

# Pestivirus seroprevalence in Irish lambs at time of slaughter



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#### Abstract:

Sera from 2059 lambs, from 196 different flocks spanning 25 counties in the Republic of Ireland were examined for pestivirus antibodies. Four lambs (0.194%) from four different flocks (2.04%) were antibody positive.

#### Introduction:

Border disease is a member of the Pestivirus genus within the Flaviviridae family, which also includes classical swine fever virus (CSFV) and bovine viral diarrhoea virus (BVDV) types 1 and 2. Classical swine fever virus is a host specific virus; however border disease can affect sheep, goats and pigs while BVDV can affect cattle, sheep, goats, deer and pigs (O'Neill et al 2004).

Due to the potential risk of cross-infection between species (Graham et al 2001), it is currently recommended that genomic characteristics, rather than the species in which they were isolated, are used to classify isolates of the pestiviruses.

Border disease can spread horizontally or vertically within a population. It causes mild/inapparent disease in healthy adults and primarily manifests as a reproductive issue, causing barren ewes, abortions, stillbirths, low birth weight, neonatal ill-thrift and the distinctive "Hairy shaker" syndrome in lambs (Nettleton et al 1998).

The latter results in lambs having excessively coarse fibres in their fleece, giving a 'halo' effect when back-lit, and a tremor as the virus affects the nervous system, leading to lesions such as hypomyelinogenesis and cerebellar dysplasia (Nettleton et al 1998, Jeffrey and Roeder, 1987).

As with other pestiviruses such as BVD, the main source of infection amongst a naïve population are the persistently infected (PI) animals, these animals are infected prior to the immune system developing inutero, leading to the foetus becoming immunotolerant towards the virus (Sandvik 2014). These PI animals may be clinically normal dependent on stage of gestation when infected, and they excrete large amounts of virus, acting as a reservoir of infection (Nettleton et al 1998).

While transmission from sheep to cattle is possible (Graham et al 2001), it is likely that the predominant flow of infection is from cattle to sheep. This is substantiated by the fact that in previous studies BVD was found to be the main circulating pestivirus in the sheep population in Northern Ireland (Graham et al 2001); and the relatively high herd prevalence (>85%) of BVD in Ireland in comparison to the 30% flock prevalence of Border Disease (Barrett et al 2011).

There has been considerable progress made in the eradication of BVD since the BVD eradication programme was initiated in 2013, as seen by the fact that animal prevalence of PIs has fallen from 0.67% in 2013 to 0.06% in 2018 (AHI statistics). Studies from Switzerland have implicated the presence of pestivirus circulating in the sheep population as a potential barrier to eradication in the latter stages of the Swiss BVD eradication programme (Kaiser, V et al 2017). Concerns were raised among the Implementation Group of the Irish BVD eradication programme that pestivirus may also be circulating in the Irish sheep population and may be a potential barrier to eradication in Ireland.

#### **Objectives:**

The first objective of this study was to investigate the seroprevalence of pestiviruses in a sentinel population of lambs; with a view towards providing an estimate of the levels of disease currently circulating in Irish sheep flocks.

A further objective was to investigate if there was an epidemiological link between the sheep flocks where pestivirus was demonstrated to be circulating and the presence of BVD in cattle on those establishments.

#### Materials and Methods:

Clotted blood samples were taken from 2,059 lambs from 196 different flocks spanning 25 counties in the Republic of Ireland (Limerick being the only exception) as part of an unrelated liver fluke surveillance survey.

The samples were then centrifuged and serum collected from each one was stored at  $-20^{\circ}$ C. They were then examined using an ELISA test for pestivirus antibodies.

The number of flocks tested per county was directly proportional to the number of sheep in that county according to the most recent sheep census in December 2016. According to information derived from the 2016 national sheep and goat census, the flocks selected had a mean size of 268, the largest of which had 2139 sheep, while the smallest had 24 sheep.

A competitive ELISA antibody test kit (details below) was used in accordance with the manufacturer's instructions to test the samples. The sensitivity of the kit using sera as the sample tested is 60 % (36-81%, 95% CI), while the specificity is 97% (88-100%, 95% CI) as reported by Baptiste-Hanon et al 2017.

