalence has ranged from 3-52% in roe deer, 8-45% in red deer, 17-21% in fallow deer (Table 1.3). Antibodies to *T. gondii* have also been detected in black-tailed deer, sika deer, and reindeer (Table 1.3). These findings show that deer, being herbivores, can pick up oocysts from environment and can get exposed to *T. gondii*. Transplacental transmission was demonstrated in white-tailed deer in USA at mid-gestation in six of 61 fetuses, and early gestation in 9 of 27 fetuses in one study (Dubey *et al.* 2008b), and one of 155 fetal brain samples in another study (Dubey *et al.* 2014) with bioassay confirmation in both studies (Table 1.2). Fetal transmission was also reported in a reindeer wherein tissue cysts were histologically identified in brain, and tachyzoites in placenta and fetal myocardium were observed together with high serum antibody titre in a dam (Dubey *et al.* 2002).

In the farmed deer population, *T. gondii* has been documented in an earlier Scottish study in which 14-51% of red deer showed antibodies to *T. gondii* between years 1972-77 on same farm (Williamson *et al.* 1980). In an another serological study involving wild and farmed deer in Czech Republic, sero-prevalences from 17-50% have been reported in sika, red, and fallow deer (Bartova *et al.* 2007). Recently, *T. gondii* was reported in New Zealand in a study involving four large deer farms, where *Toxoplasma* was suspected to cause abortions (Wilson *et al.* 2012). That study reported an overall sero-prevalence of 42% by latex agglutination test (LAT) along with DNA detection in eight of nine fetal brains recovered from fetuses in pregnant hinds with signs of abortion. The abortion rates on those farms varied between 1.2 and 16% indicating that fetal wastage does occur on New Zealand deer farms and that *Toxoplasma* may be a potential cause. These findings suggest that *Toxoplasma* may be playing a role in fetal losses that may go unobserved on other farms.

1.6 Non-infectious causes of abortion

1.6.1 Nitrate poisoning

In New Zealand, deer are generally fed chaumolier, turnips, kale and rape as a supplementary crop feed. Excessive intake of these nitrogen rich crops over a short period of time may lead to nitrate poisoning. Earlier, abortions due to nitrate poisoning from supplementary crop feeding have been reported in cattle (Sund *et al.* 1957; Pfander *et al.* 1964). The main reason for nitrate accumulation may be nitrate leaching from effluent or heavy applications of nitrogen-rich fertiliser. A study in the lower North Island of New Zealand found the majority of forage samples with nitrate levels above the recommended safe levels (Hill 1998). The major plants reported in New Zealand for nitrate poisoning are tama ryegrass, turnip, chaumolier, Italian ryegrass, young maize and oats (O'Hara and Fraser 1975). The clinical signs observed in a case of abortion in New Zealand in cows grazing on Italian ryegrass and white clover were similar to those observed in nitrate poisoning in cattle including methaemoglobinemia (Vermunt and Visser 1987). Fetal hypoxia and methaemoglobinemia after transplacental transmission of nitrate are thought to be the main causes of fetal death in pregnant dams with nitrate poisoning. However, while nitrate poisoning has not been reported in deer in New Zealand, it has been experimentally shown to cause death with methaemoglobinemia in reindeer in early 80's in Sweden (Nordkvist *et al.* 1984). Also, there is no published evidence of nitrate poisoning associated with abortion or fetal deaths in deer, but this remains a potential cause.

1.6.2 Nutrition

Inadequate nutrition is also believed to have an effect on reproduction in livestock. A study in USA has shown that putting whited-tailed deer on low-level nutrition may reduce the overall fecundity or reproductive success (Verme 1965). The nutrition during the mating season may also have an effect on the breeding date and whole gestation length (Verme 1965). The winter feeding of mated red deer is important for the maintenance of pregnancy during the cold season. Also, weight gain or loss during pregnancy in winter is thought to be important in the survival of fawns after birth. This was studied in elk cows in USA in which the Authors showed that weight lost during pregnancy was significantly related to the survival of fawns (Thorne *et al.* 1976). In New Zealand, loss in body condition score was found to be negatively associated with weaning rates in adult (>3 years) hinds in a study involving 3,364 adult and 653 yearling hinds from 15 deer herds (Audigé *et al.* 2000). A

study on red deer in New Zealand showed that nutrition levels in pregnant hinds can influence fetal development and subsequent variation in gestation length, although abortions were not observed in that study. (Asher *et al.* 2005b).

1.6.3 Trace element deficiency

Selenium deficiency in red deer can cause white muscle disease. Selenium is an important trace element for calves during the neonatal period and up to the weaning (Mackintosh *et al.* 1989). The detailed effects of Se on deer reproduction in New Zealand are not known. However, a study on captive moose (*Alces alces*) demonstrated reduction in abortion in Se deficient dams following supplementation in pelleted ration (Stephenson *et al.* 2001).

Iodine is an important element for thyroid function activity in new born deer and also for overall growth. Iodine deficiency and its subsequent effects on reproduction have been studied in cattle and sheep. Enlarged thyroid glands in lambs have been reported in several studies related to neonatal mortalities in New Zealand and Australia (Setchell *et al.* 1960; Andrews and Sinclair 1962; King 1976). In cattle, stillbirth, abortion and calf weakness were associated with Iodine deficiency in a Japanese herd of 600 cows where histological improvement in thyroid glands was observed after Iodine supplementation (Seimiya *et al.* 1991). Forages such as white clover, ryegrass, kale, and other brassicas contain thiocyanates and glucosinolates (precursor of thiocyanates) that can cause sub-clinical Iodine deficiency and subsequent congenital goitre leading to heavy reproduction losses (Watkins *et al.* 1983; Alexander *et al.* 1990; Sargison *et al.* 1997, 1998). A study involving Iodine supplementation in sheep showed increase in litter size in 2 of 10 flocks (Clark *et al.* 1998).

In New Zealand deer, Iodine deficiency was associated with stillbirth in four calves with enlarged thyroid glands and oedema of neck and thyroid hypoplasia on post-mortem examination (Smits *et al.* 2000). Thyroid hyperplasia, or goitre, was reported in neonatal mortalities in 10 of 70 wapiti calves and 10 of 120 calves from unspecified deer species in New Zealand (Smits *et al.* 2001). The effect of iodine deficiency on pregnancy is not known, but the reports of stillborn fawns with goitre like findings suggests that sub-clinical goitre may contribute to reduced reproductive performance on at least some New Zealand deer farms.

1.7 Conclusion

Abortions pose significant risk to reproduction in farmed deer. This review of the international literature of potential causes of abortion was based on those agents and factors for which there has been direct or indirect evidence of infection or exposure in deer species. There are few publications describing abortion in deer and fewer describing definitively diagnosed cause/s. Nevertheless, there is evidence that deer can be infected by or exposed to a number of infectious agents and non-infectious factors known to cause abortion in other ruminant species. Given this evidence, infectious abortifacients for other ruminants should be regarded as potential abortifacients in deer and therefore included in abortion investigation in deer species. *Toxoplasma gondii* was the only infectious agent implicated in a clinical abortion investigation in New Zealand farmed deer and more studies are needed to investigate the association, given the endemic nature of this agent. Non-infectious causes such as nitrate poisoning, inadequate nutrition, and trace element deficiency can also lead to impaired fetal development and abortion, so should similarly be included in clinical investigation of abortion.

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Chapter 2. Mating management, pregnancy and mid-term abortion rates in farmed red deer in New Zealand

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This chapter is prepared in the style format of the New Zealand Veterinary Journal.

2.1 Abstract

Reproductive performance in rising two-year-old (R2) and mixed-aged (MA) adult hinds is sub-optimal in farmed red deer in New Zealand due failure to conceive, fetal loss, stillbirth, and postnatal mortality. Abortions ranging from 1.6 to 16% were detected in a pilot study on four R2 herd and one MA herd. Thus, this study is designed to quantify fetal wastage in New Zealand farmed deer. Rising two-year-old (R2) and adult (MA) hinds from 85 farms recruited over two years were ultrasound scanned early (Scan-1) and at the end of the second trimester (Scan-2) to detect early pregnancy and mid-gestation abortion rates. An additional scan was undertaken in four herds earlier than the usual Scan-1 date as a pilot to determine how early abortions were occurring. Fifteen R2 and seven MA herds were scanned both years. Stags were joined with hinds between December to March in R2 herds and between January to March in MA herds. Stags were separated after remaining for an average period of 93 (April to July) and 72 (April to June) days with R2 and MA hinds, respectively. Overall, 85.8% of 22,130 R2 and 93.3% of 36,223 hinds were detected pregnant in 87 R2 and 71 MA herds, respectively, at Scan-1. Mid-term abortions occurred in 305 R2 hinds from 60 (73%) R2 herds and 92 hinds from 36 (61%) MA herds. The herd-level daily abortion rate, adjusted for the between-scan interval across herds, varied from 0.005% to 0.21% in R2 and 0.07% to 0.1% in MA herds. At Scan-1 46 R2 and 12 MA aborting hinds were detected from 22 herds which, combined with detection 1.2 - 7.1% fetal loss in the three early-scanned herds supports that early abortions contribute to sub-optimum pregnancy rates on many farms. Abortion rates were unpredictable and occurrence was inconsistent between years in both R2 and MA herds sampled across two years. The abortion rates reported in this study constitutes a significant cost to the New Zealand deer industry and warrants further investigation into causation.

KEY WORDS: *Red deer, mating management, abortion, fetal wastage, pregnancy, ultrasound scanning.*

2.2 Introduction

Reproductive efficiency of farmed red, wapiti and red x wapiti crossbred deer in New Zealand is limited by hinds generally being single ovulators and hence bearing singleton offspring. Therefore, the production efficiency gains reaped by the sheep industry in this country through fecundity genetics are unavailable to deer farmers. Benchmarking studies conducted in the lower North Island (Audigé *et al.* 1999a), Canterbury (Campbell *et al.* 2000), Hawke's Bay (Walker *et al.* 2000), Southland (Lawrence 2003) and Invermay (AgResearch) (Fennessy *et al.* 1986) show that there is biological potential for significantly higher reproductive efficiency than currently achieved on most deer farms. Moreover, poor reproductive efficiency remains one of the most common concerns expressed by deer farmers (Asher and Pearse 2002; Asher 2003; Asher and Wilson 2011).

Sub-optimum weaning rates (reproductive wastage) constitute a significant cost to the New Zealand deer industry (Asher 2003; Asher and Wilson 2011). The highest reproductive inefficiency occurs in primiparous hinds, an age that has the greatest negative financial impact through reducing the mean reproductive longevity of hinds (Asher 2003; Asher and Wilson 2011). Failure to conceive and loss of calves from birth to weaning have been conventionally regarded as the main areas resulting in sub-optimum reproductive efficiency in all age groups, and much research work on reproductive wastage in deer has investigated management factors aimed at optimising performance in those areas (Audigé *et al.* 1999b; Audigé *et al.* 1999c).

By contrast, embryo and fetal loss have historically been regarded as insignificant for reproductive wastage. This is likely because they are difficult to detect grossly and because hinds are rarely, if ever, checked for fetal loss by repeated ultrasound scanning or other techniques. However, there have been some attempts at characterising the extent of fetal loss between scanning for pregnancy early in gestation and calving in New Zealand farmed deer. For example, Fennessy *et al.* (1986) reported 2.6% abortion in yearlings on a research deer farm, whereas Campbell *et al.* (2000) reported a lower abortion rate of 1% in 15 mixed-age deer herds in Canterbury. An additional study by Audigé *et al.* (1999a) showed fetal loss averaging 0.6% in yearling and 0.8% in adult hinds in eight intensively monitored herds in the lower North Island. However, data are limited and based on crude measures such as abdominal and udder palpation late in gestation or post-calving, rather than repeated ultrasound pregnancy scans. One overseas report described a fetal mortality of 28% (2/7) in

yearling hinds and 10% (4/39) mixed-age hinds in an experimental farm study in Spain (Gomez-Nieto *et al.* 2011).

A recent clinical investigation that involved repeat pregnancy scanning on four large deer farms identified that 1.7%, 7%, 10%, and 16% of hinds in four rising-two-year-old (R2) herds and 4% of hinds in one mixed-age (MA, ≥3-year-old) herd had aborted in mid-pregnancy (Wilson *et al.* 2012). This pattern of fetal loss is observed in cattle and sheep and can be due to various agents, such as *Toxoplasma gondii* in sheep (Hartley *et al.* 1954; Hartley and Marshall 1957) and *Neospora caninum* in cattle (Thornton *et al.* 1991; Thornton *et al.* 1994). The abortions reported by Wilson *et al.* (2012) suggested that fetal wastage may be occurring on New Zealand deer farms at higher rates than previously believed. However, despite these studies, New Zealand data remains limited.

Thus, the aim of this study was to establish national prevalence and incidence rates of abortions in New Zealand farmed red deer. This descriptive data presented here are part of a larger study to quantify abortions and subsequently investigate potential causation and prevention. Data include animal and herd-level pregnancy rates, mid-term abortion rates, repeatability of abortions within farm between years, observations on the timing of abortions, from primiparous and older deer from 85 deer farms over two years. Data collected also allowed a descriptive analysis of mating management practices. Data on investigation of causation of abortion are presented in later chapters.

2.3 Materials and methods

2.3.1 Study design

This longitudinal observational study was undertaken over two consecutive reproductive cycles, 2012-13 and 2013-14, on commercial deer farms throughout New Zealand. According to a power analysis, with an absolute precision of 10%, 96 herds from each age group were required to determine abortion prevalence assuming that 50% of herds had at least one case of abortion with 95% confidence (Cannon et al. 1982). Commercial deer farmers throughout New Zealand were invited to take part in the study through deer and mixed practice veterinarians, commercial pregnancy test scanners, or by direct contact by the researchers. Based on initial responses, farms were selected based on suitable handling

facilities, willingness to participate, and fulfilment of the requirements of the project for additional scanning and blood, specimen and data collection.

2.3.2 Animals and pregnancy determination

This study involved red deer (*Cervus elaphus scoticus, hippelaphus)* genotypes from both R2 and MA groups although it is acknowledged that some deer may have possessed some introgressed wapiti (*Cervus elaphus nelson, roosevelti, manitobensis*) genes. Deer which phenotypically resembled cross-breds (e.g. F1 hybrids) or wapiti were excluded from the study. All manipulations with deer were approved by the Massey University Animal Ethics Committee (Protocol number: 12/34).

For determination of herd prevalence and within-herd incidence of abortion, there were two ultrasound pregnancy scanning episodes for each age-class on each farm in both year-1 and year-2 of the study. Scanning was performed by experienced operators, either veterinarians or private lay-operators with a small number of herds scanned by one of the co-supervisors. At the first scanning time point (Scan-1), which was timed 6-7 weeks after the stag removal according to the farmer's usual practice, all hinds in each herd were scanned using a rectal ultrasound linear probe as described by Revol and Wilson (1991). At the second pregnancy scanning (Scan-2), timed at the end of the second trimester or later, as determined by the farmer, a sub-sample of 100 R2 and 155 MA hinds from herds greater than those numbers were required to detect abortions of 2% and 1%, respectively, with 95% confidence interval (Cannon *et al.* 1982), or all animals in herds with fewer that those numbers were scanned mostly using the flank method. For selection, by way of example, for a herd with 500 MA hinds, the farmer was asked to initially break it down to groups of about 50, i.e. 10 batches, then to select 15-16 hinds from each batch for scanning, to make a total of 155. Occasionally owners chose to scan all hinds at Scan-2, and in those cases, all data were included for calculations.

Hinds were classified as being 'pregnant' based on the presence at least one of fetus or part thereof, amniotic membrane, and/or presence of placentomes, or as being 'not pregnant' based on absence of those signs combined with visualisation of a non-pregnant uterus. Evidence of an aborting fetus was recorded when observed. A fetus was considered aborting based on absence of heart beats, signs of tissue disintegration and mummification, and/or signs of resorption in uterus. The term "aborting" is ascribed to hinds that had ultrasound evidence of aborting fetuses at Scan-1 and Scan-2. The term "aborted", used for

calculating the daily abortion rate (DAR), is ascribed to hinds that were pregnant at Scan-1 but not pregnant at Scan-2, plus those aborting at Scan-2.

The daily abortion rate (DAR) ((number aborted/ number scanned)/between-scan interval (days)) for each herd was calculated to adjust for the variation in between-scan interval between herds. Any discrepancies in pregnancy scanning data sent by farmer in Scan-1 and vet/scanner were resolved by contacting them separately to retrieve/verify actual data.

2.3.3 Abortion repeatability

Based on year-1 DAR data, both R2 and MA herds were categorised into no, low (R2: >0-0.03%, MA: >0-0.02%), medium (R2: 0.031-0.06%, MA: 0.021-0.035%), and high (R2: >0.06%, MA: >0.035%) abortion rate groups as in Table 2.6. Randomly selected farms from each category were contacted for participation in year-2. Fifteen R2 and seven MA herds were selected and scanned twice, as above, during year-2 of the study. Data from these herds simultaneously contributed to abortion prevalence and incidence for year-2.

2.3.4 Early fetal loss

Due to the observation of aborting fetuses on some farms at the time of first scanning in year-1, a small sub-study involving three R2 herds and one MA herd was undertaken in year-2. In these selected herds, a sub-sample of up to 125, or all animals in herds of fewer, were rectal-ultrasound scanned for early pregnancy seven to 21 days after removal of the stag, on average two weeks earlier than industry practice. Hinds were considered pregnant based on criteria described above or presence of an intrauterine vesicle putatively associated with early pregnancy, herein referred to as "indicative" of pregnancy (Revol and Wilson 1991), in the absence of signs of definitive pregnancy. Hinds were categorized as 'no visible pregnancy' when the uterus was visualised in the absence of definitive or indicative signs. A second pregnancy scanning of all mated hinds per herd including the previously scanned hinds and was carried out at the farmer's chosen normal time at 28, 30, 40 days after stag removal in R2 herds and 44 days for the MA herd.

2.3.5 Farm data collection

Questionnaires (Appendix 6 and 7) aimed at collecting farm management data relevant to investigation of abortion were sent to participating deer farmers on two occasions in each year. The first questionnaire, sent after Scan-1, covered the period from joining to

Scan-1 and focused on collecting farm topography, previous reproductive performance, mating and management to first pregnancy scanning, and farm environment data. The second questionnaire, sent after Scan-2, covered topics relating to the management period from Scan-1 and collected data on grazing management, diseases, abortion, and movement of hinds in and out of the farm. Not all farmers completed each questionnaire.

2.3.6 Statistical analysis

The statistical analysis was performed in SAS software, v9.4 (SAS Institute Inc., Cary NC, USA). Differences between mean stag introduction and removal dates, mating interval, and interval from stag introduction to Scan-1 across different age groups and sampling years were analysed using analysis of variance (ANOVA). At individual animal level, the dependent variables (pregnancy at Scan-1 and abortion at Scan-2) were binary responses and resembled a binomial distribution. At herd level, the dependent variable "pregnancy proportion" at Scan-1 was available as "number pregnant per number scanned" whereas the dependent variable of "DAR" at Scan-2 was available as "number aborted between scans per number scanned". Generalised estimating equations (GEE) (Zeger and Liang 1986) with an exchangeable correlation structure, binomial distribution and logit link was used to model pregnancy rate at Scan-1 and DAR at Scan-2 to account for correlation between herds belonging to same farms and/or repeat sampled in year-2. The GEE model was executed using events/trials syntax of Proc GENMOD command in SAS using farm as repeated measurement. The natural log of between scan interval was chosen as offset to model DAR at Scan-2. Herds were categorised as small (R2 <200 and MA <500 hinds) or large (R2 ≥200 and MA ≥500 hinds) based on the presence of number of hinds at Scan-1. Effect of age, herd-size category (small or large), sampling year (year-1 or year-2), sampling island (North or South), and early scanning on pregnancy rate and DAR were examined. The Pearson correlation coefficient for correlation of incidence of pregnancy rates and DAR with herd size was assessed using Proc CORR command in SAS. The difference in paired herd-level DARs for abortion repeatability was assessed with Student's t-test using Proc TTEST command in SAS.

2.4 Results

2.4.1 Farm recruitment

A total of 85 farms were recruited over two years, with each farm comprising rising two-year-old (R2) and/or mixed-age adult (MA) herds. At the completion of the first year, 56 farms (49 R2 and 40 MA herds) were recruited with an additional 29 farms (23 R2 and 24 MA herds) recruited in the second year. Fifteen of the farms (15 R2 and seven MA herds) from the first year of the study were followed during the second year to check for the repeatability of data collected. Of the 85 farms enrolled in the study, 28 farms were located in the North Island regions (Auckland (n=2), Bay of Plenty (n=1), Gisborne (n=1), Hawke's Bay (n=5), Manawatu-Whanganui (n=13), Waikato (n=5), and Wellington (n=1)), and 57 were located in the South Island (Canterbury (n=30), Otago (n=8), Southland (n=17), and Westcoast (n=2)). Within farms, the average herd mob size was 254 for R2 herds (Range: 15 - 1648, SE=33) and 510 for MA herds (range: 60 - 2998, SE=60) (Figure 2-1). Details of each farm are presented in Appendix 1.

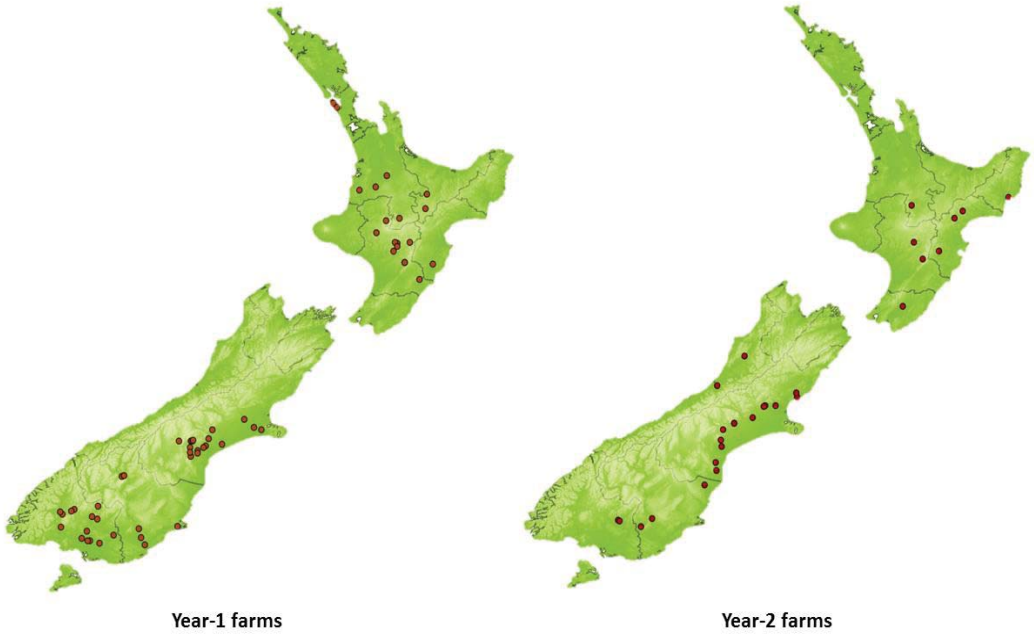

Figure 2-1: Geographical distribution of participating deer farms in year-1 and year-2.

In total, 65/85 farms (76.5%) returned completed questionnaires that included data on 57 R2 and 44 MA herds (Table 2.1). Verified scanning data for 87 R2 and 71 MA herds was collected at Scan-1 and for 82 R2 and 59 MA herds at Scan-2 (Table 2.1). Unfortunately, one R2 and MA herd had discrepancy in scanning date for Scan-1, therefore Scan-2 data could

only be used to estimate herd-level abortion prevalence but could not be used to calculate DAR.

Stag introduction (joining) data and interval between stag removal and Scan-1 are presented for 57 R2 and 44 MA herds in Table 2.2. The median date for the introduction of the stag was in early February for R2 herds (range: December - March) and in early March for MA herds (range: January – March). On average, stags remained in R2 herds for 93 days (95% CI: 86-101 days) and 72 days (95% CI: 67-78 days) in MA herds. The median date that stags were removed from hinds occurred in early May for both R2 and MA herds. The mean joining period of stags in R2 and in MA herds did not differ significantly between year-1 and year-2 (R2, p=0.98; MA, p=0.90) (Table 2.2).

The period from stag joining to Scan-1 was available in 56/57 R2 and 43/44 MA herds as scanning data for one R2 and one MA herd could not be verified. The mean time interval from stag removal to Scan-1 was 46 days (95% CI: 40-51 days) in R2 herds and 49 days (95% CI: 45-53) in MA herds and did not differ between years in either age group (R2, p=0.24; MA, p=0.09) (Table 2.2).

Table 2.1: Summary of total number of herds and animals available for mating management, Scan-1, and Scan-2 data.

Data available from	No. of herds (total no. of animals)					
	R2 year-1	R2 year-2	Total R2 herds	MA year-1	MA year-2	Total MA herds
Questionnaire	37 (10,438)	20 (4,571)	57 (15,009)	24 (13,522)	20 (11,581)	44 (25,103)
Scan-1	49[a] (14,238)	38 (7,892)	87[a] (22,130)	40[a] (19,386)	31 (16,837)	71[a] (36,223)
Scan-2	47 (8,110)	35 (2,895)	82 (11,005)	32 (4,117)	27 (3,257)	59 (7,374)

[a] Scan-1 date could not be confirmed on one R2 and MA herd.

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.

Event		R2 herds			MA herds		
		Year-1 (n=37)	Year-2 (n=20)	Total (n=57)	Year-1 (n=24)	Year-2 (n=20)	Total (n=44)
Stag joining date	Earliest	1 Dec	1 Dec	1 Dec	20 Jan	1 Jan	1 Jan
	Median	11 Feb	13 Feb	11 Feb	1 Mar	2 Mar	2 Mar
	Latest	22 Mar	15 Mar	22 Mar	10 Mar	15 Mar	15 Mar
Stag removal date	Earliest	20 Apr	26 Apr	20 Apr	20 Apr	22 Apr	20 Apr
	Median	7 May	10 May	9 May	5 May	8 May	6 May
	Latest	23 Jul	28 May	23 Jul	25 May	1 Jun	1 Jun
Joining interval (days)	Mean (95% CI)	93 (84-103)	93 (79-108)	93 (86-101)	72 (65-79)	73 (63-83)	72 (67-78)
	Median (range)	90 (50-176)	83 (54-178)	90 (50-178)	68 (49-101)	67 (51-139)	67 (49-139)
Stag removal to first scan (days)[a]	Mean (95% CI)	48 (41-55)	42 (33-50)	46 (40-51)	52 (47-58)	45 (39-52)	49 (45-53)
	Median (range)	45 (0-103)	41 (2-71)	43 (0-103)	52 (29-83)	43 (10-75)	44 (10-83)

[a] first scan date was not available from one R2 and MA herd

2.4.2 Scan-1 pregnancy results

Data are presented in Tables 2.4 and 2.5 and Figure 2-2.

2.4.2.1 Rising 2-year-old herds

A total of 22,130 R2 hinds from 87 herds underwent Scan-1 between late May to mid-August in year-1 and mid-May to late July in year-2, with the median scanning date near the end of June for both years (Table 2.3). The overall mean Scan-1 date for R2 herds was 26 June. Overall, 85.8% R2 hinds were scanned pregnant at Scan-1 (Table 2.4) and the overall mean herd-level pregnancy rate was 82.0% (median 87.4% (min 7.0%, max 100%), 95% CI=78.6-85.4%) (Table 2.5). Of note, two R2 herds achieved 100% pregnancy rate at Scan-1 and 39.1% (34/87) of R2 herds had Scan-1 pregnancy rates ≥ 90% (Figure 2-2 (1A and 1B)). The herd-level mean pregnancy rate was not different between years (p=0.13) or farm location (North vs. South, p=0.97). The correlation between herd size and herd-level pregnancy rates was positive but marginally not significant (Pearson correlation coefficient=0.21, p=0.06). The herd-level mean pregnancy rate was higher in hinds from large (n=41, ≥200 hinds) herds compared with small (n=46, <200 hinds) herds (87.3%, 78.6% respectively; p=0.006). The joining period (p=0.96) and stag removal to Scan-1 interval (p=0.45) had no significant effect on the herd-level pregnancy rates in R2 herds.

Table 2.3: Scan-1 and Scan-2 dates for each age group each year.

Scan	Age group, year	No. of herds	Mean	Earliest	Median	Latest
Scan-1	R2, year-1	48[a]	27 June	28 May	27 June	15 August
	R2, year-2	38	23 June	19 May	25 June	30 July
	MA, year-1	39[a]	28 June	6 June	27 June	31 July
	MA, year-2	31	24 June	20 May	23 June	23 July
Scan-2	R2, year-1	47	24 September	9 August	27 September	29 October
	R2, year-2	35	21 September	7 August	23 September	4 November
	MA, year-1	32	27 September	21 August	29 September	5 November
	MA, year-2	27	18 September	7 August	20 September	11 October

[a] Scan-1 date could not be confirmed on one R2 and MA herd

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.

Age group, year	No. (%) pregnant at Scan-1	No. (%) aborting at Scan-1	No. at Scan-2	No. aborted by Scan-2	No (%). aborting at Scan-2	Overall between-scan abortion prevalence % (95% CI)
R2, year-1	12,418 (87.2)	24 (0.17)	8,110	223	1 (0.01)	2.76 (2.41-3.12)
R2, year-2	6,562 (83.1)	22 (0.28)	2,895	79	2 (0.07)	2.80 (2.20-3.40)
Total	18,980 (85.8)	46 (0.21)	11,005	302	3 (0.03)	2.77 (2.46-3.08)
MA, year-1	18,093 (93.3)	5 (0.03)	4,117	50	1 (0.02)	1.24 (0.90-1.58)
MA, year-2	15,703 (93.3)	7 (0.04)	3,257	40	1 (0.03)	1.26 (0.88-1.64)
Total	33,796 (93.3)	12 (0.03)	7,374	90	2 (0.03)	1.25 (0.99-1.5)
Overall total	52,776 (90.4)	58 (0.1)	18,379	392	5 (0.03)	2.16 (1.95-2.37)

[a] calculations exclude one herd with missing Scan-1 date, [b] calculations exclude one R2 and MA herd with missing Scan-1 date.

Table 2.5: Summary of herd-level pregnancy rates at Scan-1 and daily abortion incidence rates at Scan-2.

Age group, year	Herd-level pregnancy rate			Herd-level daily abortion rate			
	No of herds at Scan-1	Mean (95% CI) pregnancy rate (%)	Range (%)	No. of herds at Scan-2	No of herds with aborted hinds (%)	Mean (95% CI) daily abortion rate (%)	DAR Range (%)
R2, year-1	49	84.3 (80.6 - 87.9)	47.0 -100	47	34/47 (72.3)	0.041 (0.028 - 0.055)[a]	0.005 - 0.213
R2, year-2	38	79.0 (72.7 - 85.3)	7.0 - 100	35	26/35 (74.3)	0.046 (0.031 - 0.060)	0.006 - 0.140
R2 Total	87	82.0 (78.6 - 85.4)	7.0 - 100	82	60/82 (73.1)	0.043 (0.034 - 0.053)[a]	0.005 - 0.213
MA, year-1	40	93.0 (90.0 - 96.1)	39.8 - 100	32	22/32 (68.8)	0.020 (0.014 - 0.026)[a]	0.007 - 0.048
MA, year-2	31	92.1 (89.4 - 94.7)	67.2 - 100	27	14/27 (51.9)	0.032 (0.018 - 0.047)	0.007 - 0.101
MA, Total	71	92.6 (90.6 - 94.6)	39.8 - 100	59	36/59 (61.0)	0.025 (0.018 - 0.032)[a]	0.007 - 0.101
Overall total	158	86.8 (84.5 - 89)	(7.0 - 100)	141	96/141 (68.1)	0.036 (0.030 - 0.043)[b]	0.005 - 0.213

[a] calculations exclude one herd with missing Scan-1 date,

[b] calculations exclude one R2 and MA herd with missing Scan-1 date.

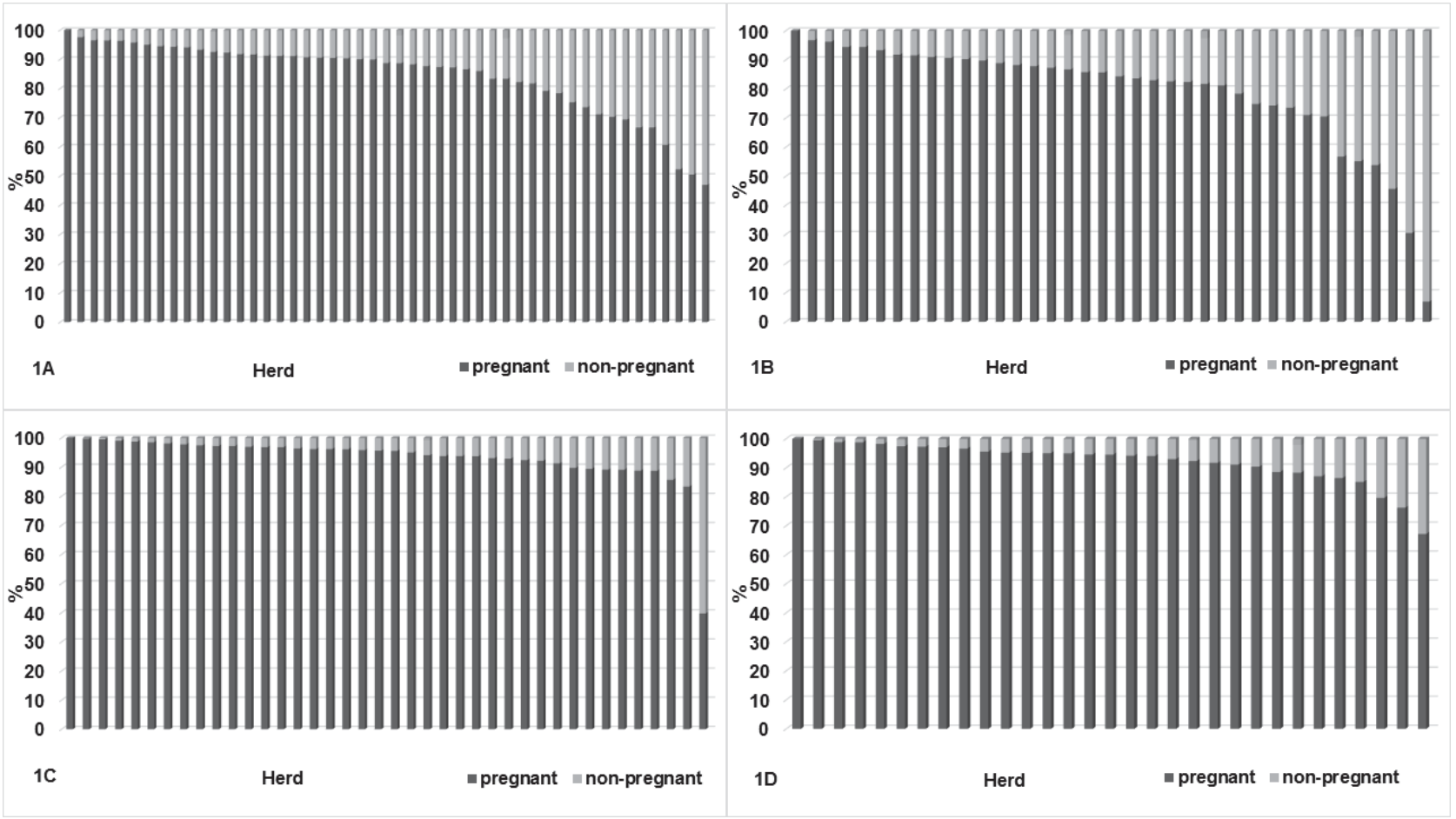

Figure 2-2: Individual R2 herd Scan-1 pregnancy rates in year-1 (1A) and year-2 (1B). Individual MA herd Scan-1 pregnancy rates in year-1 (1C) and year-2 (1D) (each bar represents one herd).

2.4.2.2 Mixed-age herds

A total of 36,223 MA hinds from 71 herds underwent Scan-1 between early June and late July in year-1 and between mid-to-late May and late July in year-2 with median scanning date during late June for both years (Table 2.3). The overall mean Scan-1 date for MA herds was 25 June. Overall at Scan-1 during both years, 93.3% of MA hinds were scanned pregnant (Table 2.4) with a mean herd-level pregnancy rate of 92.6% (95% CI: 90.6-94.6%). Moreover, two MA herds achieved 100% pregnancy rate at Scan-1 and 76.1% (54/71) of MA herds had Scan-1 pregnancy rates ≥90% (Figure 2-2 (1C and 1D)). The herd-level mean pregnancy rate was not significantly different between years (p=0.99) nor was a significant correlation between herd size and individual herd pregnancy rates observed (Pearson correlation coefficient=0.08, p=0.53). The herd-level mean pregnancy rate was significantly higher (p=0.002) in herds from South Island (mean pregnancy rate=95.5%) compared to herds from North Island (mean pregnancy rate=89.5%). The herd-level mean pregnancy rate was higher in hinds from large (n=23, ≥500 hinds) herds than small (n=48, <500 hinds) herds (95.4%, 89.9% respectively; p=0.006). The joining period and stag removal to Scan-1 interval had no effect on the herd-level pregnancy rates in MA hinds (p=0.77, p=0.19 respectively).

The mean pregnancy rate in MA herds was significantly higher than R2 herds (p<0.001). However, with all herds considered regardless of age group, the mean pregnancy rate did not differ between islands (North vs. South, p=0.15) or years (year-1 vs. year-2, p=0.67).

2.4.3 Abortion at Scan-1

A notable observation at Scan-1 was a total of 46 (0.21%) R2 and 12 (0.03%) MA hinds were found with aborting fetuses (Table 2.4). At herd level, aborting fetuses were observed in 13 (15%) of R2 and nine (13%) of MA herds.

2.4.4 Scan-2 data and abortion rates

Eighty-two of 87 R2 and 59/71 MA herds were included in the Scan-2 data over the two years of the study (Table 2.1). However, DAR could not be calculated for one R2 and one MA herd because of a discrepancy in Scan-1 date which could not be resolved, although these two herds were included in the analysis for proportion of herds without aborted hinds between age groups, years, and islands (Table 2.5). Hence, there were 46 and 35 R2 and 31

and 27 MA herds in years one and two, respectively, for which between-scan daily abortion data was able to be calculated (Table 2.1).

2.4.4.1 Rising 2-year-old herds

Scan-2 took place between early August and early November for the R2 herds with a median date at the end of September (Table 2.3). The mean interval between Scan-1 and Scan-2 for R2 hinds was 89 days in year-1 (range= 40-119, 95% CI= 83-94) and 91 days in year-2 (range= 51-131, 95% CI= 84-98) whereas it was 90 days (range=40-131, 95% CI= 85-94) for both years combined. The overall mean and median Scan-2 dates were 23 September both years. Ten R2 herds in year-1 and three in year-2 with over 100 pregnant hinds at Scan-1 scanned between 82 and 100% of hinds at Scan-2. Therefore, herd sample size at Scan-2 ranged from 14 to 1,525, averaging 173 in year-1 and 83 in year-2. Overall at Scan-2 during both years, 2.8% of R2 hinds were determined to have aborted by Scan-2 or aborting at Scan-2 with 73% of 82 R2 herds containing aborted hinds (Table 2.4 and Table 2.5). There was no difference in proportion of herds with no aborted hinds between years, 1 (28%) and 2 (26%) (p=0.98), or islands, North (29%) and South (24%) (p=0.61). The overall abortion prevalence in all herds was 2.77% (95%CI: 2.46 - 3.08%) with the mean herd-level DAR incidence in herds with aborted hinds at 0.043%/day (95% CI: 0.034 - 0.053%) (Table 2.4 and Table 2.5). The individual herd DAR incidences in herds with aborted hinds ranged from 0.005% to 0.21% (mean=0.041%, 95% CI: 0.028 - 0.055%) in 34 year-1 herds (Figure 2-3 (2A)) and 0.006% to 0.14% (mean=0.046%, 95% CI: 0.031 - 0.06%) in 26 year-2 herds (Figure 2-3 (2B)). In these herds with aborted hinds, there was no significant difference in herd-level mean DAR when the Scan-2 date was earlier or later than the mean Scan-2 date of 23 September (p=0.39).

Additionally, the herd-level mean incidence of DAR in R2 herds with aborted hinds was similar between years (p=0.50) and the herd-level mean incidence of DAR in herds with aborted hinds was higher (p=0.023) in small herds (n=28; mean=0.055%, 95% CI: 0.038 - 0.071) compared to large herds (n=32; mean=0.033%, 95% CI: 0.023 - 0.044). The herd-level mean incidence of DAR was not different between islands (p=0.98). At Scan-2, aborting fetuses were seen in three (0.03%) R2 hinds, one each from three of 82 R2 herds (Table 2.4).

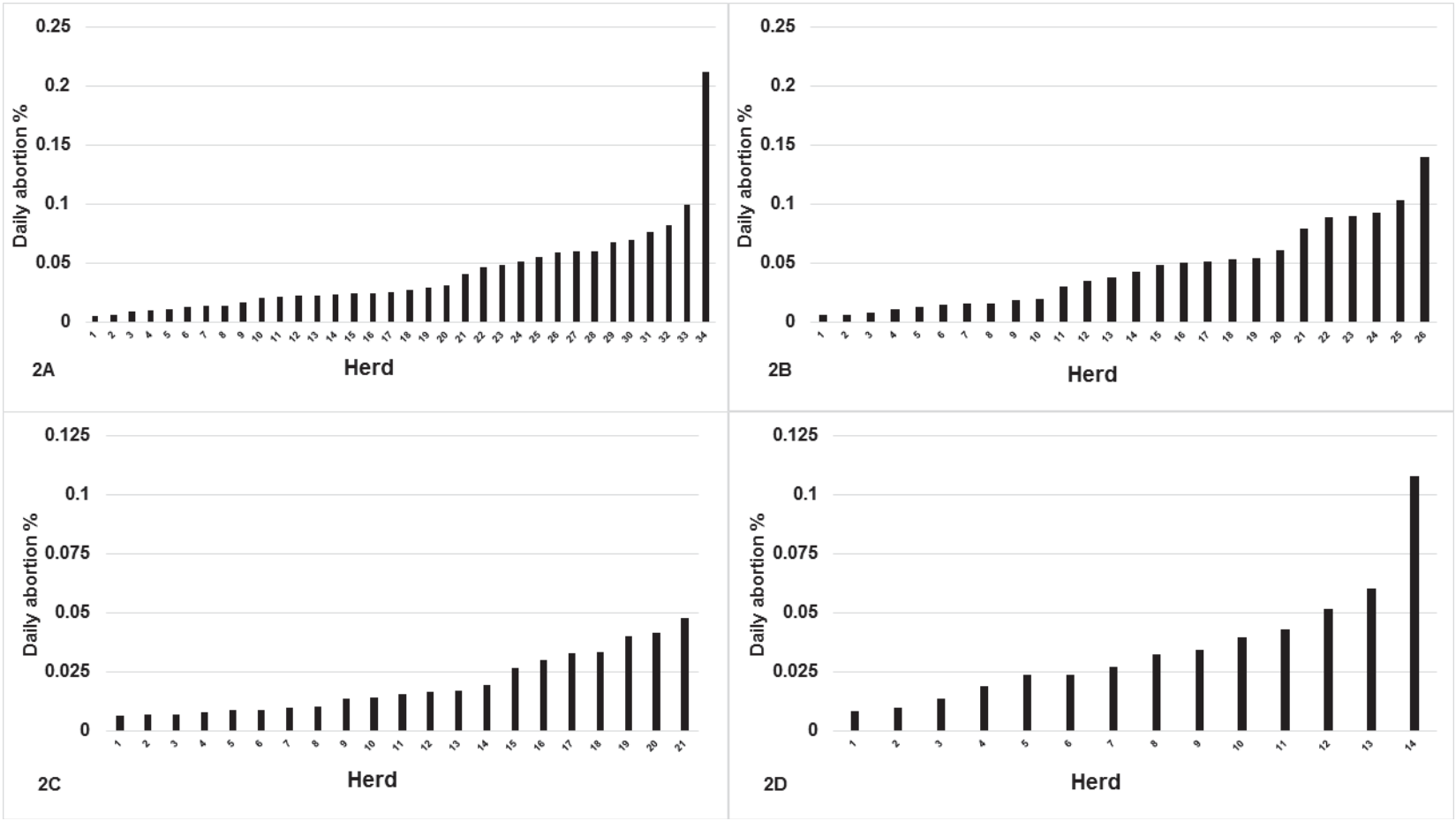

Figure 2-3: Individual herd between-scan daily abortion rates for R2 herds in year-1 (2A) and year-2 (2B). Individual herd between-scan daily abortion rates for MA herds in year-1 (2C) and year-2 (2D) (each bar represents one herd).

2.4.4.2 Mixed-age herds

For MA hinds, Scan-2 took place between early August and late October with a median date within late September for both years (Table 2.3). The mean interval between Scan-1 and Scan-2 for MA hinds was 90 days (range= 40-152, 95% CI= 82-98) and 83 days (range= 51-116, 95% CI= 77-90) whereas it was 87 days (range= 40-152, 95% CI= 82-92) for both years combined. Hinds at Scan-2 numbered from 48 to 228 between herds with a mean of 129 hinds in year-1 and 121 hinds in year-2. Three herds in year-1 and two in year-2 scanned more than the required number of 155 (n = 167 (95.4% of herd), 199 (96.6% of herd), 219 (51.4% of herd), 156 (54.7% of herd), and 228 (100% of herd)). Overall at Scan-2 during both years, 1.2% of 7,374 MA hinds were determined to have aborted by Scan -2 or aborting at Scan-2 with 61% of 59 MA herds containing at least one aborted hind (Table 2.4 and Table 2.5). There was no significant difference in proportion of herds with no aborted hinds in years, 1 (31%) and 2 (48%) (p=0.26), or islands, North (25%) and South (47%) (p=0.08). The overall abortion prevalence in all herds was 1.25% (95% CI: 0.99-1.5%)with the mean herd-level DAR incidence in herds with aborted hinds at 0.025%/day (95% CI= 0.018-0.032%). (Table 2.4 and Table 2.5). The individual herd-level DAR in herds with aborted hinds ranged from 0.007% to 0.048% (mean=0.020%, 95% CI: 0.014-0.026) in 21 year-1 herds (Figure 2-3 (2C)) and from 0.007% to 0.101% (mean=0.032%, 95% CI: 0.018-0.047) in 14 year-2 herds (Figure 2-3 (2D)). In herds with aborted hinds, no significant difference in herd-level mean DAR incidence was observed in herds with a Scan-2 date earlier or later than the mean Scan-2 date of 23 September (p=0.42).

Additionally, the herd-level mean incidence of DAR in herds with aborted hinds was significantly higher in year-2 compared with year-1 (p=0.048). The herd-level mean incidence of DAR in herds with aborted hinds was not different (p=0.84) in small herds (n=23, mean=0.026%, 95% CI: 0.016-0.035) compared to large herds (n=12, mean=0.023%, 95% CI: 0.014-0.033). The herd-level mean incidence of DAR was not different between islands (p=0.29). At Scan-2, aborting fetuses were seen in two (0.03%) MA hinds, one each from two of 59 MA herds (Table 2.4).

2.4.4.3 Age and location

The proportion of herds with aborted hinds (or the herd-level prevalence) was not different between age groups (R2 vs. MA, p=0.08), years (year-1 vs. year-2, p=0.29) or islands (North vs. South, p=0.36). In herds with aborted hinds, herd-level mean DAR

observed in R2 herds (mean 0.043, 95% CI: 0.034-0.053) was significantly higher than in MA herds (mean 0.025, 95% CI: 0.018-0.032) (p=0.009). When all herds were considered together regardless of age group, the herd-level mean DAR in herds with aborted hinds was not different between years (year-1 vs. year-2, p=0.3) or islands (North vs. South, p=0.66). At Scan-2, aborting fetuses were observed in three (0.03%) R2 hinds, one each from three of 82 R2 herds and two (0.03%) MA hinds, one each from two of 59 MA herds.

Table 2.6: Number (and %) of herds with no, low, medium, and high daily abortion rates.

Age group, year	Herds	Daily abortion rate category			
		Nil	Low (0.01-0.03%)	Medium (0.031-0.06%)	High (≥0.061)
R2, Year-1	46	12 (26.1)	19 (41.3)	8 (17.4)	7 (15.2)
R2, Year-2	35	9 (25.7)	11 (31.3)	8 (23)	7 (20)
		Nil	Low (0.01-0.02%)	Medium (0.021-0.035%)	High (≥0.036)
MA, Year-1	31	10 (32)	14 (45)	4 (13)	3 (10)
MA, Year-2	27	13 (48)	5 (18.5)	4 (15)	5 (18.5)

2.4.5 Abortion repeatability

The DARs for 15 individual R2 herds recorded in both years are presented in Table 2.7 and Figure 2-4. The majority (10/15) of herds experienced fewer abortions in year-2. Abortions in year-2 occurred in two of the three nil DAR herds (herds #2 and #3) which did not have abortions in year-1. In the low DAR group, five herds had lower abortion rates in year-2 with a difference in DAR percentage points ranging between 0.011-0.022% from year-1 (Table 2.7). Additionally, two herds (#4 and #7) had an increase in DAR between the two years. In the medium and high abortion category, abortions were lower in year-2 in all herds compared to year-1. One herd (#13) from the high DAR category experienced no abortions in year-2. Overall, the herd-level mean incidence of DAR observed in year-2 in these 15 herds was not statistically different from year-1 (p=0.15) as analysed by Student's paired t-test.

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.

Herd no.	Abortion category in year-1	% daily abortion		Difference (percentage points)
		Year-1	Year-2	
1	nil	0	0	0
2	nil	0	0.03	+0.03
3	nil	0	0.061	+0.061
4	low	0.01	0.016	+0.006
5	low	0.014	0	-0.014
6	low	0.017	0	-0.017
7	low	0.021	0.038	+0.017
8	low	0.023	0.011	-0.012
9	low	0.024	0	-0.024
10	low	0.028	0.006	-0.022
11	medium	0.051	0.019	-0.032
12	medium	0.055	0.016	-0.039
13	high	0.060	0	-0.06
14	high	0.076	0.049	-0.027
15	high	0.082	0.02	-0.060

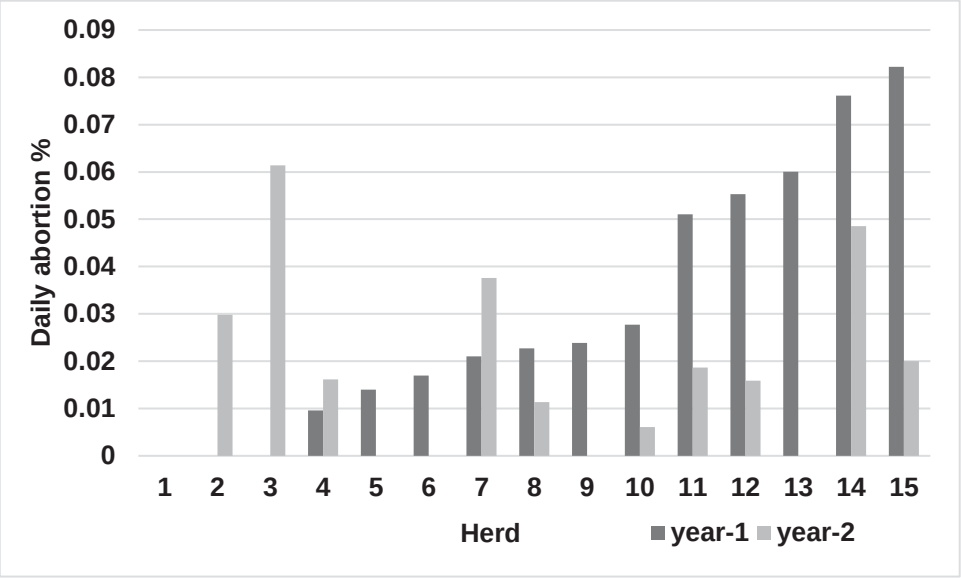

Figure 2-4: Individual herd between-scan daily abortion rates for R2 herds on farms scanned both in year-1 and year-2.

The DAR in seven MA herds recorded both years are presented in Table 2.8 and Figure 2-5. The only MA herd (#1) with nil abortions in year-1 had no abortion in year-2. From the low DAR category, two herds (#3 and #4) had no abortions and two herds (#2 and #5) had higher DAR in year-2. The only herd from the year-1 medium abortion group (herd #6) had no abortions in year-2. DAR was higher in year-2 in the only year-1 high abortion

category herd (herd #7). Overall, the year-2 herd-level mean incidence of DAR was not different from year-1 (p=0.75) as analysed by Student's paired t-test.

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.

