Pestivirus Apparent Prevalence in Sheep and Goats in Northern Ireland: A Serological Survey

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Background

Bovine viral diarrhoea virus (BVDV) and Border disease virus (BDV) can cause significant health problems in ruminants and economic impacts for farmers. The aim of this study was to evaluate pestivirus exposure in Northern Ireland in sheep and goat flocks, and to compare findings with a previous study from the region.

Methods

Up to 20 animals were sampled from 188 sheep and 9 goat flocks (n=3,418 animals; 3,372 sheep and 46 goats) for pestivirus antibodies. Differentiation of the causative agent in positive samples was inferred using serum neutralisation. Abortion samples from 177 ovine cases were tested by BVDV RT-PCR and antigen ELISA.

Results

Apparent animal and flock (one antibody positive animal within a flock) prevalence was 1.7% and 17.3%, respectively, a statistically significantly drop in apparent prevalence since a survey in 1999. 52.6% of samples testing positive had higher antibody titres to BVDV than to BDV. Of the ovine abortion samples, only one positive foetal fluid sample was detected by ELISA.

Conclusion

The present study found that, since 1999, there has been a decrease in apparent animal and flock prevalence of 3.7 and 12.8 percentage points respectively, suggesting pestivirus prevalence has decreased across Northern Ireland between 1999 and 2018.

Introduction

Three species within the family *Flaviviridae*, genus *Pestivirus*, have historically been of veterinary interest: Border disease virus (BDV), Bovine viral diarrhoea virus (BVDV) and Classical swine fever (CSF). Genetic sequencing has further classified BVDV into BVDV-1, BVDV-2 and BVDV-3 (1–3). In Northern Ireland only BVDV-1 was found to be the circulating within cattle (4). The economic costs can be substantial in countries where BVDV is endemic (5,6).

BVDV and BDV are not confined to a primary host as each is capable of infecting both sheep and cattle (7–9), as well as deer (10). Reproductive issues are the main effect of BDV in sheep including infertility, abortion, stillbirth, and birth of small and weak lambs (11,12). The virus can cross the placenta in pregnant ewes, and lambs that survive the infection may be born with the appearance of hairy "shaker" lambs. Although these lambs can occasionally appear normal (13), they are persistently infected (PI) with BDV and become lifelong shedders of virus (4). Failure to remove PI lambs early can cause outbreaks of BDV to occur in naïve flocks (14).

With the development and successful progression of eradication programmes for BVDV in cattle in multiple European countries, there is a need to evaluate possible spillover hosts and barriers to eradication. Currently, commercially available antibody tests for BDV and BVDV are unable to distinguish between the pestivirus strains, therefore a positive result can only determine exposure to a pestivirus but not which species/strain (15). The serum neutralisation test (SNT) is the gold standard for determining antibodies to the pestivirus genus (16). Graham *et al.* (17) found a pestivirus animal prevalence level of 5.3% in Northern Ireland sheep. All of the fourteen antibody positive samples were tested by serum neutralisation (SNT), all had a fourfold higher titre for BVDV- 1 than BDV. Thereby showing that BVDV-1 was the main pestivirus circulating in Northern Ireland sheep at that time.

A voluntary BVDV eradication programme commenced in Northern Ireland in 2013, becoming compulsory in March 2016. The programme requires all calves born to be ear notch sampled using

tissue sample-enabled official identity tags. Cattle are required by legislation to be tagged by 20 days of age and the sample tested for the presence of virus by a designated laboratory (18).

The aim of the present study was to evaluate pestivirus exposure in sheep and goat flocks in Northern Ireland, to determine the prevalent strain(s) in these species and to compare its apparent prevalence and geographical distribution with previous studies. Quantifying the level and types of pestivirus in sheep and goats in Northern Ireland will help inform farmers and veterinary practitioners of the current disease dynamics within flocks and could be used to inform policies supporting the BVDV eradication programme.

