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

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ABSTRACT

This study tested for association between bovine viral diarrhoea virus (BVDv) and cervid herpesvirus type-1 (CvHV-1) exposure 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 New Zealand were pregnancy tested using ultrasound early in gestation (Scan-1) and 55-89 days later (Scan-2) to detect mid-term abortion. Sera from aborted and non-aborted hinds at Scan-2 were tested for BVDv and CvHV-1 using virus neutralisation tests. Available uteri from aborted hinds and from hinds not rearing a calf to weaning were tested by PCR for herpesvirus DNA.

In herds with aborted hinds, 10.3% of 639 R2 and 17.2% of 302 MA hinds were sero-positive for BVDv and 18.6% of 613 R2 and 68.5% of 232 MA hinds were sero-positive for CvHV-1. There was no association between BVDv sero-status and abortion at animal level (R2 p = 0.36, MA p = 0.76) whereas CvHV-1 sero-positivity was negatively associated with abortion in MA hinds (p = 0.01) but not in R2 hinds (p = 0.36), MA). Eleven of 108 uteri from aborted R2 hinds but no MA 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.

Findings suggest that BVDv and CvHV-1 may not be associated with abortion in R2 hinds, but association needs to be tested further in MA hinds. The role of CRhV-2 requires clarification.

1. 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 11 years (Statistics New Zealand, 2015). Inefficiency arises from failure to conceive, fetal loss, stillbirth, and postnatal mortality (Asher, 2003; Asher and Pearse, 2002; Asher and Wilson, 2011).

While some causes and risk factors for conception failure in both R2 and MA hinds have been studied previously (Asher et al., 2005; Asher et al., 2011; Audigé et al., 1999b; Audigé et al., 1999c), bovine viral diarrhoea virus (BVDv) and cervid herpesvirus type-1 (CvHV-1) were not included in those studies. Pregnancy loss due to abortion in New Zealand deer has been reported in a few limited studies (Audigé et al., 1999a; Campbell et al., 2000; Fennessy et al., 1986; Wilson et al., 2012). However, more substantial data are reported by Patel et al. (2018) describing mid-term herd-level mean daily abortion rates of 0.043% and 0.025% in R2 and MA herds, respectively, equating 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 production and lower financial returns.

BVDv 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 BVDv infection in dairy cattle include early embryonic death, fetal resorption, mummification, conception failure, abortions and congenital malformations (Fray et al.,

Abbreviations: BVDv, Bovine viral diarrhoea virus; CvHV-1, Cervid herpesvirus type-1; CRhV-2, Cervid Rhadinovirus type-2; DAR, Daily abortion rate; DSP, Deer slaughter premise; MA, Mixed-age, two years and older; PCR, Polymerase chain reaction; PI, Persistently infected; R2, Rising-two-year-old; VNT, Virus neutralisation test

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2000; Lindberg et al., 2001; McGowan et al., 1993). Exposure to BVDv has been reported in deer populations worldwide. The infection pattern for BVDv in white-tailed deer (*Odocoileus virginianus*) was reported to be similar to that in cows under experimental conditions in USA wherein abortions, stillbirth and birth of persistently infected (PI) fawns were observed (Ridpath et al., 2008). Congenital transmission of BVDv and resultant PI fawns in white-tailed deer have been documented in the USA (Passler et al., 2010; Passler et al., 2009). Losses due to BVDv infection and subsequent fetal infections have also been reported in sika deer (*Cervus nippon*) in China where the BVDv sero-prevelence ranged from 60-87% (Gao et al., 2011; Rui et al., 2000). In New Zealand, a BVDv sero-prevalence of 9.5% in 400 samples randomly selected from a serum bank established by collecting 1,150 samples from 575 red deer herds has been reported (Motha et al., 2000). Exposure to BVDv has also been reported in red deer populations in Spain and Denmark (Fernandez-Aguilar et al., 2016; Nielsen et al., 2000; Rodriguez-Prieto et al., 2016). Given the high prevalence of BVDv in the New Zealand cattle population (Fray et al., 2000; Thobokwe and Heuer, 2004) and its potential transmission to deer, pregnant deer may also be at risk of abortion caused by BVDv.