Herd no.	Abortion group in year-1	% daily abortion		Difference (percentage points)
		Year-1	Year-2	
1	nil	0	0	0
2	low	0.007	0.01	+0.004
3	low	0.007	0	-0.007
4	low	0.010	0	-0.010
5	low	0.019	0.036	+0.017
6	medium	0.034	0	-0.034
7	high	0.048	0.101	+0.053

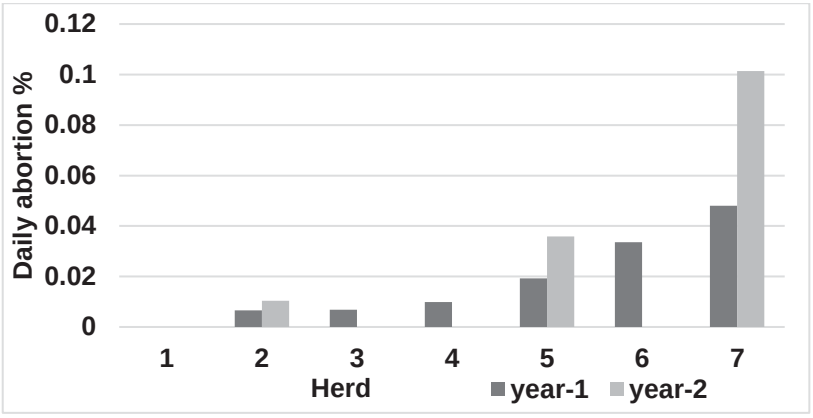

Figure 2-5: Individual herd between-scan daily abortion rates for MA herds on farms scanned both in year-1 and year-2.

2.4.6 Early fetal losses

Due to the observation of aborting fetuses on some farms at the time of first scanning in year-1, a small sub-study involving three R2 herds and one MA herd was undertaken in year-2. Early scanning took place in late May year-2 (2013) at two, seven, or 22 days after removal of stags in three R2 herds and on the day of stag removal in one MA herd (Table 2.9). The second scan was carried out in late June - mid July in year-2 and corresponded with the expected time period of first scanning of hinds nationally. The time interval between scans for R2 herds averaged 38 days and was 44 days for the MA herd. The scanning data for pregnant hinds segregated into indicative and definitively pregnant was only available from one R2 herd. In that herd, all the indicative pregnant hinds at Scan-1 were found pregnant at Scan-2, whereas three hinds from the definitively pregnant group lost their fetuses by Scan-2.

In other two R2 and MA herds, hinds were scanned as indicative, definitively pregnant or ‘no visible pregnancy’ at Scan-1, however only summary data of overall numbers in the herds but not individual hind data was provided to Author (Table 2.9).

The early fetal loss rates, combining definitively and indicative pregnancies at Scan-1, were zero, 4.9% and 7.1% in R2 herds and 1.2% in the MA herd. The DAR on those herds during this early gestation period were 0%, 0.16%, and 0.18% in the three R2 herds and 0.03% in the MA herd.

Table 2.9: Mating management, scanning, and early fetal losses recorded in the R2 and MA herds scanned for detection of early abortion.

Age group and herd		Stag out on	Date of first scan	No. at first scan	No. pregnant	No. indicative pregnant	Days to second scan	No. pregnant (definite or "indicative") present at second scan	% (no.) fetal deaths by second scan	% daily fetal death rate
R2	1	7 May	29 May	89	84	na	40	84	7.1 (6)	0.18
	2	22 May	29 May	81	50	13	30	61	4.9 (3)	0.16
	3	1 May	3 May	125	125	na	28	125	0.0 (0)	0
MA	1	20 May	20 May	84	82	na	44	82	1.2 (1)	0.03

na= not available

2.5 Discussion

This is the first comprehensive study of abortions on red deer farms in New Zealand or worldwide. Data show a wide range of mid-term abortion rates in both R2 and MA hinds, with daily rates up to 0.21% and 0.1%, respectively. Data also permitted analysis of early pregnancy rates (R2=85.8% and MA=93.3%), which were consistent with previous reports (Audigé *et al.* 1999a; Campbell *et al.* 2000; Lawrence 2003). Any abortion results in loss of productivity but some of the rates observed here would have significant impact on the economic viability of deer farms. While rates reported here are from the mid-term of pregnancy, observations during this study confirmed that abortions are to likely occur both before and after the interval studied here. Hence, whole of gestation abortion rates are likely higher than those reported here, but quantification was beyond what was achievable on the scale of this study on commercial farms. This Chapter presents descriptive data, while analysis of risk factors for abortion and potential causation will be presented in Chapters 4, 5, 6, and 7.

At the study design stage, power analysis estimated that 96 herds from each age group were required, however, the actual number of 87 R2 herds and 71 MA herds was achieved with recruitment of 85 farms across two years including repeat scanning of 15 farms in year-2. Herd- and animal-level abortion prevalence exceeded the required prevalence in power analysis for each age group and hence the statistical power provides confidence in the robustness of the findings, in the context of herd selection criteria. However, it is acknowledged that the recruitment of farms was not random, and it is possible that farmers with reproductive efficiency problems in their herds were more inclined towards participation in this type of study. However, it is logistically impossible to undertake a study of this nature in a fully randomised design. The abortion data reported here are higher than those published from studies of reproductive performance on deer farms (Audigé *et al.* 1999a; Walker *et al.* 2000; Lawrence 2003), but are similar to those reported by Wilson et al (2012). Given that undiagnosed reproductive wastage is common on NZ deer farms, the abortion rates reported here are plausible and suggest the abortion rates are a reasonable reflection of industry performance.

Stag joining information was available from 57 R2 herds and 44 MA herds. The median joining date for R2 hinds was 12 days earlier than that described previously by

Lawrence (2003) in herds from Southland and Otago regions. The joining period range was also wider than described by those Authors, with 25 days earlier joining and 10 days later stag removal in the present study. For MA hinds, the median joining date was earlier by 12 days and the joining date range was wider than those described by Lawrence (2003). The herds in this study were joined to stags earlier by 33 to 41 days for R2 and 14 to 22 days for MA compared with median joining dates from Audigé *et al.* (1999a). However, the joining date range in that study was narrower than reported here and the joining dates were reported collectively after combining both age groups and therefore less comparable to the dates reported here.

The median stag removal date for R2 herds was 11 days earlier and the mean range of 94 days was wider than the mean of 16 days reported in the study by Lawrence (2003). Additionally, the median stag removal dates for MA herds were earlier by nine days whereas the mean range of 43 days was wider than the 29 days reported by Lawrence (2003). The median stag removal dates were two to nine days earlier for R2 herds and five to 12 days earlier for MA herds and the stag removal date range for R2 and MA herds was wider than those described by Audigé *et al.* (1999a). Taken together, the mating data reported in this two-year study suggests changes in management practices in terms of earlier stag introduction and removal in herds from both age groups over time, consistent with advice from research into factors contributing to improved reproductive performance.

The 85.8% Scan-1 pregnancy rate in R2 hinds was higher than 83% reported by Campbell *et al.* (2000) on 16 South Canterbury/North Otago deer farms, 84.7% reported by Audigé *et al.* (1999a) on 15 lower North Island deer farms and 81.2%, 82.2% on 11 trial farms over two years and 61.2% to 83.3% on other farms from 1995 to 1999 in Hawke's Bay as reported by Walker *et al.* (2000). However, the pregnancy rate was lower than 95% reported on 50 Southland and Otago farms (Lawrence 2003). It should be noted that there was a serious drought throughout New Zealand in year-2 of this study leading to low quality/shortage of pasture and/or heat stress, which could have affected pregnancy rate, as observed in cattle (Nardone *et al.* 2010).

The Scan-1 pregnancy rate for MA hinds averaged 93.3% which was lower than 96.8% reported by Audigé *et al.* (1999a) in the lower North Island, 95% reported by Campbell *et al.* (2000) on South Canterbury/North Otago farms, 95% and 97.5% reported by Walker *et al.* (2000) on 11 Hawke's Bay farms over two years, and 97% reported on

Southland and Otago deer farms (Lawrence 2003). However, it was higher compared with 82.4%, 89.3%, 90.7, and 92.3% and similar to 93.1% as reported by Walker *et al.* (2000) in Hawke's Bay farms from 1995 to 1999. Nearly 75% of MA herds achieved more than 90% Scan-1 pregnancy rate. In addition, the observation of higher pregnancy rate (mean R2=87.3% and MA=95.4%) in large herds in both age groups may be indicative of a better farm management practice compared with small herds.

Detection of aborting fetuses at Scan-1 was consistent with the observation of aborting fetuses on one of the four R2 herds in the study by Wilson *et al.* (2012) in which some fetuses were as young as 42 days. These findings confirm that abortions do occur very early in gestation but may go unnoticed. These early abortions are likely to be a contributing factor in the sub-optimum pregnancy scanning results for this age group observed on many deer farms. A hind may take as many as 2-3 days to expel an aborting fetus (pers. comm.)[1], therefore, the proportion of aborting hinds at Scan-1 may be higher than reported here since early stage abortion may not be detectable on ultrasound scan. These early fetal losses also suggest that the pregnancy rates for both age groups reported here may not be a reliable measure of conception *per se.* Observations reported in this study strongly suggest that abortions may occur before the usual scan date on commercial farms. Further evidence supporting early abortions were occurring was the reporting of very early fetal deaths in the pilot study undertaken in year-2 in two of three R2 herds and one MA herd.

The proportion of R2 hinds that aborted reported here is higher than the whole of gestation abortions of 2.1% reported by Audigé *et al.* (1999a) in eight R2 lower North Island herds and 2.3% reported by Fennessy *et al.* (1986) in one South Island R2 herd. However, the data from studies by Audigé *et al.* (1999a) and Fennessy *et al.* (1986) came from limited numbers of hinds (303 and 88 hinds respectively) and therefore the power for the rates reported in this study is comparatively higher. Unfortunately, DARs reported in this study could not be directly compared with abortion rates from previous studies as abortions in those studies were estimated using the number of pregnant hinds that failed to give birth to a live calf. However, using a similar protocol for detecting abortions between early- and mid-pregnancy scans as used in this study, Wilson *et al.* (2012) reported abortions between 1.7% and 16% on four R2 herds.

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The mean DAR was not different between years in R2 herds. This suggests that on a population basis, abortions occur on a consistent manner across years. At the herd-level, 73% of R2 and 61% of MA herds were found to have at least one aborted hind. The proportion of R2 herds with aborted hinds was similar in year-1 and year-2, as were the mean DARs. However, repeatability study showed abortion was not predictable between years within a farm. The DAR in MA herds with aborted hinds was higher in year-1 compared with year-2. This observation may be confounded by the fact that year-2 experienced a severe drought in New Zealand and might have had an effect on pregnancy and/or abortion rates in both R2 and MA age groups. For both years and regardless of age group, almost all regions included in the study had at least one herd with abortion except Bay of Plenty. However, while the study was not designed as a regional prevalence study, data demonstrate that abortions were widespread in New Zealand.

The 1.2% mid-gestation abortions in MA hinds from this study was similar to the 1% reported by Campbell *et al.* (2000) in Canterbury, higher by 0.41 percentage points reported by Audigé *et al.* (1999a) in Lower North Island, and lower by 2.8 percentage points as reported by Wilson et al (2012) in Southland. However, it should be noted that the sample size was generally lower in previous studies and that the estimates other than that by Wilson et al (2012) were for the whole of gestation. Regionally, abortion was recorded in all regions except West Coast demonstrating that fetal losses in MA hinds was widespread.

While this study calculated mid-term abortion rates, should that rate be consistent throughout pregnancy, the whole of gestation losses could be estimated at 6.9% and 3.3% for R2 and MA hinds, respectively, but further research would be needed to verify this assumption. At herd-level, the mean abortion incidence rates for whole of gestation in herds with aborted hinds could be 3.9% and 2.2% in R2 and MA herds, respectively. However, it is feasible that abortions continue to occur through late gestation, given the disparity between pregnancy rates observed on most commercial deer farms and the number of calves weaned, and the failure to find dead calves on many farms (Audigé *et al.* 2001).

The DAR rates observed in R2 hinds was significantly higher than in MA hinds. This is consistent with the hypothesis of an infectious cause to which hinds are exposed to at a young age, and for which they develop an enduring immunity, protecting them from abortion later in life. Further evidence for this hypothesis is presented in Chapter 4 in relation to *Toxoplamsa gondii* associated fetal loss. Additionally, the observation of a lower pregnancy

rate and higher DAR in small R2 herds suggests that these hinds were at a higher risk of abortion and subsequent reduction in overall reproduction compared with hinds from large herds.

The abortion rates reported in this study in deer are comparable those reported in cattle herds and sheep flocks in New Zealand although not much data is available on abortion incidences in those species. A study in late 1960's showed abortion averaging 1.2% of cows in 192 cattle herds in the Lower North Island (Moller *et al.* 1967) and an abortion outbreak study reported overall abortion incidence ranging from 2 to 9% across two years in a dairy cattle herd (Pfeiffer *et al.* 2002). In sheep, abortion rates of 15% during abortion storms in the early 1950s (Hartley *et al.* 1954), 1.2% (of 1,477) and 6.7% (of 240) in 2005 (West *et al.* 2006) and 38% (of 115) mixed-aged ewes on one flock (Howe *et al.* 2008) have been reported. Abortion investigations in cattle have reported the involvement of *Neospora caninum* (Thornton *et al.* 1991; Thornton *et al.* 1994; Thobokwe and Heuer 2004) *Leptospira* spp. (Mackintosh *et al.* 1980) and bovine viral diarrhoea virus (BVDV) in cattle (Thobokwe and Heuer 2004) whereas *Toxoplasma gondii* (Hartley *et al.* 1954; Hartley and Marshall 1957), *Campylobacter* spp. (McFarlane *et al.* 1952), and *Neospora caninum* (West *et al.* 2006; Howe *et al.* 2008; Howe *et al.* 2012) have been reported in sheep. Therefore, potential infectious causes of abortions in deer warrant investigation including *T. gondii, N. caninum,* BVDV, Cervid herpesvirus-1 (CvHV-1) and *Leptospira* spp. and findings are presented in Chapters 4, 5, and 6.

The R2 and MA herds sampled in both years (2012 and 2013) had no significant difference in mean DAR. However, the higher DAR in low or nil abortion R2 herds and lower rate in medium and high abortion herds in year-2 highlights the variation in occurrence across years within herd. Hence, abortions were unpredictable, which would make the evaluation of potential control measures difficult. The variation in DAR could also be consistent with exposure to a pathogen which is subjected to seasonal variation. For example, *T. gondii* DNA was implicated in abortions in R2 herds in which *T. gondii* antibodies and DNA were detected in blood and tissues, respectively, from aborting hinds (Wilson *et al.* 2012). However, if R2 hinds were vaccinated against *T. gondii*, then the vaccine cost-effectiveness would be affected by the *T. gondii* exposure rate and the age at exposure on a given deer farm. *T. gondii* exposure depends on the population density of the feral cats which will depend on the environmental conditions in that area or region. Recruitment of herds with

a wide range of abortions in year-1 for the repeatability study was difficult to achieve, limiting numbers, as the majority of the herds were drought affected in year-2 and many farmers were reluctant to repeat participation in the study.

The herds in the early fetal loss pilot study were restricted to the lower and Central North Island. The first scan was as early as the earliest of the other herds, and the second was earlier than about 50% of first scan dates on other herds. Despite this, it would have been preferable to have scanning even earlier than that achieved, however, this was not possible due to the disruption of normal farm management practices. The absence of fetal losses in pregnant hinds from one R2 herd and in indicatively pregnant hinds in another R2 herd shows that the indicative pregnancy diagnosis at first scan was accurate early in gestation in those two herds. This observation provides confidence that the data from the other two herds with non-availability of pregnancy data segregated into indicative and definite pregnancies is likely to be accurate. Early abortions as reported in this study before Scan-1 were suspected by Audigé *et al.* (1999a), although confirmatory diagnosis was not performed in that study. Evidence from this early scanning component of the study, when combined with observation of aborting fetuses at the time of first scanning in 22 herds, supports that abortion does indeed occur prior to first scanning on many properties, hence contributing to sub-optimum apparent pregnancy scan rates. Hence, the conception rate *per se* is likely to be higher on many farms than apparent from scanning, suggesting that abortion could be a more economically limiting factor on farms than hind fertility *per se*. These findings confirm that early fetal losses occur in both R2 and MA hinds and there is a need for a wider study to further determine its prevalence and incidence.

2.6 Conclusion

This is the first study to systematically detect and quantify fetal wastage on a large scale on New Zealand deer farms. It was prompted by persistent sub-optimum reproductive performance across the deer industry coupled with data from a pilot clinical report of abortion by Wilson et al. (2012). The findings from both study years confirm that fetal losses occur at least in mid and early phases of gestation at a rate that could be economically significant on many deer farms. This study has provided evidence that mid-gestation abortions on many deer farms may be contributing more significantly to sub-optimum reproductive performance than previously believed and the abortion rates reported here are an under-estimate of the actual abortion rate. Additionally, the finding reported in this study suggest that the long-held

belief of farmers that most losses were attributable largely to perinatal and postnatal losses may not be correct in some cases. Similarly, the early fetal loss observed suggests that early abortion at less than 4-6 weeks of gestation may be contributing to the sub-optimum pregnancy rates at scanning in many commercial herds. The estimated whole-of-gestation abortions rates, assuming abortion risk was same throughout the gestation, could mean that overall fetal loss rate could be double those observed in mid-gestation as observed in this study. Taken together, these finding justify that an investigation into whole of pregnancy abortion rates and its causation is warranted to determine whether these losses can be prevented in part or in full.

2.7 Acknowledgements

This study is supported by the Deer Reproductive Efficiency Group based in Southland. It is funded by AgResearch, Agmardt, DEEResearch, MSD Animal Health, Southland Deer Farmers Association, Massey University and individual farmers, particularly Landcorp Farming Ltd. The in-kind contribution of all participating farmers is gratefully acknowledged, as is the assistance of a large number of veterinary practices, and scanners for scanning and blood sample collection.

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Chapter 3. Sensitivity and specificity of ELISA, latex agglutination test, and Western blot to detect *Toxoplasma gondii* antibodies in farmed red deer sera

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This chapter is prepared in the style format of the New Zealand Veterinary Journal.

3.1 Abstract

The reproductive performance in New Zealand (NZ) farmed red deer has been sub-optimal. Abortion due to *Toxoplasma gondii* infections during pregnancy has been suspected in rising-two-year-old (R2) and mixed-aged (MA) hinds. No test for *T. gondii* has been validated for use in deer although sero-prevalence of 40% for *T. gondii* using latex agglutination test (LAT) has been reported in R2 herds with two to 16% mid-term abortion rates. The aim of this study was to assess the sensitivity (Se) and specificity (Sp) of an immunoglobulin G (IgG) enzyme linked immunosorbent assay (ELISA) 'Chekit-Toxotest' (IDEXX, Switzerland) and the 'Toxoreagent' LAT (Eiken Chemical & Co, Japan) for use in deer. Evaluations used both the western blot (WB) gold standard and Bayesian latent class (BLC) statistics assuming absence of a gold standard. For LAT Bayesian analysis estimated the Se and Sp to be 88.7% and 74.3%, respectively, 95.8% and 95.1%, respectively, for WB, and 98.8% and 92.8%, respectively, for ELISA at an optimised cut-off S/P(%) of 15.5. Although, sero-prevalence estimation was not an aim of this study, the true sero-prevalence from its posterior distribution from BLC in the study population was estimated at 33.0% (95% credible interval: 27.3% to 38.9%). The apparent *T. gondii* sero-prevalence from ELISA at S/P(%) cut-off of 15.5 was 38.5%. The validated ELISA with optimised cut-off can be used to detect *T. gondii* exposure in deer to determine the sero-prevalence and assess a association between serology and reproductive performance.

KEY WORDS*: Deer, pregnancy, abortion, ELISA, latex agglutination test, Western blot, Bayesian analysis, validation, sensitivity, specificity.*

3.2 Introduction

In New Zealand (NZ), sub-optimum reproductive performance of farmed rising two-year-old red deer (*Cervus elaphus)* hinds (R2) has been a persistent problem (Audigé *et al.* 1999a; Asher and Wilson 2011). Reproductive efficiency (number of calves weaned / number of hinds mated) in R2 and mixed-age groups is estimated to be about 75% and is a significant concern for the NZ deer industry (Statistics New Zealand 2016b). A recent study by Wilson *et al.* (2012) reported mid-term abortion rates ranging from 2% to 16% across four R2 large deer herds and 4.5% in one mixed-aged (MA) hinds' herd. More recent studies in NZ farmed deer reported mid-term mean daily abortion rates of 0.043% in R2 herds (n=59) and 0.025% in MA herds (n=36) (Chapter-2).

Causes of reproduction losses in deer may include farm management practices, such as crop poisoning (Nordkvist *et al.* 1984), poor nutrition during pregnancy (Thorne *et al.* 1976) and infectious causes including *Toxoplasma gondii*, *Leptospira* spp, cervid herpes virus (CvHV), and bovine viral diarrhoea virus (BVDV) infections (Patel *et al.* 2012). *Toxoplasma gondii* DNA has been identified from brain tissue of aborted fetuses found at the time of scanning, and uteri of non-pregnant and aborted R2 hinds on NZ deer farms (Wilson *et al.* 2012). A *T. gondii* sero-prevalence of 40% using latex agglutination test (LAT) was reported in the same study, suggesting a possible *T. gondii* exposure association with abortion. Earlier, Reichel *et al*. (1999), also using LAT, reported a sero-prevalence of 52.5% with increasing sero-prevalence with age in NZ farmed deer. Collectively, these observations suggest that *T. gondii* might play a role in reducing reproductive performance of farmed deer in NZ.

Toxoplasma gondii, a protozoon parasite*,* is found worldwide and infects warm blooded animals including domestic and wild animals and humans (Dubey and Beattie 1988). Ingestion of *T. gondii* oocysts from the environment and subsequent infection in animals can cause reproductive losses including abortion, fetal death, and early embryonic loss, mummification, and stillbirth leading to economic losses (Freyre *et al.* 1997; Buxton *et al.* 2007). Historically, *T. gondii* has been isolated from fetal and placental tissues of aborting sheep during the abortion storms in sheep in New Zealand (Hartley *et al.* 1954), three aborted kids and goats in USA (Dubey 1981a), and from brain and/or liver of six aborted and stillborn lambs in United Kingdom (Beverley and Watson 1961). In addition, *T. gondii* has also been isolated from fetuses of white-tailed deer in six of 15 early and nine of 27 mid-term pregnancies in the USA (Dubey *et al.* 2008b).

Further investigation of disease causation and role of suspect causative agent involved in cervid fetal loss requires tests validated specifically for species under investigation and evaluation of the sensitivity (Se) and specificity (Sp) of available tests to inform appropriate test choice. The aim of this study was to assess the sensitivity and specificity of an immunoglobulin G (IgG) enzyme linked immunosorbent assay (ELISA) 'Chekit-Toxotest' (IDEXX, Switzerland) and the 'Toxoreagent' LAT (Eiken Chemical & Co, Japan) for use in deer. Evaluations used both the western blot (WB) as the gold standard (Sohn and Nam 1999) and Bayesian latent class (BLC) statistics assuming absence of a gold standard (Joseph *et al.* 1995).

3.3 Materials and methods

3.3.1 Samples

Sera used for this study were collected from studies investigating abortion in farmed red deer (Wilson *et al.* 2012; Patel *et al.* 2013). In 2011, 152 samples were collected from two farms in association with pregnancy scanning early and again later in gestation from randomly selected pregnant and non-pregnant R2 hinds, along with hinds having been diagnosed as aborting. In 2012, 100 sera comprising 3-16 samples per farm from 15 farms in various locations throughout NZ from R2 (n=60) and mixed-aged (MA) (n=40) hinds, were randomly selected from a serum bank of early and late pregnancy samples. Blood samples had been collected by jugular venipuncture into 10 ml vacuum blood collection tubes without anticoagulant, and transported chilled to Massey University where they were centrifuged at 1,512 × g for 15 min and serum withdrawn and stored at -20ºC. The blood collection was approved by the Massey University Animal Ethics Committee (Protocol number: 12/34).

3.3.2 Serological assays

3.3.2.1 Indirect ELISA (IDEXX-ELISA)

An immunoglobulin G (IgG) based *T. gondii* ELISA test for small ruminants ('Chekit-Toxotest' IDEXX laboratories, Switzerland) was performed on all serum samples according to the manufacturer's instructions. The S/P(%) ratio was calculated for all samples and results interpreted <20 negative, ≥20 - <30 ambiguous, ≥30 - <100 weakly positive and ≥ 100 positive as recommended by the manufacturer. For the purpose of analysis, sera with S/P(%) of less than 30 was considered sero-negative whereas sera equal to and above S/P(%) 30% was considered sero-positive.

3.3.2.2 Latex agglutination test

A duplicate of all samples was sent to a commercial pathology laboratory (NZ Veterinary Pathology Ltd, Palmerston North) for *Toxoplasma gondii* antibody screening using the latex agglutination test (LAT), 'Toxoreagent' (Eiken Chemical Company, Tokyo, Japan). The antibody end-point titres to *T. gondii* used were as per manufacturer's instructions. A titre of <1:32 was called negative whereas a titre of ≥ 1:32 was considered weak positive and those ≥1:64 as positive.

3.3.2.3 Western blot assay

3.3.2.3.1 Preparation of water-soluble T. gondii antigen

See appendix 2 for detailed *T. gondii* Western blot protocol.

Live tachyzoites of an attenuated strain (S48) of *T. gondii* from Toxovax® vaccine (Schering-Plough Animal Health, Wellington, New Zealand) were used to extract crude antigen. Thirty millilitres of vaccine suspension was centrifuged at 1500 x g for 10 minutes to pellet tachyzoites. The pelleted tachyzoites were re-suspended in 2ml PBS (pH 7.4) and disrupted by three cycles of freezing/thawing. This was followed by seven cycles of sonication on ice (Sonics VibracellTM, Sonics & Materials Inc., CT, USA). The sonicated tachyzoites were centrifuged at 12,000 x g for 30 minutes at 4^{o}C to remove debris and the supernatant containing the water soluble proteins was collected. The protein content was determined using a Nano-spectrophotometer (NanoDrop ND-1000, Thermo Scientific, DE, USA) and stored in aliquots.

3.3.2.3.2 Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and western blot analysis

Prepared antigen (2.2 µg/µl) was denatured using 2X lamelli buffer (Sigma, St. Louis, USA) containing 4% SDS, 20% glycerol, 10% 2-mercaptoethanol, 0.004% bromophenol blue and 0.125M tris HCL by heating at 100^{o}C for 10 minutes. The denatured protein was run through a 12.5% Tris-HCL gel (Criterion, Bio-Rad, California, USA) for 90 minutes at 100 volts. Each western blot was run with a broad range (10-250 kD) protein marker (Precision Plus Protein^{TM}, Bio-Rad), a negative and positive control serum. After separation, the proteins were electrophoretically transferred on to polyvinylidene difluoride (PVDF) membrane (Bio-Rad) for 45 minutes at 70 volts. After confirmation of complete protein transfer through Ponceau S (Sigma) staining, membranes were blocked overnight at 4^{o}C

using 5% blotting solution (5g skim milk powder) with 1% PBS-Tween-20 (PBS-T, Sigma). Blocked membrane was cut into strips and incubated with test sera, diluted 1:50 in 5% blotting solution with 1% PBS-T, at room temperature for 60 minutes. After primary incubation with sera samples, strips were washed three times for 10 minutes using 1% PBS-T. Secondary antibody, rabbit anti-deer antibody conjugated to horseradish peroxidase (KPL, Maryland, USA), diluted 1:9000 in 5% blotting solution with 1% PBS-T, was added to each strip and incubated for 60 minutes at room temperature. Strips were washed three times as described above for wash after primary incubation and then incubated at room temperature (22^0C) for five minutes in chemiluminiscent ECL solution (Amersham™ ECL Select™ Western Blotting Detection Reagent, GE Healthcare UK Limited, Buckinghamshire, UK) and arranged on a transparent plastic film. The film was exposed for 30 seconds in LAS-1000 plus luminescent image analyser (Fujifilm, Japan) and the bands were measured against the standard markers. Presence of bands indicating immunodominant antigens (IDA) of *T. gondii* were recorded. For this study, sera that recognised two or more IDA with molecular weight (MW) between 24 to 40 kD were considered positive for *T. gondii*. The positive control serum used in the study showed consistent bands at 24kD, 31kD, 33 kD, 38 kD, and 40kD of and thus acted as an internal control loading and antibody probing.

3.3.3 Statistical analysis

3.3.3.1 Western Blot as a gold standard test

Analysis used SAS statistical software version 9.3 (SAS Inc., Cary, NC, USA). The ELISA S/P(%) values were log transformed on a natural logarithmic scale for normality and analysis purposes and were back transformed for reporting results. Sensitivity, Sp, positive predictive value (PPV), negative predictive value (PPV), likelihood ratio positive (LR+), likelihood ratio negative (LR-) for both tests were calculated using qualitative results from WB. McNemar's Chi-square test statistic was performed to check difference (to check for test bias) between sero-positive proportions from WB and ELISA, and WB and LAT. The Kappa statistic was estimated to check the level of agreement between WB and LAT and, WB and ELISA. Based on the Kappa statistic, the test agreement can be interpreted as <0.2 = slight agreement; 0.2 - 0.4 = fair; 0.4 - 0.6 = moderate; 0.6 - 0.8 = substantial; >0.8 = almost perfect (Dohoo *et al.* 2003).

3.3.3.2 Bayesian latent class (BLC) analyses in the absence of a gold standard test

BLC analysis was used to determine sensitivity (Se), specificity (Sp), positive predictive value (PPV), and negative predictive value (NPV) of the three tests and sero-prevalence (P) using a latent class model assuming that western blot was not a gold standard test. A posterior distribution was derived combining existing and prior knowledge about the parameters (Se_{ELISA}, Sp_{ELISA}, Se_{WB}, Sp_{WB}, $Se_{LAT,}$ and Sp_{LAT}). For the purpose of analysis, all sera (252 samples from 17 herds across two years) were considered as one population. All three tests were assumed to be dependent conditionally on the true *T. gondii* exposure status and with constant test accuracy in the test population.

Prior information about the test Se_{LAT} and Sp_{LAT} for LAT (Dubey *et al.* 1995; Hokmabad *et al.* 2011) and WB tests were available from data in past studies on another species and/or from expert opinion from the laboratories applying the tests for research purpose (Table 3.1). For example, *T. gondii* sero-prevalence recorded earlier in farmed red deer population in New Zealand by LAT was 52.5% in 1997 (Reichel *et al.* 1999) in 40% in 2011 (Wilson *et al.* 2012). Because LAT was suspected for lower Se and Sp and hence an over-estimated sero-prevalence in deer compared with the true sero-prevalence, it was assumed that the most likely sero-prevalence value will be 20% and greater than 10% corresponding to a beta distribution of (α=5.1, β=17.6). Similarly, the most likely Sp_{WB} value was assumed to be 95% from a previous study on pigs (Basso *et al.* 2013) and 99% from an expert opinion (personal communication) that corresponded to a beta (α=4.18, β=1.2) distribution. The priors for the mean of the log-transformed ELISA S/P(%) values for diseased group was set to three for the *T. gondii* exposed hinds and one for the *T. gondii* non-exposed hinds. The standard deviation (SD) for the mean ELISA S/P(%) of both exposed and non-exposed was set to one. Independent beta prior distributions were derived for the parameters using Betabuster[a] software to check for their uncertainty using modal or most probable values and 5^{th} and 95^{th} percentiles.

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.

Parameter	Mode	95% sure greater than	Corresponding beta prior distribution (α, β)
Prevalence %	20	10	5.1, 17.6
WB Se%	95	50	4.18, 1.2
WB Sp%	95	50	4.18, 1.2
LAT Se%	90	50	5.38, 1.49
LAT Sp%	80	50	7.55, 2.63

The model estimating P, Se_{ELISA}, Sp_{ELISA}, Se_{WB}, Sp_{WB}, $Se_{LAT,}$ and Sp_{LAT} was fitted to the animal data assuming independence across herds in WinBUGS (Lunn *et al.* 2000) using the Bayes Continuous Diagnostic Test (BCDT) software package version 3.7 (Division of Clinical Epidemiology 2015). BCDT software was developed for diagnostic tests with continuous outcomes based on the latent class analysis methods to determine disease prevalence and parameters for diagnostic test with dichotomous outcomes as described by Joseph and Gyorkos (1996) and Joseph *et al.* (1995). ELISA S/P(%) values were transformed on a natural logarithmic scale before being analysed in the BCDT software and then back transformed for reporting results. Each model was run for 50,000 iterations with a burn-in discard period of 5,000 iterations. Convergence of the posterior iterates was assessed in WinBUGS by examining history trace plots and by specifying two sets of dispersed initial values and examining the convergence statistic. The influence of the parameter priors on posterior estimates of P, Se, Sp, PPV, NPV of LAT, ELISA and WB was evaluated in a sensitivity analyses using uniform, optimistic and pessimistic priors. A cut-off for ELISA SP with optimum sensitivity and specificity was selected from the receiver operating characteristic (ROC) dataset generated by WinBUGS and Se_{ELISA}, Sp_{ELISA}, PPV_{ELISA}, and NPV_{ELISA} at selected cut-off are reported.

3.4 Results

All 252 sera samples were analysed on western blot (WB), ELISA and LAT for comparisons between all three tests.

3.4.1 Western blot

The immunoglobulin G (IgG) antibodies from 138 of 252 (54.8%) sera samples reacted with protein antigens on western blot (Figure 3-1) producing 1-5 bands (Table 3.2) ranging from 24-40 kilodaltons (kD). Sera (n=162) with one or no band were regarded as

negative for the purpose of the analyses. In WB positive sera, 10%, 30%, 33.3%, and 26.7% sera were observed with 2, 3, 4, and 5 bands, respectively. The most frequent observed band with molecular weight of 24 kD was present in 110 (43.7%) of positive sera (Table 3.2 and Figure 3-1). The estimated sero-prevalence from WB was determined to be 35.7% (90/252; 95% CI: 29.8-41.6%).

Table 3.2: Summary of sera with observation of bands with different molecular weights (kD) on western blot immunoblot image

Band molecular weight (kD)	Number (%) of sera	Mean elisa S/P(%)
24	110 (43.7)	41.0
31	72 (28.6)	55.9
33	56 (22.2)	62.2
38	87 (34.5)	54.6
40	62 (24.6)	53.5

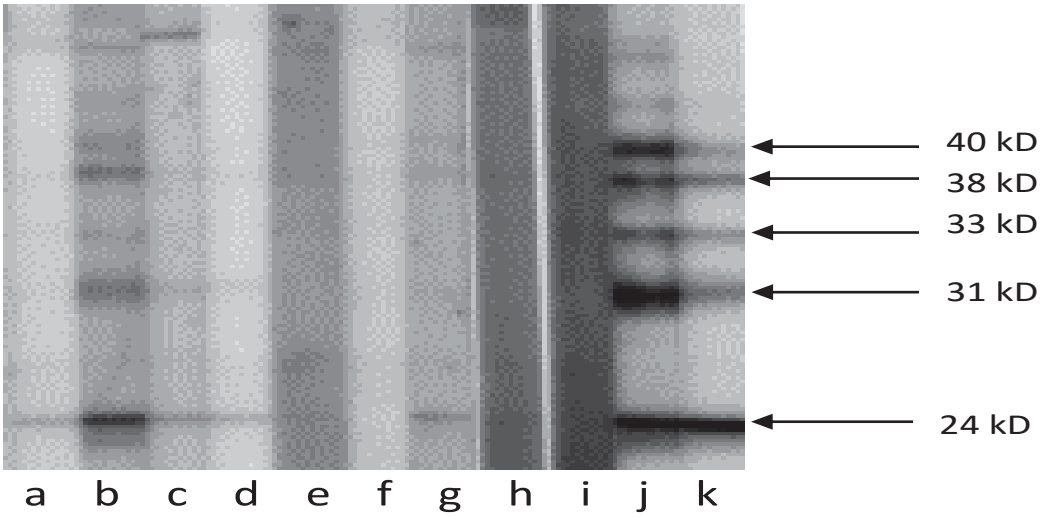

Figure 3-1. Western blot patterns of a sub-sample of sera (b to j). Positive sera samples b,c,d,g and j show bands to *Toxoplasma gondii* antigens from 24 to 40kD. Sera labelled e, f, h, and I represent negative samples. Sera labelled 'a' and 'k' represent negative and positive control sera, respectively.

3.4.2 LAT compared with Western blot

On LAT, 119 (47.2%) were positive at the manufacturer's recommended cut-off titre of 1:32 or above. Of the 119 positive sera, 76 (63.8%) of 119 were also positive on WB (Table 3.3).

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.

Assay and sero-status		WB positive		WB negative	
		LAT positive[a]	LAT negative	LAT positive	LAT negative
ELISA	Sero-positive[b]	66	5	3	1
	Sero-negative	10	9	40	118

[a] LAT positive samples were defined as titres of ≥1:32

[b] ELISA positive samples were defined as S/P(%) ≥30

The Se_{LAT} and Sp_{LAT} at cut-off titre of 1:32 by comparing it to WB was 84.4% and 73.5%, respectively. The PPV_{LAT} was 63.9% and NPV_{LAT} was 89.5%. The McNemar's Chi-square test was significant ($p<0.001$) at a value of 14.7 confirming a difference between proportion of positive samples on LAT and WB. The Kappa value was 0.54 (*Table 3.4*).

3.4.3 ELISA compared with Western blot

Seventy-five of 252 (29.8%) samples, interpreted at the manufacturer's recommended cut-off (S/P(%)≥ 30), were sero-positive on ELISA with a mean S/P(%) of 65.5 (SD= 29.3, range 30.2 to 177.3). Of the 75 ELISA positive samples, 71(95%) were in positive agreement with WB (Table 3.3). Fourteen (5.5%) sera fell in the intermediate range (S/P(%): ≥20 to <30) and nine of those were positive on WB. The remaining 163 samples were ELISA negative with a mean S/P(%) of 5.1 (SD: 4.7, range: 1.6 to 19.6).

The sera that fell in the intermediate S/P(%) range category were not regarded as positive, to allow Se and Sp calculations. At the manufacturer's cut-off S/P(%) of 30, Se_{ELISA} was 78.9% and Sp_{ELISA} was 97.5% using WB as a gold standard test. The PPV_{ELISA} and NPV_{ELISA} at this cut-off were 94.7% and 89.3%, respectively. The Kappa value was 0.79 (Table 3.4). The positive sera proportions from ELISA significantly differed to that of from WB (McNemar's Chi-square p=0.001) at manufacturer's cut-off S/P(%) of 30 whereas the positive proportions were not significantly different at chosen cut-off S/P(%) of 15.5 (McNemar's Chi-square p=0.28).

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.

Parameters	LAT and WB	ELISA and WB
	Cut off titre (Manufacturer: 1:32)	Cut off SP% (Manufacturer: 30%)
Sensitivity (%)	84.4 (74.9 - 90.9)	78.9 (68.8 - 86.5)
Specificity (%)	73.5 (65.8 - 79.9)	97.5 (93.4 - 99.2)
Apparent prevalence (%)	47.2 (41.1 - 53.4)	29.8 (24.1 - 35.4)
Positive predictive value (PPV) (%)	63.9 (54.5 - 72.3)	94.7 (86.2 - 98.3)
Negative predictive value (NPV) (%)	89.5 (82.7 - 93.9)	89.2 (83.5 - 93.2)
Positive likelihood ratio (LR+)	3.2 (2.4 - 4.2)	32.0 (12.0 - 84.6)
Negative likelihood ratio (LR+)	0.21 (0.13 - 0.34)	0.22 (0.15 - 0.32)
Kappa statistic	0.54 (0.44 - 0.64)	0.79 (0.71 - 0.87)
McNemar's Chi-square statistic	14.7 (p=0.0002)	9.8 (p=0.001)

3.4.4 Bayesian latent class analysis

The median Se, Sp, PPV, and NPV values for LAT and WB test from posterior distributions along with their 95% credible intervals using uniform priors (α=1, β=1 for all parameters) and informative priors are listed in Table 3.1. Application of weakly informed prior or good prior had little effect on the posterior median values compared with those obtained using informative priors (data not shown here).

The Se, Sp, PPV, and NPV for ELISA at manufacturer's recommended S/P(%) cut-off of 30 were 85.1%, 98.5%, 96.5%, and 93.2%, respectively. Using the ROC data, an optimised cut-off S/P(%) of 15.5 was chosen which improved the sensitivity to 98.8% and slightly lowered the specificity to 92.8% (Table 3.6). The area under the ROC curve (AUC), as determined from BLC analysis was 0.98 (95% CI: 0.97 - 0.99) (Figure 3-2). The resulting PPV and NPV at the S/P(%) cut-off of 15.5% was 87% and 99.4%, respectively. Although, sero-prevalence estimation was not an aim of this study, the true sero-prevalence from its posterior distribution from BLC in the study population was estimated at 33.0% (95% credible interval: 27.3% to 38.9%). The apparent *T. gondii* sero-prevalence at S/P(%) cut-off of 15.5 was 38.5%. In addition, from the BLC analysis, the Se and Sp of WB were 95.8% (95% credible interval: 89.4% to 99.2%) and 95.1% (95% credible interval: 90.6-98.1%), respectively whereas the Se and Sp for LAT were 88.9% (95% credible interval: 80.7% to 94.9%) and 74.3% (95% credible interval: 67.5% to 80.5%), respectively (Table 3.5). The

change in sensitivity and specificity estimates due to variation in prevalence across herds was very low (<2%, data not shown).

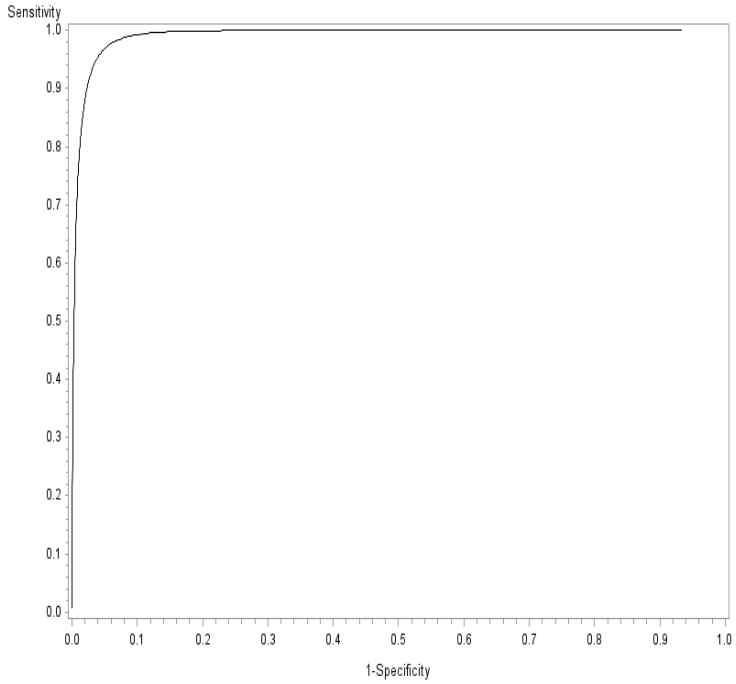

Figure 3-2. Receiver operating characteristic (ROC) curve plotted for ELISA S/P(%) from Bayesian latent class (BLC) analysis.

Table 3.5. *Toxoplasma gondii* 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.

Parameter	Gold standard analysis	Non-informative priors for all parameters	Informative priors for all parameters
Sero-prevalence (%)	35.7 (29.8 - 41.6)	33.9 (27.0 - 40.1)	33.0 (27.3 - 38.9)
Se WB (%)	na	95.9 (89.3 - 99.3)	95.8 (89.4 - 99.2)
Sp WB (%)	na	94.4 (89.6 - 97.6)	95.1 (90.6 - 98.1)
Se LAT (%)	84.4 (74.9 - 90.9)	88.7 (80.3 - 94.8)	88.7 (80.8 - 94.7)
Sp LAT (%)	73.5 (65.8 - 79.9)	74.1 (67.1 - 80.4)	74.3 (67.5 - 80.5)
PPV WB (%)	na	89.7 (81.2 - 95.7)	89.6 (81.0 - 95.5)
NPV WB (%)	na	97.8 (94.1 - 99.7)	97.8 (94.3 - 99.6)
PPV LAT (%)	63.9 (54.5 - 72.3)	63.6 (54.5 - 72.2)	63.6 (54.7 – 72.0)
NPV LAT (%)	89.5 (82.7 - 93.9)	92.8 (87.0 - 96.8)	93.0 (87.4 - 96.8)

na= not applicable

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

Parameters	Gold standard analysis	Bayesian latent class analysis	
Cut off SP%	Manufacturer - 30	Manufacturer - 30	Optimised - 15.5
Se (%)	78.9 (68.8 - 86.5)	85.1 (76.2 - 91.9)	98.8 (96.1 - 99.8)
Sp (%)	97.5 (93.4 - 99.2)	98.5 (96.9 - 99.4)	92.8 (88.9 - 95.7)
PPV (%)	94.7 (86.2 - 98.3)	96.5 (92.6 - 98.6)	87.0 (80.0 - 92.6)
NPV (%)	89.2 (83.5 - 93.2)	93.2 (88.4 - 96.4)	99.4 (97.9 - 99.9)

3.5 Discussion

This is the first validation of serological tests for *Toxoplasma gondii* in farmed red deer. Both gold standard and Bayesian latent class analyses techniques were used for the validation of the LAT and ELISA assays. Both assays had reasonable sensitivity and specificity when interpreted according to manufacturer's recommendations. Bayesian analysis determined that the optimum ELISA Se and Sp was achieved when an adjusted cut off S/P(%) of 15.5 was used.

The ELISA Se and Sp at cut-off S/P(%) of 15.5 were higher than Se and Sp of LAT and equivalent to Se and Sp of WB as determined from BLC analysis, hence demonstrating that that ELISA performed better than the LAT. The LAT assay is used by commercial diagnostic laboratories in New Zealand as it is not species specific and detects both immunoglobulin G (IgG) and immunoglobulin-M (IgM). However, the LAT requires user interpretation as it involves microscopic observation of agglutination endpoint allowing for possible intra- and inter-laboratory variation. On the other hand, the ELISA test evaluated here only detects IgG but can be modified to determine antibody avidity and hence the timing of the infection whether the antibodies raised are due to acute or chronic infection (Syed-Hussain *et al.* 2013). ELISA is less time-consuming and can be semi-automated, hence higher throughput can be achieved with less intra- and inter-laboratory variation. WB had equivalent Se and Sp to ELISA (at cut-off S/P(%) of 15.5). However, it is a labour-intensive assay which also requires user interpretation of results.

The optimum ELISA cut-off was chosen to improve the NPV and to achieve optimum Se and Sp. The ELISA cut-off S/P(%) of 15.5 improved the Se_{ELISA} (98.8%) by 16.1%, with a decrease in Sp_{ELISA} (92.8%) by 6.1%, when compared with the Se_{ELISA} (85.1%) and Sp_{ELISA} (98.5%) estimates at manufacturer's recommended cut-off S/P(%) of 30. Se and Sp ELISA estimates at cut-off S/P(%) of 15.5 were found to be higher than LAT and equivalent to WB in this study. Higher Se (98.8%) and NPV (99.4%) will help to establish a more accurate assessment of *T. gondii* exposure in hinds with and without abortion.

Western blot was chosen as the reference test for the gold standard analysis. The BLC analysis was included to obtain estimates if WB was not a reference test as to Author's knowledge, there are no reports of Se and Sp of WB for its use in deer. Western blot has been previously used as a gold standard test to detect *T. gondii* in feline (Sohn and Nam 1999) and ovine (Wastling *et al.* 1994; Wastling *et al.* 1995) species. However, no reported data exist for farmed deer. The band range observed on the immunoblot image in this study for deer was comparable to band range obtained in sheep (Wastling *et al.* 1994), pig (Al-Adhami and Gajadhar 2014), and cat (Sohn and Nam 1999) studies. The BLC analysis estimated Se_{WB} and Sp_{WB} to be 95.8% and 94.3%, respectively, which were similar to the estimates from expert opinion (pers. comm.)[2]. Uniform priors (or dis-informative prior with $\alpha=1$ and $\beta=1$) had little or no effects on the Se_{WB} and Sp_{WB} estimates.

When compared with a serological study using an ELISA at manufacturer's cut off S/P(%) of 30, and WB as a gold standard, in white-tailed deer (*Odocoileus virginainus)* in Mexico (Olamendi-Portugal *et al.* 2012), Se_{ELISA} (78.9%) and Sp_{ELISA} (97.5%) estimates from gold standard analysis were higher by 7.6% and lower by 2.6%, respectively, when compared with Se_{ELISA} of 73% and Sp_{ELISA} of 100% using WB. This difference could be attributed to use of an in-house ELISA with an anti-sheep IgG secondary antibody (Olamendi-Portugal *et al.* 2012) compared with the anti-ruminant IgG secondary used in the IDEXX commercial ELISA used in this study. A study on roe deer (*Capreolus capreolus*) in France estimated Se and Sp of a competitive-inhibition ELISA (bioMérieux, France, now called "Toxotest Antibody Test Kit", IDEXX) to be 96% and 86%, respectively, with a Kappa value of 0.8, using microscopic agglutination test (MAT) as a reference test. However, the sample size was smaller (n=61) (Gotteland *et al.* 2014) than used in this study. The

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Se_{ELISA} (78.9%) and Sp_{ELISA} (97.5%) estimates obtained from gold standard analysis here were lower by 21.7% and higher by 13.4%, respectively, as compared with the Se_{ELISA} and Sp_{ELISA} estimates from the French study. The Se_{ELISA} and Sp_{ELISA} using WB as a reference test reported here in farmed red deer are similar and comparable to above studies in white-tailed and roe deer when an ELISA test was compared with a reference test such as WB or MAT.

While sero-prevalence estimation was not the aim of the study, as the BLC also provides true sero-prevalence in the study population, appropriate priors for sero-prevalence were included in the BLC analysis. The LAT and WB overestimated the true sero-prevalence by 14.2 and 2.7 whereas the ELISA, at cut-off S/P(%) of 30, under-estimated sero-prevalence by 3.2 percentage points. The apparent sero-prevalence at ELISA cut-off S/P(%) of 15.5 was overestimated by 5.5 percentage points. These findings suggest that LAT overestimates the true sero-prevalence compared with ELISA and therefore the ELISA should be chosen as the test of choice to test for *T. gondii* antibodies in deer. The ELISA cut-off S/P(%) of 15.5 was chosen to maximise the NPV and optimise the Se and Sp compared with the similar parameters at manufacturer's cut-off.

The validated ELISA assay from this study will be used to establish the sero-prevalence of *T. gondii* and to assess the association between the presence of the parasite and fetal loss in New Zealand farmed deer.

3.6 Conclusion

It can be concluded that the ELISA test at the optimised cut-off S/P(%) of 15.5 had better Se and Sp than LAT and that it can detect *T. gondii* antibodies in red deer population with good sensitivity and specificity.

3.7 Acknowledgements

This test evaluation was part of a larger study funded by DEEResearch, Massey University Doctoral Scholarship, AgResearch, Agmardt, and MSD Animal Health (NZ Ltd). I thank deer farmers, veterinarians, farm staff, and IVABS technical staff for their participation and assistance in the study. I also thank Dr. Geoff Jones (Institute of Fundamental Sciences, Massey University) for his assistance in Bayesian latent class analyses.

3.8 Sources and manufacturers

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b. http://www.medicine.mcgill.ca/epidemiology/joseph/Bayesian-Software-Diagnostic-Testing.html

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Chapter 4. Investigation of association between *Toxoplasma gondii* and early pregnancy and abortion rates in New Zealand farmed red deer

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This chapter is prepared in the style format of the New Zealand Veterinary Journal.

4.1 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 failure to conceive, fetal loss, stillbirth, and postnatal mortality. A previous study on 22,130 rising two-year-old (R2) and 36,223 mixed-age (MA) hinds from herds throughout New Zealand indicated mid-term herd-level mean daily abortion rates (DAR) of 0.043% and 0.025% for these respective age-groups (Chapter 2). This study examined whether there was an association between *Toxoplasma gondii* and the observed mid-pregnancy abortions. Serum from pregnant and non-pregnant hinds at early gestation (Scan-1, R2 n= 861, MA n=357) and approximately the end of second trimester (Scan-2, R2 n=714, MA n=372) were tested for *T. gondii* antibodies on a validated ELISA using a S/P(%) cut-point of ≥15.5. Fetuses and uteri from hinds observed aborting at both scans, non-pregnant uteri after Scan-1 and 2, uteri from normal pregnancies at Scan-1, and uteri from hinds not rearing a calf to weaning were collected at deer slaughter premises and tested for *T. gondii* DNA using a PCR.