Methods

Samples

The blood samples tested in the present study were collected from June to November 2018 as part of the Department of Agriculture, Environment and Rural Affairs (DAERA) of Northern Ireland annual sheep and goat serological survey. The DAERA survey collected samples from 20 randomly selected sheep/goats over 1 year of age, from each of 230 random flocks across Northern Ireland, this accounted for approximately 2.3% of the total flocks. The samples were tested for a range of notifiable pathogens to comply with European legislation (19). These included *Brucella melitensis*, *Brucella ovis*, Maedi Visna virus and contagious agalactia which includes *Mycoplasma mycoides* and *Mycoplasma capricolum*. A weighted number of flocks from each Divisional Veterinary Office (DVO) was calculated by DAERA and a random sample from each DVO selected. For a flock to be eligible for inclusion, it had to contain at least 1 sheep or goat and have not been sampled in the previous three years. In flocks with 20 or fewer animals all animals were sampled, and in larger flocks, 20 sheep/goats were sampled with the first sheep/goats presented by the farmer being targeted.

The sampling strategy was not within the study remit to co-ordinate, but was consistent with other studies investigating pestivirus exposure in sheep (17,20). A retrospective analysis was performed of the sampling strategy and is reported in the supplementary data. An authorisation form and

89 questionnaire were completed with the flock owners by DAERA's Animal Health and Welfare
90 Inspectors at the time of blood sample collection; these forms were forwarded to the Agri-Food and
91 Biosciences Institute (AFBI) laboratories along with the samples for testing by antibody ELISA (enzyme-
92 linked immunosorbent assay), SNT and reverse-transcription polymerase chain reaction (RT-PCR). In
93 total, 197 (85.7%) of the 230 flock owners gave permission for inclusion within this study, representing
94 approximately 2% of Northern Ireland flocks.

95 Aborted lambs submitted to AFBI for post-mortem examination between December 2018 and May
96 2019 (typical Northern Ireland lambing season) had foetal fluid and organ samples (spleen, liver and
97 lung) taken for pestivirus investigation as part of the present study. The spleen, liver and lung samples
98 were pooled (one organ pool per animal) and, homogenised in Minimum Essential Medium (MEM)
99 and antibiotics (1,000 units Penicillin and 1000µg/ml Streptomycin) at a 1/10 dilution and centrifuged.
100 The supernatant was removed for testing by RT-PCR. Foetal fluids were centrifuged and the
101 supernatant removed for testing by antigen ELISA.

102 *Antibody ELISA*

103 Serum removed from the samples post centrifugation was tested for p80 antibody using the
104 PrioCHECK ruminant BVD and BDV p80 antibody serum enzyme linked immunosorbent assay (ELISA)
105 kit. This kit is designed to detect antibodies against a specific non-structural protein (p80) present in
106 all strains of BVDV and BDV. According to the manufacturer's validation report, the test sensitivity and
107 specificity were 95.8% and 100% respectively. The PrioCHECK ruminant BVDV & BDV p80 antibody
108 serum assay was used as per manufacturer's instructions. Percentage positivity values of <50% were
109 interpreted as negative, ≥50% as positive.

110 *Serum Neutralisation Test*

111 Samples positive for antibody to p80 were tested by SNT to determine the pestivirus strain to which
112 the antibodies were raised. Serum samples were heat inactivated at 56°C for 30 minutes and pre-

diluted to ¼ with maintenance media for each tissue culture cell line with Eagle's Minimal Essential Media for lamb kidney cell line or Glasgow Minimal Essential Medium BHK-21 for foetal calf lung cell line, Gibco. Each serum sample was titrated in duplicate in a doubling dilution series from 1/8 to 1/8192 on a dilution plate. An equal volume of 100$TCID_{50}$ of the relevant virus, BVDV (type 1a, field isolate) or BDV (Moredun isolate, APHA) was added to each serum dilution in the series and incubated in the presence of CO_2 at 37°C for 30mins. The serum/virus mix was transferred to pre-monolayered plates containing the relevant cell culture and incubated at 37°C, 5% CO_2 for 4-6 days depending on the rate of development of the cytopathogenic effect in the tissue culture. After incubation the cells were fixed by the addition of 50µl of 10% formalin per well and incubated at 37°C for 30mins. Endogenous peroxidases were neutralised by the addition of 100µl of 1% H_2O_2 to each well and incubation at 37°C for 5mins. The virus was visualised using pestivirus monoclonal antibody (WB103/105, APHA), goat anti-mouse peroxidase (Jackson Immuno Research) and Diamino benzidine (DAKO) substrate. The antibody titre of each serum sample against each of the virus types was recorded. A four-fold or higher difference in the titre of a sample against each of the virus types was used to determine which virus type was responsible for production of the antibody in the host animal. In cases where the difference in titre was not four-fold or greater, the original virus type was considered to be inconclusive.