Cervid herpesvirus-1 (CvHV-1), belonging to the Alpha herpesvirinae sub-family, has been reported in both farmed and wild deer populations (Frolich, 1996; Frolich et al., 2006; Nettleton et al., 1986; Pospisil et al., 1996; Thiry et al., 1988). 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 conjunctiva of one hind with a history of keratoconjunctivitis which later developed vaginal lesions including mucosal erosions and petechial haemorrhages, typical of an alpha herpesvirus infection, following immunosuppression using dexamethasone (Squires et al., 2012). A sero-survey in New Zealand showed that 38% of 314 farmed deer were positive for CvHV-1 (Motha and Jenner, 2001). 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 using samples from farms and animals from a large study of abortion in farmed deer in New Zealand described by Patel et al. (2018), to test for association between CvHV-1 and BVDv seropositivity and mid-term abortion as determined by repeat ultrasound pregnancy scanning in red deer hinds. This study also provided further evidence about the occurrence of CvHV-1, cervid rhadinovirus type-2, and BVDv in New Zealand farmed deer.

2. Materials and methods

2.1. Study design

The sampling frame for farms and animals used for this analysis is described in detail in the parent study by Patel et al. (2018). 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 in the first trimester of 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. All animal manipulations were approved by the Massey University Animal Ethics Committee (Protocol number: 12/34).

Hinds were classified as being 'pregnant' based on ultrasound scan observation of at least one of fetus or part thereof, amniotic membrane, and/or presence of placentomes, or as being 'non-pregnant' based on absence of these 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), 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 ((number aborted at Scan-2 / number scanned at Scan-2) / number of days between Scan-1 and Scan-2) was calculated to categorise herds 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 for stratification of sample selection. This approximated a mid-term abortion rate.

2.2. Sample collection and handling

Blood samples were collected from up to 21 aborted hinds as available and 21 randomly selected non-aborted hinds per herd from 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 °C. In total, 2,932 blood samples were collected at Scan-2 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 senior author (KP) or the DSP veterinarian. Reproductive tracts from nine hinds that did not rear a calf at weaning were also collected at DSPs as available. Numbers are summarised in Table 1. Samples were sent chilled or frozen to Massey University post-mortem facility where they were processed. Gross observations from uteri and fetal tissues were recorded at dissection. Uteri, vaginal, placental, cotyledon, and fetal samples were stored at −20 °C for PCR.

2.3. Sample selection for serology

The numbers and range of sera tested per herd from aborted and non-aborted hinds for BVDv and CvHV-1 are presented in Table 2.

2.3.1. BVDv

Sera collected at Scan-2 from all aborted hinds from all herds were tested. Sera from non-aborted hinds were selected randomly from herds under no (R2 = 17 and MA = 18 herds), low (R2 = 26 and MA = 14 herds), medium (R2 = 15 and MA = 7 herds), and high (R2 = 14 and MA = 8 herds) DAR categories.

2.3.2. 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 and MA = 17 herds), low (R2 = 28 and MA = 16 herds), medium (R2 = 15 and MA = 7 herds), and high (R2 = 14 and MA = 8 herds) DAR categories.

2.4. Virus neutralisation assays (VNT) for BVDv and CvHV-1

A virus neutralising test (VNT) was carried out at the Animal Health Laboratory, Ministry of Primary Industries, Wallaceville, Wellington,

Table 1
Summary of number (number of herds) of maternal samples from aborted rising 2-year-old (R2) and mixed age (MA) hinds at second pregnancy scan (Scan-2) and from 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 (16)	na	4 (2)
	MA	Aborted	31 (12)	5 (4)	1 (1)
Weaning	MA	No live calf at weaning	9 (1)	na	na

na = no animals in this category.

Table 2
Summary of herds and sera (and range per herd) tested for bovine viral diarrhoea virus (BVDv) and cervid herpesvirus type-1 (CvHV-1) from aborted and non-aborted rising 2-year old (R2) and mixed age (MA) hinds at second pregnancy scan (Scan-2).