Associations between sero-status and having aborted at individual animal level, sero-prevalence and DAR at herd level, were analysed. At Scan-1, 31.1% of 861 R2 and 28.3% of 357 MA hinds were sero-positive for *T. gondii*. There was non-significant association between Scan-1 sero-status and non-pregnancy at animal (R2 p=0.05, MA p=0.43) or herd level (R2 p=0.37). *Toxoplasma gondii* DNA was detected in 3/18 placenta and 4/18 fetal brains from aborting R2 hinds and 15/ 157 R2 and 3/21 MA uteri from non-pregnant hinds at Scan-1. At Scan-2, the sero-prevalence in aborted hinds (34.3% of 268) was significantly higher (Odds ratio=1.6, p=0.032) than in non-aborted R2 hinds (23.5% of 446), but not in MA hinds (p=0.21). The within-herd sero-prevalence at Scan-2 was positively associated with DAR in R2 herds having aborted hinds ($p<0.001$) but not in MA herds (p=0.07). 7.9% of abortions were attributable to *T. gondii* exposure at Scan-2 in R2 hinds from herds with aborted hinds. *Toxoplasma gondii* DNA was detected in uteri from 22/138 R2 and 5/31 MA aborted hinds, cotyledons from 1/15 R2 and 1/5 MA hinds, and fetal brains from 1/5 MA aborted hinds at Scan-2. Pinpoint and focal/petechial haemorrhages were observed on caruncles and/or uterine horn from 7/157 pregnant and 4/29 of aborting R2 hinds at Scan-1, and 68/137 aborted R2 and 3/31 aborted MA hinds at Scan-2. *Toxoplasma gondii* DNA was detected in uteri from 1/10 R2 and 4/23 MA hinds not rearing a calf to weaning.

These findings provide serological, molecular and pathology evidence that *T. gondii* causes abortion in deer, possibly in all three trimesters.

KEY WORDS: *Red deer, ultrasound scanning, pregnancy, non-pregnancy, abortion,* Toxoplasma gondii, *uterus, fetus, ELISA, PCR.*

4.2 Introduction

Reproductive performance in rising two-year-old (R2) and mixed-aged (MA) adult hinds is suboptimal in New Zealand farmed deer (Asher and Pearse 2002; Asher 2003; Asher and Wilson 2011). The reproductive efficiency (calves weaned/hinds mated) in last decade has averaged 75.2% in last 13 years and arises from failure to conceive, fetal loss, stillbirth, and postnatal mortality (Statistics New Zealand 2016a). Based on early scanning data, presented in Chapter 2, pregnancy rates of R2 and MA hinds were 85.8% and 93.3%, respectively.

Causes and risk factors for conception failure in both R2 and MA hinds have been studied previously (Audigé *et al.* 1999b; Audigé *et al.* 1999c; Asher *et al.* 2005a; Asher *et al.* 2011). Pregnancy loss due to abortions in New Zealand deer has been reported in a few studies (Fennessy *et al.* 1986; Audigé *et al.* 1999a; Campbell *et al.* 2000; Wilson *et al.* 2012). However, the most substantial data available is presented in Chapter 2 in which the mid-term herd-level mean daily abortion rates were 0.043% and 0.025% in R2 and MA herds, respectively, which calculates to 3.9% in R2 and 2.2% in MA herds for 90 mid-term gestation days. Hence abortions can pose a significant economic impact on the deer farmers and the deer industry in New Zealand in terms of lost opportunities and lower financial returns.

Abortion can be attributed to non-infectious and infectious causes. Risk factors for non-infectious causes, as in other species such as cattle and sheep, can include farm management, nutrition, health and environmental conditions as discussed later in Chapter 7 (Bartels *et al.* 1999; Fthenakis *et al.* 2012). Feeding of winter crops and supplements increased the probability of pregnancy at Scan-1 whereas Johne's disease (JD) occurrence, reflecting the JD status of herd, co-grazing of herds with beef cattle, and hay supplements increased the risk of abortion by Scan-2 (Chapter 7). Pathogens, such as *Toxoplasma gondii, Neospora caninum*, *Leptospira* spp., herpes virus, and bovine viral diarrhoea (BVD), known for causing abortions in cattle, sheep and other small ruminants have been reported in deer and have been discussed in Chapter 1 (Trainer *et al.* 1961; Dubey *et al.* 1996; Dubey *et al.* 2008c; das Neves *et al.* 2009b; Passler *et al.* 2009; Passler *et al.* 2010; Subharat *et al.* 2010; Dubey *et al.* 2013; Dubey *et al.* 2014). *Toxoplasma gondii* has been shown to be a significant cause of abortion in New Zealand (Hartley *et al.* 1954) and overseas sheep populations (Beverley and Watson 1961; Hurtado *et al.* 2001; Pereira-Bueno *et al.* 2004). In New Zealand

farmed deer, *T. gondii* DNA was detected from 8/9 fetal brains recovered from aborting R2 hinds in a clinical investigation in a herd experiencing a 1% early and 7.2% mid-term abortion rate, with serological evidence of *T. gondii* exposure (Wilson *et al.* 2012). *Toxoplsama gondii* DNA was detected in non-pregnant uteri and antibody was detected in blood from non-pregnant, pregnant, and aborting hinds from two other R2 herds affected with abortions in the same report. There are reports elsewhere of trans-placental transmission and antibodies for *T. gondii* in deer (Dubey *et al.* 2008b; Dubey *et al.* 2014). However, an association between *T. gondii* and abortion in farmed deer in New Zealand has not been thoroughly investigated.

Thus, the aim of this study was to test for association between *T. gondii* sero-status and pregnancy outcome at scanning early in gestation, and mid-term abortion as determined by repeat scanning in red deer hinds undertaken on farms and animals described in Chapter 2. Tissue samples from aborted and aborting hinds and aborting fetuses were also examined grossly and tested for *T. gondii* DNA.

4.3 Materials and methods

The sampling frame for farms and animals used for this analysis is described in detail in Chapter 2. Briefly, 22,130 R2 and 36,223 MA red deer hinds from 87 and 71 herds, respectively, throughout New Zealand were ultrasound scanned after a mean interval of 49 days of stag removal for pregnancy early in gestation (Scan-1), and a sub-sample (up to 100 R2 and 200 MA hinds per herd) was scanned again approximately at the end of the second trimester (Scan-2) after a mean between-scan interval of 89 days during the 2012 and 2013 reproductive cycles (Table 4.1).

Hinds were classified as ‘pregnant’ based on the presence of at least one fetus or part thereof, amniotic membrane, and/or presence of placentomes, or as being ‘non-pregnant’ based on absence of those signs combined with visualisation of a non-pregnant uterus at Scan-1. The term “aborting” is ascribed to hinds that had ultrasound evidence of aborting fetuses at Scan-1 and Scan-2. The term “aborted”, used for calculating the DAR, is ascribed to hinds that were pregnant at Scan-1 but not pregnant at Scan-2, plus those aborting at Scan-2. Daily abortion rate (DAR) ((number aborted at Scan-2 / number scanned at Scan-2) / number of days between Scan-1 and Scan-2) was calculated to account for differences in time between scans. This approximated a mid-trimester abortion rate.

4.3.1 Sample collection

All animal manipulations were approved by the Massey University Animal Ethics committee (Protocol numer: 12/34).

Serum samples were collected over two years, 2011 and 2012, from 21 randomly selected pregnant, non-pregnant, and all available aborting hinds per herd at Scan-1 from late May to mid-August and from up to 21 aborted hinds as available and 21 non-aborted hinds per herd in mid-September to mid-October at Scan-2. While this sampling was cross-sectional, 235 R2 and 131 MA hinds were sampled at both Scan-1 and Scan-2. Samples were collected by jugular veni-puncture into 10 ml vacuum blood collection tubes without anticoagulant, and transported chilled to Massey University where they were centrifuged at 1,512 × g for 15 min and serum withdrawn and stored at -20^{o}C until tested. In total, 4,835 (R2=2,786 and MA=2,049) and 2,932 (R2=1,780 and MA=1,152) blood samples were collected at Scan-1 and Scan-2, respectively, to provide the sample pool for selection for serological analyses.

Non-pregnant and aborting hinds at Scan-1, aborted hinds at Scan-2, and pregnant hinds at Scan-1, as available, were tracked to deer slaughter premises (DSP) where whole reproductive tracts from the posterior cervix were collected by the Author or the DSP veterinarian. Additionally, reproductive tracts from hinds that did not rear a calf to weaning were also collected at DSPs. Samples were kept in a chiller/freezer and then sent chilled or frozen to Massey University where they were processed at the post-mortem facility. Gross observations from uteri and fetal tissues were recorded at dissection. Uteri, placental, cotyledon, and fetal samples were dissected and stored at -20^{0}C (fresh specimens) for PCR, or in 10% formalin for histology. Nine subsequently *T. gondii* DNA positive uteri and one *T. gondii* DNA negative uterus from aborted R2 hinds were further processed for histopathological examination. The sections from endometrium and myometrium were stained by hematoxylin and eosin (H&E) stain and were observed under a microscope.

4.3.2 Sample selection for serology

To determine the association between non-pregnancy status and sero-status at Scan-1, sample selection for Scan-1 serology was determined after the Scan-2 serology results were available. Herds for Scan-1 serology were selected from those categorised as nil, low (R2: >0 - 0.03%, MA: >0 - 0.02%), medium (R2: 0.031 - 0.06%, MA: 0.021 - 0.035%), and high (R2: >0.06%, MA: >0.035%) DAR groups. Twenty-three sera from hinds in 10 herds (9 R2 and 1

MA) were tested at the farmer's request after Scan-1 and were included in the Scan-1 serology analysis. Sera from aborting hinds (27 R2 and 16 MA) at Scan-1 from both years and the equivalent number of sera from pregnant hinds in the same herd were tested for antibodies to *T. gondii*. Therefore, in total, sera from 861/2,786 and 357/2,049 sampled hinds were tested from 46 R2 and 18 MA herds as summarised in Table 4.1.

Sera from five pregnant and non-pregnant hinds each per herd from 15 R2 herds were tested in year-2. After analysing the results from year-1 Scan-1 serology, only sera from those aborted and the equivalent number from non-aborted MA hinds were selected for year-2 serology.

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.

Scan	Age group	Year	Herds	Total number tested (range per herd)		
				Non-pregnant	Pregnant	Total
Scan-1	R2	Year-1	23	297 (2 - 29)	389 (1 - 24)	686 (1 - 52)
		Year-2	23	76 (1 - 7)	99 (1 - 9)	175 (1 - 14)
		Total	**46**	**373 (1 - 29)**	**488 (1 - 24)**	**861 (1 - 52)**
	MA	Year-1	13	107 (1 - 23)	234 (1 - 39)	341 (2 - 42)
		Year-2	5	8 (1 - 3)	8 (1 - 3)	16 (2 - 6)
		Total	**18**	**115 (1 - 23)**	**242 (1 - 39)**	**357 (2 - 42)**
Scan-2	R2	Year-1	33	189 (1 -22)	314 (1 - 24)	503 (1 - 42)
		Year-2	24	79 (1 - 18)	132 (2 - 10)	211 (1 - 28)
		Total	**57**	**268 (1 - 22)**	**446 (1 - 24)**	**714 (1 - 42)**
	MA	Year-1	19	45 (1 - 6)	173 (10 - 39)	218 (1 - 44)
		Year-2	12	37 (1 - 8)	117 (2 - 10)	154 (1-18)
		Total	**31**	**82 (1 - 8)**	**290 (2 - 39)**	**372 (1 - 44)**

Sera from aborted hinds from all herds were tested. Sera from non-aborted hinds (10 or more per herd) were selected from herds under nil (R2=6 herds, MA=6 herds), low (R2=11 herds, MA=7 herds), medium (R2=6 herds, MA=4 herds), and high (R2=7 herds, MA=5 herds) DAR categories. Therefore, the total sera from non-aborted and aborted hinds tested per herd varied from 2-42 in 63 R2 and 1-44 in 39 MA herds.

4.3.3 ELISA

An immunoglobulin G (IgG) based commercial indirect *T. gondii* ELISA test for small ruminants ('Chekit-Toxotest' IDEXX laboratories, Switzerland) was performed as per manufacturer's recommendations, after validating it for use in deer using Bayesian latent class analyses (Chapter 3). Sera with a S/P(%)of ≥15.5 were considered positive and the term

"serological status" refers to positive or negative at that cut-point. Full details of methodology are presented in Chapter 3.

4.3.4 PCR.

DNA was extracted from myometrium and caruncles pooled together, cotyledon, fetal brain and fetal diaphragm tissue samples, using the DNeasy Tissue Kit (Qiagen, Victoria, Australia) as per the manufacturer's instructions for fresh or frozen tissue samples. Water blanks were included as sample processing controls to confirm the lack of contamination during sample DNA extraction process.

The samples were screened for *T. gondii* using a nested polymerase chain reaction (PCR) procedure described by Aspinall *et al.* (2002) (Appendix 3). To confirm successful amplification 10 µl of the final PCR product was run on a 1.5% agarose gel containing ethidium bromide prior to purification and sequencing. A known *Toxoplasma gondii* isolate (incomplete strain S48, Toxovax®, MSD Animal Health, New Zealand) confirmed by sequencing, was used as a positive control and water blanks were included as negative controls.

4.3.5 Statistical analysis

The results from serology in R2 and MA herds were analysed separately. Analysis was performed using SAS software, version 9.4 (SAS Institute Inc., Cary NC, USA). For analysis purposes, hinds aborting at Scan-1 were considered as 'non-pregnant', and hinds aborting at Scan-2 were considered as aborted, and therefore included in the DAR calculation and analysis.

4.3.5.1 Individual animal level

The dependent variable non-pregnancy (non-pregnant (1) / pregnant (0)) at Scan-1 and aborted (aborted (1) /non-aborted (0)) at Scan-2 were binary responses and resembled a binomial distribution. A logistic model with 'farm' as a random effect was used to model association, between non-pregnancy at Scan-1 and sero-status (positive or negative) at Scan-1 (Eq.1). A similar model was used to test association between aborted status at Scan-2, and sero-status at Scan-1 and Scan-2 at individual animal level. A similar model with *T. gondii* sero-status (negative (0) or positive (1)) as a binary outcome was used to test for difference in animal level sero-prevalence between age groups, islands, and year at both scans. Odds ratios with 95% confidence interval and p-value from the Chi-square's test were reported for animal

level association between non-pregnancy and serological status at Scan-1 and aborted and serological status at Scan-2.

$$Y_{ij} = A + B*\text{sero-status}_{ij} + U_i + E_{ij} \quad \text{(Eq.1)}$$

Where,

Y = Logit-transformed scanning outcome (1/0) of 'i'th hind in 'j'th herd

A = Intercept (average non-pregnancy rate of sero-negative hind)

B = Difference in non-pregnancy rate for sero-positive hinds

U_i = Random effect of 'i'th herd

E_{ij} = Random, uncorrelated error term

The association between presence/absence of haemorrhages (binary variable) in uterus and PCR positivity for *T. gondii* DNA was assessed using logistic regression model. 'Farm' was used as a random effect in the model. The odds ratio and p-value by Chi-square test were reported.

4.3.5.2 Herd-level

Herd-level analysis included herds with ≥10 sera tested per herd for estimation of sero-prevalence at herd level. The dependent variable at Scan-1 was pregnancy rate (%), (number not pregnant at Scan-1/number scanned at Scan-1) whereas DAR was the dependent variable at Scan-2. The association between within-herd non-pregnancy rate at Scan-1 and within-herd sero-prevalence at Scan-1 was assessed using a logistic model (Eq.2). A similar model was used for the association between within-herd DAR and within-herd sero-prevalence. The natural log of between-scan interval was chosen as the offset to model DAR at Scan-2.

$$Y_i = A + B1*\text{sero-prevalence}_i + E_i \quad \text{(Eq.2)}$$

Where,

Y_i = Logit-transformed non-pregnancy rate or DAR of 'i'th herd

A = Intercept (average non-pregnancy rate or average DAR of all herds)

B1 = Regression coefficient estimate for sero-prevalence in each herd at Scan-1 or Scan-2

E_i = random, uncorrelated error term

4.3.5.3 Population attributable fraction (PAF)

PAF is defined as the proportion of disease in the population that is attributable to exposure of risk factor/s. PAF in this study was calculated as the proportion of aborted hinds in the sero-studied population (total number of hinds tested for *T. gondii* antibodies) that was attributable to *T. gondii* exposure. The PAF was calculated in SAS using the Proc STDRATE based on the formula described in equation 3. The confidence intervals for PAF were estimated in SAS using the formula provided by Greenland and Rothman (2008).

$$\mathrm{PAF} = \frac{\mathrm{P} - \mathrm{Po}}{\mathrm{Po}} \qquad (\mathrm{Eq.}\,3)$$

Where,

PAF=Population attributable fraction

P =Proportion of aborted hinds in total sero-studied population

P_o = Proportion of aborted hinds in sero-negative group

4.4 Results

4.4.1 Serology

4.4.1.1 Association with pregnancy at Scan-1

Data for sero-prevalence in hinds form herds with or without aborted R2 and MA hinds are presented in Table 4.2. The sero-prevalence in R2 and MA hinds at Scan-1 was 31.1% and 28.3%, respectively. Combining both age groups, the sero-prevalence was 29.0%. The animal level sero-prevalence at Scan-1 did not differ between R2 and MA hinds (p=0.8), between islands (North vs. South) (p=0.82) or sampling years (year-1 vs year-2) (p=0.15). Within age groups, the animal level sero-prevalence was not different between islands (North vs. South) (p=0.66 in R2 and p=0.08 in MA) or sampling years (year-1 vs year-2) (p=0.08 in R2 and p=0.38 in MA). The hind-level sero-status at Scan-1 was not associated with the non-pregnancy status at Scan-1 in MA hinds (analysed only in year-1, p=0.43) whereas the association was marginally non-significant in R2 hinds (Odds ratio= 1.35, p=0.05, unadjusted model) (Table 4.3). Nine of 27 R2 and six of 16 MA hinds identified as aborting at Scan-1 were seropositive.

Table 4.2: Sero-prevalence of *Toxoplasma gondii* in pregnant and non-pregnant hinds at Scan-1 and aborted and non-aborted hinds at Scan-2 in R2 and MA herds.

Scan	Age group	Herds with or without aborted hinds at Scan-2	No. of herds	% sero-positive (no. tested)[a] hinds		
				Non-pregnant	Pregnant	All
Scan-1	R2	With	36	32.7 (294)	26.9 (379)	29.4 (673)
		Without	10	43.0 (79)	33.0 (109)	37.2 (188)
		Overall	**46**	**34.9 (373)**	**28.3 (488)**	**31.1 (861)**
	MA	With	11	35.3 (85)	28.3 (191)	30.4 (276)
		Without	7	26.7 (30)	17.6 (9/51)	21 (17/81)
		Overall	**18**	**33.0 (115)**	**26.0 (242)**	**28.3 (357)**
Scan-2	R2	With	57	34.3 (268)	24.6 (362)	28.7 (630)
		Without	6	na	19.0 (84)	19.0 (84)
		Overall	**63**	**34.3 (268)**	**23.5 (446)**	**27.6 (714)**
	MA	With	32	32.9 (82)	30.3 (225)	33.6 (307)
		Without	7	na	23.1 (65)	23.1 (65)
		Overall	**39**	**32.9 (82)**	**31.4 (290)**	**31.7 (372)**

na= Not applicable, [a] Those non-pregnant at Scan-2 are those which had aborted since Scan-1.

Table 4.3: Odds ratios and p-value for logistic models based on *Toxoplasma gondii* 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.

T. gondii sero-status association with	Age group	Unadjusted model		Adjusted model	
		Odds ratio	Chi-square p-value	Odds ratio	Chi-square p-value
Non-pregnancy at Scan-1	R2	1.35 (0.99 - 1.81)	0.05	1.33 (0.99 - 1.79)	0.06
	MA	1.25 (0.72 - 2.19)	0.43	1.24 (0.7 - 2.18)[a]	0.46
Aborted by Scan-2	R2	1.6 (1.04 - 2.48)	0.03	1.6 (1.04 - 2.48)	0.03
	MA	0.65 (0.32 - 1.28)	0.21	0.63 (0.31 - 1.27)	0.20

[a] Adjusted by island effect only

At herd-level, data from 33 R2 herds (n=821 hinds) and 10 MA herds (n=330 hinds) with ≥10 sera tested per herd were available for analyses at Scan-1 (Table 4.4). No positives were observed in one R2 herd. The mean within-herd sero-prevalence was 32.9% in R2 herds and 25.5% in MA herds. The within-herd Scan-1 sero-prevalence was not associated with the non-pregnancy% in R2 herds (p=0.37) (Table 4.5). The within-herd mean Scan-1 sero-prevalence was not different between herds with and without hinds aborted as detected at Scan-2 (p=0.22). Also, the mean sero-prevalence did not differ between years (p=0.90) or island (p=0.20).

Table 4.4: Mean, SE and range of within-herd *Toxoplasma gondii* sero-prevalence (%) at Scan-1 and Scan-2 in R2 and MA herds with and without aborted hinds.

Scan	Age group	Herds with or without aborted hinds at Scan-2	Herds	Sero-prevalence (%)		
				Mean	SE	Range
Scan-1	R2	With	25	30.4	4.4	2.4 - 80.0
		Without	7	41.9	10.0	11.8 - 80.0
		Overall	**32**	**32.9**	**4.1**	**2.4 - 80.0**
	MA	With	8	28.1	6.9	5.6 - 69.0
		Without	2	15.0	5.0	10.0 - 20.0
		Overall	**10**	**25.5**	**5.8**	**5.6 - 69.0**
Scan-2	R2	With	21	31.0	4.2	4.5 - 85.7
		Without	5	25.5	7.2	5.0 - 40.0
		Overall	**26**	**30.0**	**3.7**	**4.5 - 85.7**
	MA	With	14	36.5	5.4	7.7 - 66.7
		Without	5	24.0	5.1	10.0 - 40.0
		Overall	**19**	**33.2**	**4.3**	**7.7 - 66.7**

Table 4.5: Beta coefficient estimate and p-value based on unadjusted *Toxoplasma gondii* sero-prevalence, or sero-prevalence controlled by year and island (adjusted), for association between within-herd *T. gondii* sero-prevalence and proportion of hinds not pregnant at Scan-1 and daily abortion rate between Scan-1 and Scan-2.

Within-herd sero-prevalence association with	Age group	No. Herds	Unadjusted model		Adjusted model	
			Logarithmic beta estimate (SE)	P-value	Logarithmic beta estimate (SE)	P-value
Proportion not pregnant at Scan-1	R2	33	0.59 (0.65)	0.37	0.75 (0.67)	0.26
Daily abortion rate by Scan-2	R2	31	2.34 (0.65)	<0.001	2.27 (0.68)	<0.001
	MA	22	2 (1.11)	0.07	1.27 (1.15)	0.27

4.4.1.2 Association with hinds having aborted by Scan-2

Serology was undertaken on Scan-2 samples from 714 and 372 hinds from 63 R2 and 39 MA herds, respectively (Table 4.2). The sero-prevalence in R2 and MA hinds at Scan-2 was 27.6% and 31.7%, respectively. Combining both age groups, the sero-prevalence was 30.3%. The R2 hinds were less likely to be sero-positive than MA hinds (OR=0.65, 95% CI=0.4 - 0.99, p=0.04) in herds with aborted hinds whereas no such difference was observed among R2 and MA hinds in herds without aborted hinds (p=0.66) (Table 4.2). Overall, the animal-level sero-prevalence from herds with and without aborted hinds combined was not different in R2 and MA hinds (p=0.13). Sero-prevalence at individual animal level was not different between years (year-1 vs. year-2) (p=0.48) or island (North vs South) (p=0.86). Within age groups, animal level sero-prevalence did not differ between years (p=0.22 in R2 and p=0.25) or island (North vs South) (p=0.89 in R2 and p=0.69 in MA).

In herds with aborted hinds, the animal-level sero-prevalence at Scan-2 in aborted hinds was significantly higher than in non-aborted hinds in R2 herds (p=0.03) but not in MA herds (p=0.21) (Table 4.2). In R2 herds with aborted hinds, sero-positive hinds at Scan-2 were 1.6 times as likely to have aborted than sero-negative hinds (Table 4.3). At animal-level, Scan-2 sero-prevalence in hinds from herds with aborted hinds was not different than in hinds from herds without aborted hinds in R2 (p=0.64) or MA (p=0.44) age groups.

Paired individual animal data for Scan-1 sero-status and aborted status at Scan-2 were available for 235 R2 and 131 MA hinds which were pregnant at Scan-1 (Table 4.6). Sera from 32.8% R2 and 31% of MA hinds were positive. There was no association between sero-status at Scan-1 and the abortion status at Scan-2 in R2 (p=0.21) or MA (p=0.68) hinds. Paired serology data was available from 26 R2 and six MA hinds from this subset. Sero-conversion was observed in one MA and two R2 hinds. One R2 and one MA sero-positive hind at Scan-1 were sero-negative at Scan-2. Sixteen R2 and four MA hinds were sero-negative at both scans and seven R2 hinds were sero-positive at both scans.

Table 4.6: *Toxoplasma gondii* Scan-1 sero-status of hinds scanned pregnant at Scan-1 and their Scan-2 aborted or non-aborted status.

Age group	Aborted		Non-aborted	
	Sero-negative	Sero-positive	Sero-negative	Sero-positve
R2	18	8	159	50
MA	5	1	95	30

Herd-level analysis was done on 31 R2 (n=624 hinds) and 22 MA (n=328 hinds) herds with ≥10 sera tested per herd at Scan-2. Overall, 26 R2 and 19 MA herds were seropositive. The mean within-herd sero-prevalence was 30.0% in R2 herds and 33.2% in MA herds. The within-herd sero-prevalence at Scan-2 was positively associated with DAR in R2 herds (p=<0.001) but not in MA herds (p=0.07) (Table 4.5). The mean within-herd sero-prevalence was not different between herds with and without aborted hinds in R2 (p=0.29) or MA hinds (p=0.27). The within-herd Scan-1 sero-prevalence was not associated with DAR at Scan-2 (p=0.26) in R2 herds. The mean within-herd sero-prevalence was not different between age groups (p=0.84), years (p=0.57) or island (p=0.80). Within age groups, the herd-level mean sero-prevalence did not differ between years (p=0.94 for R2 and p=0.28 for MA) or island (p=0.84 for R2 and p=0.87 for MA).

In herds with aborted hinds, the population attributable fraction (PAF) for *T. gondii* exposure in R2 hinds tested for sero-status was estimated at 7.9% (95% CI: 1.9 – 13.5). This suggests that 7.9% of the total abortions in herds with aborted hinds could be reduced by preventing the *T. gondii* exposure in R2 hinds.

4.4.2 PCR and relationship with serology in aborting and pregnant hinds

Data from uteri and fetal tissues collected from aborting hinds at Scan-1 and aborted hinds at Scan-2 are presented in Table 4.7.

4.4.2.1 Scan-1

Uteri from 157 R2 and 21 MA non-pregnant hinds were collected after Scan-1. In total, twenty-nine R2 hinds from six herds and three MA hinds from three herds aborting at Scan-1 were tracked to a DSP. Uteri (n=35) were collected from these hinds however, only 23 fetuses were recovered from the uteri as ten R2 and two MA hinds had expelled the fetuses by the time of sampling. Additionally, 19 uteri containing a fetus from nine R2 and 11 MA cull pregnant hinds from four herds and one uterus containing fetal remnants from one R2 hind were collected at a DSP. Diaphragm (n=40) and fetal brain (n=42) were taken from collected fetuses. Diaphragm samples were not collected from fetuses recovered from one aborting R2 hind and one pregnant MA hind due to small fetus size (<26 mm). The fetal crown rump length (CRL) for fetuses recovered from R2 aborting hinds varied from 25mm (45 days) to 410 mm (age of fetus beyond 65mm CRL was indeterminable) and in cull R2 pregnant hinds from 51mm (56 days) to 450 mm at Scan-1. Crown rump length (CRL) varied from 21mm (42 days) to 495 mm in fetuses from cull MA pregnant hinds sampled whereas CRL was 160mm from the one MA aborting hind available at Scan-1.

Overall, uteri from 22.2% of 9 pregnant, 9.6% of 157 non-pregnant and 17.2% of 29 aborting R2 hinds and, 14.3% non-pregnant and 18.2% aborting MA hinds were positive for *T. gondii* DNA (Table 4.7). From R2 hinds, *T. gondii* DNA was detected in fetal brains from 22% of 18 aborting and 11% of nine pregnant hinds and placenta from 17% of 18 aborting hinds and fetal diaphragm samples from one fetus in a pregnant MA hind. Both placenta and uterus sample from one R2 hind were PCR positive (Table 4.7).

Paired serology and tissue PCR data from aborting (n=16), pregnant (n=6), and non-pregnant hinds (n=4) at Scan-1 were available from five R2 herds (Table 4.7). One serum sample from an aborting hind was available from one MA herd. Of 16 aborting R2 hinds, 8

(50%) were sero-positive of which only 2 (12.5%) were *T. gondii* PCR positive. Of six pregnant R2 hinds, two were positive sero-positive of which one was *T. gondii* PCR positive. The only aborting MA hind was sero-negative and negative on PCR.

4.4.2.2 Scan-2

Uteri from 138 R2 and 31 MA hinds having aborted were recovered from DSPs. Aborting fetuses were recovered from uteri of 11 R2 and five MA aborting hinds. Farmers from two R2 herds sent a total of four aborted fetuses from R2 hinds recovered directly from paddocks (Table 4.7). Overall, 16% of 169 uteri, 13.3% of 15 of cotyledons, and 6.7% (1/15) of fetal brain were positive for *T. gondii* DNA. Of note, the PCR positive cotyledon sample from an aborting R2 hind was also sero-positive.

Paired serology and tissue PCR data from aborted hinds (n=94) at Scan-2 were available from 15 R2 and five MA herds (Table 4.7). Overall, 38.3% of 94 were sero-positive. Of the 79 aborting R2 hinds, 30 (38%) were sero-positive of which 5 (6.3%) were *T. gondii PCR* positive. Of the 15 MA aborting hinds, 6 (40%) were sero-positive but none of them were also *T. gondii* PCR positive.

4.4.2.3 Weaning

In total, uteri samples from 10 hinds from one R2 herd and 23 hinds from three MA herds present at weaning without rearing a calf were collected at DSPs. Uteri from one R2 and four MA hinds were *T. gondii* PCR positive.

Table 4.7: Number positive/number PCR tested for *Toxoplasma gondii* 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.

Scan	Age group	Status	Uterus	Cotyledon	Fetal brain	Fetal diaphragm	Placenta	Fetal remnants	Sero-positive
Scan-1	R2	Pregnant	2^{a}/9	1/9	1/9	0/9	0/9	na	2^{a}/6
		Non-pregnant	15/157	na	na	na	na	na	2/4
		Aborting	$5^{b,d}$/29	0/18	4^{c}/18	0/17	3^{b}/18	0/1	$8^{c,d}$/16
	MA	Pregnant	2/11	0/8	0/11	1/10	0/11	na	Na
		Non-pregnant	3/21	na	na	na	na	na	Na
		Aborting	0/3	0/1	0/1	0/1	0/1	na	0/1
Scan-2	R2	Aborted	$22^{e,f,g,h}$/138	1^{i}/10	0/15	1/15	0/11	0/3	$30^{e,f,g,h,i}$/79
	MA	Aborted	5/31	1/5	1/5	0/5	0/5	na	6/15

na = not available; Tissue samples with similar superscript letter (^{a,b,c,d,e,f,g,h,i}) denote sample collection from same hind

4.4.3 Pathology

4.4.3.1 Scan-1

At gross examination of Scan-1 samples, pinpoint, focal and petechial haemorrhages were observed on caruncles and/or entire uterine horn in uteri from 4.5% (7/157) non-pregnant R2 hinds from two R2 herds and 13.8% (4/29) aborting hinds in one herd. However, none of the uteri were positive on PCR for *T. gondii* DNA.

4.4.3.2 Scan-2

At gross examination of Scan-2 samples, pinpoint, focal and petechial haemorrhages (Figure 4-1 and Figure 4-2) were observed on caruncles and/or entire uterine horns in uteri from 49.6% of 137 R2 and 9.6% of 31 MA aborted hinds from 14 R2 and three MA herds, respectively. Additionally, a fetal skull was recovered from a *T. gondii* DNA negative uterus of an aborted sero-negative MA hind indicating fetal resorption. Fifteen (22%) of 68 R2 and two of three MA uteri with haemorrhages were PCR positive. However, there was no association between PCR positivity and presence of haemorrhages on the uteri samples in pooled samples from aborted R2 and MA hinds (OR=2.43, p=0.1).

Toxoplasma gondii DNA negative uteri from three R2 hinds contained moderate to severely mummified fetuses and none of the fetal samples (diaphragm or brain) were PCR positive. Signs of mummification with presence of dark brownish fetal remnants was observed in two MA hinds, one of which had a uterine sample that was PCR positive for *T.*

gondii. The DNA negative uterus with mummified fetal remnants was from sero-negative MA hind.

The microscopic examination of endometrium section from uteri showed insufficient preservation to enable histological evaluation.

4.4.3.3 Weaning

At weaning, four of 23 uteri from MA hinds were observed with haemorrhages on caruncles and one of those was positive on PCR for *T. gondii* DNA. Pus-like fluid was observed in the uterus from one R2 and two MA *T. gondii* PCR negative hinds.

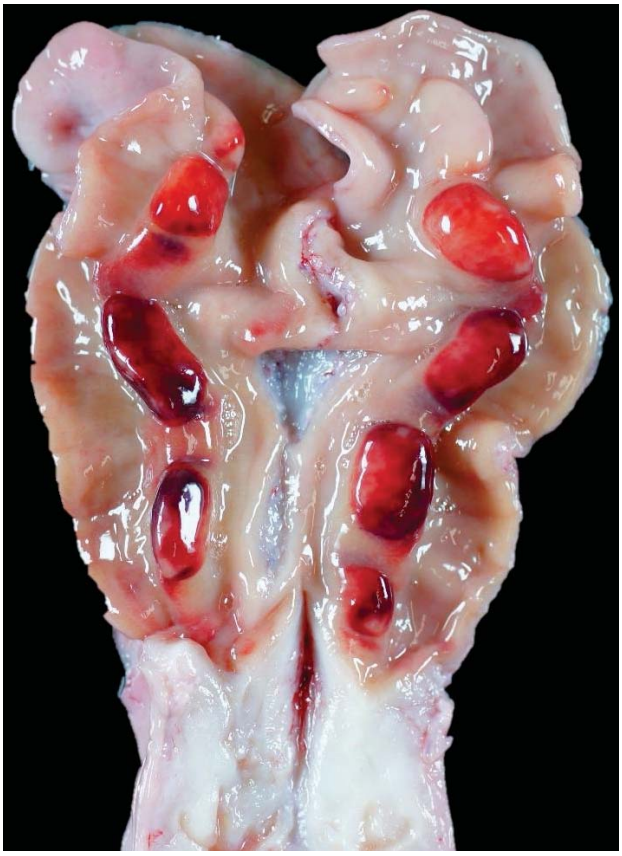

Figure 4-1: Uterus with haemorrhagic caruncles from an aborted *Toxoplasma gondii* DNA negative R2 hind at Scan-2.

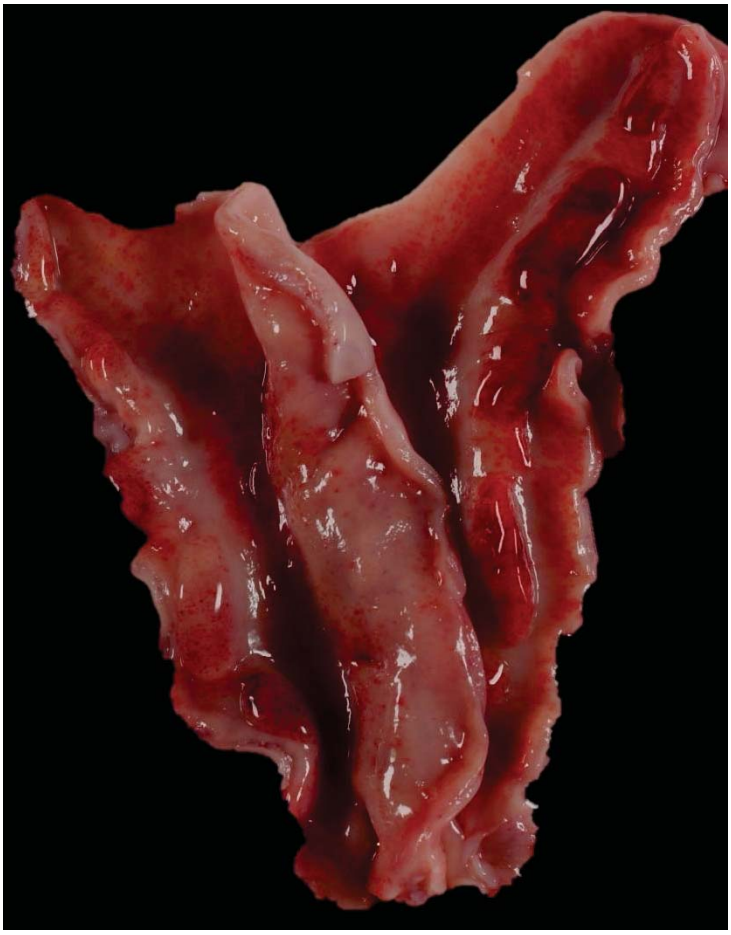

Figure 4-2: Uterus from an aborted *Toxoplasma gondii* DNA positive and sero-positive R2 hind at Scan-2 with presence of pinpoint and petechial haemorrhages on caruncles and uterine floor.

4.5 Discussion

This is the first study to extensively examine the existence of an association between infection with *T. gondii* and both early pregnancy diagnosis and abortion mid-gestation. This study followed a recent clinical investigation of abortion early and mid-gestation by Wilson *et al.* (2012) in farmed deer. The results of the current study showed that there was no association between serological or PCR data and non-pregnancy rate shortly after mating. However, *T. gondii* sero-prevalence in mid-term aborted hinds was significantly higher than in non-aborted hinds, and the odds of sero-positive hinds having aborted were significantly higher than sero-negative hinds having aborted. The association between *T. gondii* and abortion was further supported by the detection of *T. gondii* DNA in fetal tissue samples and uteri from aborting and aborted hinds sent to DSP at Scan-1, Scan-2, and after weaning. This

finding is consistent with the observation of PCR positive results in 8/9 aborting fetuses reported by Wilson et al (2012). Differences in abortion rate between R2 and MA hinds (Chapter 2), and differences in sero-prevalence between age groups suggested here, is consistent with a possible infectious or parasitic agent being involved to which the animal develops an enduring immunity. Taken together, these findings support that *T. gondii* may play a role in the sub-optimum reproductive performance in NZ farmed deer.

Association between non-pregnancy and sero-status at Scan-1 was a secondary objective of this study. Failure to show a significant association between *T. gondii* serology at herd and individual level, or PCR, and pregnancy at Scan-1 suggests that *T. gondii* may not be contributing to sub-optimum pregnancy scan rates. However, the non-significance was marginal with an actual p-value at 0.0503. This implies that the relationship between non-pregnancy and sero-status at Scan-1 in R2 hinds may have been biologically relevant at individual animal level but the significance could not be proven by statistical analysis. The presence of *T. gondii* antibodies in blood and DNA in uteri of non-pregnant R2 and MA hinds suggests that *T. gondii* may be playing a role in sub-optimum pregnancy diagnosis rates. Presence of DNA in uteri from non-pregnant hinds at Scan-1 was consistent with reports in two R2 deer herds by Wilson *et al.* (2012). Additionally, presence of DNA in fetal tissues from aborting and normal fetuses, and antibodies in aborting R2 hinds at Scan-1 indicates trans-placental transmission in pregnant hinds after infection early in gestation. Hence, despite failure to statistically confirm *T. gondii* association, data from this study does conclusively suggest that early fetal loss occurs in New Zealand farmed deer and may be contributing to sub-optimum pregnancy rates on farms, and that *T. gondii* may be a contributory cause. Early abortions in experimental studies in other species such as dairy goats in USA (Dubey 1981b) and sheep in New Zealand (Wilkins *et al.* 1987) have been reported. Further study to quantify early fetal loss, particularly in R2 hinds and its causation is therefore warranted.

The majority of the herds included in the serology at Scan-1 or Scan-2 were sero-positive. Also, the herds selected for serology were situated in different regions of New Zealand. This observation suggests that the organism is ubiquitously present in New Zealand and that the pregnant hinds are at risk of acquiring infections and subsequently abort.

The individual animal level sero-prevalence of 31.1% and 28.3% in R2 and MA hinds at Scan-1 and 27.6% and 31.7% at Scan-2 was not directly comparable with previous studies

reported in deer since different serological tests were used. The latex agglutination test (LAT) used in the previous NZ studies showed a poor test sensitivity (Se) (88.7%) and specificity (Sp) (74.3%) when evaluated using Bayesian latent class analysis (Chapter 3), compared with the ELISA used here, and therefore the prevalence data reported by Reichel *et al.* (1999) of 52.5% and Wilson *et al.* (2012) of 42.5% (Scan-1) and 69.2% (Scan-2) might be an overestimate of the true prevalence, given the low specificity of that test. The ELISA used in this study had a test Se and Sp of 98.8% and 92.8%, respectively, (Chapter 3) and therefore the sero-prevalences reported here are more robust than those reported using the LAT in those studies. Also, the sample selection of two sera per herd by Reichel *et al.* (1999) may not be truly representative of a typical herd compared to the sample selection of ≥10 sera per herd in the majority of the herds here.

A number of reports of *T. gondii* in deer have been reported from other countries. The animal-level sero-prevalence reported in this study of 29.7% for all hinds at both scans was higher than compared with 6.6% of 348 farmed deer in Ireland (Halová *et al.* 2013) using LAT. Sero-prevalence of 24.1% of 552 red and 30.4% of 92 roe wild deer has been reported in Poland (Witkowski *et al.* 2015) and 39.5% of 81 in free-ranging red deer in Italy (Formenti *et al.* 2015) using a commercial indirect ELISA with manufacturer's cut-point of S/P(%) ≥50% for sero-positivity. However, the previous studies using the indirect ELISA could not be compared with this study as the assay in those studies was not validated for its use in deer and the sero-prevalence reported based on manufacturer's cut-point may be an under-estimate of the actual sero-prevalence. Sero-prevalence of 15.6% of 441 in wild red deer (Gauss *et al.* 2006) and 39.2% of 109 in wild roe deer (*Capreolus capreolus)* (Gamarra *et al.* 2008) in Spain, and 30-60% in US in white-tailed deer (*Odocoileus virginianus)* (Humphreys *et al.* 1995; Vanek *et al.* 1996; Dubey *et al.* 2004; Dubey *et al.* 2008c; Ballash *et al.* 2014) have been reported using modified agglutination test (MAT). Using, Sabin-Feldman dye test, *T. gondii* antibodies were detected in wild red deer population of 15% of 303 in Czech Republic (Hejlicek *et al.* 1997), 12% of 99 in Norway (Kapperud 1978), and 12 of 12 pampas deer (*Ozotocerus bezoarticus*) in Uruguay (Puentes and Ungerfeld 2011). Using direct agglutination test, sero-prevalences of 7.7% of 571 in red deer, 34% of 760 in roe deer and 1% of 866 in reindeer (*Rangifer tarandus*) were reported in Norwegian wild population (Vikoren *et al.* 2004). Moreover, another Belgian study carried out in 73 roe deer using ELISA and MAT reported a sero-prevalence of 52.2% using a cut-off of mean plus 3 x

standard deviation (De Craeye *et al.* 2011). However, the sero-prevalence data reported in these studies use different tests not validated for use in deer whereas the ELISA test used in this study was validated specifically for its purpose. Nevertheless, despite lack of direct comparison the global reports from wild and farmed deer suggests ubiquitous presence of *T. gondii* in cervidae.

Serological evidence at herd and individual animal levels from R2 herds at Scan-2 shows that *T. gondii* was significantly associated with abortion. Presence of *T. gondii* antibodies in sera of aborted hinds from this study was consistent with detection of antibodies in hinds with abortion at Scan-2 as reported by Wilson *et al.* (2012). The significantly lower sero-prevalence in R2 hinds, compared with MA hinds, and higher abortion rates likely reflects the immune-naïve status of R2 hinds to *T. gondii* due to limited or delayed exposure. Progressive exposure of older deer would result in a cumulative increase in sero-prevalence. Young hinds are therefore more likely susceptible to acute infection and subsequent abortion. Enduring immunity to *T. gondii* as seen in other species, such as sheep (Buxton *et al.* 1993; Buxton and Innes 1995), would mean the likelihood of abortions due to this organism in MA hinds would be lower than in R2 hinds, as observed in this study.

The lack of sero-prevalence difference between R2 and MA hinds in herds without aborted hinds suggests that hinds from both age group in those herds may have built immunity towards acute infection and therefore acquire the ability to resist abortion. This is consistent with the proposition that exposure and infection in those situations may have been prior to the risk period of pregnancy, and that development of immunity has prevented abortions, as observed in sheep (Hartley 1961; McColgan *et al.* 1988). At herd level, the positive association between sero-prevalence and abortion rate in R2 herds suggests that the higher sero-prevalence to *T. gondii* is linked to higher abortion rates, assuming the exposure is within the period of risk. Hence both epidemiological and immunological patterns support that *T. gondii* is a cause of abortion in New Zealand farmed deer.

The association between *T. gondii* sero-positivity and abortion at Scan-2 was further supported by the presence of DNA in uteri from aborted hinds and aborting fetal tissue. Detection of *T. gondii* DNA in fetal tissues is consistent with reports of detection of *T. gondii* in fetuses during early to mid-pregnancy reported in American white-tailed deer (Dubey *et al.* 2008c; Dubey *et al.* 2014). Also, antibodies to *T. gondii* were detected in a case of an aborted fetus in New Zealand (Orr and Thompson 1993). The detection of *T. gondii* DNA in fetal

tissues from this study and detection of antibodies in fetal heart blood from an aborted fetus submitted to laboratory in New Zealand (Orr and Thompson 1993) suggests that the organism can infect the fetus and cause subsequent abortion in NZ farmed deer. Furthermore, the observation of mummified fetuses, uteri with signs of mummification and fetal remnants provides evidence that abortions are occurring, after Scan-1, and that fetuses are getting completely or partially resorbed before calving. Therefore, losses across the NZ deer industry from scanning to weaning are likely to be at least partly attributable to abortions.

Abortions due to *T. gondii* have been observed in other species. In sheep, *T. gondii* has been isolated from fetal and placental tissues during abortion storms in sheep in New Zealand (Hartley *et al.* 1954), from brain and/or liver of aborted and stillborn lambs in United Kingdom (Beverley and Watson 1961) and from various fetal tissues from aborted and stillborn fetuses in Spain (Hurtado *et al.* 2001; Pereira-Bueno *et al.* 2004) In caprines, *T. gondii* has been reported in three aborted kids and goats in USA (Dubey 1981a). These reports provide further evidence that *T. gondii* can infect pregnant animals and cause abortions in small ruminant livestock.

The majority of abortions reported here were in early to mid-gestation. Abortions due to *T. gondii* in mid-gestation have been reported in sheep (Blewett *et al.* 1982; Buxton *et al.* 1993; Buxton and Henderson 1999). Moreover, reports from these studies together with detection of *T. gondii* in fetuses at mid-pregnancy in white-tailed deer (Dubey *et al.* 2008c) suggest that *T. gondii* can cause abortion in early to mid-gestation, when the immune status of the fetus might not have developed fully as reported in sheep (Salami *et al.* 1985; Buxton and Finlayson 1986).

The population attributable fraction (PAF) is a useful measure in planning and evaluating an intervention, such as vaccination, to reduce abortions due to *T. gondii* exposure. To the Author's knowledge, this is the first report to estimate the proportion of aborted hinds that could be attributable to *T. gondii* exposure. However, more studies will be needed to evaluate the cost and benefits of any possible intervention. PAF for abortions due to exposure of other infectious pathogens have been reported in cattle. They include *N. caninum* (3% in New Zealand and 12.5% in UK) (Davison *et al.* 1999; Sanhueza *et al.* 2013), bovine viral diarrhoea virus (3.5%) and *Leptospira* spp serovars; Hardjobovis (4.7%) and Pomona (3.6%) in New Zealand (Sanhueza *et al.* 2013). The PAF reported in deer in this study and in cattle from above studies suggest that infectious agents play an important role in abortions. Also,

the PAF reported in this study was for R2 hinds at Scan-2 at which a significant association was observed between sero-status and aborted status in R2 hinds. Additionally, the observations of early fetal losses coupled with the presence of *T. gondii* DNA in aborting maternal and fetal tissues and antibodies in serum at Scan-1 suggest that the true PAF may be higher than that observed here.

Haemorrhages observed in the uteri, especially in caruncles, of aborting (Scan-1) and aborted (Scan-2) hinds may be associated with abortion. Typical necrotic lesions on placenta and cotyledons with white patches due to *T. gondii* have been observed in aborting ewes at mid-gestation (Hartley *et al.* 1954; Buxton and Finlayson 1986). The presence of *T. gondii* DNA in uteri of hinds at weaning that failed to rear a calf suggests that those hinds might have aborted before calving.

The findings from this study at both scans suggest that *T. gondii* can infect pregnant hinds at early and mid-gestation and can cause abortions. Therefore, the sub-optimal reproductive performance in farmed red deer in New Zealand may be partly due to *T. gondii* infections. However, further studies will be needed to accurately establish the causal relationship between *T. gondii* and abortion by challenging the pregnant R2 hinds with *T. gondii* and monitoring the fate of pregnancies. However, that would be logistically difficult to undertake.

4.6 Conclusion

There was marginal statistical evidence for reduction in pregnancy rates associated with *T. gondii*, but the observation of early abortions and detection of *T. gondii* DNA from aborting fetal and maternal tissues, corroborated by antibody in maternal blood suggests that at least some pregnancy losses may be attributed to *T. gondii* infections. The serological, molecular and pathology findings at Scan-2 from this study provide additional evidence that *T. gondii* is a cause of early and mid-gestation abortions in R2 hinds, and potentially in MA hinds that are first exposed to the organism at the time of pregnancy. The abortions attributable to *T. gondii* exposure in R2 hinds was 7.9%. These findings suggest that *T. gondii* may play a role in sub-optimum reproductive performance in NZ farmed deer.

4.7 Acknowledgements

This study was supported by the Deer Reproductive Efficiency Group based in Southland. It is funded by AgResearch, Agmardt, DEEResearch, MSD Animal Health,

Southland Deer Farmers Association, Massey University and individual farmers, particularly Landcorp Farming Ltd. The in-kind contribution of all participating farmers is gratefully acknowledged, as is the assistance of a large number of veterinary practices and scanners for scanning and blood sample collection and DSP staff and veterinarians for tissue sample collection. I am also thankful to the IVABS technical team and visiting veterinary science students for their assistance in laboratory work, and the staff at the post-mortem facility to allow dissection of maternal and fetal tissues in the post-mortem premises.

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Chapter 5. Investigation of association between *Leptospira* spp. serovars Hardjobovis and Pomona and *Neospora caninum*, and pregnancy and abortion in New Zealand farmed deer

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This chapter is prepared in the style format of the New Zealand Veterinary Journal.

5.1 Abstract

Sub-optimum pregnancy rates and abortion contribute to reproductive inefficiency in the New Zealand farmed deer industry. This study investigated association between *Leptospira* serovars Hardjobovis and Pomona and *Neospora caninum,* in pregnancy and mid-term abortion in farmed New Zealand red deer.

Rising two-year-old (R2, n=22,130) and mixed-age (MA, n= 36,223) hinds from 87 and 71 herds, respectively, throughout NZ were ultrasound scanned early in gestation (Scan-1) and a sub-sample of up to 100 R2 and 155 MA hinds/herd were re-scanned (Scan-2) between 55 and 89 days later, at approximately the end of the second trimester. A daily abortion rate (DAR) was calculated. Blood from 21 pregnant and non-pregnant R2 and MA hinds at both scans were collected, along with blood from hinds observed aborting at the time of scanning. For *Leptospira* spp. serology, hinds from herds with known vaccination status were excluded. A sub-sample from herds with and without aborted hinds, and all aborting hinds were tested for serovar Hardjobovis and Pomona antibodies on modified agglutination test (MAT) using a cut-point titre of 1:48. Sera from all aborting hinds were tested on a commercial *N. caninum* ELISA using the manufacturer's cut-point S/P(%) of 40 and positives were tested on Western blot assay for confirmation. The low sero-prevalence in aborted hinds did not justify testing of non-aborted hinds for *N. caninum* antibodies. Fetuses and uteri from hinds observed aborting at both scans, non-pregnant uteri after Scan-1 and 2, uteri from normal pregnancies at Scan-1, and uteri from hinds not rearing a calf to weaning were collected at deer slaughter premises and tested for *Leptospira* spp. and *N. caninum* DNA using a real-time PCR. Associations were analysed between sero-status and having aborted hinds, at individual animal level, and sero-prevalence and having aborted hinds at herd level.