RT-PCR

Negative samples from positive flocks i.e. those with at least one positive antibody result were further tested by RT-PCR. As persistently infected animals are typically seronegative and virus positive, this testing was performed to determine whether any of the seronegative animals in the sample population were virus-positive. Due to the likely low levels of PIs in flocks and especially since older animals were sampled within this survey, the chances of finding a PI animal if present were likely low, and the findings should be interpreted in light of this.

RNA from organ pools and serum samples from antibody negative animals were extracted using the MagMAX™ -96 Viral RNA Isolation Kit (Applied Biosystems). The nucleic acid extract was tested with the virotype BVDV real-time Reverse Transcription PCR (RT-PCR) kit (Qiagen, now Indical Bioscience). This kit targets the 5´non-translated region (5´NTR) which shows a high homology with all known pestivirus strains. According to manufacturers' validation report, the kit can detect BVDV-1, BVDV-2 and BVDV-3 strains as well as classical swine fever virus, border disease virus and other atypical non-bovine origin pestiviruses with a test sensitivity of 99.6 % and specificity of 99.9 %.

Antigen ELISA

Foetal fluid samples from ovine abortion post-mortems were tested for BVDV antigen by the BVDV Antigen/Serum Plus ELISA kit (IDEXX) as per manufacturer's instructions.

Statistical analyses

Statistical analyses were undertaken including Wilcoxon signed rank test, chi-square (χ^2), fishers exact test and Spearman's rank correlation in R v3.4.1 (21) for questionnaire data and p80 results. To calculate the 95% confidence intervals around animal level apparent prevalence, binomial GLMMs were performed with antibody result as the outcome and Farm ID as a random factor to account for within flock clustering of sampling. To calculate the confidence intervals for flock level apparent prevalence the package DescTools was used for confidence intervals for binomial proportions. The sheep flock and sheep (animal) level apparent prevalence from the 1999 study by Graham *et al.* (17) were mapped using GIS to compare with the data from sheep in this current study. R packages ggplot2 (22) and maptools (23) were used to produce seroprevalence maps. To test whether there was any statistically significant difference in sheep flock level apparent prevalence between both time periods, a gaussian univariable generalised linear model (GLM) was constructed with apparent prevalence as the outcome, and year as the sole predictor, entered as a categorical variable with values of either 1999 or 2018. The model was compared against a null model without year as a factor using likelihood ratio tests.

Results

Of the 230 flock owners that were approached to take part in the DAERA serological survey, 197 flock owners (188 sheep and 9 goat) agreed to participate in this study. A total of 3,418 animals were sampled including 3,372 sheep and 46 goats.

192 of the 197 flock owners completed a questionnaire with the Animal Health and Welfare Inspectors. Of the 192 flock owners questioned, 119 (62%) also kept cattle and 27 (22.7%) of these herds advised they vaccinated cattle for BVDV. Of the 119 herds that kept cattle there were 10 dairy (8.4%), 26 fattening (21.8%), 20 mixed (beef and dairy enterprises) (16.8%) and 63 suckler (52.9%) herds. 76 (39.6%) and 71 (37%) of the 192 farms respectively had common grazing and common housing for cattle and sheep. 84 (43.8%) of the farms used land away from the main farm, with only 2 (1%) farms participating in cattle "B&B" (cattle being housed over winter at another farm). 2 (1%) of the 192 flocks advised they have previously had a BDV infection. There were only 9 goat flocks sampled as part of the serological survey and unfortunately flock characteristics were not available for analysis. Therefore, it is difficult to draw any conclusions on pestivirus infection in NI goat flocks. Goat results were not included in the DVO results or when comparing with the 1999 study.

p80 antibody results

Table 1. p80 antibody ELISA results of sheep and goat serum samples **a)** animal level (total number of p80 antibody positive results) and **b)** flock level (presence of at least one antibody positive result within the flock).