Virus	Age group	Herds with or without aborted hinds	No. herds	Total number tested (range per herd)		
				Aborted	Non-aborted	Total
BVDv	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 = no animals in this category.

using a method adapted from Kirkland and Mackintosh (1993) and the OIE (2016) for BVDv. Similarly, a VNT for CvHV-1 was carried out following methodology described by the OIE (2016). According to the laboratory's BVDv test manual criteria, a titre of ≤1:4 for BVDv indicates sero-negative or a PI animal, titres of between ≥1:8 and up to and including 1:64 indicate recent infection or colostral antibody in animals up to six months of age, or a PI animal, whereas a titre > 1:64 indicates exposure to BVDv virus. For analyses, sera with titres ≥1:8 for BVDv were designated 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 positive.

2.5. Herpesvirus consensus PCR and sequencing

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 herpesvirus consensus nested PCR was carried out based on method adapted from Johne et al. (2002) and VanDevanter et al. (1996) which amplified an approximately 200bp fragment of the DNA polymerase gene. Briefly, the first round PCR reaction mixture volume of 50 µL consisted of 1x PCR buffer, 1.5 mM $MgCl_2$, 10 µL of Q solution (Qiagen), 3.75 µL of DMSO (Sigma-Aldrich, Auckland, New Zealand), 0.2 mM dNTPs, 0.2 µM DFA forward primer (5'-GAYTTYGCNAGYYTNTAYCC-3'), 0.2 µM ILK forward primer (5'TCCTGGACAAGCAGCARNYSGCNMTNAA-3'), 0.2 µM KG1 reverse primer (5'GTCTTGCTCACCAGNTCNACNCCYTT-3'), 1 unit of Platinum Taq (Invitrogen), and 5 µL (~30 ng) of DNA sample. The second-round PCR reaction volume of 50 µL consisted of 1x PCR buffer, 1.5 mM $MgCl_2$, 10 µL of Q solution (Qiagen), 3.75 µL of DMSO (Sigma-Aldrich, Auckland, New Zealand), 0.2 mM dNTPs, 0.2 µM TGV forward primer (5'TGTAACTCGGTGTAYGGNTTYACNGGNGT-3'), 0.2 µM IYG reverse primer (5'-CACAGAGTCCGTRTCNCCRTADAT-3'), 1 unit of Platinum Taq (Invitrogen), and 5 µL 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 a previous herpesvirus case (CvHV-1) in red 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.

Positive amplification products 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.

2.6. Statistical analysis

Data 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 analyses, hinds aborting at Scan-2 were considered as aborted, and therefore included in the DAR calculation and analysis.

At the individual animal level, the dependent variable aborted status (aborted (1) /non-aborted (0)) at Scan-2 was a binary response and resembled a binomial distribution. A logistic regression model with 'herd' as a random effect to adjust for within-herd clustering was used to model association between aborted status at Scan-2 and sero-status (BVDv or CvHV-1) (negative (0) or positive (1)) at Scan-2. A similar logistic regression 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 BVDv or CvHV-1 serological status at Scan-2. A p-value < 0.05 was considered to be statistically significant.

3. Results

3.1. BVDv serology

Sero-prevalence was 10.3% (66/639) in R2 hinds and 17.2% (52/302) in MA hinds and 12.5% (118/941) overall (Table 3). Titres for BVDv ranged up to 1:192 in R2 hinds and to 1:620 in MA hinds. In herds with aborted hinds, the BVDv sero-status was not associated with aborted status in R2 (p = 0.36) or MA (p = 0.76) hinds. The BVDv sero-prevalence was similar between hinds from herds with and without aborted hinds in R2 (p = 0.55) and MA (p = 0.75) age groups. The BVDv 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

Table 3
Sero-prevalence (and number sero-positive) for bovine viral diarrhoea virus (BVDv) and cervid herpesvirus type-1 (CvHV-1) in aborted and non-aborted hinds at second pregnancy scan (Scan-2) in rising 2-year-old (R2) and mixed age (MA) herds.