At Scan-1, 47.5% of 446 R2 and 37.7% of 215 MA hinds were sero-positive for Hardjobovis and 1.9% of 415 R2 and 9.5% of 232 MA hinds were sero-positive for Pomona. There was no association between Scan-1 Hardjobovis (R2 p=0.23, MA p=0.25) or Pomona (R2 p=0.61, MA p =0.45) sero-status and non-pregnancy at animal level. In sero-positive hinds at Scan-1, the geometric mean titre (GMT) was higher for Pomona in pregnant hinds than non-pregnant hinds in MA (p=0.015) but not in R2 hinds (p=0.2). For Hardjobovis at Scan-1, GMT was similar in non-pregnant and pregnant hinds (R2 p=0.24 and MA p=0.39). At Scan-2 in herds with aborted hinds, 37.2% of 1,306 R2 and 44.7% of 936 MA hinds were sero-positive for Hardjobovis and 5.4% of 1,308 R2 and 9.5% of 935 MA hinds were sero-

positive for Pomona. In herds with aborted hinds, there was no association between Scan-2 Hardjobovis (R2 p=0.91, MA p=0.9) or Pomona (R2 p=0.85, MA p=0.61) sero-status and aborted status at animal level. The within-herd sero prevalence of Hardjobovis (R2 p=0.33, MA p=0.2) and Pomona (R2 p=0.42, MA p=0.56) at Scan-2 was not associated with DAR in R2 or MA herds. R2 and MA hinds from herds with aborted hinds were less ($p<0.001$) and more ($p<0.001$), respectively, likely to be sero-positive for Hardjobovis than hinds from herds without aborted hinds. In herds with aborted hinds, the Hardjobovis GMT was higher in non-aborted hinds than in aborted hinds for (p=0.018) in MA hinds and for Pomona in R2 hinds (p=0.006). No fetal kidney, fetal aqueous humor, or uterine DNA samples were positive for leptospiral DNA.

The *N. caninum* sero-prevalence in aborted hinds was 0.4% of 267 in R2 hinds and 1.2% of 81 in MA hinds based on sera verified on WB and after excluding sera positive for both *N. caninum* and *T. gondii*. *Neospora caninum* DNA was detected in cotyledon and fetal diaphragm from an aborting R2 and a pregnant MA hind at Scan-1 whereas at Scan-2, DNA was detected in uteri, fetal brains or cotyledons from nine R2 aborted fetues and uterus sample from a MA aborted hind. Additionally, *N. caninum* DNA was detected in the uterus of 1 of 22 MA hinds that did not rear a live calf at weaning.

These findings suggest that *Leptospira* Hardjobovis and Pomona are not associated with sub-optimum pregnancy rate. Most of the evidence presented supports that these serovars appear not to play a major role in mid-term abortion in deer. The finding of *N. caninum* DNA from fetal and maternal tissues suggest that despite the apparently low sero-prevalence, *N. caninum* can infect pregnant hinds and may be a cause of abortion.

KEY WORDS: *Red deer, ultrasound scanning, pregnancy, non-pregnancy, abortion,* Leptospira borgpetersenii *serovar Hardjobovis*, Leptospira interrogans *serovar Pomona*, Neospora caninum, Toxoplasma gondii, *uterus, fetus, aqueous humor, kidney, modified agglutination test, ELISA, real-time PCR.*

5.2 Introduction

Reproductive performance in rising two-year-old (R2) and mixed-aged (MA) adult hinds has been suboptimal in New Zealand farmed deer (Asher and Pearse 2002; Asher 2003; Asher and Wilson 2011). Reproductive efficiency (calves weaned/hinds mated) in last decade has averaged 75.2% in last 13 years (Statistics New Zealand 2016a). Inefficiency arises from failure to conceive, fetal loss, stillbirth, and postnatal mortality. Based on early scanning, pregnancy rates of R2 and MA hinds in this study were 85.8% and 93.3%, respectively (Chapter 2).

Causes and risk factors for early pregnancy failure in both R2 and MA hinds have been studied previously (Audigé *et al.* 1999b; Audigé *et al.* 1999c; Asher *et al.* 2005a; Asher *et al.* 2011) but *Leptospira* spp. and *Neospora* were not included in those studies. Pregnancy loss due to abortions in New Zealand deer has been reported in a few studies (Fennessy *et al.* 1986; Audigé *et al.* 1999a; Campbell *et al.* 2000; Wilson *et al.* 2012). However, the most substantial data available is presented in Chapter 2 in which the mid-term herd-level mean daily abortion rates were 0.043% and 0.025% in R2 and MA herds, respectively, which calculates to 3.9% in R2 and 2.2% in MA herds for 90 mid-term gestation days. Hence abortions can pose a significant impact on deer farmers and the deer industry in New Zealand in terms of lost opportunities and lower financial returns.

Abortion can be attributed to non-infectious and infectious causes. Risk factors for non-infectious causes, as in other species such as cattle and sheep, can include farm management, nutrition, health and environmental conditions prevailing in herd or farms (Bartels *et al.* 1999; Fthenakis *et al.* 2012) and are addressed later in Chapter 7. Of note, feeding of autumn crops was positively related to pregnancy at Scan-1 whereas Johne's disease (JD) occurrence, co-grazing of hinds with beef cattle and hay feeding was positively related to abortion by Scan-2 (Chapter 7).

Pathogens known to cause abortions in cattle, sheep and other small ruminants, including *Toxoplasma gondii*, *Leptospira* spp, *Neospora caninum*, herpesvirus infection, and bovine viral diarrhoea virus (BVDv) have been reported in deer and have been reviewed in Chapter 1. *Toxoplasma gondii* was detected in 8/9 fetal brains recovered from aborting R2 hinds in a clinical investigation in a herd experiencing a 1% early and 7.2% mid-term abortion rate, with serological evidence of *T. gondii* exposure (Wilson *et al.* 2012). In addition, *T. gondii* was found to be positively associated with abortions with detection of *T.*

gondii antibodies in blood samples and DNA from uteri and fetal material from non-pregnant and aborted hinds (Chapter 4). Other infectious abortifacients such as *Leptospira* spp. in farmed deer and *N. caninum* in cattle and sheep have been reported in New Zealand.

Leptospira spp. infection caused by *Leptospira borgpetersenii* serovar Hardjobovis and *Leptospira interrogans* serovar Pomona is widely prevalent in New Zealand deer population (Flint *et al.* 1988; Wilson *et al.* 1998; Ayanegui-Alcérreca *et al.* 2010) and is also known for causing reproductive losses (Grooms 2006). *Leptospira* sero-prevalence has been negatively associated with reproductive performance in cattle (Dhaliwal *et al.* 1996) and pigs (Ramos *et al.* 2006). Leptospires have been demonstrated in the reproductive organs of cattle (Ellis and Thiermann 1986; Ellis 1994), pig (Ellis *et al.* 1985), sheep and goats (Lilenbaum *et al.* 2008). They have also been associated with early embryonic death, abortions, and stillbirths in cattle (Smyth *et al.* 1999) and pigs (Kazami *et al.* 2002). Leptospires were isolated from aborted fetuses in cattle in USA and Brazil (Ellis *et al.* 1982b; Langoni *et al.* 1999) and pigs (Ellis *et al.* 1985). Leptospiral abortion has been demonstrated in an experimental challenge study in white-tailed deer *(Odocoileus virginianus)* in North America wherein leptospires from fetal kidney were detected through real-time PCR assay (Trainer *et al.* 1961).

Leptospira spp. is prevalent on New Zealand deer farms with individual animal sero-prevalence up to 60.8% for Hardjobovis and 8.4% for Pomona and a herd-level sero-prevalence up to 78% for Hardjobovis and 20% for Pomona have been reported (Reichel *et al.* 1999; Ayanegui-Alcérreca *et al.* 2010). Studies in New Zealand have shown that vaccination against *Leptospira* improved weaning percentage by six (Subharat *et al.* 2011b) and nine (Ayanegui-Alcerreca 2006) percentage points but whether the effect was due to abortion or perinatal/postnatal mortality was not determined. More recently, *Leptospira* spp. DNA was detected in one of 27 fetal tissue samples in an attempt to isolate *Leptospira* spp. from hinds and normal *in-utero* fetuses from slaughtered hinds in New Zealand (Subharat *et al.* 2010). Taken together, these findings from cattle, pig and deer studies suggest that *Leptospira* spp. can reach the fetus through vertical transmission upon infection in pregnant cattle, pig or deer species. However, there has been no investigation of *Leptospira* spp. and pregnancy rate *per se.*

Neospora caninum, an apicomplexan parasite first isolated from Norwegian dogs associated with encephalomyelitis and myositis (Bjerkås *et al.* 1984) is known for causing

abortions in cattle and its congenital transmission has been documented (Dubey *et al.* 1996; Dubey 2005; Dubey *et al.* 2007; Heuer *et al.* 2007; Dubey *et al.* 2013). Typical *N. caninum* type lesions have been demonstrated in fetal brains in aborted New Zealand cattle (Thornton *et al.* 1991) and DNA has been detected in uteri of heifers experimentally infected with *N. caninum* contaminated semen in Spain (Serrano *et al.* 2006). In sheep, *N. caninum* has been indicated in abortion cases wherein DNA was detected in aborted fetal tissues with maternal serological evidence in New Zealand (Howe *et al.* 2008; Howe *et al.* 2012). The experimental studies in sheep and cattle suggest that *N. caninum* can infect pregnant cows and sheep and can cause abortions (Innes *et al.* 2002).

Moreover, *Neospora caninum* has been reported in black-tailed deer (*Odocoileus hemionus columbianus)* (Woods *et al.* 1994) and white-tailed deer (Gondim *et al.* 2004) in the USA. Sero-prevalence ranging from 40% to 48% has been documented in white-tailed deer in North America (Dubey *et al.* 1999; Lindsay *et al.* 2002b). In New Zealand deer, lesions similar to *N. caninum* infection were reported in two deer calves that aborted one month before term (Orr and Thompson 1993). These observations suggest that deer can acquire *N. caninum* infection, but no case of clinical neosporosis or its effect on reproduction (pregnancy or abortions) have been documented in New Zealand farmed deer.

This study was undertaken on farms and animals described in Chapter 2, to determine if these is an association between *Leptospira* spp. (serovars Hardjobovis and Pomona) sero-positivity and non-pregnancy at scanning early in gestation (Scan-1), and *Leptospira* spp. and *N. caninum* sero-positivity and mid-term abortion as determined by repeat scanning in red deer hinds. Tissue samples from hinds and fetuses were also tested for *Leptospira* spp. and *N. caninum* DNA.

5.3 Materials and methods

The sampling frame for farms and animals used for this analysis is described in detail in Chapter 2. Briefly, 22,130 R2 and 36,223 MA red deer hinds from 87 and 71 herds, respectively, throughout New Zealand were ultrasound scanned for pregnancy early in gestation (Scan-1), and a sub-sample was scanned again at approximately the end of the second trimester (Scan-2) during the 2011 and 2012 reproductive cycles. Blood, uteri,

aborted and normal fetuses were collected. All animal manipulations were approved by the Massey University Animal Ethics committee (Protocol numer: 12/34).

Hinds were classified as being 'pregnant' based on the presence of at least one fetus or part thereof, amniotic membrane, and/or presence of placentomes, or as being 'non-pregnant' based on absence of those signs combined with visualisation of a non-pregnant uterus. The term "aborting" was ascribed to hinds that had ultrasound evidence of aborting fetuses at Scan-1 and Scan-2. The term "aborted", used for calculating the DAR, was ascribed to hinds that were pregnant at Scan-1 but not pregnant at Scan-2, plus those aborting at Scan-2. Daily abortion rate (DAR) ((number aborted at Scan-2 / number scanned at Scan-2) / number of days between Scan-1 and Scan-2) was calculated to account for differences in time between scans. This approximated a mid-trimester abortion rate. For analysis purposes, hinds aborting at Scan-1 were considered as 'non-pregnant', and hinds aborting at Scan-2 were considered as aborted, and therefore included in the DAR calculation.

5.3.1 Sample collection and handling

Blood samples were collected from 21 randomly selected pregnant, non-pregnant, and all available aborting hinds per herd at the time of Scan-1 from late May to mid-August and from up to 21 aborted hinds as available and 21 non-aborted hinds per herd in mid-September to mid-October during Scan-2. Samples were collected by jugular venipuncture into 10 ml vacuum blood collection tubes without anticoagulant, and transported chilled to Massey University where they were centrifuged at 1,512 × g for 15 min and serum withdrawn and stored at -20°C. A 30 µL aliquot from each serum sample diluted in 150 µL of normal saline solution was placed on a 96-well plate for *Leptospira* spp. serology and was stored at -20°C until tested. In total, 4,480 and 2,932 blood samples were collected from Scan-1 and Scan-2, respectively, to provide the sample pool for selection for serological analyses.

Non-pregnant and aborting hinds at Scan-1, aborted hinds at Scan-2, and pregnant hinds at Scan-1, as available, were tracked to deer slaughter premises (DSP) where whole reproductive tracts from the posterior cervix were collected by the Author or the DSP veterinarian. Additionally, reproductive tracts from hinds that did not rear a calf to weaning were also collected at DSPs. Samples were kept in a chiller/freezer and then sent chilled or frozen to Massey University where they were processed at the post-mortem facility. Gross observations from uteri and fetal tissues were recorded at dissection. Uteri, placental,

cotyledon, and fetal samples were dissected and stored at -20°C (fresh specimens) for PCR, and in 10% formalin for histology.

5.3.2 Serology

5.3.2.1 Leptospira *spp. serology*

Samples from herds with and without abortion were tested for *Leptospira* spp. as shown in Table 1.

Herds with a history of recent vaccination against *Leptospira* spp. were excluded. *Leptospira* spp. serology at Scan-1 was carried out on request by some deer farmers. Since all serum samples had been placed into 96-well plates upon receipt in the laboratory, all sera on the plate were tested if the plate contained sera samples from farms requesting *Leptospira* spp. serology or if the plate was subsequently found to contain sera from herds with aborted hinds.

Sera from aborted and non-aborted hinds from all herds with aborted hinds were tested for *Leptospira* serovars Hardjobovis and Pomona. In total, 446 and 215 sera from 13 R2 and seven MA herds, respectively, at Scan-1 and 1,306 and 936 sera from 57 R2 and 44 MA herds, respectively, at Scan-2 were tested for Hardjobovis. For Pomona, 415 and 232 sera from 12 R2 and 8 MA herds, respectively, at Scan-1 and 1,308 and 935 sera from 57 R2 and 44 MA herds at Scan-2 were tested (Table 5.1). Due to insufficient volume of sera, five sera from aborted hinds, one each from one R2 and four MA herds were not tested for Hardjobovis whereas sera from one aborted MA hind from one herd and four aborted MA hinds from another herd were not tested for Pomona.

Table 5.1. Summary of total number (and range per herd) of sera tested for *Leptospira borgpetersenii* serovar Hardjobovis and *Leptospira interrogans* serovar Pomona from pregnant and non-pregnant hinds at Scan-1 and from aborted and non-aborted hinds at Scan-2.

Scan, serovar	Age group	Herds with or without aborted hinds at Scan-2	Herds	Total number tested (range per herd)		
				Non-pregnant	Pregnant	Total
Scan-1, Hardjobovis	R2	With	8	110 (1 - 27)	162 (14 - 23)	272 (15 - 47)
		Without	5	69 (5 - 21)	105 (21)	174 (26 - 42)
		Total	**13**	**179 (1 -27)**	**267 (14 - 23)**	**446 (15 - 47)**
	MA	With	3	50 (14 - 21)	64 (21)	114 (35 - 43)
		Without	4	32 (0 - 21)	69 (6 - 21)	101 (6 - 42)
		Total	**7**	**82 (0 - 21)**	**133 (6 - 22)**	**215 (6 - 43)**
Scan-2, Hardjobovis	R2	With	44	190 (1 - 20)	845 (9 - 24)	1,035 (8 - 41)
		Without	13	na	271 (20 - 21)	271 (20 - 21)
		Total	**57**	**190 (1 - 20)**	**1,116 (9 - 24)**	**1,306 (8 - 41)**
	MA	With	28	65 (1 - 6)	546 (13 - 24)	611 (5 - 26)
		Without	16	na	325 (7 - 26)	325 (7 - 26)
		Total	**44**	**65 (1 - 6)**	**871 (7 - 26)**	**936 (5 - 26)**
Scan-1, Pomona	R2	With	7	101 (1 - 27)	140 (14 - 23)	241 (15 - 47)
		Without	5	69 (5 - 21)	105 (21)	174 (26 - 42)
		Total	**12**	**170 (1 - 27)**	**245 (14 - 23)**	**415 (15 - 47)**
	MA	With	4	51 (1 - 21)	80 (16 - 22)	131 (17 - 43)
		Without	4	32 (0 - 21)	69 (6 - 21)	101 (6 - 42)
		Total	**8**	**83 (0 - 21)**	**149 (6 - 22)**	**232 (6 - 43)**
Scan-2, Pomona	R2	With	44	191 (1 - 20)	846 (9 - 24)	1,037 (8 - 41)
		Without	13	na	271 (20 - 21)	271 (20 - 21)
		Total	**57**	**191 (1 - 20)**	**1,117 (9 - 24)**	**1,308 (8 - 41)**
	MA	With	28	64 (1 - 6)	546 (13 - 24)	610 (5 - 26)
		Without	16	na	325 (7 - 26)	325 (7 - 26)
		Total	**44**	**64 (1 - 6)**	**871 (7 - 26)**	**935 (5 - 26)**

The sample selection for Scan-1 serology included sera from seven R2 and one MA aborting hind.

5.3.2.2 Leptospira *spp. Microscopic agglutination test (MAT)*

Antibodies to Hardjobovis and Pomona were detected using a MAT as described by Fang *et al.* (2014) based on method reported earlier by Faine (1982). Briefly, each serum sample was diluted two-fold in 0.9% saline from 1: 24 to 1:3072. The sera samples were then incubated for a period of 1.5 to 4 hours with live cultures of each serovar. Standard *L. borgpetersenii* serovar Hardjobovis and *L. interrogans* serovar Pomona antisera were obtained from the WHO/FAO/OIE Leptospirosis Reference Laboratory in Brisbane, Australia. Serovars Hardjobovis and Pomona were hamster-passaged strains that were provided by Schering-Plough, Wellington, New Zealand. The test was considered sero-positive for the dilution at which 50% or more of leptospires were agglutinated or lysed. For

analysis purposes, serum samples with ≥50% of agglutination or lysis at a dilution of ≥1:48 were considered sero-positive, for both serovars.

5.3.2.3 Neospora caninum *Indirect ELISA*

All available aborted hinds were tested with an immunoglobulin G (IgG) based commercial indirect *Neospora caninum* ELISA test for small ruminants ('Chekit' IDEXX laboratories, Switzerland) as per manufacturer's recommendations to assess for serological evidence of *N. caninum* exposure. Briefly, this assay detects *N. canunim* antibodies using *N. caninum* antigen with anti-ruminant IgG conjugates. Sera with a S/P(%) of ≥40 were considered positive and the term "serological status" refers to positive or negative at that cut-point. Based on the low prevalence of *N. caninum* antibodies in aborted hinds, no sera were tested from non-aborted hinds.

5.3.2.4 Neospora caninum *Western blot assay (WB)*

A Western blot assay was used as a confirmatory test for positive ELISA serum samples as described by Syed-Hussain *et al.* (2013). Briefly, water-soluble *N. caninum* antigen was prepared using inocula from a *N. caninum* isolate (NcNZ1) maintained by regular passages in Vero cells. Tachyzoites were harvested, suspended in PBS (pH=7.4) and pelleted by centrifuged at 1200 x g for 10 minutes. The pelleted tachyzoites were re-suspended in 2ml PBS (pH 7.4) and were strained through a 5µm filter for removal of any debris. The tachyzoites were then re-centrifuged, supernatant removed and the resultant pellet was stored at -20^{o}C. The stored pelleted tachyzoites were re-suspended in 5 ml distilled water and subjected to disruption by thrice cycling of freezing/thawing from room temperatures to -80^{o}C. This was followed by 15 cycles of sonication on ice (Sonics VibracellTM, Sonics & Materials Inc., CT, USA). The sonicated tachyzoites were centrifuged at 10,000 x g for 30 minutes at 4^{o}C to remove debris and the supernatant containing the water soluble proteins were collected. The supernatant solution was stored in aliquots after assessing the protein content using a Nano-spectrophotometer (NanoDrop ND-1000, Thermo Scientific, DE, USA).

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot analysis was carried out using the method adapted from Syed-Hussain *et al.* (2013) as described by Okeoma *et al.* (2004b) and Okeoma *et al.* (2004a). Prepared antigen (0.34 µg/µl) was denatured using 2X lamelli buffer (Sigma, St. Louis, USA) containing 4% SDS, 20% glycerol, 10% 2-mercaptoethanol, 0.004% bromophenol blue and 0.125M tris

HCL by heating at 100ºC for 10 minutes. The denatured protein was run through a 12.5% Tris-HCL gel (Criterion, Bio-Rad, California, USA) for 90 minutes at 100 volts. Each Western blot was run with a broad range (10-250 kilodaltons (kD)) protein marker (Precision Plus Protein™, Bio-Rad), a negative and positive control serum. After separation, the proteins were electrophoretically transferred on to polyvinylidene difluoride (PVDF) membrane (Bio-Rad) for 45 minutes at 70 volts. After confirmation of complete protein transfer through Ponceau S (Sigma) staining, membranes were blocked overnight at 4ºC using 5% blotting solution (5g skim milk powder) with PBS with 0.1%Tween-20 (PBS-T, Sigma). Blocked membrane was cut into strips and incubated with test sera, diluted 1:50 in 5% blotting solution with PBS-T, at room temperature for 60 minutes. After primary incubation with sera samples, strips were washed three times for 10 minutes using PBS-T. Secondary antibody, rabbit anti-deer antibody conjugated to horseradish peroxidase (HRP) (KPL, Maryland, USA), diluted 1:9,000 in 5% blotting solution with PBS-T, was added to each strip except positive control and incubated for 60 minutes at room temperature. Rabbit anti-sheep IgG: HRP (Santa Cruz Biotechnology, CA, USA) at 1:10,000 dilution in 5% blotting solution was added to positive control and incubated at room temperature as above. The strips were washed three times as described above for wash after primary incubation and then incubated at room temperature (22^0C) for five minutes in chemiluminiscent ECL solution (Amersham™ ECL Select™ Western Blotting Detection Reagent, GE Healthcare UK Limited, Buckinghamshire, UK) and arranged on a transparent plastic film. The film was exposed for 30 seconds in LAS-1000 plus luminescent image analyser (Fujifilm, Japan) and the bands were measured against the standard markers. Presence of bands indicating immunodominant antigens (IDA) of *N. caninum* were recorded. For this study, sera that recognised one or more IDA with molecular weight (MW) between 15 to 40 kD were considered positive for *N. caninum* antibodies. The positive control serum used in the study was from a naturally infected ewe with *N. caninum* and showed consistent bands at 17, 25-29, and 33-37 kD (Alvarez-Garcia *et al.* 2003) and thus acted as an internal control loading and antibody probing.

5.3.3 Molecular diagnostics

5.3.3.1 Leptospira *spp. real-time PCR (qPCR)*

The number of samples tested for leptospiral DNA are presented in Table 5.2.

Samples from fetal kidneys, aqueous humor, and uteri from aborting and/or pregnant R2 and/or MA hinds at Scan-1 and Scan-2 were tested for the presence of *Leptospira* spp. DNA by the Leptospirosis Research Laboratory, Hopkirk Research Institute, Massey University. Briefly, DNA was extracted from tissue samples using the DNeasy Tissue Kit (Qiagen, Victoria, Australia) as per the manufacturer's instructions for fresh or frozen tissue samples. Water blanks were included as sample processing controls to confirm the lack of contamination during the sample testing process. The DNA sample extracts were kept frozen at -20^{o}C until tested.

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.

Scan	Age group	Status	Uterus	Fetal kidney	Fetal aqueous humor
1	R2	Aborting	6	17	17
		Pregnant	3	9	9
	MA	Aborting	1	1	1
		Pregnant	0	10	10
2	R2	Aborted	4	15	12
	MA	Aborted	0	5	5

A real-time PCR (qPCR) was run based on the method adapted from study by Fang *et al.* (2014). The qPCR reaction solution consisted of 5 pM of primers to target the gyrB gene (Slack *et al.* 2006), of sequence 5'-TGAGCCAAGAAGAAACAAGCTACA-3' (2For) and 5'-MATGGTTCCRCTTTCCGAAGA-3' (504Rev), 2.4 µM of SYTO9 (Invitrogen Corp., Carlsbad, CA, USA), 12.5 µL of a commercial mastermix (Roche LightCycler 480 Probes Master 04707494001, Roche Diagnostics GmBH, Mannheim, Germany), 2 µL of the DNA preparation and double distilled water towards a total volume of 25 µL. The qPCR assay was run on a Rotor-Gene Q (Qiagen, Bio-Strategy Ltd, Auckland, New Zealand). The initial denaturation of 10 minutes at 95°C was followed by 40 cycles, consisting of 10 seconds of denaturation at 95°C, 20 seconds of annealing at 63°C and 10 seconds of elongation at 72°C. The melting temperature was measured every 0.2°C from 78-90°C by monitoring the fluorescence on the green channel. DNA extracted from sheep urine inoculated with a live culture of a New Zealand strain of *L. borgpetersenii* serovar Hardjobovis was used as a positive control while a negative control of double distilled water was used for each qPCR run. A sample was considered positive if the melting temperature of sample was similar to that of the positive control.

5.3.3.2 Neospora caninum *qPCR*

Tissues samples from fetal brain, diaphragm, and remnants, and uteri, cotyledon, and placenta from aborting and/or pregnant R2 and/or MA hinds at Scan-1 and Scan-2 underwent DNA extraction as above and were tested by qPCR. Uteri samples from R2 and MA hinds present at weaning without rearing a calf were collected at DSPs and were tested by qPCR.

The primers NeoF (5′-GTGAGAGGTGGGATACG-3′) and NeoR (5′-GTCCGCTTGCTCCCTA-3′) were used to amplify the Nc-5 gene (Kaufmann *et al.* 1996). The reaction mixture contained 2µl of DNA sample, 1x PCR buffer, 1.5 mM MgCl2, 0.2 mM dNTP, 0.1 µM primer NeoF, 0.15 µM primer NeoR, SYTO9 and 1 U of Platinum Taq DNA polymerase (Invitrogen) towards a 25µl of total volume. The reaction conditions were optimised for primers as described by Syed-Hussain *et al.* (2013). The reaction volume was subjected to the following cycling conditions: 95°C for 10 minutes; 45 cycles of 94°C for 10 seconds (s), 65°C for 10 seconds and 72°C for 20 seconds. The melting temperature was measured every 0.2°C from 80-90°C by monitoring the fluorescence on the green channel and were visualized on Rotor-Gene Q (Qiagen). The positive control consisted of *N. caninum* DNA extracted from cell-culture whereas the negative control consisted of distilled water and were used in each run. A DNA sample was considered positive if the sample melt curve coincided with the melt curve of the *N. caninum* positive control. See attached Appendix 4 for detailed protocol for *N. caninum*.

5.3.4 Statistical analysis

Statistical analysis was only undertaken for *Leptospira* spp. serology results since there were insufficient numbers of positives found for *N. caninum* serology. The results from *Leptospira* spp. serology for each serovar in R2 and MA herds were analysed separately. Analysis was performed using SAS software, version 9.4 (SAS Institute Inc., Cary NC, USA).

5.3.4.1 Individual animal level

The dependent variable non-pregnancy (non-pregnant (1) / pregnant (0)) at Scan-1 and aborted (aborted (1) / non-aborted (0)) at Scan-2 were binary responses and resembled a binomial distribution. A logistic model with ‘farm’ as a random effect, to adjust for clustering, was used to model association, between non-pregnancy at Scan-1 and sero-status (positive (0) or negative (1)) at Scan-1 (Eq.1). A similar model was used to test association between aborted status at Scan-2, and sero-status at Scan-2. The same logistic model with

sero-status as a binary outcome was used to test for difference in animal level sero-prevalence between age groups at both scans. Odds ratios with 95% confidence interval and p-value for the Chi-squared test were reported for animal level association between non-pregnancy and serological status at Scan-1 and aborted and serological status at Scan-2.

$$Y_{ij} = A + B*\text{sero-status}_{ij} + U_i + E_{ij}$$

(Eq.1)

Where,

Y = Logit-transformed scanning outcome (1/0) of 'i'th hind in 'j'th herd

A = Intercept (average non-pregnancy rate of sero-negative hind)

B = Difference in non-pregnancy rate for sero-positive hinds

U_i = Random effect of 'i'th herd

E_{ij} = Random, uncorrelated error term

5.3.4.2 Herd-level

Sufficient serological data for herd-level analysis was available only from Scan-2. The dependent variable was DAR. Separate models were run for Hardjobovis and Pomona. The association between within-herd DAR and within-herd sero-prevalence was assessed using a logistic model using the events/trials syntax in PROC GLIMMIX command in SAS (Eq.2). The natural log of between-scan interval was chosen as the offset to model DAR at Scan-2.

$$Y_i = A + B1*\text{sero-prevalence}_i + E_i$$

(Eq.2)

Where,

Y_i = Logit-transformed DAR of 'i'th herd

A = Intercept (average DAR of all herds)

B1 = Regression coefficient estimate for sero-prevalence in each herd at Scan-1 or Scan-2

E_i = random, uncorrelated error term

5.3.4.3 Geometric mean titre

Geometric mean titre was calculated for pregnant and non-pregnant hinds at Scan-1 and aborted and non-aborted hinds at Scan-2 (Eq.3 and Eq.4). The reciprocal titre of an individual animal was log-transformed as follows;

$$Log\ titre\ (Y) = \log_2\left(\frac{reciprocal\ titre}{12}\right)$$

(Eq.3)

The geometric mean titre (GMT) was calculated for sero-positive samples only as follows;

$$GMT = 2^{\overline{Y}}\ x\ 12$$

(Eq.4)

The GMT analysis was done on sero-positive hinds only. The difference in GMT between sero-positive pregnant and non-pregnant hinds at Scan-1 and aborted and non-aborted hinds at Scan-2 was assessed by Student's t-test.

5.4 Results

5.4.1 *Leptospira* spp. serology

The distribution of reciprocal titres at Scan-1 and Scan-2 are summarised in Table 5.3. At Scan-1, based on a cut-off of 1:48, 47.5% of R2 and 37.7% of MA hinds were sero-positive for Hardjobovis and 1.9% of R2 and 9.5% of MA hinds were sero-positive for Pomona. At Scan-2, 37.2% of R2 and 44.7% of MA hinds were sero-positive for Hardjobovis and 5.4% of R2 and 9.5% of MA hinds were sero-positive for Pomona.

Table 5.3: Number (and % of total) of sera and reciprocal titres for *Leptospira borgpetersenii* serovar Hardjobovis and *Leptospira interrogans* serovar Pomona from R2 and MA hinds at Scan-1 and Scan-2.

Scan	Reciprocal titre	Hardjobovis		Pomona	
		R2 hinds	MA hinds	R2 hinds	MA hinds
Scan-1	0	202 (45.3)	112 (52.1)	394 (95.0)	195 (84.0)
	24	32 (7.2)	22 (10.2)	13 (13.1)	15 (6.5)
	48	47 (10.5)	30 (14.0)	3 (0.7)	9 (3.9)
	96	58 (13.0)	20 (9.3)	2 (0.5)	6 (2.6)
	192	51 (11.4)	20 (9.3)	3 (0.7)	5 (2.1)
	384	31 (7.0)	6 (2.8)	na	2 (0.9)
	768	17 (3.8)	5 (2.3)	na	na
	1536	5 (1.1)	na	na	na
	3072	3 (0.7)	na	na	na
Scan-2	0	685 (52.5)	424 (45.3)	1,172 (89.6)	809 (86.5)
	24	135 (10.3)	94 (10.0)	65 (5.0)	37 (4.0)
	48	153 (11.7)	109 (11.7)	33 (2.5)	32 (3.4)
	96	150 (11.5)	128 (13.7)	20 (1.5)	38 (4.1)
	192	98 (7.5)	92 (9.8)	10 (0.8)	9 (1.0)
	384	51 (3.9)	49 (5.2)	3 (0.2)	7 (0.7)
	768	30 (2.3)	31 (3.3)	5 (0.4)	2 (0.2)
	1536	4 (0.3)	8 (0.9)	na	1 (0.1)
	3072	na	1 (0.1)	na	na

na= no animals in this category

5.4.1.1 Hardjobovis serology at Scan-1

Animal level

The sero-prevalence for Hardjobovis in non-pregnant and pregnant hinds at Scan-1 was 45.8% and 48.7%, respectively, in R2, and 45.1% and 33.1%, respectively, in MA hinds (Table 5.4). Hardjobovis sero-status was not associated with non-pregnancy status in R2 (p=0.23) or MA hinds (p=0.25) (Table 5.5). Hardjobovis sero-prevalence was not different between age groups (p=0.23) or between hinds from herds with and without aborted hinds (R2 p=0.52 and MA p=0.11).

Table 5.4: Sero-prevalence for *Leptospira borgpetersenii* serovar Hardjobovis and *Leptospira interrogans* 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.

Scan, serovar	Age group	Herds with or without aborted hinds at Scan-2	Herds	% sero-positive (number tested)		
				Non-pregnant	Pregnant	Total
Scan-1, Hardjobovis	R2	With	8	52.7 (110)	56.8 (162)	55.1 (272)
		Without	5	34.8 (69)	36.2 (105)	35.6 (174)
		Total	**13**	**45.8 (179)**	**48.7 (267)**	**47.5 (446)**
	MA	With	3	50.0 (50)	46.9 (64)	48.2 (114)
		Without	4	37.5 (32)	20.3 (69)	25.7 (101)
		Total	**7**	**45.1 (82)**	**33.1 (133)**	**37.7 (215)**
Scan-2, Hardjobovis	R2	With	44	35.8 (190)[a]	37.9 (845)	37.5 (1,035)
		Without	13	na	36.2 (271)	36.2 (271)
		Total	**57**	**35.8 (190)[a]**	**37.5 (1,116)**	**37.2 (1,306)**
	MA	With	28	49.2 (65)[a]	50.5 (546)	50.4 (611)
		Without	16	na	33.8 (325)	33.8 (325)
		Total	**44**	**49.2 (65)[a]**	**44.3 (871)**	**44.7 (936)**
Scan-1, Pomona	R2	With	7	0 (101)	0 (140)	0 (241)
		Without	5	4.3 (69)	4.8 (105)	4.6 (174)
		Total	**12**	**1.8 (170)**	**2.0 (245)**	**1.9 (415)**
	MA	With	4	15.7 (51)	15.0 (80)	15.3 (131)
		Without	4	0 (32)	2.9 (69)	2.0 (101)
		Total	**8**	**9.6 (83)**	**9.4 (149)**	**9.5 (232)**
Scan-2, Pomona	R2	With	44	4.7 (191)[a]	4.3 (846)	4.3 (1,037)
		Without	13	na	9.6 (271)	9.6 (271)
		Total	**57**	**4.7 (191)[a]**	**5.6 (1,117)**	**5.4 (1,308)**
	MA	With	28	10.9 (64)[a]	9.0 (546)	9.2 (610)
		Without	16	na	10.2 (325)	10.2 (325)
		Total	**44**	**10.9 (64)[a]**	**9.4 (871)**	**9.5 (935)**

na= no animals in this category, [a] aborted since Scan-1.

Table 5.5: The odds ratio and p-value for animal-level *Leptospira borgpetersenii* serovar Hardjobovis and *Leptospira interrogans* serovar Pomona sero-status association with non-pregnancy at Scan-1 and having aborted by Scan-2 in herds with aborted hinds.

Serovar	Association with	Age group	Unadjusted model		Adjusted by year and island	
			Odds ratio	P-value	Odds ratio	P-value
Hardjobovis	Non-pregnancy at Scan-1	R2	0.76 (0.49 - 1.19)	0.23	0.73 (0.46 - 1.15)	0.17
		MA	1.45 (0.77 - 2.72)	0.25	1.53 (0.82 - 2.83)	0.18
	Aborted status at Scan-2 (herds with aborted hinds only)	R2	1.02 (0.67 - 1.55)	0.91	0.97 (0.64 - 1.46)	0.87
		MA	0.97 (0.59 - 1.66)	0.90	0.93 (0.53 - 1.64)	0.8
Pomona	Non-pregnancy at Scan-1	R2	0.68 (0.15 - 3.00)	0.61	0.65 (0.14 - 2.90)	0.57
		MA	0.69 (0.26 - 1.80)	0.45	0.69 (0.26 - 1.85)	0.46
	Aborted status at Scan-2 (herds with aborted hinds only)	R2	1.09 (0.44 - 2.67)	0.85	1.17 (0.47 - 2.89)	0.73
		MA	1.27 (0.51 - 3.16)	0.61	1.70 (0.64 - 4.50)	0.29

The GMT in seropositive R2 hinds was significantly higher (156 vs 110, p=0.004) than in MA hinds (Table 5.6). The GMT was not different between pregnant and non-

pregnant hinds (R2 p=0.24 and MA p=0.39) or hinds from herds with or without aborted hinds (R2 p=0.85 or MA p=0.81).

Table 5.6: Mean of *Leptospira borgpetersenii* serovar Hardjobovis and *Leptospira interrogans* serovar Pomona reciprocal antibody titres (≥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.

Scan, serovar	Age	Herds with or without aborted hinds at Scan-2	Reciprocal titre in sero-positive hinds*						
			Non-pregnant		Pregnant		All		
			No.	Mean (SD)	No.	Mean (SD)	No.	Mean (SD)	Range
		With	58	192 (28)	92	146 (32)	150	156 (30)	48 - 3,072
	R2	Without	24	167 (42)	38	136 (36)	62	146 (36)	48 - 3,072
Scan-1, Hardjobovis		**Total**	**82**	**179 (32)**	**130**	**146 (32)**	**212**	**156 (32)**	**48 - 3,072**
		With	25	110 (30)	30	127 (30)	55	118 (30)	48 - 768
	MA	Without	12	90 (21)	14	110 (26)	26	103 (22)	48 - 384
		Total	**37**	**103 (28)**	**44**	**118 (28)**	**81**	**110 (28)**	**48 - 768**
		With	68	110 (26)[a]	320	118 (28)	388	118 (28)	48 - 1,536
	R2	Without	na	na	98	136 (30)	98	136 (30)	48 - 1,536
Scan-2, Hardjobovis		**Total**	**68**	**110 (26)[a]**	**418**	**118 (30)**	**486**	**118 (28)**	**48 - 1,536**
		With	32	103 (26)[a]	276	156 (32)	308	146 (30)	48 - 3,072
	MA	Without	na	na	110	110 (28)	110	110 (28)	48 - 1,536
		Total	**32**	**103 (26)[a]**	**386**	**136 (30)**	**418**	**136 (30)**	**48 - 3,072**
		With	na	na	na	na	na	na	na
	R2	Without	3	156 (18)	5	73 (22)	8	96 (22)	48 - 192
Scan-1, Pomona		**Total**	**3**	**156 (18)**	**5**	**73 (22)**	**8**	**96 (22)**	**48 - 192**
		With	8	73 (19)	12	118 (28)	20	103 (26)	48 - 384
	MA	Without	na	na	2	38 (19)	2	38 (19)	48 - 96
		Total	**8**	**73 (19)**	**14**	**110 (26)**	**22**	**96 (24)**	**48 - 384**
		With	9	48 (12)[a]	36	84 (21)	45	73 (21)	48 - 192
	R2	Without	na	na	26	146 (34)	26	146 (34)	48 - 768
Scan-2, Pomona		**Total**	**9**	**48 (12)[a]**	**62**	**103 (28)**	**71**	**96 (28)**	**48 - 768**
		With	7	78 (30)[a]	49	96 (24)	56	96 (26)	48 - 1,536
	MA	Without	na	na	33	103 (26)	33	103 (26	48 - 768
		Total	**7**	**78 (30)[a]**	**82**	**96 (26)**	**89**	**96 (26)**	**48 - 1,536**

na= no animals in this category, [a] aborted since Scan-1.

Herd-level

Ten of 13 R2 and four of seven MA herds were sero-positive. The within-herd Hardjobovis mean sero-prevalence in R2 and MA herds was 58.2% and 53.1%, respectively (Table 5.7). Statistical analysis was not performed because of insufficient number of herds.

Table 5.7: Mean, SE and range of within-herd sero-prevalence for *Leptospira borgpetersenii* serovar Hardjobovis and *Leptospira interrogans* serovar Pomona at Scan-1 and Scan-2 in R2 and MA sero-positive herds.

Scan, serovar	Age	Herds	Mean sero-prevalence %	Std error	Range
Scan-1, Hardjobovis	R2	10	58.2	8.4	2.4 - 85.7
	MA	4	53.1	11.1	20.9 - 71.4
Scan-1, Pomona	R2	2	9.5	4.5	4.8 - 14.3
	MA	3	18.5	7	4.8 - 27.9
Scan-2, Hardjobovis	R2	42	52	4.6	2.9 - 100
	MA	40	49.5	4.6	3.8 - 100
Scan-2, Pomona	R2	13	23.5	7.5	4 - 95
	MA	14	29.3	7.3	4.5 - 80

5.4.1.2 Pomona serology at Scan-1

Animal level

The sero-prevalence for Pomona in non-pregnant and pregnant hinds was 1.8% and 2%, respectively, in R2, and 9.6% and 9.4%, respectively, in MA hinds (Table 5.4). Pomona sero-status at animal level was not associated with non-pregnancy in R2 or MA hinds (Table 5.5). The sero-prevalence for Pomona, after adjusting for clustering, was 0.8% (95% CI= 0.2 - 2.9%) in R2 and 2.4% (95% CI= 0.7 - 8.3%) in MA hinds (adjusted OR=0.33, 95% CI=0.12 - 0.9, p=0.03) (Table 5.4). The sero-prevalence for Pomona was similar in herds with and without aborted hinds in MA age group (p=0.26).

The GMT was similar in seropositive R2 and MA hinds (p=0.97) (Table 5.6). The GMT was higher in pregnant than non-pregnant MA hinds (p=0.015) but there was no difference in R2 (p=0.2) hinds. The mean GMT was similar in hinds from MA herds with or without aborted hinds (p=0.48).

Herd level

Two of 12 R2 and three of eight MA herds were sero-positive. The within-herd mean Pomona sero-prevalence was 9.5% and 18.5% in R2 and MA herds, respectively (Table 5.7). Statistical analysis was not performed because of insufficient number of herds.

5.4.1.3 Hardjobovis serology at Scan-2

Animal level

Overall, 1,646 sera from herds with aborted hinds and 596 sera from herds without aborted hinds were tested for Hardjobovis antibodies at Scan-2. In herds with aborted hinds, the sero-prevalence for Hardjobovis in aborted and non-aborted hinds was 35.8% and 37.9%,

respectively, in R2, and 49.2% and 50.5% in MA hinds, respectively (Table 5.4). There was no association between Hardjobovis sero-status and aborted status in R2 (p=0.91) or MA (p=0.9) hinds in herds with aborted hinds (Table 5.5). Overall (in herds with and without aborted hinds combined), R2 hinds were less likely to be sero-positive than MA hinds (OR=0.41, 95% CI=0.31 -0.54, p<0.0001). The sero-prevalence, after adjusting for clustering, was 27% (95% CI=18.7 – 37.4%) and 47.7% (95% CI=35.8 - 59.8%) in R2 and MA hinds, respectively.

R2 hinds from herds with aborted hinds were less likely to be sero-positive than R2 hinds from herds without aborted hinds (OR=0.04, 95% CI=0.014 - 0.14, p<0.0001). Sero-prevalence, after adjusting for clustering, was 14.7% (95% CI=6.8 - 28.7%) and 79.5% (95% CI=54.7 - 92.6%) in hinds from herds with and without aborted hinds, respectively.

Mixed-age hinds from herds with aborted hinds were more likely to be sero-positive than MA hinds from herds without aborted hinds (OR=2.27, 95% CI=1.1 - 4.67, p<0.0001). Sero-prevalence, after adjusting for clustering, was 50.8% (95% CI=35.5 - 65.9%) and 31.2% (95% CI=17.7 - 49.1%) in hinds from herds with and without aborted hinds, respectively.

Overall (in herds with and without aborted hinds combined), the GMT in sero-positive MA hinds was higher than in R2 hinds (136 vs 118) (p=0.014) (Table 5.6). In herds with aborted hinds, the GMT was higher in non-aborted seropositive MA hinds than in aborted hinds (156 vs 103) (p=0.018) but there was no difference in R2 hinds (p=0.42). The GMT in sero-positive hinds was similar in those from herds with and without aborted hinds in R2 (p=0.35) or MA (p=0.07) age group.

Herd level

Overall, 42 of 57 R2 and 40 of 44 MA herds were Hardjobovis sero-positive. The within-herd mean sero-prevalence was 52% and 49.5% in R2 and MA herds, respectively (Table 5.7). The Hardjobovis within-herd sero-prevalence was not associated with DAR in R2 or MA herds (Table 5.8).

Table 5.8: Model coefficient logarithmic estimate and p-value for association between Hardjobovis and Pomona within-herd sero-prevalence and daily abortion rate at Scan-2.

Within-herd daily abortion rate association with	Age group	Herds	Model coefficient logarithmic estimate	95% CI	P-value
Hardjobovis within-herd sero-prevalence	R2	57	-0.38	-1.17 - 0.41	0.33
	MA	44	0.74	-0.32 - 1.80	0.16
Pomona within-herd sero-prevalence	R2	57	0.83	-1.25 - 2.91	0.42
	MA	44	0.52	-1.28 - 2.31	0.56

5.4.1.4 Pomona serology at Scan-2

Animal level

Overall, 1,647 sera from herds with aborted hinds and 596 sera from herds without aborted hinds were tested for Pomona antibodies at Scan-2. In herds with aborted hinds, sero-prevalence for Pomona in aborted and non-aborted hinds was 4.7% and 4.3%, respectively, in R2, and 10.9% and 9%, respectively, in MA hinds (Table 5.4). The Pomona sero-status was not associated with aborted status in R2 (p=0.85) or MA (p=0.61) hinds in herds with aborted hinds.

Overall (in herds with and without aborted hinds combined), R2 hinds were less likely to be seropositive than MA hinds (OR=0.2, 95% CI=0.12 - 0.33, p<0.0001). Sero-prevalence, after adjusting for clustering, was 0.81% (95% CI=0.38 - 1.7%) and 3.9% (95% CI=1.9 - 7.6%) in R2 and MA hinds, respectively. Sero-prevalence was similar in hinds from herds with and without aborted hinds in R2 (p=0.2) and MA (p=0.81) age groups.

Overall (in herds with and without aborted hinds combined), the GMT for Pomona was similar in both age groups (p=0.18) (Table 5.6). In herds with aborted hinds, the GMT was higher (84 vs 48) in non-aborted hinds than in aborted hinds in R2 (p=0.006) but not in MA hinds (p=0.51). The GMT was similar in hinds from herds with and without aborted hinds in R2 (p=0.17) or MA (p=0.71) hinds.

Herd level

Overall, 13 of 57 R2 and 14 of 44 MA herds were sero-positive. The within-herd mean Pomona sero-prevalence was 23.5% in R2 herds and 29.3% in MA herds (Table 5.7). The Pomona within-herd sero-prevalence was not associated with DAR in R2 (p=0.42) or MA herds (p=0.56) (Table 5.8).

5.4.2 *Neospora caninum* serology

Two of 268 R2 and four of 82 sera from aborted hinds at Scan-2 were sero-positive on ELISA. Both R2 and two of four MA ELISA positive sera were positive on Western blot (Table 5.9). Immuno-dominant antigen (IDA) bands with molecular weight between 21 to 35 kD were observed on the immunoblot image for the WB positive sera consistent with *N. caninum* infection. The two ELISA positive WB negative MA sera only had IDA bands with a molecular weight of less than 15 kD which is inconsistent with *N. caninum* infection and rather suggests antigen cross-reactivity in the ELISA assay. *Toxoplasma gondii* ELISA sero-positivity results (Chapter 4) for the sera tested for *N. caninum* were available for assessment of dual sero-positivity. The one R2 and one MA ELISA and WB positive sera were positive for *T. gondii* ELISA and were excluded from the calculation of sero-prevalence. Thus, the adjusted sero-prevalence was 0.4% of 267 R2 and 1.2% of 81 MA hinds based on sera verified on WB and after excluding sera with cross-reacting antibodies to both *N. caninum* and *T. gondii*.

Table 5.9: Paired *Neospora caninum* and *Toxoplasma gondii* serology and uterus PCR results for hinds sero-positive on *Neospora caninum* ELISA.

Animal id.	*Neospora caninum*				*Toxoplasma gondii*		
	ELISA S/P(%)	Sero-status (ELISA)	Sero-status (WB)	Uterus qPCR	ELISA S/P(%)	Sero-status (ELISA)	Uterus PCR
1	90.3	+	+	na	10.3	-	na
2	163.0	+	+	-	37.2	+	+
3	46.2	+	-	na	5.4	-	na
4	64.8	+	-	na	3.2	-	na
5	89.9	+	+	na	73.1	+	na
6	84.7	+	+	na	11.0	-	na

5.4.3 Molecular diagnostics

5.4.3.1 Leptospira *spp. qPCR*

Initial screening shown in Table 2 showed no fetal kidney, fetal aqueous humor or uterine DNA samples were positive for leptospiral DNA on qPCR. Therefore, further testing of all samples collected was not justified.

5.4.3.2 Neospora caninum *qPCR*

At Scan-1, tissue samples from 11 R2 fetuses from aborting hinds and two R2 and seven MA fetuses from hinds in normal pregnancy at Scan-1 were collected. The crown rump length (CRL) ranged from 25-306 mm and 51-450mm in fetuses from aborting and pregnant R2 hinds, respectively, at Scan-1 whereas it ranged from 21-495mm in fetuses from pregnant

MA hinds. At Scan-2, samples from 14 R2 and five MA fetuses from aborting hinds were tested The CRL ranged from 192-640 mm and 381-640 mm in fetuses from aborting R2 and MA hinds, respectively, at Scan-2.

Neospora caninum DNA was detected in cotyledon and fetal diaphragm in aborting R2 and pregnant MA hinds at Scan-1 and at Scan-2, DNA was detected in uteri, fetal brains and cotyledons from R2 aborted hinds and uterus sample from one aborted MA hind (Table 5.10). Pinpoint haemorrhages were observed on caruncles in one R2 DNA positive aborted hind at Scan-2. Additionally, *N. caninum* DNA was detected in the uterus of one of 22 MA hinds from three herds that were tested after not rearing a live calf to weaning. The 10 R2 hinds from one herd tested at weaning were *N. caninum* DNA negative. *Toxoplasma gondii* PCR results (Chapter 4) for the tissue samples tested for *N. caninum* were available for assessment of dual positivity. None of the *N. caninum* DNA positive samples were positive for *T. gondii* DNA.

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.

Sample collection at	Age group	Status	Uterus	Cotyledon	Fetal brain	Fetal diaphragm	Placenta	Fetal remnants
Scan-1	R2	Pregnant	0/2	0/2	0/2	0/2	0/2	na
		Non-pregnant	0/10	na	na	na	na	na
		Aborting	0/12	1/11	0/11	1/10	0/11	0/1
	MA	Pregnant	0/7	1/7	0/7	0/6	0/7	na
		Non-pregnant	0/1	na	na	na	na	na
Scan-2	R2	Aborted	4*/132	2/10	3/14	0/14	0/11	0/3
	MA	Aborted	1/30	0/5	0/5	0/4	0/5	na
Weaning	R2	Returned with no live calf	0/10	na	na	na	na	na
	MA		1/22	na	na	na	na	na

na = not applicable, *Pin-point haemorrhages were observed on caruncles in uterus sample from one hind

5.5 Discussion

This is the first study to investigate association between *Leptospira* spp. serology and non-pregnancy and abortions in deer in New Zealand. There was no association between Hardjobovis or Pomona sero-status and non-pregnancy at Scan-1 or aborted status at Scan-2. No fetal tissues from aborting hinds at Scan-1 or Scan-2 were positive for leptospiral DNA. The GMT for Pomona in sero-positive MA pregnant hinds at Scan-1 was higher than in non-

pregnant hinds. In herds with aborted hinds, the GMT for Hardjobovis and Pomona in sero-positive MA and R2 non-aborted hinds, respectively, was higher than in MA and R2 aborted hinds, respectively. While few hinds were sero-positive for *N. caninum* some fetal and uterine tissues were positive on PCR.

The non-significant association between Hardjobovis sero-status and non-pregnancy status at Scan-1 in this study was similar to that observed by Dreyfus (2013). However, the findings described by Dreyfus (2013) could not be directly compared with this study as the association between within-herd sero-prevalence and pregnancy rate in that study was assessed at herd-level and the deer were called sero-positive if the serum sample was positive for either Hardjobovis or Pomona. Therefore, the estimates reported by Dreyfus (2013) were for exposure for serovars Hardjobovis and Pomona combined and pregnancy rate.