	Result	Sheep	Goats	Total
Animal Level				
a)	Negative	3,316	45	3,361
	Positive	56	1	57
	Apparent Prevalence	1.7% (95%CIs 1.4 – 4.2%)	2.2% (95%CIs 0.0 – 23.8%)	1.7% (95%CIs 1.4 – 3.9%)
	Total Tested	3,372	46	3,418
Flock Level				
b)	Negative	155	8	163
	Positive	33	1	34

Apparent Prevalence	17.6% (95%CIs 12.4 – 23.8%)	11.1% (95%CIs 0.3 – 48.2%)	17.3% (95%CIs 12.3 – 23.2%)
Total Tested	188	9	197

The results from the p80 antibody testing from sheep and goats are shown in **Table 1**. The mean apparent prevalence in the flocks with positive antibody results was 9.7% (range 5% to 40%). There was no evidence of a relationship between antibody flock status and keeping cattle (χ^2=0.170, df = 1, p = 0.68), BVDV vaccinating cattle (χ^2= 2.0, df = 1, p = 0.157), cattle type (χ^2= 2.447, df = 3, p-value = 0.485), number of breeding cows (Wilcoxon Rank Test W = 1016, p-value = 0.726), common grazing (χ^2= 0.316, df = 1, p-value = 0.574), common housing (χ^2= 0, df = 1, p-value = 1), use of conacre (χ^2= <0.001, df = 1, p-value = 0.981), B&B (χ^2 = 0, df = 1, p-value = 1). There was a statistically significant difference when a flock had previously had border disease on the farm; however only 2 of the 192 flocks that completed the questionnaire indicated that they had had BDV previously and both were positive for pestivirus during this study, so we would advise caution interpreting this result.

Table 2 shows the sheep results of the antibody testing by Divisional Veterinary Office (DVO), reflecting the geographical apparent prevalence across Northern Ireland. Samples were not submitted from the Dungannon DVO area, therefore it was not included in the analysis.

Table 2. Results of sheep p80 antibody ELISA testing at animal and flock level per DVO area throughout Northern Ireland. *95% CIs not available due to low quantity of test positive sheep in DVO.

Region	Number of Flocks	Number of Sheep	Positive Flocks (%)	Flock 95% CIs	Positive Sheep (%)	Sheep 95% CIs
Armagh	18	347	2 (11.1%)	1.4 – 34.7%	2 (0.6%)	0.0 – 21.6%
Ballymena	10	187	3 (30%)	6.7 – 65.2%	5 (2.7%)	0.0 – 6.2%
Coleraine	27	458	7 (25.9%)	11.1 – 46.3%	9 (2.0%)	0.0 – 2.4%
Enniskillen	37	603	7 (18.9%)	8.0 – 35.2%	12 (2.0%)	0.9 – 7.0%
Londonderry	18	344	1 (5.6%)	0.1 – 27.3%	1 (0.3%)	N/A*
Mallusk	9	173	1 (11.1%)	0.3 – 48.2%	2 (1.2%)	0.0 – 98.3%
Newry	34	578	8 (23.5%)	10.7 – 41.2%	17 (2.9%)	1.1 – 6.9%
Newtownards	14	264	1 (7.1%)	0.2 – 33.9%	2 (0.8%)	N/A*
Omagh	21	418	3 (14.3%)	3.0 – 36.3%	6 (1.4%)	1.0 – 21.9%
Total	**188**	**3,372**	**33 (17.6%)**	**12.4 – 23.8%**	**56 (1.7%)**	1.4 – 4.2%

Figure 1 shows the geographical differences (aggregated to DVO regions) in pestivirus exposure in sheep and flocks between the Graham *et al.* (17) study (1999 data) and the present study (2018 data). We found that at DVO level, there was a mean decrease of 14.1% in the percentage of sheep flocks with a positive pestivirus result between 1999 and 2018. This was significant when compared to a null model (coef = -14.111, p = 0.045, Likelihood Ratio Test p = 0.044). Ballymena and Enniskillen were the only DVOs which had an increase in apparent prevalence between the two studies.

Of the 119 flocks that kept cattle, records of BVDV results in the cattle could only be obtained for 48 farms where a herd number was available. 10 of the 48 herds had reported one or more positive BVDV antigen results since the start of the compulsory phase of the Northern Ireland BVDV eradication programme (March 2016). A Spearman's correlation was performed to assess any correlation between the number of BVDV positive ear notch results on a farm and the number of positive p80 antibody results in the flock. No correlation was found ($r_s = -0.12$, p= *0.936*).

SNT results

Of the 57 serum samples tested by SNT to determine whether the antibodies present were of BDV or BVDV origin, 52.6% (30 of 57) had a four-fold or higher titre against BVDV-1 than BDV and 21.1% (12 of 57) had a four-fold or higher titre against BDV than BVDV-1. 26.3% (15 of 57) did not have a four-fold or greater titre difference between the two viruses, and therefore had an unknown definitive BDV or BVDV result and were classified as inconclusive.