Virus	Age group	Herds with or without aborted hinds	% (no.) sero-positive hinds		
			Aborted	Non-aborted	Overall
BVDv	R2	With	9.3 (24)	11.5 (30)	10.4 (54)
		Without	na	10.1 (12)	10.1 (12)
		Total	9.3 (24)	11.1 (42)	10.3 (66)
	MA	With	15.4 (12)	19.8 (22)	18.0 (34)
		Without	na	15.9 (18)	15.9 (18)
		Total	15.4 (78)	17.9 (40)	17.2 (52)
CvHV-1	R2	With	20.3 (54)	17.1 (42)	18.8 (96)
		Without	na	17.6 (18)	17.6 (18)
		Total	20.3 (54)	17.3 (60)	18.6 (114)
	MA	With	57.5 (46)	78.3 (47)	66.4 (93)
		Without	na	71.7 (66)	71.7 (66)
		Total	57.5 (46)	74.3 (113)	68.5 (159)

na = no animals in this category.

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).

3.2. CvHV-1 serology

The overall sero-prevalence for CvHV-1was 32.3% (273/845) whereas it was 18.6% (114/613) in R2 and 68.5% (159/232) in MA hinds (Table 3).

In herds with aborted hinds, the CvHV-1 sero-positivity was negatively associated with aborted status in MA (OR = 0.37, 95% CI: 0.17-0.81, p = 0.01) but not in R2 (p = 0.36) hinds.

The CvHV-1 sero-prevalence was similar between hinds from herds with and without aborted hinds in R2 (p = 0.61) and MA (p = 0.23) groups. 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).

3.3. Herpesvirus consensus PCR

Eleven (10.2%) of 108 R2 and none of 31 MA uteri from aborted hinds were positive for herpesvirus DNA on PCR. Placenta and vaginal samples were negative for herpesvirus DNA.

Seven of the 11 R2 PCR positive uteri were selected for sequencing based on the presence of a strong positive amplicon. The seven isolates had a 99% sequence homology to Elk gammaherpesvirus isolate Wo-1 (GenBank KY612412) and Type 2 ruminant rhadinovirus isolate C050 (GenBank KY462772). The herpesvirus DNA positive samples came from three R2 herds (1, 6, and 4 samples/herd). Paired PCR and serology data were available from the seven of 11 PCR positive R2 hinds. Four were sero-positive for CvHV-1 antibodies whereas the remaining three R2 PCR positive hinds were sero-negative.

4. Discussion

This is the first study to assess association between BVDv and CvHV-1 and abortion in farmed deer in New Zealand or elsewhere. There was no association between BVDv 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 both BVDv and CvHV-1 was lower in R2 than in MA hinds. This study also contributes to understanding of exposure to BVDv and CvHV-1, given limited study of these viruses in the NZ farmed deer population to date.

This study assessed association between sero-status and abortion at a single time point. The parent study (Patel et al., 2018) involved blood collection at both Scan-1 and Scan-2, but it was not logistically feasible to repeat sample the same animals at both scans. Hence it has not been possible to test for association between sero-conversion *per se* and abortion.

The statistical test for association suggests that BVDv is not a significant cause of abortion in New Zealand farmed red deer. However, the non-significance of BVDv sero-prevalence difference between R2 and MA hinds in herds with aborted hinds, as opposed to significantly lower sero-prevalence in herds without aborted hinds, could lead to the inference that BVDv exposure in R2 hinds in those herds might be associated with abortion. In sika deer, fetal loss and subsequent economic losses have been attributed to BVDv infection (Gao et al., 2011; Rui et al., 2000). The higher sero-prevalence in MA than R2 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 OIE standards, which detected specific BVDv 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 the 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. Another possibility could be that the MA hinds might have developed immunity from previous exposures and therefore the sero-positive hinds were less likely to abort. However, that possibility needs to be tested further given the lack of association between CvHV-1 sero-status and abortion in R2 hinds. Alternatively, this association may be due to the low number of sera tested in non-aborted hinds compared with the 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). The CvHV-1 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. This is the second report of ruminant Rhadinovirus type-2 (CRhV-2) from deer in New Zealand, providing confirmatory evidence for the report by 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 it 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 to transmit trans-placentally, but without adverse effects upon 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, and this virus should not be discounted in investigation of individual herd abortion outbreaks.

The sero-prevalence reported here for BVDv 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. Participating farms were possibly biased toward those with on-going reproduction problems, though Patel et al. (2018) discuss that there was limited evidence to support that selection resulted in biased. Nevertheless, the sero-prevalence of BVDv 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.

5. Conclusion

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

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