The lack of association between Hardjobovis sero-status and aborted status is consistent with similar reports in cattle in New Zealand (Carter *et al.* 1982), Australia (Elder *et al.* 1985; Chappel *et al.* 1989) and USA (Guitian *et al.* 1999). However, by contrast, experimental studies from UK and USA in cattle have shown that exposure to Hardjobovis can cause abortions (Ellis and Michna 1977; Thiermann 1982). In addition, increased risk of abortions due to Hardjobovis exposure, at a cut-off titre of 1:384, were reported by Sanhueza *et al.* (2013) in New Zealand beef cattle. Natural infection by Hardjobovis in cattle has been linked to abortion in an Australian study wherein sero-prevalence (cut-off ≥1:300) of 17.8% and 8.4% were reported in aborting and non-aborting cows, respectively (Corbould 1971; Hoare and Claxton 1972). In a UK study, a wide variation of titres (<1:10 to 1:3,000) was observed between aborting cows (Ellis *et al.* 1982c). However, that study did not test non-aborting cows for *Leptospira* spp. antibodies.

In MA hinds, the observation of higher sero-prevalence in hinds from herds with aborted hinds compared to hinds from herds without aborted hinds suggest that, on an overall basis, the Hardjobovis may be associated with abortion. However, in herds with aborted hinds, the GMT for Hardjobovis in aborted MA hinds was lower than in non-aborted hinds at Scan-2 suggesting drop in titres after abortion. A similar drop was observed by Hoare and Claxton (1972) in aborting cattle following a clinical episode of leptospiral mastitis. Non-aborted hinds with high titres might have aborted after Scan-2. However, this may have occurred due to small numbers for GMT calculation in each group. These findings together with reports from cattle suggest that the causal relationship between Hardjobovis and

abortion is also variable in the deer population given the endemic status of Hardjobovis in New Zealand deer (Ayanegui-Alcerreca *et al.* 2007; Ayanegui-Alcérreca *et al.* 2010).

There was no association between Pomona sero-status and non-pregnancy or aborted status. The significantly higher Scan-1 Pomona GMT in MA pregnant hinds compared with non-pregnant MA hinds could be a result of timing of Pomona exposure, wherein the hinds were exposed after the time of risk for pregnancy or the GMT difference may have occurred due to smaller numbers. A similar explanation can be put forward for the observation of higher GMT in non-aborted hinds than in aborted hinds from R2 herds with aborted hinds. Experimental infection of Pomona has been shown to cause abortion in deer (Trainer *et al.* 1961). In cattle, natural infection with Pomona caused abortions in a dairy cattle herd in Canada wherein antibodies to Pomona were demonstrated in fetal tissues by fluorescent antibody test (Kingscote and Wilson 1986). In New Zealand, the risk of abortions due to Pomona exposure (titre cut-off 1:768) in beef cattle has been reported by Sanhueza *et al.* (2013).

The effects of vaccination of hinds against both Hardjobovis and Pomona on weaning percentage in deer herds have been studied by Subharat *et al.* (2011b) and Ayanegui-Alcerreca (2006). Those studies reported significantly improved weaning percentages in *Leptospira* spp. vaccinated hinds than in non-vaccinated hinds and confirming that *Leptospira* spp. exposure was associated with reduced reproductive efficiency. However, it was likely these losses were peri- or post-natal. Despite that those studies could not show an effect of *Leptospira* spp. exposure on abortion, the finding of non-significant association between Hardjobovis and Pomona, and abortion in this study corroborates the reports for effect of *Leptospira* spp. exposure on peri or post-natal losses by Subharat *et al.* (2011b) and Ayanegui-Alcerreca (2006).

Leptospira spp. DNA was not detected in uteri collected at Scan-1 or Scan-2. This observation was consistent with the report by Subharat *et al.* (2010) in uteri from 120 normal MA deer. Our study used the same protocol for *Leptospira* spp. DNA detection as used by Subharat *et al.* (2010) and therefore the qPCR used in this study had the same sensitivity and specificity making the results directly comparable. While no maternal or fetal tissue samples were positive on PCR from this study, one fetal kidney sample was positive in the study by Subharat *et al.* (2010) from a fetus in relatively early pregnancy whereas the fetal samples in this study came from hinds in both early and mid-pregnancy. This provides evidence of

absence of leptospiral infection during mid-gestation in fetuses, at least in the sampled tissues. Furthermore, the fetuses from Subharat *et al.* (2010) were in normal pregnancy whereas the fetuses tested in this study were from both normal pregnant and aborted hinds. Leptospiral DNA has been demonstrated in genital and urinary tracts of cattle (Ellis *et al.* 1986; Ellis and Thiermann 1986). Hardjobovis is thought to cause abortion in cattle (Ellis *et al.* 1982b) and was isolated from naturally aborted fetuses (Ellis and Michna 1977; Langoni *et al.* 1999) and fetuses in normal pregnancy (Ellis *et al.* 1982a) demonstrating transplacental transmission. The findings from this study suggest absence of leptospiral transplacental transmission in red deer but experimental studies have shown that pregnant white-tailed deer are at risk of abortion due to leptospiral infection as demonstrated by Trainer *et al.* (1961).

Bacterial culture of fetal or maternal tissues was not possible because most of the tissues came frozen or were kept at least 4-5 hours in a chiller (4^{o}C). The qPCR used in this study was genus specific, detecting pathogenic *Leptospira* spp. (Slack *et al.* 2006) and is not serovar specific and detects DNA from lysed or inactive leptospires. Thus, this qPCR can be a useful tool for *Leptospira* spp. DNA screening and is informative of likely serovar when read in parallel with serology because of the limited number of serovars in New Zealand.

Taken together, while the findings from *Leptospira* spp. serology and qPCR suggests that *Leptospira* spp. may not be associated with non-pregnancy or abortion in deer, given the endemic status of serovar Hardjobovis in New Zealand, these results do not fully discount *Leptospira* spp. as an indirect cause of abortion since pregnant deer may abort after clinical infection as observed in cattle species.

The sero-prevalence for *N. caninum* using an ELISA test, after confirmation with WB, in aborted hinds was 0.4% in R2 and 1.2% in MA hinds. This sero-prevalence in aborted hinds did not justify the testing of sera from non-aborted hinds. Additionally, *N. caninum* DNA was found in fetal tissues from a pregnant MA and aborting R2 hind at Scan-1 and aborted hinds at Scan-2. Of note, cross-reaction between the *N. caninum* and *T. gondii* antibodies was observed and therefore the cross-reactive sera were excluded in calculation of sero-prevalence, despite that it may be possible that they were dual infected. The *N. caninum* ELISA used in this study was not validated for use in deer and therefore the sero-prevalence estimated could be an under- or over- estimate of the true sero-prevalence. However, the positives from ELISA were tested on Western blot for confirmation taking into consideration that validation was not possible due to few positives. Moreover, the uterus sample from one

R2 hind sero-positive to *N. caninum* (ELISA and WB) and *T. gondii* (ELISA) was negative for *N. caninum* DNA and positive for *T. gondii* DNA suggesting that the sero-positivity to *N. caninum* observed on the ELISA and WB may be due to cross reaction.

A Belgian study reported a similar observation in a wild red deer population wherein no *N. caninum* antibodies were detected in seven red deer using ELISA (De Craeye *et al.* 2011). Using WB as a confirmatory test, sero-prevalence of 11% (12/106) in farmed and 13% (6/46) in free-ranging red deer has been reported using the ISCOM-ELISA (*N. caninum* iscom antigen) for *N. caninum* antibodies in a Polish study from an area with documented evidence of neosporosis in cattle (Gozdzik *et al.* 2010). Antibodies to *N. caninum* using different tests (ELISA, IFAT, WB, ISCOM-ELISA) in wild deer have been reported in white-tailed deer (*Odocoileus virginianus*), mule deer (*Odocoileus hemionus hemionus)*, black-tailed deer (*Odocoileus hemionus columbianus)*, Vietnam sika deer (*Cervus nippon pseudaxis*), roe deer (*Capreolus capreolus*), fallow (*Dama dama*) deer, and caribou (*Rangifer tarandus*) ranging from 17-88% prevalence in North America and up to 14% in Europe (Dubey and Schares 2011). From these reports, it appears that wild deer in overseas conditions may be exposed to *N. caninum*. However, tests used in those studies were not validated for use in deer and therefore the sero-prevalences reported may also be an under- or over- estimate of the true sero-prevalence. However, regardless of this limitation, the serology results from this study in New Zealand farmed deer population suggests lower exposure to *N. caninum*.

Despite low sero-prevalence, *N. caninum* DNA was detected in fetal and maternal tissue using a qPCR. Fetal or maternal tissue samples positive for *N. caninum* DNA were negative for *T. gondii* DNA confirming that the qPCR was not misclassifying *T. gondii* DNA as *N. caninum* DNA. To the Author's knowledge this is the first report of *N. caninum* DNA in fetal brains and uteri in pregnant and aborted hinds in farmed deer in New Zealand. The observation of *N. caninum* DNA in fetal brains was consistent to that reported by Dubey *et al.* (1996) in still born Eld deer (*Cervus eldi siamensis*). However, the fetus in the latter study was a still born and the timing of *N. caninum* infection was unknown. Observations from this study were from hinds and fetuses aborted at mid-gestation demonstrating that *N. caninum* can infect a fetus as early as in mid-gestation. These findings suggest that antibody to *N. caninum* may diminish from maternal blood in rapidly after transplacental transmission of *N. caninum* to fetus and subsequent abortion. A similar observation has been reported in sheep

by Weston *et al.* (2009) wherein sera from *N. caninum* inoculated ewes were DNA negative at parturition but the lambs were DNA positive for brain and/or serum samples. Also, the presence of DNA in fetal tissue and absence of maternal sero-positivity suggest imperfect sensitivity of the ELISA and further justifies use of Western blot as a confirmatory test.

Neospora caninum has been known to cause abortion in cattle (Thornton *et al.* 1991; Thornton *et al.* 1994; Thobokwe and Heuer 2004; Dubey 2005; Dubey *et al.* 2007; Heuer *et al.* 2007; Weston *et al.* 2012) and sheep (West *et al.* 2006; Howe *et al.* 2008; Howe *et al.* 2012). In New Zealand cattle, 28% of 320 fetal brains submitted for abortion investigation had typical *N. caninum* type lesions on histological examination (Thornton *et al.* 1991). In another study in cattle, *N. caninum* typical abortion diagnosis was made in 26.5% (of 34) aborted fetuses submitted for histopathological examination (Weston *et al.* 2012). In New Zealand sheep, *N. caninum* DNA has been detected from seven of 13 brains of aborted fetuses (Howe *et al.* 2008) and from 18 of 138 normal and aborted fetuses combined, and 11 of 71 cotyledon samples (Howe *et al.* 2012). *Neospora caninum* DNA was detected in two of nine uteri from heifers experimentally inoculated with semen contaminated with *N. caninum* tachyzoites in Spain (Serrano *et al.* 2006). These findings in cattle and sheep show that *N. caninum* can localise in cotyledon, fetal brains, and uteri. However, the PCR method used in above studies was different to that used in this study and targeted different locations of the *N. caninum* DNA. The qPCR used in this study amplified Nc5 gene on *N. caninum* DNA.

5.6 Conclusion

Lack of significant associations between *Leptospira* spp. serology and non-pregnancy or abortions and absence of DNA evidence from aborted fetal material and uteri suggest that *Leptospira* spp. was not a significant cause of sub-optimum pregnancy rate or mid-term abortion in deer in this study. Serological evidence for *N. caninum* was limited, suggesting that this pathogen may not be playing a significant role in impaired reproductive performance in farmed deer, although no definitive conclusions can be drawn, given the lack of validation of the ELISA. However, the findings of *N. caninum* DNA from fetal and maternal tissues confirms that *N. caninum* can infect pregnant hinds. Nevertheless, these observations do suggest that a small proportion of abortions in NZ deer may be due to *N. caninum.* Additional studies are needed to fully understand the role of *N. caninum* in farmed deer in New Zealand.

5.7 Acknowledgements

This study was supported by the Deer Reproductive Efficiency Group based in Southland. It is funded by AgResearch, Agmardt, DEEResearch, MSD Animal Health, Southland Deer Farmers Association, Massey University and individual farmers, particularly Landcorp Farming Ltd. The in-kind contribution of participating farmers is gratefully acknowledged, as is the assistance of a large number of veterinary practices and scanners for scanning and blood sample collection and DSP staff and veterinarians for tissue sample collection. The technical assistance in the laboratory from the IVABS technical team is well appreciated. I am also thankful to the staff at the post-mortem facility to allow dissection of maternal and fetal tissues in the post-mortem premises. I am also thankful to Dr. Emilie Valleé for processing and testing of fetal kidney and aqueous humor samples.

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Chapter 6. Investigation of association between bovine viral diarrhoea virus and cervid herpesvirus type-1, and abortion in New Zealand farmed deer

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This chapter is prepared in the style format of the New Zealand Veterinary Journal.

6.1 Abstract

Sub-optimum pregnancy rates and abortion contribute to reproductive inefficiency in the New Zealand farmed deer industry. Bovine viral diarrhoea virus (BVD) and cervid herpesvirus type-1(CvHV-1) are known abortifacients and have been reported in deer. Thus, this study investigated whether there was an association between BVD and CvHV-1 and abortion in New Zealand farmed red deer.

Rising two-year-old (R2, n=22,130) and mixed-age (MA, n= 36,223) hinds from 87 and 71 herds, respectively, throughout NZ were ultrasound scanned early in gestation (Scan-1) and at approximately the end of the second trimester, a subsample of up to 100 R2 and 155 MA hinds/herd were re-scanned (Scan-2). A daily abortion rate (DAR) was calculated. Blood from 21 pregnant and up to 21 aborted R2 and MA hinds as available at Scan-2 were collected, along with blood from hinds observed aborting at the time of scanning. A sub-sample of non-aborting hinds from herds with and without aborted hinds, and all aborting hinds were tested for BVD and CvHV-1 antibodies using virus neutralisation tests (VNT). Cut-off titres for BVD and CvHV-1 were ≥1:8 and ≥1:1, respectively. Fetuses and uteri from hinds observed aborting at Scan-2, non-pregnant uteri after Scan-2, and uteri from hinds not rearing a calf to weaning were collected at deer slaughter premises and tested for herpesvirus DNA. A sub-sample of PCR positive uteri DNA samples were sequenced. Associations were analysed between sero-status and abortion, at individual animal level.

In herds with aborted hinds, 10.3% of 639 R2 and 17.2% of 302 MA hinds were sero-positive for BVD and 18.6% of 613 R2 and 68.5% of 232 MA hinds were sero-positive for CvHV-1. In herds with aborted hinds, there was no association between BVD sero-status and aborted status at animal level (R2 p=0.36, MA p=0.76) whereas CvHV-1 sero-positivity was negatively associated with aborted MA hinds but not in R2 hinds (R2 p=0.36, MA p=0.01). Eleven of 108 R2 and none of 31 uteri from aborted hinds were positive for herpesvirus DNA. Vaginal samples from four R2 and one MA aborted hinds tested were negative for herpesvirus DNA. A Cervid Rhadinovirus type-2 (CRhV-2) was identified in seven PCR positive uteri samples.

These findings suggest that BVD and CvHV-1 (in R2 hinds only) may not be associated with abortion. In MA hinds, the negative association between CvHV-1 and abortion may be due to lower sample size or that the hinds had a higher level of immunity

from previous exposures and therefore resisted abortions. The CRhV-2 may be associated with abortion in R2 hinds.

KEY WORDS: *Deer, abortion, bovine virus diarrhoea virus, BVD, Cervid herpesvirus type-1, virus neutralisation test, serology, PCR, Cervid Rhadinovirus type-2.*

6.2 Introduction

Reproductive performance in rising two-year-old (R2) and mixed-aged (MA) adult hinds has been sub-optimal in New Zealand farmed deer with reproductive efficiency (calves weaned/hinds mated) averaging 75.2% over the last 13 years (Statistics New Zealand 2016a). Inefficiency arises from failure to conceive, fetal loss, stillbirth, and postnatal mortality (Asher and Pearse 2002; Asher 2003; Asher and Wilson 2011). Based on early scanning of this study population, pregnancy rates of the R2 and MA hinds were 85.8% and 93.3%, respectively (Chapter 2).

Causes and risk factors for conception failure in both R2 and MA hinds have been studied previously (Audigé *et al.* 1999b; Audigé *et al.* 1999c; Asher *et al.* 2005a; Asher *et al.* 2011) but bovine viral diarrhoea virus (BVD) and cervid herpesvirus type-1 (CvHV-1) were not included in those studies. Pregnancy loss due to abortions in New Zealand deer has been reported in a few studies (Fennessy *et al.* 1986; Audigé *et al.* 1999a; Campbell *et al.* 2000; Wilson *et al.* 2012). However, the most substantial data available is presented in Chapter 2 in which the mid-term herd-level mean daily abortion rates were 0.043% and 0.025% in R2 and MA herds, respectively, which calculates to 3.9% in R2 and 2.2% in MA herds for 90 mid-term gestation days. Hence abortions can pose a significant impact on the deer farmers and deer industry in New Zealand in terms of lost opportunities and lower financial returns.

Abortion can be attributed to non-infectious and infectious causes (Bartels *et al.* 1999; Fthenakis *et al.* 2012). Chapter 7 described non-infectious risk factors such as farm management, nutrition, health and environmental conditions prevailing in herd or farms which can contribute to poor reproductive performance in the study population. Additionally, infectious causes such as *Toxoplasma* was found to be positively associated with abortions with detection of *T. gondii* antibodies in blood and DNA samples from uteri and fetal material in non-pregnant and aborted hinds (Chapter 4). Additionally, *Neospora caninum*, a known abortifacient of cattle and sheep species in New Zealand (Thornton *et al.* 1991; Thornton *et al.* 1994; Howe *et al.* 2008; Howe *et al.* 2012) and reported in overseas wild deer population (Lindsay *et al.* 2002b; Gondim *et al.* 2004), although herds only had a low sero-prevalence, DNA was detected in fetal brains, cotyledon, and uteri of aborted hinds suggesting potential involvement in deer abortion (Chapter 5). However, the presence of *Leptospira borgpetersenii* serovar Hardjobovis, *Leptospira interrogans* serovar Pomona,

which are endemic in New Zealand deer (Ayanegui-Alcérreca *et al.* 2010), had no association with non-pregnancy or aborted status (Chapter 5).

Viruses such as CvHV-1 and BVD have been reported in deer. BVD is known for causing reproduction losses in cattle and is prevalent on New Zealand cattle farms (Fray *et al.* 2000; Thobokwe and Heuer 2004). Consequences of BVD infection in dairy cattle include early embryonic death, fetal resorption, mummification, conception failure, abortions and congenital malformations (McGowan *et al.* 1993; Fray *et al.* 2000; Lindberg *et al.* 2001). The infection pattern for BVD in white-tailed deer (*Odocoileus virginianus*) was reported to be similar to that in cows under experimental conditions in USA where in abortions, stillbirth and birth of persistently infected (PI) fawns were observed (Ridpath *et al.* 2008). Congenital transmission of BVD and resultant PI fawns in white-tailed deer has been documented in the USA (Passler *et al.* 2009; Passler *et al.* 2010). Losses due to BVD infection and subsequent fetal infections have been reported in sika deer (*Cervus nippon*) in China where the BVD sero-prevelence ranged from 60-87% (Rui *et al.* 2000; Gao *et al.* 2011). Exposure to BVD in red deer has been reported in sero-surveys in New Zealand (Motha *et al.* 2000) and overseas deer population (Nielsen *et al.* 2000; Fernandez-Aguilar *et al.* 2016; Rodriguez-Prieto *et al.* 2016). In New Zealand, sero-prevalence of 9.5% of 400 samples collected from 575 deer herds has been reported. The majority of New Zealand deer farmers also rear cattle and sheep along with deer. Given the high prevalence of BVD in the New Zealand cattle population and its potential transmission to deer, pregnant deer may also be at risk of BVD infections and subsequent losses.

CvHV-1, belonging to alphaviridae family, has been reported in both farmed and wild deer populations (Nettleton *et al.* 1986; Thiry *et al.* 1988; Frolich 1996; Pospisil *et al.* 1996; Frolich *et al.* 2006). In New Zealand deer, CvHV-1 has been reported in semen of a red deer stag (Tisdall and Rowe 2001), cases of balanoposthitis in stags (Morgan *et al.* 2010), and ocular and vaginal lesions (Squires *et al.* 2012). In the latter study, a Cervid Rhadinovirus, a gamma herpesvirus, was reported in vaginal lesions including mucosal erosions and petechial haemorrhages from a dexamethasone immunosuppressed hind with a history of keratoconjunctivitis (Squires *et al.* 2012)(Squires *et al.* 2012)(Squires *et al.* 2012)(Squires *et al.* 2012)(Squires *et al.* 2012)(Squires *et al.* 2012)(Squires *et al.* 2012). CvHV-1 has also been isolated from nasal swab of healthy wild red deer fawn in Belgium (Thiry *et al.* 2007) and from genital tract of captive red deer in France (Thiry *et al.* 2011). A sero-survey in New

Zealand showed that 38% of 314 farmed deer were positive for CvHV-1 (Motha and Jenner 2001). CvHV-1 and bovine herpesvirus (BoHV) shares antigenic similarities (Motha and Jenner 2001; Thiry *et al.* 2006). Motha *et al.* (2000) reported a sero-prevalence of 32% to BoHV in New Zealand farmed deer. The finding from sero-surveys, together with reports of isolation of CvHV-1 from genital organs of both male and female deer populations suggest that CvHV-1 may be a risk for impaired reproduction in females.

This study was undertaken on farms and animals described in Chapter 2, to test for association between CvHV-1 and BVD serology and mid-term abortion as determined by repeat scanning in red deer hinds. Maternal tissue samples were tested for CvHV-1 DNA. This study also provided further evidence about the occurrence of CvHV-1 and BVD in New Zealand farmed deer.

6.3 Materials and methods

The sampling frame for farms and animals used for this analysis is described in detail in Chapter 2. Briefly, 22,130 R2 and 36,223 MA red deer hinds from 87 and 71 herds, respectively, throughout New Zealand were ultrasound scanned for pregnancy early in gestation (Scan-1), and a sub-sample was scanned again approximately at the end of the second trimester (Scan-2) during the 2011 and 2012 reproductive cycles. Blood, uteri, aborted and normal fetuses were collected. All animal manipulations were approved by the Massey University Animal Ethics committee (Protocol numer: 12/34).

Hinds were classified as being 'pregnant' based on the presence at least one of fetus or part thereof, amniotic membrane, and/or presence of placentomes, or as being 'non-pregnant' based on absence of those signs combined with visualisation of a non-pregnant uterus at Scan-1. The term "aborting" is ascribed to hinds that had ultrasound evidence of aborting fetuses at Scan-1 and Scan-2. The term "aborted", used for calculating the daily abortion rate DAR ((number aborted at Scan-2 / number scanned at Scan-2) / number of days between Scan-1 and Scan-2), is ascribed to hinds that were pregnant at Scan-1 but not pregnant at Scan-2, plus those aborting at Scan-2. Daily abortion rate was calculated to account for differences in time between scans. This approximated a mid-term abortion rate.

6.3.1 Sample collection and handling

Blood samples were collected over two years, 2011 and 2012, from up to 21 aborted hinds as available and 21 non-aborted hinds per herd in mid-September to mid-October

during Scan-2. Samples were collected by jugular venipuncture into 10 ml vacuum blood collection tubes without anticoagulant, and transported chilled to Massey University where they were centrifuged at 1,512 × g for 15 minutes and serum withdrawn and stored at -20^{o}C. An aliquot (30 µL) from each serum sample diluted in 150 µL of normal saline solution was stored on a 96-well plate and was stored at -20^{o}C until tested. In total, 4,480 and 2,932 blood samples were collected from Scan-1 and Scan-2, respectively, to provide the sample pool for selection for serological analyses.

Aborted hinds at Scan-2, as available, were tracked to deer slaughter premises (DSP) where whole reproductive tracts from the posterior cervix and vagina were collected by the Author or the DSP veterinarian. Additionally, reproductive tracts from hinds that did not rear a calf at weaning were also collected at DSPs. Samples were kept in a chiller/freezer and then sent chilled or frozen to Massey University where they were processed at the post-mortem facility. Gross observations from uteri and fetal tissues were recorded at dissection. Uteri, vaginal, placental, cotyledon, and fetal samples were dissected and stored at -20^{0}C (fresh specimens) for PCR, or in 10% formalin for histology.

6.3.2 Virus neutralisation assay (VNT) for bovine viral diarrhoea virus (BVD) and cervid herpesvirus type-1 (CvHV-1)

The sera for detection of neutralising antibodies to BVD and CvHV-1 virus were sent to Animal Health Laboratory (AHL), Ministry of Primary industries (MPI), Wallaceville, Wellington. A virus neutralising test (VNT) was carried out using method adapted from Kirkland and Mackintosh (1993) and OIE (2016) for BVD VNT. Similarly, a VNT for CvHV-1 was also carried out following methodology outlined by OIE (2016). According to the BVD test manual outlined by MPI, Wallaceville, a titre of ≤1:4 for BVD VNT indicated sero-negative or a PI animal, titres of between 1:8 and up to and including 1:64 indicated recent BVD infection or colostral antibody in animals up to six months of age, or a PI animal, whereas a titre >1:64 indicated that the animal had been exposed to BVD virus. For analyses, sera with titres ≥1:8 for BVD were called sero-positive. For CvHV-1, the test was set up to detect neutralising antibodies up to a titre of 1:64 only and sera with a titre ≥1:1 were considered sero-positive according to the test manual.

6.3.3 Sample selection for serology

The numbers and range of sera tested per herd from aborted and non-aborted hinds for BVD and CvHV-1 are presented in Table 6.1. Herds at Scan-2 were categorised as nil, low (R2: >0 - 0.03%, MA: >0 - 0.02%), medium (R2: 0.031 - 0.06%, MA: 0.021 - 0.035%), and high (R2: >0.06%, MA: >0.035%) DAR.

6.3.3.1 BVD

Sera collected at Scan-2 from aborted hinds from all herds were tested. Sera from non-aborted hinds were selected randomly from herds under no (R2=17 herds and MA= 18 herds), low (R2=26 herds, MA=14 herds), medium (R2=15 herds, MA=7 herds), and high (R2=14 herds, MA=8 herds) DAR categories. Therefore, the total sera from non-aborted and aborted hinds tested per herd varied from 1-41 in 72 R2 and 1-18 in 47 MA herds.

6.3.3.2 CvHV-1

Sera collected at Scan-2 from aborted hinds from all herds were tested. Sera from non-aborted hinds were selected randomly from herds under no (R2=17 herds and MA= 17 herds), low (R2=28 herds, MA=16 herds), medium (R2=15 herds, MA=7 herds), and high (R2=14 herds, MA=8 herds) DAR categories. Therefore, the total sera from non-aborted and aborted hinds tested per herd varied from 1-40 in 74 R2 and 1-18 in 48 MA herds.

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.

Virus	Age group	Herds with or without aborted hinds at Scan-2	Herds	Total number tested (range per herd)		
				Aborted	Non-aborted	Total
BVD	R2	With	55	259 (1 - 21)	261 (0 - 22)	520 (1 - 41)
		Without	17	na	119 (7)	119 (7)
		Total	**72**	**259 (1 - 21)**	**380 (0 - 22)**	**639 (1 - 41)**
	MA	With	29	78 (1 - 8)	111 (0 - 10)	189 (1 - 18)
		Without	18	na	113 (6 - 11)	113 (6 - 11)
		Total	**47**	**78 (1 - 8)**	**224 (0 - 11)**	**302 (1 - 18)**
CvHV-1	R2	With	57	266 (0 - 21)	245 (0 - 20)	511 (1 - 40)
		Without	17	na	102 (2 - 7)	102 (2 - 7)
		Total	**74**	**266 (0 - 21)**	**347 (0 - 20)**	**613 (1 - 40)**
	MA	With	31	80 (1 - 8)	60 (0 - 10)	140 (1 - 18)
		Without	17	na	92 (3 - 9)	92 (3 - 9)
		Total	**48**	**80 (1 - 8)**	**152 (0 - 10)**	**232 (1 - 18)**

na= Not applicable

6.3.4 Herpesvirus consensus PCR.

DNA was extracted from myometrium and caruncles pooled together, placenta, and vaginal samples, using the DNeasy Tissue Kit (Qiagen, Victoria, Australia) as per the manufacturer's instructions for fresh or frozen tissue samples. Water blanks were included as sample processing controls to confirm the lack of contamination during sample testing process. A summary of DNA samples tested for CvHV-1 DNA is presented in Table 6.2. Uteri samples came from 108 R2 and 31 MA aborted hinds from 16 R2 and 12 MA herds at Scan-2 and nine hinds with no live calf at weaning from one MA herd. The vaginal samples came from four R2 and 1 MA aborted hinds from two R2 and one MA herds at Scan-2, respectively, whereas the placental samples came from five MA aborted hinds at Scan-2 from four MA herds.

A herpes consensus PCR was carried out based on method adapted from Johne *et al.* (2002) and VanDevanter *et al.* (1996) (Appendix 5). The first round PCR reaction mixture volume of 50 µL DNA consisted of 20.55µL of sterile distilled water, of 5µL 10x PCR buffer, 1.5 µL of 50 mM $MgCl_2$, 10 µL of Q solution (Qiagen), 3.75µL of DMSO (Sigma-Aldrich, Auckland, New Zealand), 1.0 µL of 10mM dNTPs, 1.0 µL of 10µM DFA forward primer (5'-GAYTTYGCNAGYYTNTAYCC-3'), 1.0 µL of 10µM ILK forward primer (5'TCCTGGACAAGCAGCARNYSGCNMTNAA-3'), 1.0 µL of 10µM KG1 reverse primer (5'GTCTTGCTCACCAGNTCNACNCCYTT-3'), 1 unit of Platinum Taq (Invitrogen), and 5µL of DNA sample. The second-round PCR reaction volume of 50 µL consisted of 21.55µL of sterile distilled water, 5µL of 10x PCR buffer, 1.5 µL of 50 mM $MgCl_2$, 10 µL of Q solution (Qiagen), 3.75µL of DMSO (Sigma-Aldrich, Auckland, New Zealand), 1.0 µL of 10mM dNTPs, 1.0 µL of 10µM TGV forward primer (5'TGTAACTCGGTGTAYGGNTTYACNGGNGT-3'), 1.0 µL of 10µM IYG reverse primer (5'-CACAGAGTCCGTRTCNCCRTADAT-3'), 1 unit of Platinum Taq (Invitrogen), and 5µL of DNA sample from first round of amplification.

The thermal cycling conditions were as described by VanDevanter *et al.* (1996). All PCR products were resolved by ultra-pure agarose gel electrophoresis (1.5% w/v, Invitrogen) containing ethidium bromide and visualised under UV light on a transilluminator. DNA isolated from previous case of herpesvirus case (CvHV-1) in deer was used as a positive control. The negative control consisted of distilled water. The negative and positive control were included in each PCR run.

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.

Samples from hinds at	Age group	Status	Uterus	Placenta	Vagina
Scan-2	R2	Aborted	108	-	4
	MA	Aborted	31	5	1
Weaning	MA	No live calf at weaning	9	-	-

6.3.4.1 Herpesvirus DNA sequencing

In total, positive amplification products from seven R2 hinds from two herds with aborted hinds were purified using PureLink PCR purification kit (Invitrogen, Carlsbad, California, USA) and subjected to automatic dye-terminator cycle sequencing with BigDye™ Terminator Version 3.1 Ready Reaction Cycle Sequencing kit and the ABI3730 Genetic Analyser (Applied Biosystems Inc, Foster City, California, USA) using the nested forward and reverse primers for confirmation of the genomic sequence. The sequenced products were aligned using the Geneious Pro 4.8.5 (Biomatters Ltd, Auckland, New Zealand) software and submitted to the National Centre of Biotechnology Information (NCBI) blast nucleotide database for confirmation of correct amplification and species identification of CvHV-1 or related herpesvirus strains.

6.3.5 Statistical analysis

The results from serology in R2 and MA herds were analysed separately. Analysis was performed using SAS software, version 9.4 (SAS Institute Inc., Cary NC, USA). For analysis purposes, hinds aborting at Scan-2 were considered as aborted, and therefore included in the DAR calculation and analysis.

6.3.5.1 Individual animal level

The dependent variable aborted status (aborted (1) /non-aborted (0)) at Scan-2 had binary response and resembled a binomial distribution. A logistic model with 'farm' as a random effect was used to model association, between aborted status at Scan-2 and sero-status (BVD or CvHV-1) (negative (0) or positive (1)) at Scan-2 (Eq.1). A similar model with sero-status (negative (0) or positive (1)) as a binary outcome was used to test for difference in animal level sero-prevalence between age groups and herds with or without aborted hinds. Odds ratios with 95% confidence interval and p-value from the Chi-square test were reported for animal level association between aborted status and BVD or CvHV-1 serological status at Scan-2.

$Y_{ij} = A + B*\text{sero-status}_{ij} + U_i + E_{ij}$

(Eq.1)

Where,

Y = Logit-transformed scanning outcome (1/0) of 'i'th hind in 'j'th herd

A = Intercept (average non-pregnancy rate of sero-negative hind)

B = Difference in non-pregnancy rate for sero-positive hinds

Sero-status= BVD or CvHV-1 serological status

U_i = Random effect of 'i'th herd

E_{ij} = Random, uncorrelated error term

6.4 Results

6.4.1 Bovine viral diarrhoea virus (BVD) serology

BVD serology was carried out on 639 R2 and 302 MA hinds from 72 R2 and 47 MA herds, respectively (Table 3). Titres ranged up to 1:192 in R2 hinds and to 1:620 in MA hinds (Table 6.3). Sero-prevalence was 10.3% in R2 and 17.2% in MA hinds and 12.5% overall (Table 6.4).

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).

BVD reciprocal titre	R2 hinds		MA hinds	
	Aborted	Non-aborted	Aborted	Non-aborted
2	233 (90)	335 (88.2)	65 (83.3)	183 (81.7)
3	na	1 (0.3)	na	Na
4	2 (0.8)	2 (0.5)	1 (1.3)	1 (0.4)
8	2 (0.8)	na	1 (1.3)	Na
12	1 (0.4)	3 (0.8)	1 (1.3)	3 (1.3)
16	3 (1.2)	5 (1.3)	2 (2.6)	3 (1.3)
24	1 (0.4)	4 (1.1)	1 (1.3)	1 (0.4)
32	2 (0.8)	1 (0.3)	1 (1.3)	3 (1.3)
48	2 (0.8)	3 (0.8)	2 (2.6)	3 (1.3)
64	4 (1.5)	7 (1.8)	1 (1.3)	3 (1.3)
96	2 (0.8)	5 (1.3)	na	4 (1.8)
128	7 (2.7)	13 (3.4)	1 (1.3)	20 (8.9)
192	na	1 (0.3)	na	Na
384	na	na	1 (1.3)	Na
620	na	na	1 (1.3)	Na

na= No animals in this category

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.

Virus	Age group	Herds with or without aborted hinds at Scan-2	% sero-positive (number tested)*		
			Aborted	Non-aborted	Total
BVD	R2	With	9.3 (259)	11.5 (261)	10.4 (520)
		Without	na	10.1 (119)	10.1 (119)
		Total	**9.3 (259)**	**11.1 (380)**	**10.3 (639)**
	MA	With	15.4 (78)	19.8 (111)	18 (189)
		Without	na	15.9 (113)	15.9 (113)
		Total	**15.4 (78)**	**17.9 (224)**	**17.2 (302)**
CvHV-1	R2	With	20.6 (266)	17.1 (245)	18.8 (511)
		Without	na	17.6 (102)	17.6 (102)
		Total	**20.6 (266)**	**17.3 (347)**	**18.6 (613)**
	MA	With	57.5 (80)	78.3 (60)	66.4 (140)
		Without	na	71.7 (92)	71.7 (92)
		Total	**57.5 (80)**	**74.3 (152)**	**68.5 (232)**

na=Not applicable

Overall, the sero-prevalence for BVD at animal level in aborted and non-aborted hinds was 9.3% and 11.1%, respectively, in R2 and 15.4% and 17.9%, respectively, in MA hinds. In herds with aborted hinds, the BVD sero-status was not associated with aborted status in R2 (p=0.36) or MA (p=0.76) hinds (Table 6.5). The BVD sero-prevalence was similar in hinds from herds with and without aborted hinds in R2 (p=0.55) and MA (p=0.75) age groups. The BVD sero-prevalence was lower in R2 hinds than in MA hinds in herds without aborted hinds (OR=0.28, 95%CI: 0.09 - 0.85, p=0.02) but not in herds with aborted

hinds (p=0.13). Overall (in herds with and without aborted hinds), the sero-prevalence in R2 hinds was significantly lower than in MA hinds (OR=0.45, 95%CI: 0.25 - 0.79, p=0.006).

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.

Sero-status association with aborted status	Age group	Unadjusted model		Adjusted model	
		Odds ratio	Chi-square p-value	Odds ratio	Chi-square p-value
BVD	R2	1.37 (0.70 - 2.69)	0.36	1.39 (0.70 - 2.74)	0.34
	MA	0.87 (0.37 - 2.09)	0.76	0.85 (0.34 - 2.10)	0.72
CvHV-1	R2	1.23 (0.79 - 1.93)	0.36	1.23 (0.79 - 1.93)	0.36
	MA	0.37 (0.17 - 0.81)	0.01	0.41 (0.19 - 0.90)	0.02

6.4.2 Cervid herpesvirus type-1 (CvHV-1) serology

CvHV-1 serology was carried out on 613 R2 and 232 MA hinds from 74 R2 and 48 MA herds, respectively. Distribution of titres for R2 and MA sera are presented in Table 6.6. The overall sero-prevalence for CvHV-1 antibodies was 32.3% in all hinds (R2 and MA combined) whereas it was 18.6% in R2 and 68.5% in MA hinds (Table 6.4).

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).

CvHV-1 reciprocal titre	R2 hinds		MA hinds	
	Aborted	Non-aborted	Aborted	Non-aborted
0	212 (79.7)	220 (85.6)	34 (42.5)	35 (27.8)
1	5 (1.9)	8 (3.1)	na	3 (2.4)
2	13 (4.9)	na	1 (1.3)	na
3	5 (1.9)	2 (0.8)	6 (7.5)	2 (1.6)
4	3 (1.1)	1 (0.4)	7 (8.8)	4 (3.2)
6	1 (0.4)	1 (0.4)	6 (7.5)	na
8	2 (0.7)	na	2 (2.5)	na
12	2 (0.7)	na	1 (1.3)	2 (1.6)
16	2 (0.7)	1 (0.4)	na	3 (2.4)
24	3 (1.1)	na	na	2 (1.6)
32	5 (1.9)	3 (1.2)	4 (5)	8 (6.4)
48	6 (2.3)	3 (1.2)	3 (3.8)	4 (3.2)
64	7 (2.6)	18 (7)	16 (20)	63 (50)

na= no animals in this category

Overall, the sero-prevalence for CvHV-1 at animal level in aborted and non-aborted hinds was 20.6% and 17.3%, respectively, in R2 and 57.5% and 74.3%, respectively, in MA hinds. In herds with aborted hinds, the CvHV-1 sero-positivity was negatively associated

with aborted status in MA (p=0.01) but there was no association between sero-status and abortion in R2 (p=0.36) hinds. The OR for association between CvHV-1 sero-positivity and aborted status in MA herds with aborted hinds was confounded by year and island. The first-level interactions between CvHV-1 sero-positivity and year (p=0.39) and CvHV-1 sero-positivity and island were not significant in MA hinds from herds with aborted hinds.

The CvHV-1 sero-prevalence was similar in hinds from herds with and without aborted hinds in R2 (p=0.61) and MA (p=0.23) groups. The sero-prevalence in R2 hinds was significantly lower than in MA hinds from herds with (OR=0.02, 95%CI: 0.004-0.07, p<0.001) and without aborted hinds (OR=0.09, 95%CI: 0.05-0.17, p<0.001). Overall (combining herds with and without aborted hinds), the sero-prevalence in R2 hinds was significantly lower than in MA hinds (OR=0.07, 95%CI: 0.04-0.12, p<0.001).

6.4.3 Herpesvirus consensus PCR

Eleven of 108 R2 and none of 31 MA uteri from aborted hinds were positive for herpesvirus DNA on PCR. Initially seven R2 PCR positive uteri were sent for sequencing. Cervid Rhadinovirus strain type-2 (CRhV-2) (accession number: AY237365.1) was identified from those seven selected amplification products of the R2 PCR positive uteri samples from two herds. The herpesvirus DNA positive samples came from three R2 herds (1, 6, and 4 samples/herd). Placenta samples (n=5) from aborted MA hinds were negative. Paired PCR and serology data were available from the seven PCR positive R2 hinds. Three were sero-positive for CvHV-1 antibodies and one PCR positive R2 hind was also positive for *Toxoplasma gondii* DNA whereas the remaining three R2 PCR positive hinds were sero-negative. Vaginal samples from four R2 and one MA aborted hinds tested were negative for herpesvirus DNA.

6.5 Discussion

This is the first study to assess association between BVD and CvHV-1 and abortion in farmed deer in New Zealand. There was no association between BVD sero-status and aborted status in R2 or MA hinds. There was no association between CvHV-1 and abortion in R2 hinds, but there was a negative association between sero-positivity and abortion in MA hinds. The sero-prevalence for BVD in R2 and MA hinds was 10.3% and 17.2%, respectively. The sero-prevalence for CvHV-1 was 18.6% in R2 and 68.5% in MA hinds. The sero-prevelence of CvHV-1 in MA hinds (68.5%) was higher than in R2 (18.6%) hinds.

The findings from this study suggest BVD is not a significant cause of abortion in New Zealand farmed deer. However, in other species such as sika deer, fetal loss and subsequent economic losses have been attributed to BVD infection (Rui *et al.* 2000; Gao *et al.* 2011). The lower sero-prevalence in R2 than MA hinds is consistent with continuous exposure. The sero-prevalence of 12.5% in R2 and MA hinds combined was similar to 9.5% reported by Motha *et al.* (2000) in New Zealand. However, the sero-prevalence data reported in this study are likely more robust than those of Motha *et al.* (2000) as the selection in that study was based on collection of blood samples from two deer per farm. Motha *et al.* (2000) also used a blocking antibody ELISA which had not been validated for deer, whereas this study used VNT, based on validated OIE standards, detected specific CvHV-1 neutralising antibodies. In studies on red deer elsewhere, a sero-prevalence of 3.9% of 77 wild red deer was reported by Nielsen *et al.* (2000) in Denmark, and 10.8% of 65 wild red deer was reported in Spain (Fernandez-Aguilar *et al.* 2016). However, it should be noted that the Danish and Spanish studies also used the blocking ELISA as a screening test and used the VNT as a confirmatory test. Additionally, the Spanish study used a cut-off neutralising antibody titre of ≥1:10 for the VNT titres compared with ≥1:8 used in this study. In another Spanish study, a sero-prevalence of 19.5% of 267 wild red deer, which used an indirect ELISA as screening test and blocking ELISA as confirmatory test, was higher than 12.5% in farmed deer in this study (Rodriguez-Prieto *et al.* 2016).

Sero-positivity to CvHV-1 was negatively associated with aborted status in MA hinds but not R2 hinds. This could be related to time of exposure such that non-aborted hinds may have been infected later in gestation and not aborted whereas aborted hinds may have been infected earlier in gestation leading to abortion and subsequent post-exposure reduction in antibody titres. Alternatively, this association may also be due to the low number of sera tested in non-aborted hinds compared with aborted group. For logistic and financial reasons, it was not possible to test a larger number of samples.

The CvHV-1 sero-prevalence of 32.3% reported in this study was moderately lower than 38% reported by Motha and Jenner (2001). Sero-prevalence from this study could not be directly compared to the 32% reported for bovine herpesvirus (BoHV) by Motha *et al.* (2000) using VNT as the sero-prevalence from the latter study may be result of cross-reaction between CvHV-1 and BoHV-1, which are antigenically similar. The sero-prevalence reported in this study was higher than the 5.2% reported in roe deer, 5.7% in fallow deer and 20.5%

red deer in Germany (Frolich 1996; Frolich *et al.* 2006), 7.1% and 2% in red deer in Belgium and France, respectively, (Thiry *et al.* 1988), whereas it was lower than 71% in an imported deer population in Scotland (Pospisil *et al.* 1996). However, the sero-prevalence in this study was similar to 33% reported in farmed red deer whereas it was lower than 40% reported from wild red deer in Scotland and higher than 15.1% in farmed red deer in England (Nettleton *et al.* 1986).

Although there did not appear to be an association between CvHV-1 sero-positivity and aborted status in R2 hinds. Herpesviral DNA was detected in uteri of 11 R2 hinds, although no alpha herpesvirus (CvHV-1) was identified in the tissues, a gamma herpesvirus (CRhV-2) was detected by sequencing. The detection of herpesvirus DNA from uteri was consistent with the report from a captive red deer in France by Thiry *et al.* (2011). However, the sample used in that study was a genital swab whereas those used in the present study were from myometrium. It is proposed that sexual transmission would likely result in the virus manifesting in the uterus. Herpesvirus, CvHV-1 in particular, has also been reported in conjunctiva of a deer hind with keratoconjunctivitis in New Zealand (Squires *et al.* 2012) and Elk (*Cervus elaphus nelsonii*) in North America (Li *et al.* 2005). CvHV-1 was also isolated from semen of stags in New Zealand (Tisdall and Rowe 2001).

This is the second report of ruminant Cervid Rhadinovirus type-2 (CRhV-2) from deer in New Zealand, providing confirmatory evidence for the report of Squires *et al.* (2012) describing CRhV-2 from conjunctival swabs and buffy coat from normal hinds from two farms with known cases of keratoconjunctivitis. CRhV-2 has also been reported in Elk in North America (Li *et al.* 2005). To date, this virus has not been associated with clinical disease or production effects, however, the presence of CRhV-2 in uteri suggests that CRhV-2 could potentially be associated with abortion. Bovine herpesvirus type-4, belonging to same gamma herpesvirinae sub-family and in the Rhadinovirus genus, has been reported for transplacental transmission, however, without any adverse effects in the fetuses (Egyed *et al.* 2011). Serology in this study was done to detect CvHV-1 antibodies and not CRhV-2 antibodies and therefore testing the association between CRhV-2 serology and abortion was outside the scope for this study. However, identification of CRhV-2 does suggest that this virus should be included in future investigations of reproductive losses.

Observations from this study demonstrate that CvHV-1 may be widespread on New Zealand deer farms. Although, this study could not rule out a role of CvHV-1 in abortions or

other reproductive loss in farmed deer in New Zealand. Since this class of virus has been shown to cause disease and reproductive loss in other species, further research is warranted.

The sero-prevalence reported here for BVD and CvHV-1 may be useful in future studies of virus exposure in farmed red deer in New Zealand. However, the primary aim of this study was to assess the association between these viruses and abortion and not determination of sero-prevalence *per se*. The sero-prevalence data reported here, despite being informative, cannot be considered definitive as the participation of farms in the study was not completely random, possibly being biased toward farms with on-going reproduction problems. Nevertheless, the sero-prevalence of BVD suggests its role in clinical disease warrants further investigation, since it has not been reported as a cause of clinical disease in New Zealand farmed deer to date.

The findings from this study suggest that BVD and CvHV-1 may not be contributing to abortions and complement data suggesting that *Leptospira* Hardjobovis and Pomona and *Neospora caninum* as reported in previous studies in this thesis (Chapter 5) are also not significant causes of abortion in farmed deer in New Zealand. By contrast, *Toxoplasma gondii* was estimated to be the cause of 7.9% of abortions in R2 hinds (Chapter 4).

6.6 Conclusion

BVD was not associated with abortion in R2 or MA hinds. Although there was no serological evidence that CvHV-1 was significantly associated with abortion, there was gamma herpesviral DNA evidence in fetal and uterine tissues that will need to be further explored to determine if there is an association between Rhadinovirus herpesvirus infection and abortion.

6.7 Acknowledgements

This study was supported by the Deer Reproductive Efficiency Group based in Southland. It is funded by AgResearch, Agmardt, DEEResearch, MSD Animal Health, Southland Deer Farmers Association, Massey University and individual farmers, particularly Landcorp Farming Ltd. The in-kind contribution of all participating farmers is gratefully acknowledged, as is the assistance of a large number of veterinary practices and scanners for scanning and blood sample collection and DSP staff and veterinarians for tissue sample collection. I am also grateful to Wlodek Stainslawek (Senior Virologist, AHL, MPI, Wallaceville) and technical team at MPI virology laboratory (Smriti, Nair, Sylvia Ohneiser

and Rana Fathigaran) for undertaking testing of sera-samples for BVD and CvHV-1, and to MPI for funding this diagnostic testing. The technical assistance from the IVABS technical team at Massey University is well appreciated. I am also thankful to the staff at the post-mortem facility to allow dissection of maternal and fetal tissues in the post-mortem premises.

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Chapter 7. Analysis of farm, environment, health and management risk factors for pregnancy and abortion in New Zealand farmed red deer

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This chapter is prepared in the style format of the New Zealand Veterinary Journal.

7.1 Abstract

Sub-optimum pregnancy rates and abortion contribute to reproductive inefficiency in the New Zealand farmed deer industry. Previous studies have identified individual animal and management factors for improvement of pregnancy and weaning rates but not abortion. The aim of this study was to evaluate farm, environment, health and management factors for pregnancy, determined by ultrasound scanning early in gestation (Scan-1), and abortion, determined by a second ultrasound scan (Scan-2) around the end of the second trimester of pregnancy (approximating to mid-term abortion) in rising-two-year-old (R2) and mixed-age (MA) deer herds in New Zealand.

Rising two-year-old (R2, n=22,130) and mixed-age (MA, n= 36,223) hinds from 87 and 71 herds, respectively, throughout NZ were ultrasound scanned early in gestation (Scan-1) in a 2-Year follow-up study. A sub-sample of up to 100 R2 and 155 MA hinds per herd from 81 and 59 herds, respectively, were re-scanned (Scan-2) between 55 and 89 days later, at approximately the end of the second trimester. One questionnaire for the period from mating to Scan-1 (Q1, autumn), and one from Scan-1 to Scan-2 (Q2, winter), were sent to 85 participating farmers. The response rate was 76.5% for Q1 and 55.3% for Q2. In total, data from 61 R2 and 54 MA herds from Q1, and 40 R2 and 33 MA herds from Q2 were available for analysis. Q1 risk factors were evaluated for pregnancy rate at Scan-1 and daily abortion rate (DAR) at Scan-2. Q2 risk factors were evaluated for DAR at Scan-2. Data from R2 and MA herds were analysed separately. Univariate analysis for Q1 and Q2 data was performed using logistic regression and those risk factors with p-value ≤0.2 were entered into a multivariate model. Least square (LS) mean pregnancy rate and DAR from multivariate model are reported for comparisons.

Autumn Q1-analysis showed that mean pregnancy rates were higher in R2 herds with sheep on-farm and in MA herds fed maize and chaumolier. Mean pregnancy rate was lower in R2 herds from year-2, co-grazed with beef cattle, treated with Copper, with dairy stock on-farm, and fed lucerne hay, and in MA herds in the North than South Island and when there were dairy stock on-farm. Mean DAR was higher in R2 and MA herds co-grazed with beef cattle. The mean DAR was higher and lower in R2 and MA herds, respectively, with notification of JD from slaughterhouses. R2 herds from year-2 and MA herds fed meadow hay, co-grazed with beef cattle and with sheep on-farm had higher mean DAR. The DAR was lower in R2 herds sequentially grazed with beef cattle and managed by experienced deer

farmers, and in MA herds where Copper was added to fertiliser and where abortions had occurred in previous years.

Winter Q2-analysis showed that R2 herds reporting JD had a higher mean DAR whereas feeding bailage, and being in the North Island, or having received a leptospirosis notification from slaughterhouses were associated with a lower mean DAR. MA herds fed meadow hay and sequentially grazed with sheep had higher DAR. Higher mean DAR in herds fed meadow hay may be due to presence of mould in hay or may be due to hinds being nutritionally deficient and therefore supplemented with hay.

While the associations identified here do not necessarily have a causal interpretation most are biologically plausible. However, proof of causation would require more research.

KEY WORDS: *Red deer, abortion, fetal wastage, pregnancy, ultrasound scanning, farm management, nutrition, forage, supplements, crops, health, environment, risk analysis.*

7.2 Introduction

The deer industry in New Zealand contributes significantly to the New Zealand economy. However, reproductive performance in farmed red deer has long been sub-optimum, with estimated reproductive efficiency (calves weaned/hinds mated) averaging 75.2% for the last 13 years (Statistics New Zealand). Reproductive inefficiency may be due to failure to conceive, early embryonic loss, abortion, and perinatal and postnatal mortality. Previous studies have shown that adoption of best management practices can improve the reproductive performance of farmed deer (Beatson *et al.* 2000; Wilson 2002b; Asher and Wilson 2011), particularly with respect to pregnancy rate (Audigé *et al.* 1999b; Audigé *et al.* 1999c).