RT-PCR Results

To evaluate the presence of pestiviruses in the study flocks, antibody negative samples from 34 flocks where seropositive animals were also present, were tested by RT-PCR for pestivirus RNA. Of a total of 568 p80 negative animals, 4 samples had insufficient material left for this further testing. Samples were tested in pools of 25 by RT-PCR. All testing pools returned a negative result suggesting that there were no animals shedding pestivirus at the time of sampling within the sample population.

Of the 177 lamb abortion cases submitted, all had RT-PCR performed on organ pools and 145 had an antigen ELISA carried out on foetal fluid. All 177 cases were negative when tested by RT-PCR on organ pools. Of the 145 samples tested by antigen ELISA, a single sample (0.689%; 95%CI: 0.017-3.757) was positive.

Discussion

The apparent prevalence of sheep and sheep flocks with positive pestivirus antibodies in Northern Ireland in this study were 1.6% and 17.6%, respectively. Due to the large number of samples collected as part of the DAERA serological survey, the minimum number of samples needed for inference to be taken at animal-level for apparent prevalence was exceeded. In regard to the number of flocks required to calculate the flock-level apparent prevalence, the total number was not met. We would advocate that even though this number was not obtained the study still is informative as it represents a large number of flocks from across Northern Ireland and constitutes a larger sample size than a previous study (17). Therefore, we consider this study to be a good indicator of the apparent prevalence within Northern Ireland.

The sampling strategy in this study followed the same protocol as the 2001 Graham *et al* (17) study using samples from DAERA's sheep serology survey (20 animals randomly selected per farm across Northern Ireland). Unfortunately, the sensitivities and specificities of the antibody tests previously used are not stated and therefore, a direct comparison of true prevalence could not be carried out. Due to low numbers of goats surveyed, the seroprevalence in goats (<0.1%) and goat flocks (11.1%) is not a reliable estimate and future targeted sampling of goats should be considered.

The present study obtained a lower apparent prevalence in both animal and flock levels than those found in previous studies on the island of Ireland. A 2001 study in the Republic of Ireland reported an antibody seroprevalence of 5.6% and 46.0% at animal- and flock-level, respectively (24). A similar study performed during 1999 in Northern Ireland by Graham *et al* (17) found sheep and flock apparent prevalence of 5.3% and 30.4%, respectively. Graham *et al* (17) suggested this to be comparable to the

246 results in the indigenous sheep population in Northern Ireland in 1984 (25). From these earlier studies,
247 it can be inferred that the positive antibody levels between 1984 and 1999 had probably remained
248 stable.

249 The present study has found that since the Graham *et al.* (17) study, there has been a decrease of 3.7
250 and 12.8 percentage points in sheep (animal) and sheep flock apparent prevalence in Northern
251 Ireland, respectively. These results suggest that the apparent prevalence has decreased across
252 Northern Ireland between 1999 and 2018. The reason for this decrease in levels of pestivirus antibody
253 in animals and flocks may be due to several factors. The main change in the Northern Ireland farming
254 industry during this time has been the introduction of a voluntary BVDV eradication programme in
255 2013 based on tissue tag testing for pestivirus antigen in calves that moved into a compulsory
256 programme in 2016 which requires all new born calves to be tested for pestivirus (26). During the first
257 full year of the programme 0.66% of calves tested positive for pestivirus. This had reduced to 0.34%
258 of all calves tested in 2019 (27).

259 The majority of antibodies found in the animals in this study were against BVDV-1a. This finding would
260 support that sheep are more often infected with BVDV than with BDV. In Northern Ireland, cattle and
261 sheep are known to be frequently grazed and housed together, with bovine tuberculosis having been
262 confirmed in sheep in contact with cattle (28). The removal of PI cattle from farms reduces the
263 circulating virus and, as older animals leave the herd, the number of antibody positive animals
264 gradually decreases (29). As the eradication programme makes progress, the cattle population will
265 become increasingly naïve and consequently susceptible to the introduction of new infections.
266 Therefore, virus-positive sheep present a risk for the reintroduction of pestiviruses to cattle herds.
267 However, the low levels of virus positive animals found in this study would suggest a low risk.