#### **Results**

#### Test:

Serological analysis was carried out using a commercially available IDEXX BVDV/MD/BVD p80 Protein Antibody Test Kit, in accordance with the manufacturer's instructions.

For most part 10 sera were tested per flock, unless there were fewer than 10 animals presented for slaughter.

Figure 1.3 BDV Results

**Total number of sheep tested** = 2059 **Total number of individual positives** = 4

**Total Number of flocks tested** = 196 **Total Number of positive flocks** = 4

**Apparent animal level prevalence** = 4/2059 = 0.00194 (0.194% (95% CI 0.07% - 0.49%))

#### **Methodological Issues:**

While the sensitivity of the test used was undoubtedly imperfect in this instance, and as such we must interpret the relatively low seroprevalence with caution, one might also make the inverse argument: given that the specificity of the test used was 97% one might have even expected a greater amount of positives allowing for the imperfection of the test.

Therefore, given the extremely low seroprevalence, future studies whereby follow up testing using PCR based methods as an adjunct to the initial ELISA test would undoubtedly yield interesting results as regards presence/absence of disease.

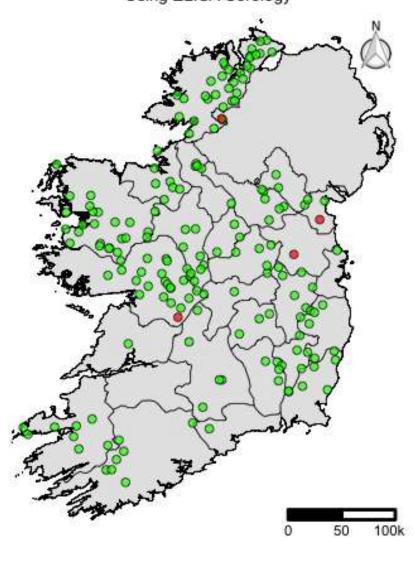
## **Border Disease Flock** seroprevalence map:

In this study DED's were used to map the flock prevalence data. District electoral divisions (DEDs) are relatively small local areas in Ireland, chosen for the purposes of organising elections. Each DED comprises an area of approximately 24km<sup>2</sup>. Each flock in Ireland is linked to one of a total of 3440 DEDs through its flock number.

Using DEDs to map disease data links each flock to a relatively small local area, but also anonymises the locations of the flocks being mapped. The flocks in this study were plotted using the R software package to generate a flock prevalence map. Each positive flock on the map, denoted by a red point, included one positive test result as well as several negative results.

Overall, the low animal level (0.194%) and flock level (2.04%) prevalence's of detectable antibodies to pestivirus suggest that there is an extremely low level of pestivirus circulating amongst the Irish lamb population.

#### BDV Flock Seroprevalence Using ELISA Serology



### Test results: Negative Positive

#### Discussion

Our main finding was that there is a very low pestivirus antibody seroprevalence amongst the Irish lamb population at the time of slaughter (0.194%), given that in previous pestivirus studies in the sheep population, seroprevalence levels were estimated at 5.3% (Graham et al 2001) and 5.8% (O'Neill et al 2004).

This indicates a low level of lambs born persistently infected (PI) with pestivirus in Irish flocks, as the presence of PI animals amongst the cohort would lead to transient infection, an antibody response and seroconversion.

A further interesting finding, as outlined above is that 2/4 seropositive lambs came from sheep-only enterprises, while the other 2/4 came from mixed enterprises (i.e. sheep and cattle on the same holding).

#### **Conclusions**

The main finding of this study was that there is very low seroprevalence (0.194%) of pestiviruses amongst the Irish lamb population. While we are trying to draw conclusions from an extremely small cohort, there also seems to be no significant epidemiological link between the sheep flocks where pestivirus was demonstrated to be circulating and the presence of BVD in cattle on those establishments.

Thus, in conclusion, while cross infection between species is possible, the results of this study should aid in allaying fears of sheep acting as a potential reservoir of infection in the eradication programme of BVD.