Pregnancy rates at ultrasound scanning described in Chapter 2 of 85.8% for rising-two-year-old (R2) hinds and 93.3% for mixed-age (MA) hinds are consistent with other data showing that the pregnancy rates are lower in R2 hinds compared with MA hinds (Audigé *et al.* 1999a; Campbell *et al.* 2000; Walker *et al.* 2000; Lawrence 2003). There is room for improvement of pregnancy rates in most R2 and MA herds. Audigé *et al.* (1999b; 1999c) identified individual animal factors including higher growth rate and bodyweight, higher proportion of NZ blood line, use of back up and experienced sires, higher body condition score, and management factors such as less frequent yarding, increased access to warm areas of paddocks, less frequent mob changes were associated with higher pregnancy rates. Those findings suggest that changes in management practices and animal factors can lead to improvement in pregnancy rates in both R2 and MA hinds.

Embryo and fetal loss have historically been regarded as insignificant problems in farmed deer in New Zealand, likely because they are difficult to detect. There has been limited quantification or characterisation of these losses on commercial farms since hinds are rarely, if ever, checked for fetal loss by repeated ultrasound scanning or other techniques. Studies characterising the extent of fetal loss between scanning for pregnancy early in gestation and calving in New Zealand farmed deer reported 2.6% abortion in yearlings (Fennessy *et al.* 1986) on a research station deer farm, 1% abortion in 15 mixed-age deer herds in Canterbury (Campbell *et al.* 2000), and 0.6% in yearling and 0.8% in adult hinds in eight intensively monitored herds in the lower North Island (Audigé *et al.* 1999a). However, data are limited and based on crude measures such as abdominal and udder palpation late in gestation or post-calving, rather than repeated ultrasound pregnancy scans. A Spanish report

described a fetal mortality in two of seven yearling hinds and four of 39 adult hinds at an experimental farm study (Gomez-Nieto *et al.* 2011). A recent clinical investigation that involved repeat pregnancy scanning on four large deer farms identified that 1.7-16.0% of R2 hinds and 4% of MA hinds in one herd had aborted in mid-pregnancy (Wilson *et al.* 2012). To the Author's knowledge, there are no studies on assessment of risk factors for abortion in New Zealand farmed deer. A comprehensive study of abortion reported in Chapter 2 showed mid-term herd level daily abortion rates ranging from 0.005-0.21% and 0.007-0.1% in R2 and MA herds, respectively.

The aim of this study was to evaluate farm, management, health and environmental risk factors for pregnancy, determined by ultrasound scanning early in gestation, and abortion, determined by a second ultrasound scan around the end of the second trimester of pregnancy (approximating to mid-term abortion) in R2 and MA deer herds throughout New Zealand.

7.3 Materials and methods

The sampling frame for farms and animals used for this analysis is described in detail in Chapter 2. Briefly, 22,130 R2 and 36,223 MA red deer hinds from 87 and 71 herds, respectively, throughout New Zealand were ultrasound scanned, after a mean interval of 49 days of stag removal, for pregnancy early in gestation (Scan-1), and a randomly selected sub-sample (up to 100 R2 and 200 MA hinds per herd) was scanned again approximately at the end of the second trimester (Scan-2) after a mean between-scan interval of 89 days (approximating mid-term) during the 2012 and 2013 reproductive cycles. Blood, uteri, and aborted and normal fetuses were collected. All animal manipulations were approved by the Massey University Animal Ethics committee (Protocol numer: 12/34).

Hinds were classified as 'pregnant' at Scan-1 based on the presence at least one fetus or part thereof, amniotic membrane, and/or presence of placentomes, or as being 'non-pregnant' based on absence of those signs combined with visualisation of a non-pregnant uterus at Scan-1. The term "aborting" is ascribed to hinds that had ultrasound evidence of aborting fetuses at Scan-1 and Scan-2. The term "aborted", is ascribed to hinds that were pregnant at Scan-1 but not pregnant at Scan-2, plus those aborting at Scan-2. Daily abortion

rate (DAR) ((number aborted at Scan-2 /number pregnant at Scan-1 undergoing Scan-2) / number of days between Scan-1 and Scan-2) was calculated at herd-level.

Eighty-five participating deer farmers were contacted through veterinary practices, lay scanners or the Author to take part in two-year fetal wastage study as described in Chapter 2. Deer farms comprised R2 and/or MA herds. Two questionnaires, one each for the period from mating to Scan-1 (Q1, designated "Autumn"), and Scan-1 to Scan-2 (Q2, designated "Winter"), were designed to cover farm, environment disease and management factors at herd-level. The questionnaires were sent by mail in July (Q1) and in August (Q2). The questionnaires were completed by deer farmers and returned using a postage-paid envelope. Questionnaires covered R2 and MA herd data individually where appropriate and farm level data common to both age groups. Farmers who did not return a questionnaire were sent another copy by mail or via their scanning providers and followed up using mail and/or telephone and/or via their scanning provider.

Questionnaires are presented in Appendices 6 and 7. Briefly, information on general topography, reproduction and abortion history, forage management, health management, and environmental factors were asked in Q1 aimed to cover information between mating (January 1) to Scan-1. General information included farm-level data on topography, location, and number of years of deer farming, number of deer and breeds present, and presence of other livestock. With reference to feeding management, data on time spent on each pasture species, feeding of supplement and autumn crops, and their grazing pattern (co- or alternate grazing with sheep/beef cattle/dairy cattle) was collected. Nutritional management data included forage species, and crops and their management. Deer health data included deer slaughter premises (DSP) notifications for JD and leptospirosis, on-farm disease occurrence (JD, toxoplasmosis, leptospirosis, neosporosis, bovine viral diarrhoea, cervid herpes virus), vaccination, and anti-parasitic treatment/s in previous and/or participation year. Environment data were collected on water source, presence of wildlife, and unusual weather conditions including any extreme conditions such as drought. Additionally, farmers were asked to provide source (memory and/or written records) of information used to complete the questionnaires.

Questionnaire-2 addressed change in deer numbers such as number culled, sold, or purchased in addition to health, forage management and environment similar to Q1, but

specific to the between-scan (winter) period. Farmers were also asked about any observation of dead fetuses.

7.3.1 Statistical analysis

Statistical analysis was done in SAS software, version 9.4 (SAS Institute Inc., Cary NC, USA). The data from both sampling years were pooled. Analyses were conducted for R2 and MA herds independently. Analyses were performed to test association between both farm demographics and autumn risk factors and pregnancy at Scan-1, and abortion by Scan-2, and both farm demographics and winter risk factors and abortion by Scan-2.

7.3.1.1 Autumn risk factors (Q1)

7.3.1.1.1 Univariate analysis

Data from Q1 are referred to as winter risk factors. The response variables, proportion pregnant (number pregnant/number scanned) at Scan-1 and daily abortion rate (DAR) ((number pregnant/number pregnant from Scan-1 and scanned at Scan-2)/between-scan interval)) at Scan-2 in R2 and MA herds, were logit transformed to allow an approximation of normal distribution. A general linear model was used for univariate analysis of risk factors from Q1 using Proc GLIMMIX.

7.3.1.1.2 Multivariate analysis

Multivariate analysis was carried out to assess the association between autumn risk factors from Q1 and pregnancy rate and DAR using logistic regression with events/trials syntax of Proc GENMOD. The response variable at Scan-1 was number pregnant/number scanned (pregnancy rate) whereas the response variable at Scan-2 was number aborted between scans per number pregnant at Scan-1 and present at Scan-2. The natural log of between-scan interval (days) was chosen as an offset to model abortion at Scan-2. Generalised estimating equations (GEE) (Zeger and Liang 1986) with an exchangeable correlation structure, binomial distribution and logit link were used to model pregnancy rate at Scan-1 and DAR at Scan-2, to account for correlation between herds belonging to same farms and/or repeat sampled in year-2 in five instances. Those risk factors with a p-value of ≤ 0.2 from Student's t-test in the univariate analysis were entered in a multivariate model. Manual backward elimination method was followed starting with a full model including confounders (year and island effect). Risk factors significant at p-value of <0.05 were retained in the final model. Confounders were not removed at any step of backward

elimination of risk factors. Least square (LS) means for pregnancy rate at Scan-1 and DAR at Scan-2 are reported for comparison purposes.

7.3.1.2 Winter risk factors (Q2)

7.3.1.2.1 Univariate analysis

Data from Q2 are referred to as winter risk factors. The response variable DAR at Scan-2 in R2 and MA herds, was logit transformed to allow an approximation of normal distribution as above. A general linear model was used for univariate analysis of risk factors from Q1 using Proc GLIMMIX.

7.3.1.2.2 Multivariate analysis

Those risk factors with p-value of ≤0.2 from Wald's Chi-square test were entered in a multivariate GEE model. Manual backward elimination regression was followed starting with a base model including confounders only (year and island effect). Risk factors significant at p-value of <0.05 were retained in the final model. Confounders were not removed at any step of backward elimination of risk factors. Least square (LS) means from multivariate model for pregnancy rate at Scan-1 and DAR at Scan-2 are reported for comparison purposes.

7.4 Results

Completed Q1 were received from 65 (76.5%) of 85 farms comprising 56 R2 and 49 MA herds. There were 43 farms with both R2 and MA herds and six and 16 farms with R2-only and MA-only herds, respectively. Additionally, questionnaires from five farms with both R2 and MA herds participated in the study for abortion repeatability assessment returning questionnaires in both years. Therefore, in total, data from 61 R2 and 54 MA herds were available for analysis of risk factors from Q1.

The herd-level pregnancy scanning summary data at Scan-1 and Scan-2 for R2 and MA herds that returned Q1 and Q2 are presented in Table 7.1. In R2 herds with Q1 data, the herd-level pregnancy rate averaged 81.4% (95% CI=77-85.8%). In MA herds with Q1 data, the herd-level pregnancy rate averaged 92.4% (95 CI=89.9-94.9%). The mean pregnancy rate was not different in herds with and without Q1 data in R2 (p=0.15) and MA (p=0.65) age groups, based on scan data from non-responder farms.

Data from Q1 suitable for risk factor analysis for abortion was from 59 R2 and 45 MA herds. In R2 herds with aborted hinds and Q1 data, the herd-level DAR averaged 0.043% (95% CI=0.031-0.054%). In MA herds with aborted hinds and Q1 data, the herd-level DAR

averaged 0.026% (95 CI=0.017-0.034%). The mean herd-level abortion rate for the average between-scan interval of 90 days, in herds with aborted hinds, was 3.9% for R2 and 2.3% for MA hinds. The mean DAR by Scan-2 was not different in herds with and without Q1 data in R2 (p=0.28) and MA (p=0.5) age groups.

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.

Age	Herds with	Herds at Scan-1	Mean (range) of pregnancy (%) at Scan-1	Herds at Scan-2	Mean (range) of abortion % at Scan-2		
					Herds	Daily	90 days
R2	Q1 data	61	81.4 (7.0 - 100)	59	43	0.043 (0.005 - 0.213)	3.8 (0.4 - 19.1)
	Q2 data	40	84.3 (80.0 - 88.7)	40	27	0.044 (0.006 - 0.213)	4.0 (0.5 - 19.1)
	All herds[a]	87	82.0 (7.0 - 100)	81	60	0.043 (0.005 - 0.210)	3.9 (0.4 - 19.1)
MA	Q1 data	54	92.4 (39.8 - 100)	45	27	0.026 (0.007 - 0.101)	2.3 (0.6 - 9.1)
	Q2 data	33	90.1 (86.1 - 94.2)	33	19	0.027 (0.007 - 0.101)	2.4 (0.6 - 9.1)
	All herds[a]	71	92.6 (39.8 - 100)	59	35	0.025 (0.007 - 0.101)	2.2 (0.6 - 9.1)

[a] These include data from those herds providing Scan-1 and Scan-2 data (described in Chapter 2), including those not returning questionnaires.

7.4.1 Univariate autumn risk factors analysis for pregnancy rate at Scan-1 and daily abortion rate (DAR) at Scan-2.

A summary of univariate analysis of autumn risk factors, relating to nutrition, and grazing management, previous reproduction history, health, trace mineral supplementation, and environment, and associations with pregnancy rate at Scan-1 and DAR at Scan-2 are presented in Tables 7.2 to 7.5.

7.4.1.1 General description

The mean period of deer farming experience was 17 years (range: 2-44) including the five farms repeated in year-2 which were considered as independent. In univariate analysis, deer farming experience was not associated with pregnancy rates (R2 p=0.18, MA p=0.62) or DARs (R2 p=0.14, MA p=0.87).

7.4.1.2 Autumn forages, crops, and supplements

Data for autumn forages grazed was missing for two R2 and one MA herds. Hinds from 59 R2 and 53 MA herds grazed mostly on ryegrass/white-clover and native grass. The mean pregnancy rate or DAR was not associated with percentage of time spent grazing autumn pastures. Crops fed in autumn included brassicas (chaumolier, kale, rape), swedes,

sugar beet. Most farmers fed swedes and kale to both R2 and MA herds. The mean pregnancy rate or DAR was not different between herds fed and not fed autumn crops in either age group. On an individual crop basis, MA herds fed swedes had a higher pregnancy rate than herds not fed swedes (p=0.003).

Farmers also fed autumn supplements such as meadow hay, peavine hay, lucerne hay, bailage, silage, bailage, maize, and barley to R2 and MA hinds. Bailage and silage were the most commonly fed supplements. Deer nuts were fed to R2 and one MA herd whereas palm kernel extract (PKE) was fed to one R2 and three MA herds. Feeding of deer nuts and PKE were included in the 'others' category for supplement feeding. Overall, autumn supplement feeding, after pooling data from all supplements or on individual supplement basis, to R2 or MA herds was not significantly associated with either pregnancy rates or DARs.

Mouldy feed including silage, bailage, and grains was fed in autumn to R2 and/or MA hinds. Overall, after pooling data for all supplements, feeding of mouldy supplements, was not associated with either pregnancy rates or DAR in R2 and MA hinds.

7.4.1.3 Autumn grazing management

Co-grazing (same pasture at the same time) or alternate (same pasture grazed at different time) autumn grazing of hinds with sheep, beef cattle or dairy cattle was carried out for both R2 and MA herds. Overall, after pooling data from sheep, beef cattle, and dairy cattle, the mean pregnancy rate in autumn co-grazed R2 herds was lower than in those not co-grazed with either of those species (p=0.02). Based on individual species, the mean pregnancy rate was lower in R2 herds co-grazed with beef cattle (p=0.01). Overall, the mean DAR was lower in R2 herds alternate-grazed with sheep/beef cattle/dairy cattle than in herds not alternate-grazed with those species (p=0.02). Based on individual species, the mean DAR was higher in MA herds alternate grazed with sheep (p=0.01).

Table 7.2: Univariate associations between pasture type fed in autumn and deer farming experience and the pregnancy rate at Scan-1.

Forage	R2 herds				MA herds			
	Data from herds	% of time spent	Model coefficient	P-value	Data from herds	% of time spent	Model coefficient	P-value
Ryegrass/White clover	59	80.5	0.0003	0.96	53	72.1	-0.006	0.17
Red clover	59	1.7	-0.008	0.79	53	1.8	0.009	0.73
Native grass	59	9.7	0.003	0.72	53	18.9	0.008	0.11
Chicory	59	2.8	-0.004	0.84	53	2.1	0.002	0.92
Plantain	59	2.8	-0.006	0.80	53	1.8	-0.005	0.86
Lucerne	59	0.1	-0.014	0.96	53	0.4	0.005	0.93
Others	59	1.4	-0.010	0.64	53	1.9	0.000	1.00
Deer farming experience (years)	60	18[a]	0.019	0.18	53	18[a]	0.008	0.62

[a] average deer farming experience (years).

Table 7.3: Univariate associations between pasture type fed in autumn and deer farming experience and daily abortion rate by Scan-2.

Forage	R2 herds				MA herds			
	Data from herds	% of time spent	Model coefficient	P-value	Data from herds	% of time spent	Model coefficient	P-value
Ryegrass/White clover	57	79.5	-0.001	0.93	45	71.2	0.007	0.64
Red clover	57	1.8	-0.117	0.13	45	2.1	0.020	0.81
Native grass	57	10.2	0.007	0.74	45	18.4	-0.012	0.47
Chicory	57	2.9	0.005	0.94	45	2.5	0.040	0.57
Plantain	57	3.0	-0.009	0.87	45	2.0	-0.002	0.99
Lucerne	57	0.1	0.200	0.79	45	0.4	-0.232	0.23
Others	57	1.5	0.005	0.93	45	2.2	-0.006	0.91
Deer farming experience (years)	59	18[a]	-0.071	0.14	45	17[a]	0.009	0.87

[a] average deer farming experience (years).

Table 7.4: Univariate associations between nutritional management, environment, health, year and island risk factors in autumn and pregnancy rate at Scan-1.

Category of variable	Variable	R2 herds					MA herds				
		Data from herds	Risk factor present in herds	Mean pregnancy rate (%)		P-value	Data from herds	Risk factor present in herds	Mean pregnancy rate (%)		P-value
				Risk factor present	Risk factor absent				Risk factor present	Risk factor absent	
Crops	Overall[a]	22 (61)	22	83.3	80.4	0.97	54	22	93.2	91.8	0.27
	Chaumolier	22 (61)	3	90.1	81.0	0.38	54	3	98.1	92.1	0.11
	Kale	61	5	86.8	80.9	0.80	54	11	95.5	91.6	0.21
	Rape	61	2	86.8	81.2	0.95	54	2	87.2	92.6	0.23
	Swedes	61	7	86.7	80.7	0.90	54	8	96.6	91.7	0.003
	Sugar beet	61	1	95.0	81.2	0.44	54	3	93.1	92.4	0.64
	Others	61	7	71.4	82.7	0.15	54	5	86.0	93.1	0.18
Supplements	Overall[a]	61	40	81.2	81.7	0.57	54	43	93.3	89.0	0.53
	Meadow hay	61	6	71.3	82.5	0.33	54	8	91.9	92.5	0.83
	Peavine hay	61	1	85.8	81.3	0.97	54	1	95.0	92.4	0.39
	Lucerne hay	61	2	67.4	82.1	0.20	54	1	93.9	92.3	0.70
	Bailage	61	23	82.6	80.6	0.17	54	24	93.3	91.6	0.81
	Silage	61	15	77.3	82.8	0.16	54	15	92.9	92.2	0.70
	Maize	61	2	68.7	81.8	0.45	54	2	97.5	92.2	0.27
	Barley	61	8	82.3	81.3	0.78	54	11	93.1	92.2	0.45
	Others	61	2	87.0	81.1	0.78	54	3	92.2	92.4	0.58
	Mouldy feed	61	8	80.6	81.5	0.54	54	13	90.2	93.1	0.91
Grazing	Co-grazing (overall[a])	61	17	71.2	85.4	0.02	54	18	91.9	92.7	0.78
	Co-grazing sheep	61	4	67.8	82.4	0.17	54	4	89.6	92.6	0.75
	Co-grazing beef	61	16	69.7	85.6	0.01	54	16	92.0	92.6	0.59
	Co-grazing dairy	61	2	70.7	81.8	0.61	54	1	89.4	92.5	0.48
	Alternate	61	29	79.8	82.9	0.15	54	26	90.7	94.0	0.87

	grazing (overall[a])										
	Alternate grazing sheep	61	13	78.1	82.3	0.37	54	11	89.1	93.2	0.93
	Alternate grazing beef	61	23	78.6	83.1	0.18	54	19	92.3	92.5	0.64
	Alternate grazing dairy	61	6	79.0	81.7	0.48	54	5	91.5	92.5	0.38
	Abortions in previous years	61	8	84.1	81.0	0.87	54	6	95.0	92.1	0.91
Johne's disease	DSP notification	61	20	79.4	82.4	0.35	54	16	93.3	92.0	0.66
	On-farm detection	61	21	81.6	81.3	0.42	54	9	94.2	92.0	0.61
Vaccination	*Leptospira* spp.	61	15	79.9	81.9	0.54	54	8	85.4	93.6	0.27
	Clostridia spp.	61	6	80.8	81.5	0.67	54	4	93.4	92.3	0.42
	Anti-parasitic treatment	61	35	78.8	85	0.04	54	11	88.3	93.4	0.02
Trace mineral supplement	Copper to hinds	61	19	75.1	84.3	0.04	54	17	89.2	93.9	0.15
	Selenium to hinds	61	15	82.8	81	0.87	59	11	92.9	92.3	0.72
	Cobalt to hinds	61	1	95.7	81.2	0.38	59	0	-	92.4	-
	Multimin to hinds	61	10	78.1	82.1	0.41	54	8	92.7	92.3	0.87
	Copper to fertiliser	61	12	77.5	82.4	0.40	54	12	94.0	92.0	0.85
	Selenium to fertiliser	61	34	83.6	78.7	0.90	54	29	94.5	89.9	0.07
	Cobalt to fertiliser	61	9	85.7	80.7	0.57	54	8	95.6	91.8	0.17
Environment	Drought	61	24	78.8	83.1	0.16	54	21	92.3	92.4	0.31
Wildlife	Wild deer	61	43	82	80	1.00	54	38	91.9	93.6	0.45
Other livestock on farm	Sheep	61	42	83	77.9	0.13	54	37	91.8	93.7	0.94
	Beef cattle	61	33	81	81.9	0.40	54	29	93.8	90.8	0.82
	Dairy cattle	61	10	75.5	82.6	0.17	54	9	87.0	93.5	0.15
Year	Year-2 vs Year-	61	22	76.7	84.1	0.30	54	21	91.5	93.0	0.12

	1										
Island	North vs South	61	24	81.1	81.6	0.76	54	21	90.4	93.7	0.75

[a] Where used, this term describes a combination of all individual variables in each category

Table 7.5: Univariate association between nutritional management, environment, health, year and island risk factors in autumn and daily abortion rate at Scan-2.

Category of variable	Variable	R2 herds					MA herds				
		Data from herds	Risk factor present in herds	Mean daily abortion rate (%)		P-value	Data from herds	Risk factor present in herds	Mean daily abortion rate (%)		P-value
				Risk factor present	Risk factor absent				Risk factor present	Risk factor absent	
Crops	Overall[a]	59	21	0.029	0.032	0.59	45	18	0.012	0.018	0.96
	Chaumolier	59	3	0.008	0.032	0.69	45	3	0.018	0.015	0.75
	Kale	59	5	0.016	0.032	0.40	45	9	0.011	0.016	0.32
	Rape	59	2	0.026	0.031	0.54	45	2	0.003	0.016	0.63
	Swedes	59	7	0.027	0.032	0.55	45	7	0.012	0.016	0.63
	Sugarbeet	59	1	0.028	0.031	0.57	45	3	0.009	0.016	0.92
	Others	59	6	0.042	0.03	0.24	45	4	0.009	0.016	0.69
Supplements	Overall[a]	59	38	0.031	0.032	0.24	45	36	0.017	0.009	0.65
	Meadow hay	59	6	0.034	0.031	0.58	45	7	0.039	0.011	0.07
	Peavine hay	59	1	0.008	0.031	0.81	45	1	0	0.016	0.23
	Lucerne hay	59	2	0.056	0.03	0.93	45	1	0.033	0.015	0.96
	Bailage	59	22	0.031	0.031	0.82	45	20	0.018	0.013	0.46
	Silage	59	14	0.025	0.033	0.81	45	13	0.015	0.015	0.45
	Maize	59	2	0.044	0.031	0.62	45	1	0.007	0.016	0.60
	Barley	59	8	0.046	0.029	0.08	45	9	0.012	0.016	0.30
	Others	59	2	0.025	0.031	0.69	45	3	0.015	0.015	0.62
	Mouldy feed	59	8	0.028	0.032	0.96	45	11	0.012	0.016	0.84
Grazing pattern	Co-grazing (overall)[a]	59	16	0.041	0.028	0.10	45	14	0.020	0.013	0.10
	Co-grazing sheep	59	4	0.046	0.030	0.16	45	2	0.061	0.013	0.14
	Co-grazing beef	59	15	0.040	0.028	0.14	45	12	0.022	0.013	0.06
	Co-grazing	59	2	0.034	0.031	0.38	45	1	0.010	0.015	0.53

	dairy										
	Alternate grazing (overall[a])	59	28	0.017	0.044	0.02	45	20	0.019	0.012	0.52
	Alternate grazing sheep	59	13	0.019	0.035	0.23	45	7	0.033	0.012	0.01
	Alternate grazing beef	59	22	0.020	0.038	0.14	45	14	0.023	0.012	0.94
	Alternate grazing dairy	59	6	0.011	0.033	0.16	45	5	0.005	0.017	0.77
	Abortions in previous years	59	8	0.037	0.03	0.28	45	6	0.004	0.017	0.13
Johne's disease	DSP notification	59	20	0.041	0.026	0.14	45	13	0.007	0.019	0.01
	On-farm detection	59	21	0.041	0.025	0.46	45	9	0.012	0.016	0.11
Vaccination	*Leptospira* spp.	59	15	0.028	0.032	0.27	45	7	0.021	0.014	0.89
	Clostridia spp.	59	2	0.031	0.031	0.61	45	1	0.002	0.017	0.11
	Anti-parasitic treatment	59	34	0.036	0.024	0.72	45	10	0.031	0.011	0.02
Trace mineral supplement	Copper to hinds	59	18	0.029	0.032	0.84	45	15	0.024	0.011	0.36
	Selenium to hinds	59	15	0.043	0.027	0.17	45	9	0.023	0.013	0.94
	Cobalt to hinds	59	1	0.005	0.032	0.93	45	0	-	0.015	-
	Multimin to hinds	59	10	0.047	0.028	0.50	45	7	0.019	0.015	0.38
	Copper to fertiliser	59	12	0.037	0.03	0.76	45	12	0.009	0.018	0.11
	Selenium to	59	34	0.027	0.037	0.96	45	25	0.013	0.018	0.25

	fertiliser										
	Cobalt to fertiliser	59	9	0.023	0.033	0.59	45	7	0.009	0.016	0.76
Unusual environment	Drought	59	24	0.031	0.031	0.83	45	18	0.019	0.013	0.80
Wildlife around farm	Wild deer	59	42	0.036	0.019	0.22	45	31	0.018	0.01	0.15
Other livestock on farm	Sheep	59	40	0.028	0.037	0.34	45	29	0.020	0.006	0.00
	Beef cattle	59	33	0.033	0.029	0.65	45	25	0.021	0.008	0.34
	Dairy cattle	59	10	0.019	0.034	0.82	45	7	0.006	0.017	0.87
Year	Year-2 vs Year-1	59	21	0.034	0.029	0.98	45	19	0.017	0.014	0.51
Island	North vs South	59	24	0.031	0.031	0.52	45	17	0.020	0.013	0.03

[a] Where used, this term describes a combination of all individual variables in each category

7.4.1.4 Previous abortions and disease history

Abortions in previous years ranged from 0.6 to 46.6% in R2 herds (n=8) and 0.2 to 15.3% in MA herds (n=6) (Table 7.6). There was no significant association between occurrence of abortions in previous years and either pregnancy rates or DARs in either age group.

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.

Farm	Age	Abortion rate (%) in previous years			Participation year		
		Year-1	Year-2	Year-3	Pregnancy (%)	Daily abortion rate (%)	Whole of gestation abortion[b] (%)
1	R2[a]	0	0	1.7	87.4	0.024	5.6
2	R2	0	0.6	0.0	86.7	0	0.0
	MA	0	0.2	0.8	97.1	0	0.0
3	R2[a]	0	10.1	8.7	90.5	0.014	3.3
4	R2[a]	0	17.2	9.8	79.2	0.070	16.3
8	R2[a]	0	0	7.2	95.0	0.028	6.5
9	R2	15.8	21.4	0	69.4	0.047	11.0
22	R2	0	0	46.7	82.2	0.023	5.4
	MA	0	0	6.7	96.9	0	0.0
39	MA	0	15.3	0	93.8	0	0.0
41	MA	0	0.7	0	96.2	0.009	2.1
61	MA	0	0	1.0	91.1	0	0.0
66	MA	0	0	0.7	95.0	0.015	3.5
83	R2	0	0	16.2	82.6	0.090	21.0

[a] Toxoplasmosis was diagnosed with laboratory confirmation from aborting hinds.

[b] Assuming that abortion rate was constant throughout gestation.

Toxoplasmosis was diagnosed, with laboratory confirmation in four R2 herds for which abortions had been experienced previously (Table 7.6).

In previous years, notification of JD from deer slaughter premises (DSP) was reported for twenty R2 and 16 MA herds and 1 R2 and 1 MA herd had leptospirosis notified. One deer farmer received DSP notification of leptospirosis and JD from both R2 and MA herds in 2011. JD notification in previous years was not associated with pregnancy rate in R2 or MA herds. In the current study years however, the mean DAR was lower in MA herds with JD DSP notifications than in herds with no JD DSP notifications ($p=0.01$).

Diagnosis of JD on-farm was reported by deer farmers for 21 R2 and nine MA herds. Diagnosis of BVD and leptospirosis each were reported by deer farmers from one farm each without reference to age group. The diagnosis of JD in previous years was not associated with

either pregnancy rate or DAR in either age group. There were insufficient data to allow analysis of association between leptospirosis, BVD or toxoplasmosis, and pregnancy rate at Scan-1 and DAR by Scan-2.

7.4.1.5 Deer health management

7.4.1.5.1 Vaccination

R2 and MA hinds were vaccinated against *Yersinia pseudotuberculosis, and Leptospira*, and in the study year/s. Fifteen percent of 87 R2 and 10% of 70 MA herds were vaccinated for *Leptospira borgpetersenii* serovar Hardjobovis and *Leptospira interrogans* serovar Pomona. *Leptospira* and *Clostridia* spp. vaccination was not associated with either pregnancy rate or DAR in either age group. There were insufficient data to allow analysis of associations with *Yersinia* vaccination.

7.4.1.5.2 Anti-parasitic treatment

Overall, 35 R2 and 11 MA herds were treated with anti-parasitic treatment. The mean pregnancy rate was lower in R2 and MA herds given anti-parasitic treatment than in non-treated herds (R2 $p=0.04$ and MA $p=0.02$). The mean DAR was higher in MA herds treated with anti-parasitic treatment than in non-treated herds ($p=0.02$).

7.4.1.5.3 Trace mineral supplementation

Trace mineral supplementation directly to hinds including Copper, Cobalt, Selenium, Iodine, and multi-mineral mixture was reported for 28 R2 and 24 MA herds. The mean pregnancy rate was lower in Copper treated hinds from R2 herds than in non-treated herds ($p=0.04$). The mean DAR was not different in R2 or MA herds treated or not treated with either of trace minerals.

7.4.1.6 Presence of other livestock

Sheep farming was also carried out in parallel with deer farming (i.e. separate from deer fenced area) on 48 farms comprising 42 R2 and 37 MA herds. Thirty-three R2 and 29 MA herds were farmed in parallel with beef cattle whereas 10 R2 and 9 MA herds were farmed in parallel with dairy cattle. The mean pregnancy rate was not different between herds with and without presence of other livestock species (sheep, beef cattle, and dairy cattle) on farm. The mean DAR in MA herds with presence of sheep on-farm was higher than those without sheep ($p=0.002$).

7.4.1.7 Environment

Drought

Data on unusual environmental conditions was collected at farm level. Twenty-Four R2 and 21 MA herds experienced drought in the year of participation. The mean pregnancy rate or DAR were not different between herds with and without drought conditions on farm in either age group.

Wildlife

Wildlife including feral cats, wild deer, possums, rabbits and hedgehogs were observed on deer farms. The mean pregnancy rate was not different in R2 or MA herds with and without presence of wild deer.

7.4.1.8 Other risk factors

The mean pregnancy rate was not different between North and South Islands. The mean DAR was higher in MA herds from the North Island (p=0.03). The mean pregnancy rate and DAR were not different between sampling years one and two.

7.4.1.9 Source of information

Data on source of information was available from 61 of 70 farm-years in Q1 and 40 of 49 farm-years in Q2 and are presented in Table 7.7.

Table 7.7: Summary of source of information as provided by deer farmers to complete the questionnaires.

Questionnaire	Source of information	Farm-years no. (%)	R2 herds no. (%)	MA herds no. (%)
Q1	Written records of farm data	12 (20)	12 (22)	8 (18)
	Memory	6 (10)	5 (9.5)	5 (11)
	Mostly memory and few recorded data	16 (26)	13 (24)	12 (27)
	Mostly recorded data and memory	27 (44)	24 (44.5)	20 (44)
	Total	61	54	45
Q2	Written records of farm data	8 (20)	6 (19)	6 (22)
	Memory	4 (10)	3 (9)	4 (15)
	Mostly memory and few recorded data	7 (17)	6 (19)	3 (11)
	Mostly recorded data and memory	21 (53)	17 (53)	14 (52)
	Total	40	32	27

7.4.2 Multivariate autumn risk factor analysis for pregnancy rate at Scan-1

Significant risk factors in the multivariable GEE logistic regression model are presented in Table 7.8. In R2 herds, least square (LS) mean pregnancy rate was lower in herds co-grazed with beef cattle, given Copper treatment, fed lucerne hay, and with dairy stock on-farm. LS mean pregnancy rate was higher in herds from the North Island and with sheep on-farm.

In MA herds, the LS mean pregnancy rate was higher in herds fed chaumolier and maize whereas it was lower in herds from the North Island, sampled in year-2, and with dairy stock on-farm.

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.

Age group	Risk factor	LS mean pregnancy % (95% CI) in herds with risk factor		P-value
		Present	Absent	
R2	North Island	62.0 (54.0-69.3)	58.4 (48.5-67.7)	0.441
	Year-2 (2013)	54.0 (44.2-63.4)	66.1 (5.6-74.5)	0.043
	Co-grazing with beef cattle	46.5 (35.8-57.4)	72.5 (66.5-77.8)	<.0001
	Trace mineral Copper to hinds	52.9 (41.9-63.5)	67.1 (60.4-3.2)	0.004
	Presence of sheep	67.2 (60.6-73.2)	52.8 (40.9-64.4)	0.015
	Presence of dairy cattle	44.4 (30.5-59.4)	74.1 (71.1-76.9)	<.0001
	Lucerne hay	50.3 (41.5-59.1)	69.3 (60.5-6.9)	<.0001
MA	Year-2 (2013)	97.3 (96.2-98.1)	98.2 (97.7-98.7)	0.037
	North Island	96.4 (95.9-96.9)	98.7 (97.9-99.2)	<.0001
	Maize	99.0 (98.0-99.5)	95.5 (93.6-96.8)	0.002
	Chaumolier	99.1 (98.6-99.3)	95.1 (94.2-95.9)	<.0001
	Presence of dairy cattle	96.7 (95.1-97.8)	98.6 (97.5-99.2)	0.047

7.4.3 Multivariate autumn risk factor analysis for daily abortion rate (DAR) at Scan-2

Significant risk factors in the multivariable model are presented in Table 7.9.

In R2 herds, LS mean DAR was lower in herds grazed alternately with beef cattle and from the North Island. The LS mean DAR was lower on farms managed by farmers with more years deer farming experience. The LS mean DAR was higher in herds co-grazed with beef cattle, with DSP notifications of JD in previous years and from year-2.

In MA herds, the LS mean DAR was lower in herds with DSP JD notification, abortions in previous years, and application of Copper in previous years, and in herds from

the North Island. The LS mean DAR was higher in herds co-grazed with beef cattle, fed meadow hay and with sheep on-farm.

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.

Age group	Risk factor	LS mean DAR% (95% CI) in herds with risk factor		P-value
		Present	Absent	
R2	North Island	0.026 (0.019-0.035)	0.035 (0.027-0.045)	0.149
	Year-2	0.041 (0.030-0.057)	0.022 (0.018-0.027)	0.002
	JD DSP notification	0.037 (0.030-0.047)	0.024 (0.018-0.033)	0.024
	Alternate grazing with beef cattle	0.019 (0.013-0.026)	0.048 (0.037-0.062)	<.001
	Co-grazing with beef cattle	0.039 (0.031-0.049)	0.023 (0.018-0.030)	0.002
MA	North Island	0.003 (0.002-0.007)	0.007 (0.004-0.012)	0.003
	Year-2	0.005 (0.003-0.011)	0.004 (0.002-0.007)	0.125
	Sheep stock on farm	0.008 (0.005-0.014)	0.003 (0.001-0.006)	0.004
	JD DSP notification	0.003 (0.001-0.007)	0.008 (0.005-0.013)	0.019
	Co-grazing beef cattle	0.006 (0.004-0.011)	0.004 (0.002-0.007)	0.015
	Meadow hay	0.009 (0.005-0.016)	0.003 (0.001-0.005)	<.001
	Copper to fertiliser	0.003 (0.001-0.005)	0.009 (0.004-0.017)	<.001
	Abortions in previous years	0.002 (0.001-0.006)	0.009 (0.006-0.016)	0.001

7.4.4 Univariate analysis of winter risk factors for daily abortion rate (DAR) at Scan-2

A summary of Scan-2 data and DAR in herds for which Q2 data was available is presented in Table 7.1. A summary of Q2 risk factors and results for univariate analyses are presented in Table 7.10 and Table 7.11.

Completed Q2 were received from 47 (55.3%) of 85 farms comprising 38 R2 and 31 MA herds. There were 22 farms with both R2 and MA herds and 16 and nine farms with R2-only and MA-only herds, respectively. Additionally, questionnaires from two farms with both R2 and MA herds participated in the study for abortion repeatability assessment returning questionnaires in both years. Therefore, in total, data from 40 R2 and 33 MA herds were available for analysis of risk factors. Abortions occurred in 27 R2 and 19 MA herds. In R2 herds with aborted hinds DAR ranged from 0.006% to 0.213% averaging 0.044% (95% CI=0.028-0.061%). In MA herds with aborted hinds DAR varied from 0.007 to 0.101% averaging 0.027% (95 CI=0.016-0.038%). The mean DAR was not different in herds with or without Q2 data in R2 (p=0.88) or MA (p=0.68) age groups.

7.4.4.1 Winter forage, crops and supplements

Data on winter forages are presented in Table 7.10. Data for forages grazed were missing for one R2 and four MA herds. Plantain was fed only to R2 herds. No forage was significantly associated with DARs in either R2 or MA hinds.

Winter crops were fed to 10 R2 and 13 MA herds. Feeding of crops except turnips, analysed either individually or all crops pooled, was not significantly associated with DAR in either R2 or MA herds. The mean DAR in R2 herds fed turnips was significantly higher than in those not fed turnips (p=0.002).

Supplements were fed to 30 R2 and 27 MA herds. Overall, the mean DAR in MA herds fed supplements was significantly higher than those not fed supplements (p=0.02). Mean DAR was significantly higher in herds fed meadow hay (p<0.001) whereas it was lower in R2 herds fed bailage (p=0.01). Mouldy silage or bailage was fed to three R2 and three MA herds but the mean DAR was not different in herds fed or not fed either of those feedstuffs.

Table 7.10: Univariate associations between pasture types grazed in winter and the daily abortion rate at Scan-2.

Forage	R2 herds				MA herds			
	Data from herds	% of time spent	Model coefficient	P-value	Data from herds	% of time spent	Model coefficient	P-value
Ryegrass/ White clover	39	64.8	0.0004	0.86	29	64.4	-0.001	0.82
Red clover	39	1.8	-0.176	0.97	29	2.8	-0.149	0.97
Native grass	39	21.9	-0.003	0.33	29	18.6	0.002	0.58
Plantain	39	0.3	0.073	0.48	29	0	-	-

7.4.4.2 Grazing management

Co-grazing with sheep, beef cattle, or dairy cattle occurred for seven R2 and six MA herds. MA hinds did not co-graze with dairy cattle. Co-grazing, all species combined, or by individual species, was not associated with DAR in R2 or MA herds. Alternate grazing of hinds with sheep, beef cattle, or dairy cattle was reported for six R2 and two MA herds. MA hinds did not graze alternatively with dairy cattle. The mean DAR was higher in herds alternate grazed with sheep and beef cattle, both species combined, or by individual species, in MA but not R2 age group.

7.4.4.3 Diseases

Notification of leptospirosis from deer slaughter premises (DSP) was reported for one R2 and MA herd each whereas JD notification was reported for two MA herds. DSP notification for leptospirosis or JD was not associated with DAR in R2 or MA herds. On-farm JD was reported for five R2 and two MA herds. The mean DAR was higher in on-farm JD positive R2 herds ($p<0.001$) but not in MA herds.

7.4.4.4 Environment

Deer farmers from 10 R2 and four MA herds reported unusual weather conditions, mainly cold and wet conditions. The mean DAR was lower in R2 herds ($p<0.02$) reporting wet and cold weather but not in MA herds.

7.4.4.5 Other risk factors

Other potentially confounding risk factors such as Island and year were not associated with DAR in R2 or MA herds. In R2 herds, the mean DAR was higher in year-1 than in year-2 ($p<0.04$) whereas mean DAR was higher in the South Island than in the North Island ($p<0.02$).

Table 7.11: Univariate associations between risk factors in winter and daily abortion rate at Scan-2.

Category of variable	Variable	R2 herds					MA herds				
		Data from herds	Risk factor present in herds	Mean daily abortion rate (%)		P-value	Data from herds	Risk factor present in herds	Mean daily abortion rate (%)		P-value
				Risk factor present	Risk factor absent				Risk factor present	Risk factor absent	
Winter crops	Overall[a]	40	10	0.022	0.033	0.54	33	13	0.014	0.017	0.76
	Chaumolier	40	1	0.011	0.030	0.31	33	1	0	0.016	0.97
	Kale	40	2	0.019	0.031	0.19	33	6	0.014	0.016	0.88
	Tumips	40	2	0.054	0.029	0.002	33	1	0	0.016	0.97
	Swedes	40	6	0.019	0.032	0.57	33	6	0.018	0.015	0.50
	Sugarbeet	40	1	0.028	0.030	0.58	33	2	0.013	0.016	0.81
	Others	40	0	-	0.030	-	33	3	0.017	0.016	0.40
Supplements	Overall[a]	40	30	0.032	0.024	0.65	33	27	0.018	0.004	0.02
	Meadow hay	40	6	0.041	0.028	0.09	33	4	0.054	0.010	<0.001
	Bailage	40	18	0.025	0.034	0.01	33	25	0.018	0.014	0.22
	Silage	40	13	0.033	0.028	0.57	33	11	0.015	0.016	0.98
	Barley	40	3	0.032	0.030	0.06	33	2	0.015	0.016	0.89
	others	40	0	-	0.030	-	33	1	0	0.016	0.97
	Mouldy feed	40	3	0.029	0.030	0.81	33	3	0.010	0.016	0.56
Grazing	Co-grazing (overall[a])	40	7	0.028	0.030	0.98	33	6	0.014	0.016	0.81
	Co-grazing sheep	40	3	0.021	0.031	0.79	33	1	0.021	0.015	0.62
	Co-grazing beef cattle	40	4	0.025	0.030	0.86	33	5	0.013	0.016	0.62
	Co-grazing dairy cattle	40	1	0.031	0.030	0.94	33	-	-	0.016	-
	Alternate grazing (overall[a])	40	6	0.019	0.032	0.10	33	2	0.061	0.013	<0.001
	Alternate grazing sheep	40	5	0.017	0.032	0.07	33	2	0.061	0.013	<0.001
	Alternate grazing beef cattle	40	3	0.020	0.031	0.10	33	1	0.101	0.013	<0.001
	Alternate grazing dairy cattle	40	1	0.031	0.030	0.94	33	-	-	0.016	-

DSP notification	Leptospirosis	40	1	0.006	0.031	0.12	33	1	0	0.016	0.97
	Johne's disease	40	-	-	0.030	-	33	2	0.013	0.016	0.78
Johne's disease	detection on farm	40	5	0.066	0.025	<0.001	33	2	0.015	0.016	0.84
unusual environment	Wet and cold weather	37	10	0.024	0.034	0.02	31	4	0.018	0.016	0.80
Year	Year-2 vs Year-1	40	15	0.024	0.033	0.04	33	15	0.018	0.014	0.79
Island	North vs South	40	19	0.023	0.036	0.02	33	19	0.018	0.014	0.84

[a] Where used, this term describes a combination of all individual variables in each category

7.4.5 Multivariate winter risk factor analysis for daily abortion rate (DAR) at Scan-2

A summary of results is presented in Table 7.12.

In R2 herds, the LS mean DAR was lower in herds fed bailage, those in the North Island and in herds with previous DSP notices for leptospirosis. The LS mean DAR was higher in R2 herds with on-farm diagnosis of JD. In MA herds, the LS mean DAR was higher in herds grazed alternately with sheep and fed meadow hay.

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.

Age group	Risk factor	LS mean DAR% (95% CI) for risk factor		P-value
		Present	Absent	
R2	North Island	0.009 (0.003-0.027)	0.016 (0.006-0.046)	0.001
	Year (2013 vs 2012)	0.010 (0.003-0.031)	0.014 (0.005-0.040)	0.197
	On-farm Johne's disease diagnosis	0.020 (0.006-0.061)	0.007 (0.003-0.021)	<.0001
	Bailage supplementation	0.008 (0.003-0.024)	0.018 (0.006-0.052)	<.0001
	DSP Leptospirosis notice	0.004 (0.001-0.032)	0.034 (0.025-0.047)	0.039
MA	North Island	0.034 (0.024-0.046)	0.036 (0.017-0.075)	0.879
	Year (2013 vs 2012)	0.031 (0.020-0.046)	0.039 (0.019-0.081)	0.580
	Meadow hay supplementation	0.061 (0.042-0.089)	0.020 (0.009-0.042)	0.017
	Alternate grazing with sheep	0.068 (0.028-0.167)	0.018 (0.011-0.028)	0.028

7.5 Discussion

This is the first study of risk factors for abortion in New Zealand farmed deer. Analysis of risk factors for pregnancy *per se,* shortly after mating, was undertaken because the data were suitable for this purpose, and the outcome supplemented earlier studies (Audigé *et al.* 1999b; Audigé *et al.* 1999c) contributing further to understanding of attainment of pregnancy in farmed deer. Only risk factors significant in multivariate models are discussed.

7.5.1 Autumn risk factors for pregnancy rate at Scan-1 or daily abortion rate at Scan-2

7.5.1.1 Pregnancy

In R2 herds, the LS mean pregnancy rate was higher in herds from the North Island and those with sheep on-farm whereas herds co-grazed with beef cattle, fed Lucerne hay, with dairy cattle on-farm, treated with Copper and from year-2 had a lower LS mean

pregnancy rate. In MA herds, LS mean pregnancy rate was higher in herds fed maize and chaumolier, whereas it was lower in herds with dairy cattle on-farm, from year-2 and North Island.

Deer farms with sheep had higher pregnancy rates. This phenomenon is not readily explainable. One proposition is sheep grazing may result in better pasture quality. Rising two-year-old and mixed-age hinds from herds with presence of dairy stock on farm had lower pregnancy rates suggesting that farmers with dairy cattle stock may be paying less management attention to yearling hinds because of higher financial returns from dairy cattle. Conversations with deer farmers by the Author indicated that deer farmers from farms with dairy cattle stock on farm were spending less time managing the deer stock.

Overall, feed supplementation to R2 herds was not associated with pregnancy rates. Conventionally, supplements during summer and/or autumn would be expected to improve and compensate the body condition of hinds during mating. A Spanish study has shown that yearling red deer hinds supplemented with protein rich pellets had higher pregnancy rates (Rodriguez-Hidalgo *et al.* 2010). In R2 herds, on an individual supplement basis, R2 herds supplemented with lucerne hay had lower pregnancy rates. This negative association may be due to presence of mould. Mouldy feed may contain mycotoxins such as zearalonone, deoxynivalenol, ochratoxin, and ergot that can impair reproduction and growth (Diekman and Green 1992). *Fusarium* spp. can produce zeralenone and are commonly found on forages (di Menna *et al.* 1987). In New Zealand, high concentration of zearalenone has been observed in forages during autumn (Sprosen and Towers 1995) which is the mating season for deer. In cattle, concentrations of zearalenone in forages and zearalenol in urine and blood samples collected in summer from cattle in herds with fertility problems were higher compared with those from herds with normal reproductive performance (Sprosen and Towers 1995). An alternative explanation is that farmers feeding lucerne hay in autumn may do so only after their hinds have failed to reach target weights or body condition, and that to achieve optimum performance these factors are more critical for young rather than older hinds.

That co-grazing of hinds with beef cattle was negatively associated with pregnancy rate in R2 herds is consistent with a previous observation of reduced early conception in yearling hinds co-grazed with stock other than deer (Audigé *et al.* 1999b). The presence of beef cattle in the same paddock may be causing a social disturbance during mating and therefore less chances of conception.

The LS mean pregnancy rate was lower in R2 herds treated with Copper. Deer farmers may have supplemented R2 hinds with Copper in herds with lower reproductive performance in earlier years, in search of a solution. Another proposition is that Copper toxicity may be associated with non-pregnancy or failure to conceive. More studies will be needed to establish causation.

Mixed-age hinds fed maize and chaumolier were more likely to be pregnant, supporting that good nutrition plays an important role in conception and/or maintenance of pregnancy. The LS mean pregnancy rate difference between island suggests that MA hinds were better managed on South Island farms, or perhaps climatic effects in the North Island favour young animals. Determination of these effects was beyond the scope of this study.

Herds in year-2 had lower LS mean pregnancy rate than sampled in year-1. This difference may be due to the confounding effect of drought which occurred in year-2.

The exposure to *Toxoplasma gondii* was not considered as a risk factor in this analysis as the data provided by deer farmers for the period that pregnancy and abortion data were collected, was not adequate. However, some herds with abortion in previous years did report confirmed *T. gondii* exposure in aborting hinds by blood test. Also, *T. gondii* sero-status was positively associated with abortion as reported in Chapter 4.

7.5.1.2 Abortion

Autumn risk factor analysis was done based on the hypothesis that autumn management may affect events such as abortion later in the reproductive cycle.

The LS mean DAR at Scan-2 was higher in R2 and MA herds co-grazed with beef cattle. Also, LS mean pregnancy rate at Scan-1 was lower in R2 herds co-grazed with beef cattle. Both these findings suggest that co-grazing of hinds with beef cattle is a risk for pregnancy. In contrast, R2 herds alternate grazed with beef cattle had a lower LS mean DAR supporting the previous positive association between co-grazing with beef cattle and DAR in this study. Therefore, co-grazing of beef cattle may be associated with non-conception and subsequent non-maintenance of pregnancy after conception.

The LS mean DAR was higher in R2 herds with DSP JD notification. Johne's disease, caused by *Mycobacterium avium* subspecies paratuberculosis (MAP) is prevalent on New Zealand deer farms (de Lisle *et al.* 1993; de Lisle *et al.* 2003) with overall animal and herd

level prevalence estimated at 45% and 59%, respectively (Stringer *et al.* 2013). Since there is little biological plausibility of a direct causal effect of JD on abortion, the association here may result from common causal factor/s. In contrast, MA herds with JD DSP notification had a lower DAR. This observation may be due to improvement in herd management/JD control options/practices followed by deer farmers in order to reduce the occurrence of JD on farm.

Increase in deer farming experience was negatively associated with DAR in R2 herds. This association is plausible as experienced deer farmers might be managing their deer stock better than less experienced or new deer farmers. The LS mean DAR in year-2 herds was higher than in year-1 herds and can be due to the confounding effect of the drought experienced by most of the herds in year-2.

MA herds with sheep on-farm had a higher DAR. This observation was in contrast to the finding of higher pregnancy rate in R2 herds with sheep on-farm. The biological plausibility of positive association of sheep stock and DAR is difficult to explain and may be a spurious result.

Mixed-age herds being fed meadow hay had a higher LS mean DAR. Optimum feeding during mating may have a persistent nutritional effect contributing to maintenance of pregnancy. Lucerne hay feeding to R2 and meadow hay feeding to MA hinds in autumn was a risk factor for pregnancy and abortion, respectively, but it is difficult to explain this adverse effect. It could be associated with mould as discussed above for pregnancy or it may be that hinds fed hay were nutritionally deficient and therefore were supplemented with hay. The higher LS mean DAR in herds fed hay also suggest that pregnancies were not well maintained in those herds.

MA herds supplemented with Copper had lower DAR. Copper deficiency is widespread on New Zealand deer farms (Grace and Wilson 2002). Since Copper helps immunity and growth, hinds grazing on pasture on Copper treated paddocks may be heavier and in better health.

The LS mean DAR in North Island herds was lower than South Island herds, possibly related to different management practices, or possibly climatic effects. Prevalence of JD in North Island deer herds has been reported lower than in South Island herds (Verdugo *et al.* 2014) and therefore the island effect observed in this study may be a reflecting surrogate effect of JD on abortion.