268 In the Northern Ireland Agricultural census for the year 2000 there were 10,848 sheep farms and
269 2,740,586 sheep (30), and in the 2018 census there were 9,984 sheep farms and 2,005,998 sheep (31).
270 The 2018 census report attributed these changing figures to the decline of breeding ewe numbers

since 1998. Sheep numbers fell by 40 per cent to a low in 2010. Since then, numbers have increased by 11 per cent but have seen fluctuations in response to volatile lamb prices (32). The 2001 foot and mouth disease outbreak resulted in large numbers of sheep being culled across Northern Ireland. The removal of large numbers of stock since 1999 would have changed the dynamics within and between flocks in Northern Ireland; this in turn may have influenced the circulating pestivirus within flocks.

There was a 97.5% return of completed questionnaires to accompany the pestivirus sampling of sheep and goats in this study. This high rate of return allowed analysis of relationships between farming practices and pestivirus antibody presence on farm, and as such this study constitutes the first comprehensive assessment of the ovine and caprine pestivirus exposure prevalence in the Northern Ireland population since 1999. The results of this analysis found that the majority of factors were not associated with pestivirus antibody presence within a flock. The only significant factor found in this study was a previous BDV infection in the flock as reported by the flock owner. There was no means to quantify how long ago the BDV infection had been identified, as only 2 of the 192 flocks had a previous BDV case, we would advise further work be performed to validate the epidemiological significance of this small population as we feel no inference can be made. Further investigation of a wider range of factors and larger flock numbers may help to identify risk factors linked to pestivirus exposure. The trading of sheep between flocks may affect the risk of pestivirus infection, this should be considered in future studies.

At flock- and animal- levels both Ballymena and Enniskillen DVOs had an increase in prevalence between 1999 and 2018; the levels in all remaining DVOs decreased. Future sampling should be considered to assess if this trend is continuing and if it is statistically and/or epidemiologically significant. This increase in prevalence in these two DVOs has no clear driving factor and further research could be conducted to evaluate potential factors, for example, sheep density changes, different breeds, climate and other diseases. As this was a random sample of flocks, it would be worth considering monitoring the same flocks over time to determine if there is a changing prevalence. There

296 was a 42.7% decrease in seroprevalence in the Coleraine DVO, again there are no clear drivers for this,
297 however it should be noted that the sample size in the 1999 study was small (2018 - 29 flocks vs 1999
298 - 10 flocks). Charoenlarp *et al.* (26) produced a hot spot map of BVDV antigen positive calves from the
299 first year of the BVDV eradication programme in 2016. The DVO areas of higher seroprevalence in
300 sheep and goats in this study did not correspond to the areas of higher BVDV antigen positive calves
301 from 2016.

302 Graham *et al.* (17) found that BVDV-1 was the predominant pestivirus circulating within sheep flocks
303 in Northern Ireland. The current study also found that BVDV-1 was the main pestivirus circulating
304 within the sheep population with 52.6% (30 of 57) of samples producing a fourfold or higher titre for
305 BVDV than BDV. This suggests that BVDV-1 rather than BDV is still the major pestivirus circulating
306 within the population. Similarly, BVDV-1 has been found to be the predominant circulating pestivirus
307 within cattle herds in Northern Ireland during 1999-2011 (4).

308 To identify current circulating pestivirus among Northern Ireland sheep, the seronegative samples
309 from flocks with a positive p80 antibody result were tested for pestivirus RNA by RT-PCR. As 568
310 animals in 33 flocks tested negative, we concluded that virus-positive adult animals in flocks in
311 Northern Ireland are rare. Our results agree with previous work showing that the survival of PI sheep
312 to adulthood is rare (33).

313 The ovine abortion samples submitted to the post-mortem unit between December 2018 and May
314 2019 were tested for the presence of pestivirus. None of the organ samples tested positive by RT-PCR
315 for pestivirus RNA and only 1 of the foetal fluids gave a positive result by antigen ELISA. From these
316 results, we suggest that pestivirus infection of pregnant ewes is not a major factor in abortions on
317 sheep farms in Northern Ireland.

318 This study has shown that apparent pestivirus seroprevalence within sheep flocks has decreased in
319 Northern Ireland in the last 20 years, during which time the BVDV eradication programme in Northern

Ireland was developed and rolled out. We would suggest, given our findings, that the eradication programme in cattle has also reduced the burden of infection in sheep.

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Competing Interests

None

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416 **Figure 1.** Apparent prevalence of pestivirus in DVOs across Northern Ireland in sheep flocks in **a)** 1999 and **b)**
417 2018 and sheep (animals) in **c)** 1999 and **d)** 2018.