Epidemiological investigation into abortion in farmed red deer in New Zealand

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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.

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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

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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. *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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Chapter 2

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.