Mixed-age herds with previous history of abortion had a lower LS mean DAR. Deer farmers with herds with a history of abortion may have applied better management. However, it is possible that abortions could have gone unnoticed to deer farmers on farms with no history of abortion, hence the effect may be due to observational bias. Also, abortion history data sent by deer farmers did not include the means of detection of abortions. Therefore, the data on abortions in previous years may not be particularly informative.

7.5.2 Winter risk factors for daily abortion rate at Scan-2

The LS mean DAR was higher in R2 herds with diagnosis of JD on farm. This observation was consistent with the finding of higher LS mean DAR in herds with previous JD DSP notifications. Since there is little biological plausibility of a direct causal effect of JD on abortion, the association here may result from common causal factor/s.

Rising-2 herds fed bailage in winter had a lower LS mean DAR supporting that a nutritional contribution to maintenance of pregnancy is important. On the other hand, MA herds fed meadow hay in winter had a higher DAR than herds not fed meadow hay and this factor also was a risk factor in the analysis of autumn risk factors. This observation may be due to presence of ergot or mould on meadow hay fed to hinds. Feeding of hay infested with ergot has been shown to cause abortions in cattle (Appleyard 1986; Ilha *et al.* 2003). The mouldy feed was not a risk factor for abortion although the non-significance of association may be due to lower sample size. An alternative explanation could be that farmers fed only after hinds had lost weight and body condition, and having experienced nutritional stress on pregnancy.

Rising-2 herds with previous leptospirosis DSP notification had a lower DAR. *Leptospira* spp. is prevalent on deer farms and has been shown to affect reproduction (Ayanegui-Alcérreca *et al.* 2010; Subharat *et al.* 2010, 2011b) though those Authors were not able to demonstrate that abortion rather than perinatal/postnatal losses were the cause. Vaccination may be a proxy for overall better health management practices.

That R2 herds from the North Island had lower LS mean DAR than South Island herds, consistent with the finding from the autumn risk factor analysis, possibly associated with environment, climate or management factors as above.

7.5.3 Possible bias

Recall bias (Elwood 2007) may be present for some of the information provided by deer farmers in this study as some questions were based on historic occurrence of abortion and risk factors. However, using one questionnaire for each risk analysis period was designed with the intention of limiting delay between events and recording, to minimise recall bias. Furthermore, data for source of information showed 64% of data in Q1 and 73% of data in Q2 was provided based on written records. Therefore, the impact of re-call bias may be less than expected in this type of observational study. By contrast, response bias (Etter and Perneger 1997) may be present since not all farmers responded to the questionnaires. However, since pregnancy and abortion rates were similar in herds with and without Q1 or Q2 data, there is likely low limited response bias.

7.6 Conclusion

Risk factor analysis for pregnancy suggests that autumn crop feeding improves conception and/or early pregnancy. Farming with other livestock species is associated with higher pregnancy rate in R2 hinds. North Island R2 herds had a higher pregnancy rate whereas MA hinds from South Island herds had a higher pregnancy rate. Maize supplementation in R2 hinds and rape feeding in MA hinds also appear to improve pregnancy rate.

Hay supplementation to R2 and MA hinds in autumn increased the risk of abortion whereas alternate grazing of R2 and MA hinds with other species reduced the risk of abortion. Abortion rate was lower in R2 hinds fed with kale and native grass in autumn. Historic Copper supplementation with fertiliser in MA herds reduced risk of abortion. MA hinds from herds with abortions in previous years had reduced the risk of abortions. The risk of abortion was lower in R2 hinds from North Island herds.

Winter risk factor analysis showed abortion was higher in hinds from JD positive herds. Supplement feeding reduced risk of abortion in R2 hinds. Alternate grazing, suggesting minimal interaction between pregnant hinds and other livestock species lowered risk of abortions. MA herds fed meadow hay and sequentially grazed with sheep had higher abortion rates. The observation of higher abortion in herds fed meadow hay may be due to presence of mould in hay or may be due to hinds being nutritionally deficient.

One of the key overall findings from this study suggests that optimum nutrition, both in terms of feedstuffs and trace minerals was associated with higher pregnancy rate and lower DAR.

The associations identified here cannot be interpreted as causation, despite that there is biological plausibility for many of them. More research would be needed to establish causation *per se*.

7.7 Acknowledgements

This study was supported by the Deer Reproductive Efficiency Group based in Southland. It is funded by AgResearch, Agmardt, DEEResearch, MSD Animal Health, Southland Deer Farmers Association, Massey University and individual farmers, particularly Landcorp Farming Ltd. The in-kind contribution of all participating farmers is gratefully acknowledged, as is the assistance of a large number of veterinary practices and scanners for scanning and blood sample collection and DSP staff and veterinarians for tissue sample collection.

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Chapter 8. General discussion

8.1 Introduction

The aim of this study was to undertake an epidemiological evaluation of abortion in farmed deer in New Zealand which can be used to quantify abortion rates, attempt to identify cause/s, to inform farmers and the industry about abortion contributing to reproductive wastage, and to inform potential control measures to reduce abortion rates in future.

This study was prompted by preliminary findings of abortion in R2 (1.2 - 16%) and MA (4.5%) deer (Wilson *et al.* 2012). It involved investigation of prevalence and incidence of early and mid-term abortions in R2 and MA hinds, risk factors for pregnancy and abortion, and association with potential infectious abortifacients such as *Toxoplasma gondii, Neospora caninum*, *Leptospira* spp., bovine viral diarrhoea virus (BVD), and cervid herpes virus type-1 (CvHV-1).

The individual chapters in this thesis are presented in a format for submission to refereed scientific journals. Therefore, discussion and critique was limited in those chapters to conform to conventions for journal publications, and it was not possible to include broad-reaching discussion of further implications arising from those individual chapters. This chapter, therefore, includes more discussion and critique on the design and methodology, implications of the study to the Deer Industry and suggestions for further research in future.

8.2 History of fetal wastage

There are only few studies reporting abortions in farmed deer in New Zealand (Fennessy *et al.* 1986; Audigé *et al.* 1999a; Campbell *et al.* 2000). However, those studies were not designed primarily to focus on detection of abortions. Rather, they aimed at benchmarking reproductive efficiency and reported estimates of abortion rates as one the study findings. These initial investigations were justified at the time by the belief among deer farmers that abortions were rare and not thought to significantly affect reproductive efficiency. However, a later preliminary investigation suggested otherwise, indicating occurrence of abortions and significant loss in reproduction (Wilson *et al.* 2012). Wilson et al (2012) detected mid-term abortions at an additional scan after the usual ultrasound post-mating early pregnancy scan suggesting that abortions may be going unnoticed on other farms and that some proportion of lower weaning percentages observed on other farms may be attributed to abortions.

The preliminary investigations for abortions by Wilson *et al.* (2012) implicated involvement of *Toxoplasma gondii* wherein *T. gondii* DNA was detected in fetal brains of aborting fetuses, and serological evidence, albeit from a non-validated test, suggested exposure to this organism was common in affected herds. This finding prompted focus on involvement of infectious causes for abortion. Other infectious pathogens reported to cause abortion in cattle and sheep have also been reported in deer. Therefore, a literature review was carried out to review evidence that those pathogens may cause abortion in deer. The review included pathogens such as *Toxoplasma gondii, Neospora caninum*, bovine viral diarrhoea virus (BVD), *Leptospira* spp. and cervid herpes virus type-1 (CvHV-1) and were included in the investigation of infectious causes of abortion in this study.

8.3 Selection of farms

The deer farmers across the country were invited to take part in this "fetal wastage" study. In year-1, deer and mixed practice veterinarians were contacted for farm recruitment. In year-2, the majority of farms were recruited by contacting the deer farmers directly. Another method of recruitment could have been to send out questionnaires to deer farmers throughout the country and then selecting farms with high and low reproduction rates. Although this approach could have had lower selection bias than the method used for in this study, it could not be used as the pregnancy scanning and sampling frame was fixed and not adjustable. Further, it would not necessarily avoid bias since those farmers with sub-optimum performance in their herds might more readily volunteer involvement anyway. Also, as this study involved an additional scan, it was necessary to ensure that famers participating in the study properly understood and agreed to an additional scan.

The optimum means of recruitment would have been truly random by applying a random selection strategy from the New Zealand deer farm database. However, that approach was not feasible given the issues around the privacy and access to the database, requiring complex procedures to contact farmers. Also, that approach could have largely attracted those farms with on-going reproductive problems leading to the same issue of selection bias present with the approach used in the study. Also, a non-response bias could have been present in that approach as not all approached deer farmers would have agreed to participate. Therefore, regardless of approach used for recruitment, the recruited population could not have fully represented the actual total population and that a selection bias in form of non-response bias or volunteer bias could have been present.

Hence, it was expected that the recruitment of deer farmers would involve those with on-going/historical reproductive problems in hinds. However, in reality, some deer farmers, with good reproduction rates in deer stock were interested to know if abortions were occurring on their farm and hence participated in the study. This would have reduced the level of bias. The key point to note is that the pregnancy rates in R2 hinds and MA hinds at Scan-1 were similar to the pregnancy rates reported in previous studies (Audigé *et al.* 1999a; Campbell *et al.* 2000; Deer Industry Manual 2000; Lawrence 2003) and therefore, R2 and MA herds from deer farms recruited for this study are representative of R2 and MA herds across the country, at least for pregnancy rate early in gestation. Within the deer industry, it is also known that a proportion of deer farmers do not prefer to scan their hinds for pregnancy post-mating. This was also noticed by the Author when talking to deer farmers and scanning operators for farm recruitment in this study. This implies that the losses on those farms, without any pregnancy scanning, would be known only at weaning leaving the deer farmer unable to determine the point of loss (pre, peri, or post-natal).

8.4 Timing of scanning

The first pregnancy scan (Scan-1) was carried out by the deer farmer according to their normal practice, so it was not possible to synchronise Scan-1 on all study herds within a particular date range. Hence there was variation in Scan-1 date. Also, efforts were made to try and standardise a 90 day between-scan interval. However, it was impossible to convince farmers on many farms to conduct Scan-2 90 days after Scan-1. Due to this, a daily abortion rate, rather than a preferred 90-day mid-gestation abortion rate was calculated and used throughout the study to avoid the confounding effect of different between-scan interval.

However, this variation did allow for an observation that a standardised timing would not, since a comparison of abortion rate from early vs. later scanned farms became possible. This helps to inform whether abortion rates were similar throughout a wider time period than would otherwise have been the case. There was no difference in abortion rate for scanning done after or before the mean Scan-2 dates (Chapter 2). This observation suggests that despite some herds with between-scan interval earlier in gestation than other herds, there was no difference in abortion rates and that the abortions were likely to be occurring for a period wider than recorded in this study. Furthermore, it justifies the proposition that the abortions were occurring after Scan-2 beyond those periods measured. This observation, together with

the observation of aborting fetuses at Scan-1 and Scan-2, justifies the extrapolation of abortion rates based on the observed abortion rates.

8.5 Timing of abortions

This study observed hinds aborting at Scan-1, and the early abortion study showed abortions before the usual time for Scan-1. It also observed aborting hinds at Scan-2 suggesting that abortions may continue to occur after Scan-2 and that the losses observed from scanning to weaning will include abortions in addition to peri- and post-natal losses. Combined, this evidence supports that abortions do occur throughout gestation. However, losses between Scan-2 and calving could not be recorded as the deer farmers did not want to disturb hinds around calving and also it is difficult to access hinds undergoing parturition in high hill country. Additionally, the weaning data, as reported by some deer farmers, were not completely robust owing to absence of reliable data on the purchase/sale of any pregnant hinds after Scan-2. Hence, true weaning percentages could not be computed. By contrast, weaning data and change in deer number was followed closely by Audigé *et al.* (1999a) in around 2700 hinds in 15 herds, giving accurate reproductive efficiency estimates. However, the purpose of the study by Audige et al. (1999) was the establishment of a reproduction profile in farmed deer compared to this study which focused on detection of abortions. The former study was undertaken on a small number of farms at a time in which farmers were prepared to commit to extremely intensive recording. Due to the changing deer farming climate, few farmers are prepared to do this presently. However, those earlier estimates are similar to the percentages observed in this study.

This study targeted mid-term abortion rates. While the study design did not allow calculation of possible whole of gestation rates, this can be crudely estimated assuming the abortion occurred at same rate throughout gestation. The possible whole of gestation abortion rates were calculated by multiplying the daily abortion rate and total gestation length days (233 days) and were 6.9% (95% CI: 6.1-7.6%) in R2 hinds and 3.3% (95% CI: 2.6-4.0%) in MA hinds. The hinds used for the detection of abortion at Scan-2 were those detected pregnant at Scan-1. Considering that abortions also occur before Scan-1, as indicated by early fetal loss detections in this study, a proportion of non-pregnant hinds at Scan-1 could have lost their fetuses after conception before being detected as non-pregnant and therefore underestimating the extrapolated losses. The extrapolated rates can be affected by the

cull/sale/purchase of hinds at Scan1 or Scan-2. However, these propositions need to be confirmed.

The selection of pregnant hinds from Scan-1 at Scan-2 may not have been fully random as some deer farmers with large R2 and MA herds culled some pregnant hinds, usually those conceiving late, without notifying Author of this study. The cull pregnant hinds in some herds were confirmed as late conception hinds. Therefore, the selection pool at Scan-2 was lower than the number of total hinds detected pregnant at Scan-1, and therefore could have been slightly biased by this culling policy, albeit that it was likely practised by only a small number of farmers. This implies that the abortion rates reported in this study may not be representative for use in predicting abortion in late conceiving hinds.

8.6 Prevalence and incidence of abortion

The population abortion prevalence for the average 90 gestation day interval in R2 (2.8%) and MA (1.2%) hinds reported in this study was higher than the assumed 2% in R2 and 1% in MA hinds used for the sample size calculation at the design stage of study. Therefore, the abortion rates reported in this study were robust and higher power was achieved than that of the sample size calculation. However, the number of herds for both age groups was lower than calculated in the sample size calculations. Nevertheless, the proportion of herds with abortions exceeded the assumed proportion of 50% herds with at least one aborted hind for both age group herds. Therefore, at both animal and herd-level, the abortion rates reported in this study achieve the requirement for robustness. Therefore, extrapolation of the estimates from this study to the national deer herd is justifiable.

The within herd abortion incidence rates varied in both R2 and MA herds. Further, the predictability and repeatability of abortions in consecutive years was poor, although the repeatability study was done on only 15 R2 and seven MA herds. Assuming that abortions continued at similar rate within herds, the whole of gestation rates could range from 1.2 to 51.1% in R2 herds and 1.7 to 24.2% in MA herds. The variation in abortion rate between herds and its lack of predictability within herds in consecutive years makes evaluation of a possible control strategy difficult.

The prevalence and incidence of abortions reported in this study will help benchmarking reproductive performance in farmed deer for the Deer Industry, deer farmers and their professional advisors. The Deer Industry (DI) can use this data to prioritise whether

they wish to undertake more research into means of reducing fetal losses in order to address one of the goals of increasing farm productivity under the 'Passion2Profit (P2P)' programme initiative (Deer Industry New Zealand).

The pregnancy rate and abortion rate estimates reported in this study were from phenotypically red deer as were the estimates reported by previous studies (Fennessy *et al.* 1986; Audigé *et al.* 1999a; Campbell *et al.* 2000). However, other farmed deer breeds present in New Zealand include fallow deer, wapiti deer, and hybrid deer (red x fallow, or wapiti x red). Given that some deer farmers use terminal wapiti males for breeding with red female deer (*Pers. comm.*[3]), the introgression of wapiti genes in hybrid (red x wapiti) can also have an effect on overall reproduction traits including abortion occurrence, for example increase in threshold live weight for mating in R2 hinds as observed by Asher *et al.* (2005a). The abortions in wapiti and fallow or other farmed species of deer in New Zealand was out of scope for this study but remains in question given the detection of abortions in red deer.

8.7 Economic impact of abortion

Abortions pose a financial risk to deer farmers and are a loss to the industry and the national economy as a whole. The economic burden due to abortions can be estimated using the following assumptions.

- Whole of gestation rates of 6.9% and 3.3% for R2 and MA hinds, respectively;
- Total red deer herd population of 55,000 R2 and 310,000 MA breeding hinds (DINZ *pers. comm.*[4]);
- Purchase of replacement of a weaner at weaning in early March at $260 including transport, cost;
- That the aborted hind is culled at weaning and therefore the cost of replacement is included (difference between slaughter value and replacement value including costs, est. approximately $100);
- The average time between abortion and sale was 6 months and cost of feeding the aborted hind at 10c/kgDM, assuming no supplements, was $40 during that period.

The economic burden due to abortions was estimated at $1.51 million in R2 and $4.08 million in MA herds.

[3] Personal communication with deer farmers, veterinarians, and scanning operators during recruitment of deer farms in year 2012 and 2013.

[4] Personal communication with Innes Moffat (Passion2Profit Manager, Deer Industry, New Zealand) on 15 September 2016.

Most deer farmers realise abortion and peri- or post-natal losses only at weaning as most of them don't record losses until weaning. Therefore, cost of weaner deer was used as a measure for estimating financial losses as the hinds aborting during pregnancy would only be identified as having aborted when checked at weaning for live calves.

While the above estimates are based on assumption of a constant abortion rate during the entire pregnancy, and are therefore speculative, the losses due to abortion from the period of data collection from this study alone was estimated at $0.58 million in R2 and $1.52 million in MA herds. Hence, cost is at least $2.10 million overall, and could range up to $5.58 million. The estimated economic losses are substantial and warranted further investigation into abortions. Assuming there are 2000 deer farms, the average loss per farm could range from $1,052 - $2,791.

8.8 Limitations on sampling and questionnaire data collection

Sampling involved collection of blood samples from pregnant, non-pregnant and aborting hinds, along with uteri from non-pregnant and aborting hinds as well as aborting fetuses. Some deer farmers chose to retain the aborted hinds and therefore tissue collection from those hinds was not possible. Also, sampling from non-aborted hinds was not possible as this study did not include provision of incentives to deer farmers for aborted or non-aborted hinds.

It was necessary to blood sample the predicted number of hinds for both age groups according to the sample size analysis. However not all samples were used for analysis. It was preferable to sample the same hinds for blood collection at both pregnancy scans. However, it was impossible given the logistics involved and hence, a longitudinal serology study was not possible. There was also loss to follow up with this approach due to hinds being sold/culled/lost by Scan-2. Also, the presence of aborting hinds at Scan-1 would lower the power for detecting abortions by Scan-2. However, 4,835 blood samples at Scan-1 and 2,932 samples were enough to create a sample pool to allow robust findings from serology tests.

The collection of tissues from deer slaughter premises (DSP) was determined by the farmer's willingness to cull hinds and the Author's ability to get them collected. A tissue collection network was established after co-ordinating with the DSP vets and traced the majority of the aborted and aborting hinds sent to DSP from the study farms. However, on a few occasions, the deer farmers failed to notify the researcher before sending the

aborting/aborted hinds to DSPs. Therefore, it was not possible to sample tissues from all aborted/aborting hinds. However, the data available from tissue testing provided enough evidence to support the evidence provided by the serology.

In practical situations arising from a cross-sectional study, often it is not possible to implement and achieve the targeted sample size. However, the numbers achieved in terms of blood and tissue samplings were enough to allow adequately robust diagnosis and analysis of association between serology and pregnancy rates at Scan-1 and DARs at Scan-2.

The questionnaires were designed to assess the association between farm, management, health and environmental factors and abortion. It was decided to design two questionnaires for the period up to Scan-1 and Scan-2 to enable collection of current or very recent data rather than re-call data. The response rate for questionnaire-1 (Q1) and questionnaire-2 (Q2) was less than desired. To improve response rates, the Author sent frequent reminders to deer farmers via telephone, mail, and through the veterinary practice/ scanning operators for assistance with completion. The response rate could have been improved by personal visit to deer farms but this was not possible considering the logistics, cost and time involved. Also, some herds withdrew the participation from study after Scan-1. This added to the higher non-response rate for Q2.

The response rate for Q1 and Q2 would have affected the analysis of some factors and may have affected parameter estimates, such as increasing standard errors. Therefore, the estimates from the risk factor analysis in Chapter 7 may not be fully robust. However, the estimates reported in this study at least pointed towards a direction of those associations with abortion.

While the risk factor analysis included herds from the recruited study farms, a similar analysis from herds from farms outside of this study would have given a better idea of selection bias. However, as most deer farmers do not undertake a second pregnancy scan, there was no means of estimating abortions in those herds.

8.9 *Toxoplasma gondii* ELISA validation for use in deer

Based on the preliminary study by Wilson *et al.* (2012) and the abortion losses from the present study, there was need to validate a serology test for its use in deer for the purposes of this study as the latex agglutination test (LAT), used in commercial laboratories and by Wilson et al. (2012), was suspected for having a low sensitivity (Se). A commercial *T. gondii*

ELISA test was validated to assess sero-status of sub-sample of hinds included in the study. Western blot (WB) is considered gold standard test for detecting *T. gondii* antibodies (Sohn and Nam 1999), and for alternative test validation. WB is a time consuming and labour intensive test, so was not chosen as the primary test for choice for screening sera samples in this study. In addition to validation of the ELISA, there was an opportunity to assess the Se and specificity (Sp) of the LAT test using WB. Therefore, the samples tested on LAT from the preliminary study by Wilson *et al.* (2012) were also included for validation of ELISA.

There was also opportunity to assess performance of WB using Bayesian analysis. This method of validating a test in the absence and presence of a gold standard test was undertaken following the OIE standards for validation of tests through latent class analysis (OIE 2013). Results demonstrated that WB can be used in future as a gold standard test since its Se and Sp were higher than 0.95.

The sera samples from the Wilson et al (2012) study used in the validation were available from two herds, constituting more than half of the total sera assessed on ELISA for validation, whereas the samples used from the current study came from 15 herds. Clustering of sera from two herds may have had affected the sero-prevalence estimate. However, as the ELISA validation study did not aim to determine sero-prevalence *per se*, the selection of sera from those two herds should not have affected the Se and Sp estimates of the validated ELISA.

The Se and Sp of ELISA were 98.8% and 92.8%, respectively. *T. gondii* exposure misclassification bias may be present due to less than perfect Se and Sp of validated ELISA. The misclassification bias here is non-differential meaning that the Se and Sp of *T. gondii* ELISA is similar in the aborted and non-aborted hinds. The bias resulting from misclassification of aborted and non-aborted hinds by ELISA can be calculated by the formula described by Lash *et al.* (2009). Using that formula, the adjusted odds-ratio for association between *T. gondii* sero-positivity and aborted status in R2 hinds was 1.77 (95% CI: 1.22 - 2.57). The *T. gondii* exposure misclassification by validated ELISA on the OR was -10.6%. Hence misclassification bias had a little impact on the OR, or conclusions from the study.

8.10 Investigation of infectious causes of abortion

After undertaking a review of infectious causes of abortion, it was decided to screen sera for *T. gondii*, as indicated in the preliminary study (Wilson et al 2012), and other pathogens including *N. caninum, Leptospira* spp, BVD, and CvHV-1.

The sera from aborted hinds tested for *T. gondii* serology differed to that tested for BVD and CvHV-1. The average volume of blood sample collected by deer veterinarians and technicians varied from herd to herd. Although the majority were able to collect the required amount of sera as specified in the blood sampling protocol, some blood samples were low in volume and therefore yielded less volume of sera. For example, blood samples from one MA herd were semi clotted and dark red suggesting haemolysis. However, no abortions were observed in that herd and the blood samples were not used for serology.

The sera and tissue samples collected at Scan-1 were tested only for *T. gondii* and *Leptospira* spp. antibodies and DNA, respectively, and not for *N caninim*, BVD, and CvHV-1. The Scan-1 serology and PCR tests were done only if the association between pathogen sero-status at Scan-2 and aborted status of hinds at Scan-2 was significant. After Scan-1 *T. gondii* serology, available tissue samples collected at Scan-1 were tested for *T. gondii* DNA.

The overall *T. gondii* sero-prevalence in this study at Scan-2 was estimated at 27.6% in R2 and 31.7% in MA hinds. At animal level, the sero-prevalence reported in aborted R2 and MA hinds of 34.3% and 32.9%, respectively, possibly reflecting natural exposure due to regular fecal shedding of *T. gondii* oocysts by feral cats. Data in Chapter 7 show the majority of the deer farmers responding to questionnaire-1 reported presence of feral cats which are a likely risk factor for exposure causing abortions. The prevalence data in New Zealand reported by Reichel *et al.* (1999) of 52.5%, and Wilson *et al.* (2012) of 42.5% (Scan-1) and 69.2% (Scan-2), albeit in a small number of herds were higher than reported in this study. However, those sero-prevalences might be an overestimate of the true prevalences, given the low specificity of LAT used in those studies (Chapter 3). On the other hand, the ELISA used in this study had a test Se and Sp of 98.8% and 92.8%, respectively, (Chapter 3) and therefore the sero-prevalences reported here are more robust than those reported using the LAT in those studies. Also, the sample selection of two sera per herd by Reichel *et al.* (1999) may not be truly representative of a typical herd.

In herds with aborted hinds, higher *T. gondii* sero-prevalence in MA than R2 hinds and non-significant association between *T. gondii* sero-status and abortion in MA hinds suggests that MA hinds might have acquired persistent immunity to *T. gondii* or been re-exposed, and resisted abortions. This observation supports the hypothesis that *T. gondii* vaccination in R2 hinds, if effective, could reduce abortions as claimed by the vaccine manufacturers for sheep.

8.11 *Toxoplasma gondii* sero-status and pregnancy

The validation of *T. gondii* ELISA allowed assessment of association between sero-status and pregnancy at Scan-1 and abortion at Scan-2.

The association between *T. gondii* sero-status and non-pregnancy was marginally non-significant in R2 hinds (Chapter 4). However, the association showed a trend that herds with sero-positive R2 hinds had higher non-pregnancy rates. This observation was parallel to the detection of *T. gondii* DNA in fetal samples from aborting fetuses at Scan-1. In addition, abortions earlier than the usual time of scanning (Scan-1) were detected in this study confirming that fetal/embryonic losses were also occurring before Scan-1. Hence, it is plausible that the higher non-pregnancy rates are likely due to *T. gondii*. Also, lack of association between *Leptospira* spp. serovars Hardjobovis or Pomona sero-status and non-pregnancy at Scan-1 suggests that *T. gondii* may be causing early abortions which may be going unnoticed with hinds being detected as non-pregnant at Scan-1. Therefore, the association between *T. gondii* and early abortions, affecting pregnancy rates at the normal time of scanning needs to be investigated in future, since conventional perception is that pregnancy rate at early scanning is a direct measure of conception. Data from this study supports the proposition that early abortions do contribute to sub-optimal pregnancy rates at scanning.

Data from this study also suggest that MA hinds were resistant to abortions due to *T. gondii,* which is consistent with the hypothesis that immunity developed from previous exposure/s is enduring, as observed for sheep in New Zealand. However, R2 *T. gondii*-naïve hinds were more susceptible to *T. gondii* infection and subsequent abortion and therefore a vaccination strategy to reduce abortions is more justifiable in R2 than MA hinds.

Analysis for difference in *T. gondii* ELISA S/P(%) ratio between aborted and non-aborted hinds was considered but not done because this was a cross-sectional study largely

sampling different hinds at Scan-1 and Scan-2. While a small number of hinds were serendipitously sampled at both scans, there were insufficient samples to permit this comparison. Further, the timing of exposure and timing of abortion could not be known as the study was set up to detect abortion between two scans.

8.12 *Toxoplasma gondii* vaccination cost-benefit

The findings from this study that approximately 7.9% of abortions in R2 hinds may be attributable to *T. gondii* can help inform vaccination cost benefit analysis. A positive significant association between *T. gondii* sero-status and aborted status was observed in R2 hinds only and therefore a cost-benefit analysis of *T. gondii* vaccination was undertaken only for this age group. Below are the assumptions for the *T. gondii* vaccination cost-benefit analysis in R2 hinds.

- Whole of gestation rate was 6.9% and 7.9% of total abortions were attributable to *T. gondii*;
- *Toxoplasma gondii* vaccine efficacy of 0.8;
- Total red deer herd population of 55,000 R2 breeding hinds;
- The estimate is based on purchase of replacement of a weaner at weaning in early March at $260 including transport, cost;
- That the aborted hind is culled at weaning and therefore the cost of replacement is included (difference between slaughter value and replacement value including costs, est. $100);
- The average time between abortion and sale was 6 months and cost of feeding the aborted hind at 10c/kgDM was $40 during that period.

The total losses from abortions due to *T. gondii* in total population of approximately 55,000 R2 hinds present in New Zealand was estimated at $101,152 whereas the losses per hind was estimated at $1.8 (Table 8.1). On the other hand, the total cost of vaccinating a hind for *T. gondii* is $2.5/hind assuming $2/dose (2mls) of vaccine cost and $0.5/hind towards labour cost for vaccination. Therefore, at a population level, vaccination of R2 hinds against *T. gondii* could not be beneficial. However, at herd-level, the abortion rates were variable and the effect of vaccination can be observed in herds with whole of gestation abortion rates ≥10%. Hence, since the repeatability of abortions was poor at herd-level, the cost-benefit at herd-level would be variable in consecutive years and that the true cost-benefit of *T. gondii*

vaccination should be evaluated over several years. From this study, the within-herd sero-prevalence of *T. gondii* varied over years from 0 to 66.7% and 0 to 40% in R2 herds with and without aborted hinds, respectively (Chapter 4) suggesting that the cost-benefit of *T. gondii* vaccination would vary depending on natural exposure of *T. gondii* on farm.

Table 8.1: The cost analysis of vaccination against *Toxoplasma gondii* in approximated red R2 hinds in New Zealand.

Srn.	Total red R2 hinds (N)	55,000
1	Whole of gestation abortions @6.9% (55,000 x 0.069)	3,770
2	Abortions attributed to *T. gondii* @7.9% (3,770 x 0.079)	298
3	Total loss Nx298x400*x0.85**	$101,152
4	Loss per hind	$1.8

*Total loss per hind including feeding cost ($40) and, hind ($100) and weaner ($260) replacement cost as per assumptions. ** vaccine efficacy

8.13 Other pathogens and sero-prevalence estimates

There was no association between *Leptospira* spp. serovars Hardjobovis and Pomona, BVD, and CvHV-1 sero-status, and aborted status at Scan-2 in R2 hinds. The sero-prevalence of *Leptospira* serovars Hardjobovis, Pomona, BVD, and CvHV-1 in R2 and MA hinds suggests that the deer are indeed exposed to these potential abortifacients. However, the abortion rate would depend on the degree of pathogen exposure, stage of gestation, and the level of immunity from previous exposures. Dual infection by *T. gondii* and *Leptospira* spp, *N. caninum,* BVD, or CvHV-1 was ruled out as only *T. gondii* had significant association with abortion.

No significant association was found between *N. caninum* and CvHV-1 serology and abortions, detection of *N. caninum* DNA in aborting fetal (Chapter 5) and maternal tissues and Cervid Rhadinovirus type-2 from uteri tissues (Chapter 6). This suggests that those pathogens may be contributing to abortions, but not at a rate able to be detected as significant using the design of this study. Further, given the absence of tissue sampling from non-aborted R2 hinds, a causal role of those pathogens in abortions in R2 hinds cannot be ruled out, and needs more investigation.

This study was not designed as a sero-prevalence study *per se*, but the data are of use in informing understanding and adding to existing data on the pathogens recorded. Little is known about the exposure to BVD and CvHV-1 in deer in New Zealand. The BVD sero-

prevalence of 12.5% in R2 and MA hinds combined was similar to 9.5% reported by Motha *et al.* (2000). However, the BVD sero-prevalence data reported in this study are likely more robust than those of Motha *et al.* (2000) as the selection in that study was based on collection of blood samples from two deer per farm. Also, Motha *et al.* (2000) used blocking antibody ELISA which was not validated for deer, whereas this study used VNT, based on OIE standards, detecting neutralising antibodies. The CvHV-1 sero-prevalence of 32.3% reported in this study was moderately lower than 38% reported by Motha and Jenner (2001). Sero-prevalence from this study could not be directly compared to 32% reported for bovine herpesvirus (BoHV) by Motha *et al.* (2000) using VNT as the sero-prevalence from the latter study may be result of cross-reaction between CvHV-1 and BoHV-1, which are antigenically similar. The sero-prevalences reported for BVD and CvHV-1 will help understand the exposure of those pathogens in deer.

The BVD exposure suggests that there could be persistently infected (PI) animals within the national deer herd population. The PI animal can continuously shed virus and could lead to clinical cases or outbreak in deer as for cattle. CvHV-1 has been detected in tissue from genital organs of deer (Tisdall and Rowe 2001), and coupled with the sero-prevalence and detection from this study, it might be playing a role in infertility in some herds. Data from this study justifies that it needs to be studied more in future. The detection of Cervid Rhadinovirus type-2 (CRhV-2) was consistent with the reports by Squires *et al.* (2012) in New Zealand. Given the exposure of these potential abortifacients, they should be included in future investigations of reproductive inefficiency in deer.

8.14 Farm, environment, health and management risk factors for pregnancy at Scan-1 and abortion by Scan-2

Herd-level risk factors from mating to Scan-1 and Scan-1 to Scan-2 were analysed for pregnancy and abortion, respectively. Risk factors from mating to Scan-1 were also analysed for abortion as some factors may take time to have effect.

Analysis of risk factors for pregnancy rates at Scan-1 was opportunistic and was done as the pregnancy scanning data from Scan-1 was available. Risk factors identified for pregnancy rates at Scan-1 in R2 and MA herds reflects that good management practices including proper nutrition and absence of other stock (dairy) are associated with high pregnancy rates. Overall, there were not many management associated risk factors except for abortion, suggesting that an infectious pathogen may be playing a greater role in abortion.

However, the analysis of risk factors may have been affected by the low response rates and non-response bias as discussed earlier in 'Limitations on sampling and questionnaire data collection'.

The presence of feral cats was not associated with DAR in R2 or MA herds. However, the findings on *T. gondii* serology association with abortion (Chapter 4) does support that *T. gondii* causes abortions in R2 hinds. Further, this study did not aim to quantify *T. gondii* contamination of environment and the resulting natural exposure in pregnant hinds. These apparently contradictory findings may be due to varied level of natural challenge and unpredictable biological cycle of *T. gondii* which may depend on the environmental conditions. Therefore, the *T. gondii* prevalence on a farm and its exposure to R2 and MA can be different in successive years. Another reason for this observation could be lower sample size for the Q1 data and that the serology findings are based on a sub-sample of the sera collected or that the presence of cats was noted almost ubiquitously, and therefore their presence alone can't be used to assess association with abortion.

Non-sporulated *T. gondii* oocysts, excreted by cats, can survive for at least three months on the ground and become infectious after sporulation under suitable conditions (Lindsay *et al.* 2002a). Mating in deer occurs in summer and autumn and the temperatures during mating (range: 10-25^{o}C) are ideal for the survival (up to 200 days) of sporulated *T. gondii* oocysts. Therefore, there is increased risk of *T. gondii* exposure to hinds through environment by consumption of oocyst contaminated pastures/forages/feed during mating. Sporulated oocysts can remain infective for 54 months at 4^{o}C suggesting that the pregnant hinds post-mating are also at risk of *T. gondii* exposure (Dubey 1998).

In MA herds, the risk of abortion was lower in those with occurrence of abortion in previous years than in herds with no abortions in previous years (Chapter 7). The lower risk of abortions in those herds may be due to improvement in herd management factors. Low repeatability of abortion in R2 and MA herds was also observed in this study (Chapter 2). These observations confirm that prediction of abortion is difficult. This confounds interpretation of the validity or success of control measures since they may be effective some years but not others. Hence the true benefit of control measures on an individual farm must be evaluated over several reproduction cycles.

Herds fed hay had lower pregnancy rates in R2 herds at Scan-1 and higher abortion rates in MA herds at Scan-2. It was difficult to explain this adverse effect; however, it may be due to presence of ergot or mould in hay or it may be that the hinds were nutritionally deficient and therefore were fed those supplements. Additionally, the co-grazing of R2 and MA herds with beef cattle was identified as a risk factor for pregnancy and abortion. This association may be due to social disturbance and therefore, change in grazing management may help to reduce those losses. The time period spent by hinds co-grazing with beef cattle may also be playing important role in this association, however, data on co-grazing time period was insufficient to allow any analysis.

The association between Johne's disease and abortion needs to be interpreted carefully. Although JD was not examined in this study, risk factors leading to occurrence of JD may be similar to those causing abortion. For example it is known the risk of Johne's disease is higher in poorly managed cattle herds (Goodger *et al.* 1996). The association between feeding and abortion discussed above, combined with the nutrition/management risk for Johne's disease supports that this association reflects common causality rather than direct causation. Also, the herd-level immunocompetence in JD positive herds may be lower than JD negative herds due to nutrition, management and concurrent disease effects, and that the pregnant R2 hinds from JD positive herds are more susceptible to acquire *T. gondii* or other pathogen infection. Additionally, it is alo known that prevalence of JD is higher in South Island than South Island, and therefore the higher least square (LS) mean DAR observed in South Island might be reflecting the JD causality and abortion association as discussed above.

From studies in other species, it is known that at a population or herd level, abortions cannot be attributed to a single cause: i.e. only a proportion of abortions can usually be ascribed to one cause (Sanhueza *et al.* 2013). Instead, the causation of abortion is a combination of different possible causes or factors creating a possibility of abortion while those factors are in effect. For example, a recent study on causes of fetal losses in beef cattle by Sanhueza *et al.* (2013) reported that 14% of total abortions could be attributed to four different pathogens namely *Leptospira* spp. (serovars Hardjobovis and Pomona), *Neospora caninum*, and bovine viral diarrhoea (BVD). Investigations into infectious causes from this study revealed that 7.9% abortions in R2 hinds could be attributed *T. gondii* whereas risk factor analysis showed lower DAR in better fed and healthy hinds. These observations indicate that the control of abortions is a collective measure which may include control of the

T. gondii exposure, and concurrent improvement in farm management practices. However, a major proportion of abortions in deer still remain unexplained and hence research into abortions on a larger scale may highlight more risk factors than identified in this study.

8.15 Limitations of the present study

- This study could not explain the higher proportion of abortions occurring on deer farms. For example, only 7.9% abortions in R2 hinds could be attributed to *T. gondii.*
- The prevalence and incidence of abortion reported in this study may have been affected by the selection and volunteer bias as discussed earlier. Selection of farms with low and high reproduction from deer farms database could have resulted in lesser selection bias than the current study.
- This study could not assess the effect of body condition score (BCS) and average weight at mating on pregnancy and abortion rates. The determination of BCS would likely have been confounded given the number of veterinarians involved in the study and the likely variation in scoring. Also, weighing of hinds was not a standard practice on many of the study farms with only few farmers providing average weights of the mating hinds.
- Fetal aging at Scan-1 would have been helpful to more closely estimate the timing of abortion relative to conception than permitted by knowing joining dates only, as used. However, it would not have been possible to get robust data given the number of operators and variation in skill-level for fetal ageing. Further, it is unlikely that farmers would have been prepared to commit the additional time required for aging, or to pay the additional fee.
- The study was not set up as regional study and therefore this study could not assess difference in abortion rates by regions. However, this study could detect difference in abortion rates between islands.
- Individual animals were not followed in this study and therefore it was not possible to blood sample the same animals at both scans. Therefore, a sero-conversion/sero-status change study was not possible.
- The losses between Scan-2 and calving could not be estimated or detected due to logistics reasons and restricted access to hinds undergoing parturition.
- This study was not able to provide estimates on reproductive efficiency *per se* due to lack of accurate weaning data largely due to poor response rate.

- The study could not provide robust data for whole of gestation rate because of farmer resistance to late gestation scanning or other methods for determination of carriage of fetus to term
- It was impossible to convince farmers to perform Scan-2 90 days after Scan-1 as targeted at the start of the study. Therefore, a daily abortion rate was calculated and used for the analysis rather than a 90-days abortion rate.
- Tissue sampling was not possible from all aborting/aborted hinds. Therefore, the *T. gondii* PCR prevalence in aborted hinds could have been higher or lower than that estimated.
- It was not possible to collect tissues from non-aborted hinds at DSPs. Therefore, the DNA data from tissue samples could not be analysed for difference in prevalence of *T. gondii* in aborted and non-aborted hinds.
- The non-response bias could have affected the estimates for the abortion risk factor analysis.
- The investigation of genetic and developmental anomalies leading to abortion was outside the scope of this study.

8.16 Implications of this research for the Deer Industry

The findings from this study have following implications for the Deer Industry.

- The observed mid-term abortion rates alone pose an economic burden of $2.10 million directly upon deer farmers. If abortions continue at this rate through the whole of gestation, losses can be up to $5.58 million. There are additional costs to the deer industry as a whole beyond the farm gate as a result of fewer animals for transporting, processing and export.
- The abortion rates reported by this study can be used as benchmarking data by stakeholders throughout the production sector of the industry, to target where interventions may be warranted.
- The deer industry can now determine whether more investment is justified in determining causes other than those shown in this study, which explain a limited proportion of abortions.
- This study has provided evidence that early abortion contributes to sub-optimum early pregnancy scanning rates, conventionally believed to be associated with conception *per se.* This suggests that more research needs to be done to quantify these losses to

justify whether or not management practices need to be targeted to this early gestation period in addition to the present focus on achieving conception.

- Because this study proves *Toxoplasma gondii* can be a cause of abortion in R2 hinds, the industry needs to consider whether research into developing an effective vaccine is warranted.
- This study draws attention to *T. gondii* in deer by showing a significant herd and individual animal sero-prevaence and demonstration of the presence of the organism by detection of DNA in tissues. This is a risk to the deer industry since this organism is zoonotic. The industry may be prudent to consider development of a strategy to deal with this potential issue around food safety.
- The findings from this study should be made available to deer farmers, perhaps through the Deer Industry New Zealand (DINZ) "Deer Hub" website, and by workshops or presentations to farmer groups. This would educate farmers about abortions and potential causes identified in this study. It would also inform farmers of the value of conducting a second pregnancy scan later in gestation in farms with persistent sub-optimum Scan-1 to weaning rates, to better identify areas contributing to losses and therefore to target control measures, particularly nutritional management.

8.17 Proposed future research

While this study determined the national prevalence and incidence rates of abortion in red deer, it was not able to identify all factors associated with abortion or affecting reproductive efficiency. While providing some biologically plausible associations, causation could not be robustly established. Other research methodology would be required for that purpose. Further, it was beyond the scope of this research to investigate means for control of abortion. More detailed research is suggested in the following areas.

- The association between *T. gondii* and abortion needs to be studied further to irrefutably confirm causation. A challenge study using an artificial dose is needed to understand the potential disease process of *T. gondii* in pregnant deer. The relationship between the immune response and abortion needs to be studied which could further help evaluate the effect of artificial and natural challenge in immune naïve and exposed animals.

- Feral cats were reported in abundance around deer farms in this study. An interventional study to assess reduction in *T. gondii* exposure by control of feral cats followed by longitudinal study on case and control farms would help understand the relationship between feral cats and *T. gondii* abortion in deer.
- A vaccination against *T. gondii* may help in reducing abortions. More studies will be needed to actually assess and monitor use of vaccine against *T. gondii* and its ability to reduce abortions in R2 herds. Studies parallel to this thesis research project have attempted to evaluate the efficacy of the Toxovax® (vaccine against *T. gondii*) in preventing abortion. Also, studies have been done to show there is a measurable immunological response, but there is equivocal data about its ability to prevent abortion. Therefore, more work needs to be done to establish if the currently available sheep vaccine is actually efficacious in deer or whether there is need to develop a deer specific vaccine.
- Determination of vaccine dose for protection of hinds against *T. gondii* exposure. A clinical study will need to be done to identify proper dose of vaccine required to provide immunity against *T. gondii* infections and reduction in abortion rate.
- The majority of the abortions remained unexplained in this study. However, ongoing studies of abortion outbreaks will provide more data to evaluate the range of potential causes of abortion, and to quantify their importance. The tissue and blood sampling from ongoing outbreaks in future can be investigated for *T. gondii, N. caninum*, and alpha and gamma herpesviruses. Intensive monitoring of farm management on case farms and selected control farms can help understand role and contribution of infectious and management causes for abortion. Data from this study has informed such approaches, and has validated one serology test for this purpose.
- This study was not designed as a regional or island oriented study. However, this study showed that abortions were widespread and the rates were different between islands. A further deeper study is needed to assess the island specific factors responsible for this difference e.g. climatic, management, topography etc. Similar studies could be carried out to quantify fetal losses in wapiti and fallow farmed deer.
- This study included naturally mated hinds for detection of abortions. Given that hinds from a proportion of herds or a proportion of hinds from those herds are artificially inseminated (AI) at high cost and often with poor pregnancy rates, study of both early

embryonic and fetal loss, along with later abortion could be undertaken to quantify these losses in AI hinds.

- This study detected abortions earlier than the normal time of scanning on 2/3 R2 and one MA herd. A wider study needs to be done for establishment of prevalence and incidence of early abortions, and quantify these against conception failure *per se*.
- A further study, depending on the incidence and prevalence of early abortion, could be needed to identify causes and risk factors for early abortion.
- The losses between Scan-2 and calving need to be quantified. Also, depending on the prevalence and incidence, a further study may be needed to identify risk factors associated with those losses.
- This study reported presence of *N. caninum* and Cervid Rhadinovirus type-2 (CRhV-2) DNA in fetal and maternal tissues. More studies are needed to investigate their role in abortions and in the case of CvHV-1, in other reproductive outcomes, and respiratory or ocular disease.
- More studies need to be done to validate the association between farm environment and management risk.
- Further assessment of control measures will be required to evaluate their cost-benefit.
- This study is the first to confirm *T. gondii* in NZ deer using PCR. Tissue cyst containing bradyzoites in processed venison meat pose risk of toxoplasmosis in humans. Therefore, a study assessing the presence of *T. gondii* in venison is justified to inform of the likelihood of transmission from venison to humans.

8.18 Conclusion

This study provided an insight into the prevalence, incidence and causation of abortions on red deer farms. The prevalence and incidence of abortions shows that early and mid-term abortions are occurring on New Zealand deer farms which are likely to have been unnoticed. While abortions were occurring at both early and mid-gestation stages, the pregnancy rates reported at Scan-1 in this study were affected by early abortion, and therefore scan pregnant rates commonly used do not accurately reflect conception rates *per se*. Primiparous hinds were at higher risk of abortion than mixed-age hinds, consistent with proposed infectious agent causation (*T. gondii*), and nutritional and management effects that are likely to affect younger animals than adults. Abortions pose an economic burden on deer farmers and are contributing to reduced reproductive efficiency observed on deer farms.

Based on diagnostic investigations, *T. gondii* may be causing abortion so an effective vaccine against *T. gondii* may help in reducing abortion in herds with high abortion rates. However, more study is needed to investigate the effectiveness, timing and dose of a *T. gondii* vaccine, and predictability that a cost-effective response will be achieved. The role of *N. caninum,* CvHV-1 and Cervid Rhadinovirus type-2 in abortions were not significant compared with *T. gondii* but needs further investigation. BVD and *Leptospira* spp. were not associated with abortion. Risk factor analysis suggests that attention to good nutrition and health reduces the risk of abortion. Results contribute to understanding of the occurrence of these organisms in farmed deer in New Zealand.

The research undertaken in this study effectively contributes to the knowledge on the prevalence, incidence, and causation of abortion. It also leads to further studies that will help deer industry plan and implement measures to reduce abortions.

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Appendices

Appendix 1: Description of farms

Table: Location, size, stag introduction (stag-in), stag removal (stag-out), number pregnant, dry, and aborting at Scan-1 and number scanned and aborted by Scan-2 for participating farms both years. (R2 = rising 2-year old, MA = 3-years and older, year-1, 2012, year-2, 2013).

Region	farm	Deer fenced area (ha)	Age	Year	Stag-in	Stag-out	Scan-1					Scan-2		
							Date	No. scanned	No. pregnant	No. dry	No aborting	Date	No. scanned	No. aborted
Auckland	1	270	R2	1	1-Feb	2-May	12-Jun	108	102	6	0	20-Sep	71	1
			MA	1	7-Mar	2-May	12-Jun	432	414	18	0	20-Sep	130	0
	2	400	R2	1	5-Mar	14-May	19-Jun	106	78	28	0	27-Sep	78	2
			R2	2	.	.	27-Jun	83	59	24	0	.	.	.
			MA	1	6-Mar	5-May	19-Jun	456	380	76	0	27-Sep	145	1
			MA	2	.	.	19-Jun	471	401	70	0	.	.	.
Bay of Plenty	1	700	R2	1	25-Jan	24-Apr	8-Jun	226	218	8	0	3-Oct	110	0
			R2	2	30-Jan	26-Apr	6-Jun	218	189	26	3	26-Sep	100	0
			MA	1	25-Jan	25-Apr	8-Jun	1227	1212	15	0	5-Oct	100	4
			MA	2	2-Mar	22-Apr	5-Jun	1125	1092	32	1	26-Sep	130	0
Canterbury	1	250	R2	1	1-Feb	15-May	21-Jun	266	236	29	1	21-Sep	120	1
	2	.	R2	1	.	.	21-Jun	306	255	51	0	2-Sep	100	6

		R2	2	.	.	27-Jun	297	160	137	0	17-Sep	122	2
		MA	1	.	.	21-Jun	212	208	4	0	2-Sep	101	1
3	110	R2	1	1-Mar	11-May	18-Jun	349	287	62	0	14-Sep	100	2
		R2	2	.	.	13-Jun	280	254	26	0	9-Sep	100	1
		MA	1	1-Mar	11-May	21-Jun	544	527	17	0	14-Sep	100	0
		R2	1	.	.	27-Jun	332	324	8	0	29-Sep	100	2
4	165	R2	1	1-Feb	3-May	2-Jul	109	100	9	0	12-Sep	93	4
		MA	1	5-Mar	3-May	2-Jul	417	401	16	0	.	.	.
5	300	R2	1	15-Feb	14-Jun	3-Jul	119	107	12	0	21-Sep	100	17
6	930	R2	1	26-Feb	2-May	4-Jul	693	632	61	0	4-Oct	120	0
		MA	1	4-Mar	5-May	4-Jul	1988	1983	5	0	4-Oct	85	0
7	.	R2	1	.	.	4-Jul	250	218	32	0	10-Oct	100	4
8	1007	MA	1	.	.	4-Jul	223	206	17	0	12-Oct	199	6
9	.	MA	1	.	.	5-Jul	130	121	9	0	3-Sep	118	1
10	96	MA	1	2-Mar	3-May	5-Jul	480	450	30	0	1-Oct	130	0
11	270	MA	1	14-Feb	10-May	9-Jul	156	150	6	0	2-Oct	130	1
12	595	R2	1	5-Jan	15-May	12-Jul	238	222	16	0	16-Oct	100	3
		MA	1	3-Mar	15-May	12-Jul	195	173	22	0	16-Oct	130	5

13	1000	R2	1	.	.	16-Jul	370	187	183	0	29-Oct	50	0
		MA	1	.	.	16-Jul	136	133	3	0	29-Oct	100	0
14	565	R2	1	1-Feb	15-May	16-Jul	111	98	13	0	9-Oct	98	0
15	80	R2	1	1-Feb	10-May	17-Jul	50	43	7	0	15-Oct	43	0
		MA	1	1-Feb	1-May	17-Jul	291	278	13	0	15-Oct	131	0
16	.	R2	1	.	.	24-Jul	115	111	4	0	12-Oct	100	1
17	900	R2	2	1-Dec	28-May	30-May	260	223	37	0	26-Sep	100	1
		MA	2	10-Mar	1-Jun	5-Jul	1452	1380	71	1	29-Sep	130	0
18	52	MA	2	1-Mar	5-May	10-Jun	326	297	29	0	19-Sep	100	0
19	240	R2	2	7-Mar	9-May	19-Jun	68	48	20	0	7-Oct	46	4
		MA	2	14-Mar	9-May	23-Jul	340	314	24	2	7-Oct	60	3
20	200	R2	2	1-Mar	18-May	19-Jun	54	51	3	0	19-Sep	51	2
		MA	2	10-Mar	8-May	19-Jun	137	129	8	0	19-Sep	110	3
21	40	MA	2	28-Feb	15-May	18-Jun	94	83	9	2	23-Sep	83	0
22	.	MA	2	.	.	24-Jun	292	264	28	0	21-Aug	130	2
23	.	MA	2	.	.	17-Jul	295	279	16	0	24-Sep	130	0
24	110	R2	2	.	.	2-Jul	68	60	8	0	28-Aug	50	1

			MA	2	.	.	2-Jul	188	179	9	0	28-Aug	80	0
	25	.	R2	2	.	.	8-Jul	15	14	1	0	28-Aug	14	1
			MA	2	.	.	8-Jul	148	143	5	0	28-Aug	114	0
	26	.	R2	2	.	.	9-Jul	144	130	14	0	24-Sep	100	0
	27	220	R2	2	15-Jan	10-May	12-Jul	100	83	17	0	20-Sep	84	3
	28	58	R2	2	5-Mar	10-May	11-Jul	43	3	40	0	.	.	.
			MA	2	15-Mar	10-May	11-Jul	183	123	60	0	3-Oct	121	2
	29	270	R2	2	15-Mar	21-May	30-Jul	49	41	8	0	4-Nov	38	0
Gisborne	1	.	R2	2	.	.	1-Jul	151	113	38	0	20-Sep	113	5
Hawke's Bay	1	.	R2	1	.	.	14-Jun	86	45	41	0	.	.	.
			MA	1	.	.	14-Jun	220	212	8	0	.	.	.
	2	160	R2	2	20-Feb	10-May	13-Jun	91	75	16	0	27-Sep	75	1
	3	800	R2	2	10-Jan	16-May	16-Jun	273	155	118	0	17-Oct	141	18
	4	316	R2	2	.	.	25-Jun	140	64	76	0	24-Sep	62	5
	5	114	R2	2	10-Jan	10-May	19-May	62	60	2	0	23-Sep	52	1
			MA	2	3-Mar	10-May	20-May	269	253	16	0	.	.	.
Manawatu-	1	140	MA	1	18-	10-May	8-Jun	289	278	11	0	11-Sep	134	1

Whanganui					Feb								
	2	120	R2	1	1-Jan	4-May	14-Jun	105	92	13	0	30-Aug	82	0
	3	230	R2	1	1-Feb	1-May	13-Jun	111	77	34	0	12-Sep	70	3
			MA	1	1-Mar	1-May	13-Jun	440	438	2	0	.	.	.
	4	.	MA	1	.	.	14-Jun	183	178	5	0	7-Oct	100	1
	5	152	R2	1	8-Mar	8-May	22-Jun	90	60	30	0	13-Oct	52	3
			R2	2	.	.	17-Jun	74	58	16	0	19-Sep	57	1
			MA	1	8-Mar	1-May	22-Jun	504	479	24	1	13-Oct	130	1
			MA	2	.	.	17-Jun	472	360	112	0	19-Sep	130	0
	6	176	R2	1	1-Mar	6-May	28-Jun	124	112	12	0	21-Sep	112	2
			R2	2	.	.	28-Jun	83	70	13	0	12-Sep	70	2
			MA	1	1-Mar	6-May	28-Jun	478	426	51	1	21-Sep	219	5
	7	80	R2	1	20-Jan	30-Apr	27-Jun	36	30	4	2	21-Aug	26	0
			MA	1	20-Jan	30-Apr	27-Jun	198	192	6	0	21-Aug	130	0
	8	68	R2	1	11-Feb	19-May	28-Jun	36	33	3	0	27-Sep	30	0
			MA	1	11-Feb	19-May	28-Jun	60	60	0	0	.	.	.
	9	200	R2	1	1-Mar	1-May	9-Jul	135	90	45	0	20-Sep	90	5
			R2	2	.	.	19-Jun	96	53	41	2	30-Sep	40	2

			MA	1	.	.	9-Jul	64	59	5	0	.	.	.
	10	1886	R2	1	20-Feb	10-May	20-Jul	375	346	29	0	29-Aug	100	4
			MA	1	10-Mar	28-Apr	20-Jul	1574	1412	162	0	29-Aug	150	1
	11	.	R2	2	.	.	8-Jul	84	72	12	0	.	.	.
	12	403	MA	2	8-Mar	7-May	19-Jun	300	285	15	0	11-Sep	156	2
	13	190	R2	2	10-Jan	15-May	23-Jun	63	55	8	0	5-Sep	51	2
			MA	2	1-Feb	15-May	23-Jun	392	339	53	0	5-Sep	127	2
Otago	1	.	R2	1	.	.	16-Jun	1300	1170	130	0	13-Oct	100	7
	2	.	R2	1	.	.	19-Jun	450	399	42	9	5-Oct	160	0
	3	250	R2	1	20-Feb	20-May	26-Jun	245	192	53	0	8-Oct	100	1
			R2	2	1-Feb	1-May	25-Jun	280	206	74	0	26-Sep	200	3
			MA	1	20-Feb	20-May	26-Jun	539	531	8	0	8-Oct	100	2
			MA	2	1-Feb	1-May	25-Jun	550	535	15	0	26-Sep	120	4
	4	.	R2	1	.	.	6-Jul	90	82	8	0	.	.	.
			MA	1	.	.	6-Jul	590	553	37	0	.	.	.
	5	56	R2	1	4-Mar	25-May	.	31	31	0	0	14-Oct	31	0
			MA	1	3-Mar	25-May	.	118	115	3	0	14-Oct	115	3

	6	.	MA	1	.	.	6-Jun	230	197	33	0	5-Nov	133	0
	7	100	MA	2	1-Mar	1-May	11-Jun	175	155	20	0	13-Sep	120	0
	8	97	R2	2	.	.	1-Jul	249	185	64	0	18-Sep	100	4
			MA	2	1-Feb	1-May	1-Jul	205	188	17	0	18-Sep	150	0
Southland	1	2300	R2	1	1-Feb	1-May	11-Jun	858	750	104	4	19-Sep	670	16
			R2	2	.	.	6-Jun	832	757	75	0	20-Sep	100	0
			MA	2	.	.	6-Jun	950	939	11	0	20-Sep	120	2
	2	1200	R2	1	1-Mar	20-Apr	8-Jun	419	379	40	0	9-Aug	230	2
			R2	2	.	.	29-May	307	251	46	10	22-Sep	100	0
			MA	1	1-Mar	20-Apr	15-Jun	972	963	9	0	.	.	.
			MA	2	.	.	29-May	1206	1200	6	0	.	.	.
	3	354	R2	1	12-Mar	7-May	13-Jun	216	171	45	0	13-Sep	171	11
			MA	1	.	.	13-Jun	760	739	21	0	13-Sep	130	5
	4	126	R2	1	1-Dec	8-May	18-Jun	84	51	33	0	28-Sep	49	3
			R2	2	11-Mar	14-May	9-Jul	49	44	5	0	27-Sep	44	0
			MA	1	8-Mar	8-May	18-Jun	183	175	8	0	28-Sep	167	0
			MA	2	11-Mar	14-May	11-Jul	170	167	3	0	24-Sep	99	0

5	.	R2	1	.	.	21-Jun	64	45	18	1	27-Sep	45	3
		MA	1	.	.	21-Jun	303	270	33	0	27-Sep	130	2
6	235	R2	1	10-Jan	7-May	25-Jun	373	357	16	0	8-Oct	200	1
7	180	R2	1	.	.	25-Jun	117	55	62	0	7-Sep	55	1
		MA	1	.	.	25-Jun	284	254	30	0	7-Sep	130	1
8	400	R2	1	.	.	27-Jun	320	292	28	0	29-Sep	290	8
		MA	1	.	.	27-Jun	764	740	24	0	.	.	.
9	500	R2	1	22-Mar	21-May	28-Jun	110	106	4	0	27-Sep	100	1
		MA	1	.	.	28-Jun	448	409	39	0	27-Sep	130	2
10	30	R2	1	10-Mar	23-Jul	23-Jul	93	70	23	0	1-Oct	70	0
		MA	1	.	.	23-Jul	197	183	14	0	1-Oct	130	3
11	320	R2	1	1-Mar	1-May	31-Jul	280	264	16	0	8-Oct	230	1
		MA	1	.	.	31-Jul	1120	1050	69	1	8-Oct	100	0
12	4500	R2	1	1-Dec	25-May	30-May	827	750	76	1	18-Sep	616	33
13	1950	R2	1	1-Jan	1-May	28-May	843	730	111	2	18-Sep	722	20
14	260	R2	1	20-Feb	4-May	15-Aug	295	210	85	0	12-Oct	187	6
		R2	2	.	.	26-Jun	250	203	47	0	28-Aug	100	1

	15	.	R2	2	.	.	8-Jul	61	56	5	0	23-Sep	56	4
			MA	2	.	.	8-Jul	140	140	0	0	23-Sep	131	4
	16	1200	R2	2	8-Mar	1-May	11-Jul	276	228	48	0	7-Oct	151	12
			MA	2	8-Mar	1-May	13-Jun	544	506	38	0	7-Oct	130	1
	17	.	MA	2	.	.	19-Jul	860	813	47	0	10-Oct	130	5
Waikato	1	1871	R2	1	1-Feb	30-Apr	13-Jun	1618	1537	79	2	25-Sep	1525	44
			R2	2	23-Jan	1-May	31-May	1648	1556	85	7	9-Oct	125	1
	2	466	R2	1	16-Jan	4-May	27-Jun	282	261	19	2	6-Sep	252	4
	3	500	R2	1	15-Feb	21-May	27-Jun	268	219	49	0	23-Oct	100	2
			R2	2	25-Feb	21-May	10-Jul	261	232	29	0	23-Sep	100	0
			MA	1	8-Mar	16-May	27-Jun	1262	1120	142	0	23-Oct	130	1
			MA	2	15-Mar	8-May	11-Jul	1394	1214	180	0	23-Sep	130	1
	4	320	R2	1	16-Feb	8-May	4-Jul	135	127	8	0	20-Sep	95	0
			R2	2	.	.	19-Jun	107	103	4	0	13-Sep	78	2
			MA	1	10-Mar	14-May	4-Jul	465	185	280	0	20-Sep	130	1
			MA	2	.	.	21-Jun	234	228	5	1	13-Sep	228	0

	5	130	R2	1	1-Feb	2-May	12-Jul	74	67	7	0	29-Aug	67	0
			R2	2	1-Jan	20-May	4-Jul	72	22	50	0	16-Sep	22	1
			MA	1	1-Feb	2-May	12-Jul	254	239	13	2	29-Aug	130	3
			MA	2	1-Jan	20-May	4-Jul	280	223	57	0	16-Sep	120	9
Wellington	1	21	R2	2	8-Mar	20-May	1-Jul	19	19	0	0	11-Oct	19	0
			MA	2	8-Mar	21-May	2-Jul	82	81	1	0	11-Oct	48	1
West Coast	1	459	R2	2	15-Feb	6-May	26-May	225	206	19	0	3-Oct	134	1
			MA	2	19-Feb	6-May	11-Jun	565	540	25	0	.	.	.
	2	1296	R2	2	11-Feb	3-May	10-Jun	460	404	56	0	7-Aug	100	0
			MA	2	13-Feb	2-May	11-Jun	2998	2853	145	0	7-Aug	130	0

Link to online repository containing the raw data used for this thesis

https://www.dropbox.com/s/kk11i4hqwxpnyad/alldata.csv?dl=0

Appendix 2: *Toxoplasma gondii* Western blot protocol

Western blotting for the detection of *Toxoplasma gondii* antibodies in deer sera

Procedure

Preparation of water soluble *Toxoplasma gondii* antigen preparation

Live tachyzoites of an attenuated strain (S48) of *Toxoplasma gondii* from Toxovax vaccine (currently available for sheep) were used to extract *T. gondii* antigen. The vaccine suspension (30mls) was centrifuged at 1500 x g for 10 minutes to obtain tachyzoites in form of a pellet at the bottom. The pellet, after removal of supernatant was stored at -20^{o}C until used. The pelleted tachyzoites were re-suspended in 2ml PBS and disrupted by three cycles of freezing and thawing by cycling them from room temperature to -80^{o}C. This was followed by seven cycles of sonication on ice (Sonics Vibracell^{TM}, Sonics & Materials Inc., CT, USA). The sonicated tachyzoites were centrifuged at 12,000 x g for 30 min at 4^{o}C to remove debris and the supernatant containing the water soluble proteins were collected. The protein content was determined using a Nano-spectrophotometer (NanoDrop ND-1000, Thermo Scientific, DE, USA) and stored in aliquots.

Pre-day 1

Prepare 2 L of Transfer buffer on day previous to day1 and leave at 4^{o}C overnight.

Day 1

Electrophoretic separation

- Prepare the SDS-PAGE running buffer. Remove the pre-cast gel from packet. Remove the tape and the comb as well. Flush the plate with distilled water.
- Insert the gel into one of the slots in the criterion tank, ensuring the upper gel chamber is facing the centre. Fill the tank (inner compartment, upper and outer chambers) with running buffer.
- Prepare the sample/s. Calculate volume of sample required. For 1 large well use 100μg, so for 10 wells = 10 μg/well.
- In a microcentrifuge tube, add required sample volume plus equal volume of loading buffer (lamelli buffer). Eg 100 μl antigen + 100 μl loading buffer
- Close tube and boil (100^{o}C) on a heating block for 10 min.

- Load the sample into the large well (uniwell gel) with pipette tips. Load 10 μL of marker into the marker well. Place lid on the gel running tank and run the gel at 100V for about 1.5 hrs.
- At end of gel run, remove the gel from the cassette and rinse out the tank with distilled water with multiple washes.

Electrophoretic Transfer

- Freeze the ice block prior to preparation of the blot assembly.
- Using forceps to handle PVDF membrane, soak the membrane in 100% methanol for 10 seconds, and then immerse it in transfer buffer in the gel/blot assembly tray.
- Soak the filter (soak) pads and the filter paper separately in transfer buffer. Equilibrate the gel in transfer buffer for approximately 15 mins.
- Fill the criterion blotter tank to half level with transfer buffer and place a magnetic flea (stirrer) on the bottom.

Use the gel blot assembly tray to assemble the gel blot sandwich:

- Pour chilled transfer buffer into each compartment of the gel/blot assembly tray. Place the PVDF membrane in the small compartment of the tray. Keep it soaked in transfer buffer while preparing the rest.
- Equilibrate the gel in transfer buffer for 10 min.
- Place the red and black cassette into the large compartment of the gel/blot assembly tray. Open the cassette so that the red side (anode) is vertical and the black side (cathode) is horizontal submerged in buffer.
- Place the fibre (soak) pad on top of the black side of the cassette, submerged in buffer. Push pad into buffer for thorough soaking.
- Place filter paper over this pad.
- Gently place the pre-equilibrated gel on the filter paper. Use the roller to remove air bubbles.
- Take the membrane from the front compartment and place on top of gel. Do not adjust membrane after it makes contact with the gel. Roll out air bubbles if any.
- Place filter paper on top of the membrane. Dip a second fibre pad in the front compartment (with transfer buffer) and then place on top.
- Lower the clamp side of the cassette and lock in the closed position.
- Place locked cassette into the groove of the blotter tank, aligning red side with red electrode.
- Transfer at a constant voltage at 70mV for 45 minutes. (Note- if voltage does not reach to 70mV, and reaches within 50-60mV range, then increase the time by 15-25 minutes accordingly.

Marking the membrane

After transfer, remove assembly and discard the gel. Carefully immerse the membrane in Ponceau solution for 1 min to stain. Rinse the membrane thrice with distilled water, place on a glass surface and mark the positions of the bottom of the protein and the protein standards (label the marker strips A & B). Also mark out and label the 24 strips to be cut after blocking.

Blocking
Cut the membrane into two just for blocking. Block the membranes in 5% Blotto in the 4ºC room overnight. (15g skimmed milk powder in 300 mL PBST). (Note – Strip cutting can be done next day after blocking it overnight in blotting solution).

Day 2

Incubating with primary antibody (serum)

- Make up 300 mL Blotto in the morning.
- Thaw out the serum at 37ºC
- Label microcentrifuge tubes 1-24 according to samples, and the positive and negative control sera to be used. Vortex the sera properly. Dilute sera into tubes: 990 μL Blotto + 10 μL serum.
- Place blocked membranes on glass surface and cut the strips. Place strips into the numbered western trays. Add sera into the trays. Put the protein standard strips into PBST until used. Cover the trays and incubate for 1 hour at 21ºC in the shaking incubator.

Washing-1

- Put each strip into a 50 mL Falcon tube filled with PBST.

If using 20x PBS, dilute to 1x (100 mL 20x to 2L distilled water) + 1 mL tween.

Place tubes in rack and place the rack on a plate shaker. Shake vigorously for 10 min. Pour off wash solution and refill with PBST. Wash 3 times, 10 min each wash.

(Note - Alternatively, washing can be done in the western trays, pouring required (2-3mls) PBST and placing it in shaking incubator)

Incubating with secondary antibody

- Place all the strips into one container and add 2º ab. at 1: 8,000 dilution (6.25 μL ab into 50 mL Blotto, mix first in a 50 mL Falcon tube and vortex). Cover container and incubate in the shaking incubator for one hour.
- Alternatively, put 2 mls of 2º ab / strip in to each well of tray and incubate in the shaking incubator for one hour.

Washing-2
Remove the 2º ab from the container and fill the container with PBST. Put all the strips into one reservoir and wash 3x for 10 min intervals.

(Note - Alternatively, washing can be done in the western trays, pouring required (2-3mls) PBST and placing it in shaking incubator.)

Detection

- Prepare chemiluminescent reagents. ECL should be prepared just prior to use.
- Open the X-ray cassette and place a clean transparency plastic inside. Can cut A4 size into two and use half of it.
- Add in 2 mL of detection reagent (1 mL each of solutions 1 & 2) into a reservoir.

- Using forceps, soak one strip at a time in the reagent, drain excess fluid onto tissue paper and place strip (face-up) onto the transparency plastic. Repeat with all strips, and place the standard markers on each side, aligning well on all sides. Place a second transparency plastic on top, ensuring excess fluid is drained out and strips are properly in place. Close the cassette.
- Go to Fuji visualising machine (IMBS), turn on the machine first and then the computer. Click on IRLAS icon on desktop, select tray position no-3. Click on focusing to adjust focal length of camera by putting 'focus on this' and then click on 'increment' from the same window which had focusing as an option. After that set the exposure (usually 30 sec) and click on start. Now patiently wait for results (bands) and don't panic.

Buffers and Reagents

SDS-PAGE

(Criterion 1-well precast 12.5% Tris-HCl gel cat# 345-0017)

Sample loading buffer

Sigma 127K6052 Laemmli Sample buffer 2x concentrate

SDS-PAGE Loading buffer

(if making own loading buffer)

0.5M Tris-HCl pH 6.8.....1.0 mL

glycerol .....0.8 mL

10% SDS.....1.6 mL

β-mercaptoethanol .....0.4 mL

1% bromophenol blue.....0.4 mL

dH_20 .....3.8 mL

0.5M Tris HCl

Tris (Trizma base).....60.55 g

dH2O.....800 mL

Adjust pH with 6N HCl (add about 37 ml, add last 1 mL very slowly, checking pH). Make up to 1 L.

Protein Standard (marker)

BioRad 161-0374 Precision Plus Dual Colour Protein Standards (no boiling necessary)

Ponceau S stain

Ponceau S .....1 g

Acetic acid .....50 mL

dH_20 .....to 1 L

SDS-PAGE running buffer

(BioRad 10x Tris/Glycine/SDS buffer; 5L Cat# 161-0772)

10x buffer.....100 mL

dH_20 .....to 1L

Western transfer buffer

(BioRad 10x Tris/Glycine buffer; 5L Cat# 161-0771)

10x transfer buffer.....100 mL

Methanol .....200 mL

dH_20 .....to 1L

Store @ 4ºC overnight

5% Blotto in PBST

Pam's skim milk powder .....2.5 g

Add into 50 mL PBST (make 15 g in 300 mL)

Secondary antibody

Rabbit anti-deer antibody conjugated to horseradish peroxidase (KPL, Maryland, USA), diluted 1:9000 in 5% blotting solution with 1% PBS-T.

Chemiluminescence reagent

ECL solution (AmershamTM ECL SelectTM Western Blotting Detection Reagent, GE Healthcare UK Limited, Buckinghamshire, UK).

Extra: If doing SDS-PAGE

SDS-PAGE stain

Coomassie Blue 0.4g

40% methanol .....200 mL (80 mL methanol + 120 mL dH_20 + 0.4g Coomassie blue)

20% Acetic acid .....200 mL (40 mL acetic acid + 160 mL dH_20)

Add both into 500 mL bottle

Destain

Methanol .....300 mL

Acetic acid .....100 mL

dH_20 to 1 L

PBS 1x

137 mM NaCl (MW 58.44)

2.7 mM KCl (MW 74.55)

10.32 mM Na_2HPO_4 (MW 141.95)

1.47 mM KH_2PO_4 (MW 136.08)

pH 7.4

PBST

1X Phosphate Buffered Saline Tween-20 (PBST) Recipe

1. Dissolve the following in 800 ml of distilled H_2O (dH_2O)
    - 8g of NaCl
    - 0.2g of KCl
    - 1.44g of Na_2HPO_4
    - 0.24g of KH_2PO_4
    - 2ml of tween-20
2. Adjust pH to 7.2
3. Adjust volume to 1L with additional distilled H_2O
4. Sterilize by autoclaving

PBS 20x Recipe

20X Phosphate Buffered Saline (20X PBS)

NaCl 160 gms

KCl 4 gms

KH_2PO4 4 gms

Na_2HPO4 23 gms

To weigh each of these and add into a 1 litre bottle and then add 1 litre of dH_2O. Add a magnet ball on the stirrer and let it to dissolve

1X Phosphate Buffered Saline (1X PBS)
Add 100mls of 20X PBS into a 2 litre bottle, and add dH_2O till full

pH should be around 7.6

0.05% Tween in PBS (PBST)
Add 0.25 ml of Tween to a 500ml bottle, add 1X PBS till full.

(0.05% X 500ml) = (100% X A); therefore, A = 0.23ml.

Tween is very viscous, to pipette slowly.

Appendix 3: *Toxoplasma gondii* (FOOD) nested PCR

Toxoplasma (FOOD) Nested PCR

Nucleic acid extraction	Kit
DNA	Qiagen Blood and tissue DNA extraction kit.

Primers	Name	Sequence (5'-3')	Size	Position	Target[1]
Forward	Food1	GGAACATCCGCTGAAGCTCATGG	491bp	1169	*Pppk-dhps* gene
Reverse	Food2	CAGAGAATCCAGTTGTTTCGAGG		1660	
Forward	Food3	CAGTCCAGACTCGTTCACCGATC	400bp	1201	
Reverse	Food4	CCGGAATAGTGATATACTTGTAG		1616	

PCR kit: Invitrogen Platinum Taq Polymerase

Reagent mix	First Round Volume (25µL)
Sterile distilled water	17.55ul
10x PCR buffer	2.5ul
MgCl2 (50mM)	0.75ul
dNTPs	0.5ul
10 uM Food1 primer	0.5ul
10uM Food2 primer	0.5ul
Taq	0.2ul
DNA	2.5ul from extracted DNA

Reagent mix	Second Round Volume (50µL)
Sterile distilled water	38.3
10x PCR buffer	5.0ul
MgCl2 (50mM)	1.5ul
dNTPs (10mM)	1.0ul
10 uM Food3 primer	1.0ul
10 µM Food4 primer	1.0ul
Taq	0.2ul
DNA	2ul from first round PCR

PCR controls	Description
Positive	Toxoplasma DNA extracted from Toxovax
Negative	Nuclease free water

L.Howe 13/12/2010

PCR Program Name: Toxo food

Cycling parameters: ~~RT-PCR 2nd Round~~ 1st	Temp (°C)	Time	No. cycles
Hold	94	5 min	1
Denature	94	30 sec	40
Anneal	57	30 sec	
Extension	72	1 min	
Hold	72 4	10 min ∞	1
Cycling parameters: ~~G-typing~~ 2nd	**Temp (°C)**	**Time**	**No. cycles**
Hold	94	5 min	1
Denature	94	30 sec	40
Anneal	57	30 sec	
Extension	72	30 sec	
Hold	72 4	10 min ∞	1

Electrophoresis	Description	Size of amplicon(s) (bp)
Agarose gel	1.5%	400bp
MW marker	100 bp	

References

1. Aspinall et al (2002) Prevalence of Toxoplasma gondii in commercial meat products as monitored by polymerase chain reaction – food for thought? Int. J. Parasitology 32:1193-1199.

LHowe 13/12/2010

Appendix 4: *Neospora* spp. real-time PCR

Neospora Real-Time PCR

Nucleic acid extraction	Kit
DNA	Qiagen DNeasy Blood and Tissue kit.

Primers	Name	Sequence (5'-3')	Size	Position	Target
Forward	Neo F	GTGAGAGGTGGGATACG	170bp		NC5 gene
Reverse	Neo R	GTCCGCTTGCTCCCTA			

PCR kit: Invitrogen Platinum Taq Polymerase + Syto 9 (green fluorescent nucleic acid stain, 5mM solution, Invitrogen cat# S34854)

Reagent mix	Volume (25µL)
Sterile distilled water	15.3ul
10x PCR buffer	2.5ul
MgCl2	0.75ul
dNTPs	1ul
10 µM Neo F primer	1ul
10 µM Neo R primer	1.5ul
Syto 9 (1:100 dilution of stock)	0.75ul
Taq	0.2ul
DNA	2ul

PCR controls	Description
Positive	*Neospora* NZNC1 from cell culture with know concentration. Include: 1:1, 1:10, 1:100, 1:1000, 1:10,000 dilutions in at least duplicate for standard curve generation
Negative	Nuclease free water

PCR program name: "Laryssa Neo HRM" on Corbett Rotor-Gene 6000 (little red one in Hopkirk lab)

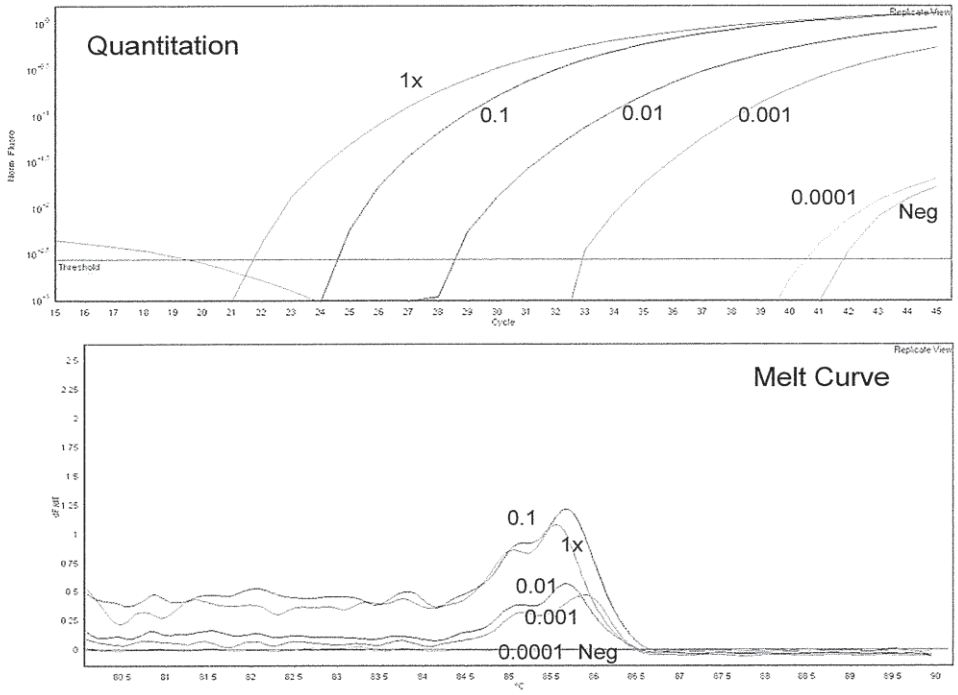
Cycling parameters:	Temp (°C)	Time	No. cycles
Hold	95	10 min	1
Denature	95	10 sec	45
Anneal	65	10 sec	
Extension	72	20 sec	
High Resolution Melt (HRM)	80-90 Over 0.2deg increments	Wait for 90 seconds pre-melt conditioning on first step Wait 5 sec after each temp change	1

References

LH 23/11/2010

Results:

Neospora NCNZ1 DNA from Culture: 3.6ng/ml



Interpretation:

Positive: 1x, 0.1, 0.01, 0.001 due to clear amplification and clear melt curve
Negative: 0.0001, negative due to no melt curve.

Assay Limit: 0.004ng/ml or $4x10^{-5}$ ng/ul Neo DNA

LH 23/11/2010

Appendix 5: Deer herpes consensus PCR

Deer Herpes - Consensus PCR

Nucleic acid extraction	Kit
DNA	Qiagen DNeasy Blood and Tissue kit.

Primers	Name	Sequence (5'-3')	Size	Position	Target
Forward	DFA	5'-GAYTTYGCNAGYYTNTAYCC-3'	~700	2149	DNA polymerase gene
Forward	ILK	5'TCCTGGACAAGCAGCARNYSGCNMTNAA-3'	~450	2405	
Reverse	KG1	5'GTCTTGCTCACCAGNTCNACNCCYTT-3'		2857	
Forward	TGV	5'TGTAACTCGGTGTAYGGNTTYACNGGNGT-3'	~200	2440	
Reverse	IYG	5'-CACAGAGTCCGTRTCNCCRTADAT-3'		2647	

PCR kit:

Reagent mix	First Round Volume (50µL)
Sterile distilled water	20.55ul
10x PCR buffer	5.0ul
MgCl2 (50mM)	1.5ul
Q solution	10ul
DMSO	3.75ul
dNTPs (10mM)	1.0ul
10 µM DFA primer	1.0ul
10 µM ILK primer	1.0ul
10 um KG1 primer	1.0ul
Taq	0.2ul
DNA	5ul

Reagent mix	Second Round Volume (50µL)
Sterile distilled water	21.55ul
10x PCR buffer	5.0ul
MgCl2 (50mM)	1.5ul
Q solution	10ul
DMSO	3.75ul
dNTPs (10mM)	1.0ul
10 µM TGV primer	1.0ul
10 µM IYG primer	1.0ul
Taq	0.2ul
DNA	5ul from First Round

PCR controls	Description
Positive	*Herpes (Deer)* cell culture stocks in -80
Negative	Nuclease free water

PCR Program Name:

Cycling parameters: First Round	Temp (ºC)	Time	No. cycles
Hold	94	5 min	1
Denature	94	30 sec	45 (1st round and 2nd round)
Anneal	46	60 sec	
Extension	72	60 sec	
Hold	72	7 min	1
	4	∞	

Electrophoresis	Description	Size of amplicon(s) (bp)
Agarose gel	1.5%	200-700 depending on PCR round and primer sets.
MW marker	100 bp	

References

1. Johne, R., et al. (2002) Herpesviral, but no papovaviral sequences, are detected in cloacal papillomas of parrots. Arch. Of Virology. 147:1869-1880

2. VanDevanter, D.R., et al (1996) Detection and analysis of diverse herpesviral species by consensus primer PCR. Journal of Clinical Microbiology 34:1666-1671

Appendix 6: Questionnaire 1 (autumn risk factors)

in conjunction with

The Deer Reproductive Efficiency Group New Zealand

Fetal wastage in farmed deer

Questionnaire -1

- *National Incidence rate and prevalence*
- *Investigation of infectious and other causes*
- *Research into solutions*

PhD candidate: Kandarp Patel BVSc & Ah, PGDip VPH

Supervisors: Prof Peter Wilson (Massey University)
Dr Geoff Asher (AgResearch Ltd)
Dr Laryssa Howe (Massey University)
A/Prof Cord Heuer (Massey University)

Dear Deer Farmer,
Thanks for being involved in the 'Fetal Wastage Study'. Your effort is well appreciated. Please complete and return this questionnaire - your information will help understanding of factors affecting reproduction in farmed deer population in New Zealand.

SAMPLING:

Please read and sign the following statement.

I, _____ of, _____ _____agree for the serum from blood samples taken on my property to be included in the Massey University Deer Serum Bank. I agree for the samples to be used at the discretion of the Massey University researchers to increase knowledge of deer diseases as appropriate in the future. I understand that every effort will be made to inform me of the results of any tests completed on these samples if they have relevance to my farming operation. I also understand that any published results will not identify my property or herd.

Owner/Manager:

(Signature) _____

(Print name) _____

Date: _____

OR □ (tick) I do **NOT** agree for the serum samples collected from my animals on the date outlined above to be included in the Massey University deer serum bank

---

Confidentiality
All information will be held strictly confidential to the researchers. No information will be released that is in any way identifiable to individual farms, owners or personnel.

This survey contains 9 sections:

- **Sections1 to 4 and 9**: General information of your farm
- **Sections 5 to 8**: Specific information about deer, sheep, and cattle. For cattle and sheep, please fill those that apply to your farm.
- **Section 10**: Source of data

Contact details

Peter Wilson
Professor, Deer Health and Production
Institute of Veterinary, Animal and Biomedical Sciences
Massey University
Palmerston North
(06) 3569099 extn 7619, Email- P.R.Wilson@massey.ac.nz
http://ivabs.massey.ac.nz/centres/deer/default.asp

Kandarp Patel
PhD candidate,
Institute of Veterinary, Animal and Biomedical Sciences
Massey University
Palmerston North
(06) 3569099 extn 2854, Mob – 021 028 75151, Email- K.K.Patel@massey.ac.nz

Questionnaire

Section 1: Contact details

1.1 Property name: _____

1.2 Contact person: _____

Owner/manager (please circle)

1.3 Farm address: _____

_____

_____

1.4 Phone (home): _____

1.5 Phone (work): _____

1.6 Fax: _____

1.7 Mobile: _____

1.8 E-mail: _____

Section 2: Farm information

General farm information

2.1 How many years have you farmed deer on the present property? _____ year(s)

Please indicate whether area values are in acres or ha

2.2 What is the **total area** of your farm (including bush)? _____ acre/ha

2.3 What is the **effective area** of your farm? _____ acre/ha

2.4 What area of your farm has been **fenced for deer**? _____ acre/ha

2.5 What **number of paddocks** are deer fenced? _____

Section 3. Deer numbers

3.1 How many of each deer class will you **winter (from July 1) in 2012 ?**

Deer breeds	**Number of each deer class**			
	R2YO		**Adult Stags (2+ years)**	**Adult Hinds (2+ years)**
	Female	**Male**		
Red – NZ origin				
Red- European origin				
Wapiti type*				
Fallow*				
Elk*				

* Not to be scanned for this research

Section 4: Forage

4.1 Describe the percentage (%) of time your hinds grazed each pasture species from **1st January 2012 to first scanning in 2012**

Please tick those fed

Age	Forage						
	Ryegrass / White Clover	Red Clover	Native grass	Chicory	Plantain	Lucerne	Others
R2YO hinds							
MA hinds							

4.2 Provide information on **winter crops fed** to your hinds over the past three years (2009, 2010, 2011) and from January 2012 to first scanning in 2012.

Please tick those fed

Forage		R2YO				MA			
		2009	2010	2011	2012	2009	2010	2011	2012
Brassicas	Chaumolier								
	Kale								
	Rape								
Tumips									
Swedes									
Sugar beet									
Other _____									

4.3 Describe any unusual environmental conditions experienced by your deer herd from **1st January 2012 to first scanning in 2012** (eg: drought, flood, other event) that you think may have affected reproduction.

……
……
……
……
……
……
……
……
……
……

OR: □ (tick) No unusual conditions observed from **1st January 2012 to first scanning in 2012**

4.4 Have you fed any supplement to breeding hinds from **1st January 2012 to first scanning in 2012** ?

Yes / No (please circle)

If YES, *please tick those fed*

Supplement type	**Hind age group**	
	R2YO	**MA**
Meadow hay		
Peavine hay		
Clover hay		
Lucerne hay		
Bailage		
Silage		
Maize		
Barley		
Other		

4.5 Pasture grazing management

Have different livestock species **shared pasture**, either **co-grazed** (same paddock, same time) **or alternate grazed (**same paddock, different time) with pregnant hinds from **1st January 2012 to first scanning in 2012 ?**

Yes / No (please circle)

If YES, please tick relevant

Age group	**Grazing pattern**	**Sheep**		**Beef cattle**		**Dairy cattle**	
		(✓)	**No of weeks**	**(✓)**	**No of weeks**	**(✓)**	**No of weeks**
R2YO	Co-grazed*						
	Alternate grazed*						
MA	Co-grazed						
	Alternate grazed						

* Co-grazed = same paddock, same time; alternate grazed = same paddock grazed in succession

Section 5: Deer

5.1 Previous reproduction history**:** please enter if accurate numbers are available

Year	**Hinds**	**No. Mated**	**No. Scanned pregnant**	**No. hinds aborting**	**No stocked at calving**	**No. of progeny at Weaning (or Feb/March tally if weaned later)**
2009-2010	R2YO					
	MA					
2010-2011	R2YO					
	MA					
2011-2012	R2YO					
	MA					

5.2 2012 mating: Please provide details of mating this year **(2012)**

Stock class	No. Mated	Scanning		Stag dates	
		No. scanned pregnant	Scanning date	In	Out
R2YO					
MA					

5.3 Have you had **confirmed abortions** over the past three years (2009, 2010, 2011).
Yes / No (please circle)
If YES, please provide details

Month & year	Stock class (R2YO or MA)	No aborted	Diagnosis (disease)	Abortion diagnosis confirmed by veterinarian or Lab. test ? (tick (✓) as many apply)	
				Lab	
				Blood	Tissue
Eg June 2010	R2YO	3	Leptospirosis	✓	

5.4 Have you ever **fed partially mouldy hay/silage/bailage/grain** to pregnant hinds over the past three years (2009, 2010, 2011) and from January 2012 to first scanning in 2012?
Yes / No (please circle)
If yes, please tick (✓) those fed and mention month

Mouldy feed	R2YO				MA			
	2009	2010	2011	2012	2009	2010	2011	2012
Mouldy hay								
Mouldy silage								
Mouldy bailage								
Mouldy grain								
Other _____								

5.5 Have you ever received **<u>any notification from a DSP regarding any diseases</u>** in hinds sent to slaughter?

Yes / No (please circle)

If YES, tick (✓) as appropriate

Disease	R2YO		MA	
	(✓) for yes	**Year**	**(✓) for yes**	**Year**
Toxoplasmosis				
Neospora				
Leptospirosis				
Cervid Herpesvirus - 1				
Bovine Viral Diarrhoea (BVD)				
Johne's disease				
Others _____				

5.6 Have any of your deer been diagnosed **on-farm** with **toxoplasmosis, neosporosis, listeriosis, leptospirosis, cervid herpes virus-1 infection, bovine viral diarrhoea (BVD), or Johne's Disease** by your veterinarian and/or through laboratory testing, over the past **three years**?

Yes / No

(Please circle)

If YES, please list in the table below

Month and year (2009-12 only)	Age group (R2YO/MA)	No. of cases	Disease / observations	Mortality (if any)
June 2009	**R2YO**	**4**	**Neosporosis**	**3 deaths**

5.7 Have any of your **breeding hinds been vaccinated** for **any disease** at any time in the past three years (2009, 2010, 2011) and current year?

If "YES", then please briefly describe vaccination regime in the table provided below

Vaccination information**- 2009-2011 only**

Year	Name of vaccine	Deer class					
		R2YO			MA		
		First	First booster	Annual booster	First	First booster	Annual booster
2009							
2010							
2011							

Vaccination information – **2012**

Name of vaccine	Deer class					
	R2YO			MA		
	First	First booster	Annual booster	First	First booster	Annual booster
Eg 5 in 1	Jan 2012					

5.8 Parasites: Did you give worm treatment to breeding hinds any time between **1st Jan 2012 to scanning** this year? **Yes / No (please circle)**

If "YES", please provide information about **drench regime**

Drench name	**Deer class**	
	R2YO	**MA**
	Month	**Month**

5.9 Were any deer given **trace mineral** supplementation from **1st January 2012 to first scanning?** **Yes / No (please circle)**

If **Yes** then please provide information about the **mineral supplementation**

Trace mineral supplementation	**Age group**	
	R2YO	**MA**
	Month	**Month**
Copper		
Selenium		
Cobalt		
Iodine		
Others (Please mention) _____		

5.10 Were supplements applied with fertiliser from 2009 – the present. **Yes / No (please circle)**

If **YES,** which minerals (tick): Copper □ Selenium □ Cobalt □

Other: (Please state). ……..

5.11 Have you any trace element monitoring data from your deer (liver and/or blood) from 2009 – the present **Yes / No (please**

circle)

If **yes**, please attach a copy of results to this questionnaire, or if you have them stored electronically, alternatively send them electronically by attachment to:

K.K.Patel@massey.ac.nz

5.12 Have you had any veterinary diagnosis of any disease in R2YO or MA hinds from 1st January 2012 to first scanning in 2012 ? **Yes / No**

(please circle)

If YES, please give details

……

……

……

……

……...

5.13 Do you have a weigh scale on your farm? **Yes / No**
If yes, have you weighed yearling and/or adult hinds since January 1 2012? **Yes / No**

If yes, please attach a copy of weights to this questionnaire, or if you have them stored electronically, alternatively send them electronically by attachment to:
K.K.Patel@massey.ac.nz

Section 6: Sheep (If you do not have sheep, go to section 7)

6.1 How many breeding ewes have you wintered for current year on your farm?

Sheep class	**Number**
Hoggets	
Adult ewes	

6.2 Have any of your breeding ewes **vaccinated** for *Toxoplasma gondii, Campylobacter spp.*, or *Leptospira* spp. at any time in the current year? **Yes / No (please circle)**

If "YES", then please give details

Vaccination against	**Age group**			
	Hoggets		**Adult ewes**	
	(✓)	**Month**	**(✓)**	**Month**
Toxoplasma gondii				
Campylobacter spp.				
Leptospira spp.				

6.3 Have you observed or suspected any ewe abortion over the past three years (2009, 2010, 2011)?

Yes / No (please circle)

If YES, then please fill in the information below

Ewe stock class (Hogget, Adult ewes)	**No aborted**	**Year**	**Diagnosis (disease)**	**Abortion diagnosis confirmed (tick (✓) as applicable)**	
				Lab	
				Blood	**Tissue**
Eg- MA	**7**	**2009**	**Toxoplasmosis**		✓

Section 7: Beef cattle (If you do not have beef cattle go to section 8)

7.1 How many breeding cows have you wintered for current year on your farm?

Cow class	Number
Heifers	
Mixed-aged (MA) cows	

7.2 Have any of your breeding cows **vaccinated** for BVD or *Leptospira* spp.at any time in the current year?

Yes / No

(please circle)
If "YES", then please give details

Vaccination against	Age group			
	Heifers		MA cows	
	(✓)	Month	(✓)	Month
Leptospira spp.				
BVD				

7.3 Have you observed or suspected any heifer/cow aborting over the past three years (2009, 2010, 2011)?

Yes / No (please circle)

If **yes**, please fill in the information below

Stock class (heifers or MA cows)	No aborted	Year	Diagnosis (disease)	Abortion diagnosis confirmed by veterinarian or Lab. test ? (tick (✓) as appropriate)	
				Lab	
				Blood	Tissue
Eg - heifers	**3**	**2009**	**Unknown**	✓	

Section 8: Dairy cattle including heifer rearing/grazing (If you do not have dairy cattle, go to section 9)

8.1 How many breeding dairy cattle cows have you wintered for current year on your farm?

Cow class	**Number**
Heifers	
Mixed-aged (MA) cows	

8.2 Have any of your breeding cows **vaccinated** for BVD or leptospirosis at any time in the current year?

Yes / No

If "YES", then please give details

Vaccination against	**Age group**			
	Heifers		**MA cows**	
	(✓)	**Month**	**(✓)**	**Month**
Leptospira spp.				
BVD				

8.3 Have you observed or suspected any heifer/cow aborting over the past three years (2009, 2010, 2011)?

Yes / No

If **yes**, please fill in the information below

Stock class (heifers or MA cows)	**No aborted**	**Year**	**Diagnosis (disease)**	**Abortion diagnosis confirmed by veterinarian or Lab. test ? (tick (✓) as many apply)**	
				Lab	
				Blood	**Tissue**
Eg - heifers	**3**	**2009**	**Neosporosis**	✓	

Section 9: Environment

9.1 Indicate the source(s) of water to your yearling and MA hinds from **January 2012 to first scanning in 2012**
Please tick all that apply

Water source	R2YO	MA
Troughs		
Dams		
Wallows/surface water		
Natural springs		
Stream		
River		
Irrigation facilities		

Other: _____

9.2 If troughs are used, how often are troughs cleaned?

- ☐ Once a month
- ☐ Once every 6 months
- ☐ Annually
- ☐ Once every two years
- ☐ Never

9.3 How often do you see water accumulation on flat part of deer paddocks?

- ☐ Once a week
- ☐ Fortnightly
- ☐ Once a month
- ☐ Once every 6 months
- ☐ Never

9.4 Do you spread animal faeces on your deer paddocks?

- ☐ Yes (from which species: _____)
- ☐ No

9.5 Please indicate the abundance of wildlife species on or immediately surrounding your deer fenced area

	Abundant:	**Seen occasionally:**	**Seen rarely:**	**Never seen:**
Feral cats	☐	☐	☐	☐
Wild deer	☐	☐	☐	☐
Possums	☐	☐	☐	☐
Rabbits	☐	☐	☐	☐
Hedgehogs	☐	☐	☐	☐

Please mention presence or otherwise of domestic cats and dogs on your farm

Pets	Number
Domestic cats	
Farm dogs	

Section 10: Source of information

The information provided in this questionnaire was based on (tick one option only):

- ☐ Written records of farm data
- ☐ Memory
- ☐ Mostly memory + a few recorded data
- ☐ Mostly recorded data + memory

Section 11: Personal comment

Please give any personal comment or describe any details of event/s which you think may have been important in your personal experience, in affecting reproductive outcomes in your deer herd. Comments about this questionnaire are also welcome:

Thank you. Your participation is much appreciated.

PLEASE NOTE: Data from the entire reproductive cycle is ultimately needed for this study. While this questionnaire has provided background to the first scan and sampling period, a second and third (much reduced) questionnaire will follow to cover the period between first and second scanning, and then between second scanning and February/early March (effectively weaning data).

.

Please return the completed questionnaire in the enclosed postage-paid envelope to the researchers, Massey University.

Appendix 7: Questionnaire 2 (winter risk factors)

in conjunction with

The Deer Reproductive Efficiency Group New Zealand

Fetal Wastage in Farmed Deer

Re-scan Questionnaire

- *National Incidence rate and prevalence*
- *Investigation of infectious and other causes*
- *Research into solutions*

PhD candidate: Kandarp Patel BVSc & Ah, PGDip VPH

Supervisors: Prof Peter Wilson (Massey University)
Dr Geoff Asher (AgResearch Ltd)
Dr Laryssa Howe (Massey University)
A/Prof Cord Heuer (Massey University)

Dear Deer Farmer,

Thanks for completing and returning the first questionnaire for the 'Fetal Wastage Study'. Your effort is well appreciated. While the previous questionnaire gave us information about your herd's previous reproductive performance and mating and management to first scanning, this questionnaire is focused on the management between first and second scan. A third questionnaire will be sent early next year to cover from second scanning to weaning. These questionnaires provide data that may highlight management and farm factors that contribute to fetal loss, and are essential to understanding reproductive wastage in total.

Please complete and return this questionnaire - your information will help understanding of factors responsible for pregnancy loss (if any) on your farm.

Confidentiality

All information will be held strictly confidential to the researchers. No information will be released that is in any way identifiable to individual farms, owners or personnel.

This survey contains 6 sections:

- General information
- Change in deer numbers
- Pasture management
- Abortion and diseases
- Environmental effects
- Source of data

Contact details

Peter Wilson
Professor, Deer Health and Production
Institute of Veterinary, Animal and Biomedical Sciences
Massey University
Palmerston North
(06) 3569099 extn 7619, Email- P.R.Wilson@massey.ac.nz
http://ivabs.massey.ac.nz/centres/deer/default.asp

Kandarp Patel
PhD candidate,
Institute of Veterinary, Animal and Biomedical Sciences
Massey University
Palmerston North
(06) 3569099 extn 2854, Mob – 021 028 75151, Email- K.K.Patel@massey.ac.nz

Questionnaire

Section 1: Contact details

1.1 Property name: _____

1.2 Contact person: _____

Owner/manager (please circle)

1.3 Preferred contact method (please circle): E-mail / telephone / mail

Section 2: Change in deer numbers

2.1 Have you had recent addition/changes in your hind herd **between first and second scan** in 2012?

Yes / No (please circle)

If YES, please give details

Stock Class	Number sold/culled		Number dead/lost		Number purchased	
	Wet	Dry	Wet	Dry	Wet	Dry
R2YO hinds						
MA hinds						

Section 3: Forage

3.1 Describe the percentage (%) of time your pregnant hinds grazed each pasture species from **first scan to re-scan in 2012**

Please tick those fed

Age	**Forage**						
	Ryegrass / White Clover	Red Clover	Native grass	Chicory	Plantain	Lucerne	Others (state)
R2YO hinds							
MA hinds							

3.2 If you fed winter crop to your pregnant hinds from **first scan to re-scan in 2012**, please provide on the dates that the **crops were fed**

Please enter date/s fed

Forage		**R2YO**	**MA**
		Date/s	Date/s
Brassicas	Chaumolier		
	Kale		
	Rape		
Tumips			
Swedes			
Sugar beet			
Other _____			

3.3 Have you fed any supplement to breeding hinds from **first scan to re-scan in 2012,** please provide on the dates that the **crops were fed** ?

Yes / No (please circle)

If YES*, please enter date/s fed*

Suplement type	**R2YO**	**MA**
Meadow hay		
Peavine hay		
Clover hay		
Lucerne hay		
Bailage		
Silage		
Maize		
Barley		
Other		

3.4 Have you **fed mouldy hay/silage/bailage/grain** to pregnant hinds from **first scan to re-scan in 2012 ?**

Yes / No (please circle)

If yes, *please enter date/s fed*

Mouldy feed	**R2YO**	**MA**
Mouldy hay		
Mouldy silage		
Mouldy bailage		
Mouldy grain		
Other_____		

3.5 Pasture grazing management

Have different livestock species e.g. shee/cattle, **shared pasture**, either **co-grazed** (same paddock, same time) **or alternately grazed (**same paddock, different time) with pregnant hinds from **first scan to re-scan in 2012?**

Yes / No (please circle)

If YES, please tick (✓**)** relevant

Age group	**Grazing pattern**	**Sheep**		**Beef cattle**		**Dairy cattle**	
		(✓)	**No of weeks**	**(✓)**	**No of weeks**	**(✓)**	**No of weeks**
R2YO	Co-grazed*						
	Alternatively grazed*						
MA	Co-grazed						
	Alternatively grazed						

* Co-grazed = same paddock, same time; alternately grazed = same paddock grazed in succession

Section 4: Abortion and diseases

4.1 2012 re-scan summary: Please provide **2012** re-scan summary,

Stock class	Scanning date	No. scanned	No. pregnant	No. aborting
R2YO				
MA				

4.2 Have you observed or suspected any hind's (other than those re-scanned) abortion from **first scan to re-scan in 2012?**

Yes / No

(please circle)
If YES, then please fill in the information below

Hind class (R2YO or MA)	No	Abortion diagnosis (if any)	Confirmed by	
			Blood	**Tissue**
Eg- R2YO	**2**	**Leptospirosis**	✓	✓

4.3 Did you find any dead fetus lying in the paddock/farm before re-scan?

Yes / No (please circle)

If **YES**, please give details

Hind class (R2YO or MA)	No	Abortion Diagnosis (if any)	Confirmed by	
			Blood	**Tissue**
Eg. R2YO	**1**	**Leptospirosis**	✓	✓

4.4 Have you received **any notification from a DSP regarding any diseases** for hinds scanned dry (or pregnant hinds sent for culling) sent to slaughter after first scan?

Yes / No (please circle)

If YES, tick (✓) as appropriate

Disease	R2YO		MA	
	(✓) for yes	**Month**	**(✓) for yes**	**Month**
Toxoplasmosis				
Neospora				
Leptospirosis				
Cervid Herpesvirus - 1				
Bovine Viral Diarrhoea (BVD)				
Johne's disease				
Others _____				

4.5 Have any of your deer been diagnosed **on-farm** with **toxoplasmosis, neosporosis, listeriosis, leptospirosis, cervid herpes virus-1 infection, bovine viral diarrhoea (BVD), or Johne's Disease** by your veterinarian and/or through laboratory testing from **first scan to re-scan in 2012 ?**

Yes / No

(Please circle)

If YES, please list in the table below

Month	Age group (R2YO/MA)	No. of cases	Disease / observations	Mortality (if any)
June 2012	**R2YO**	**4**	**Johne's disease**	**3 deaths**

Section 5: Environment

5.1 Have you noticed any abnormal activity (e.g. trespassing of wild deer, possum or feral cats) through the deer paddocks or surrounding the deer farm **from first scan to re-scan in 2012**?

If YES, please give details

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5.2 Describe any unusual environmental conditions experienced by your deer herd from **first scan to re-scan in 2012** (eg: drought, flood, other event) that you think may have affected reproduction.

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OR: □ (tick) No unusual conditions observed from **first scan to re-scan in 2012**

SECTION 6: The information provided in this questionnaire was based on (tick one option only):

- ☐ Written records of farm data
- ☐ Memory
- ☐ Mostly memory + a few recorded data
- ☐ Mostly recorded data + memory

SECTION 7: Personal comment:

Please give any personal comment or describe any details of event/s which you think may have been important in your personal experience, in affecting reproductive outcomes in your deer herd. Comments about this questionnaire are also welcome:

Thank you. Your participation is much appreciated.

Please return the completed questionnaire in the enclosed postage-paid envelope to the researchers, Massey University