A survey of antibodies to pestivirus in sheep in the Republic of Ireland

Ronan G. O’Neill, Michael O’Connor and Patrick J. O’Reilly

Department of Agriculture and Food, Central Veterinary Research Laboratory, Abbotstown, Castleknock, Dublin 15, Ireland.

Sera from 1,448 adult ewes in 91 flocks, representing all 26 counties in the Republic of Ireland, were examined for pestivirus antibodies using a commercially available ELISA which detected IgG, antibody to border disease virus. Eighty-one sheep (5.6%) in 42 flocks (46.0%) were antibody-positive. Within infected flocks, the mean seroprevalence level was 11.4% with a range of 6.3% to 30.0%. The highest antibody prevalence was detected in sheep from central lowland counties of Ireland. Comparative neutralisation testing of 42 ELISA-positive sera detected geometric mean antibody titres of 136 to the NADL strain of bovine viral diarrhoea virus (BVDV), 92 to the Moredun strain of border disease virus and 21 to the 137/4 strain of border disease virus. These results suggest that BVDV may be the major ruminant pestivirus infecting sheep in Ireland. Although there are high numbers of infected flocks, many sheep within such flocks remain antibody-negative and are at risk of giving birth to lambs with congenital border disease.

Key words
Sheep, Pestivirus, Border disease, Republic of Ireland, Antibody

Abbreviations
BDV Border disease virus
BVDV Bovine viral diarrhoea virus
CI95 95% confidence interval
COD Corrected optical density
CSFV Classical swine fever virus
ELISA Enzyme-linked immunosorbent assay
FBK Foetal bovine kidney
FMD Foot-and-mouth disease
HRPO Horseradish peroxidase
NI Northern Ireland
SNT Serum neutralisation test
reduce productivity in individual flocks (Bonniwell et al., 1987). Genomic, antigenic and serological studies have shown that BDV is more closely related to CSFV than to BVDV (Vilcek et al., 1997). BDV infection of pigs complicates the serological diagnosis of CSFV, a Class A notifiable disease (Pearson, 1992), and is a potential cause of antibody false-positives with serious implications for animal movement and trade. In addition, congenital infection of pigs with BDV can mimic clinical infection with low virulence strains of CSFV (Paton and Done, 1994). The objectives of the present study were to assess the serological prevalence of border disease virus in the Irish sheep population, and to explore the relationship and cross-reactions between antibodies to BDV and the other important pestivirus, BVDV. Similar studies have been performed in the UK (Sands and Harkness, 1978), Norway (Loken et al., 1991), Sweden (Lunden et al., 1992), Denmark (Tegtmeier et al., 2000) and Northern Ireland (Graham et al., 2001).

Materials and methods

Animals

Sera from 1,448 adult ewes, collected from 91 flocks, were examined by ELISA for BDV antibodies. Flocks were tested from every county in the Republic of Ireland, the number selected for each county was weighted to represent the respective proportion of the national flock farmed there – according to the 2001 National Sheep Census figures (Costelloe et al., 2002). The flock selected had a mean size of 172; the largest had 744 sheep and the smallest had 18 sheep. Table 1 shows how these sheep flocks were distributed, both by size and by county.

Sixteen sera per flock were tested with the exception of one flock in Co Kildare, with eight sera only. The blood samples were collected as part of the surveillance for foot-and-mouth disease (FMD) virus antibodies over a six-week period: May 17, 2001 to June 26, 2001. At this time serum was collected, centrifuged and stored at –20°C. Eight nonadjacent flocks were identified as having high seroprevalence to pestivirus (≥20%) and underwent a further targeted round of testing, using all available sera.

Tests

Border disease ELISA

A commercially available solid-phase indirect ELISA (BDV-Ab, SVANOVA Biotech, Sweden) was used according to the manufacturer’s instructions. Results were read at 450nm. Corrected optical density (COD) values of less than 0.25 were regarded as negative.

### Table 1: Numbers of sheep flocks tested, categorised on size and county of origin

| Flock size | 0 - 49 | 50 - 99 | 100 - 199 | 200 - 299 | 300+ |
|------------|--------|---------|-----------|-----------|------|
| No. of flocks | 11 | 20 | 36 | 16 | 8 |
| No. of different counties | 7 | 14 | 18 | 12 | 6 |

### Table 2: Numbers and percentages of flocks and individual sheep seropositive to pestivirus by ELISA per county in the Republic of Ireland

| County | Number of flocks tested | Number of sheep tested | Number positive | Percentage positive |
|--------|-------------------------|------------------------|-----------------|--------------------|
| Carlow | 3 | 48 | 2 | 4 | 100 | 8 |
| Cavan | 2 | 32 | 2 | 4 | 100 | 13 |
| Clare | 1 | 16 | 1 | 2 | 100 | 13 |
| Cork | 5 | 80 | 3 | 7 | 60 | 9 |
| Donegal | 8 | 128 | 3 | 3 | 38 | 2 |
| Dublin | 1 | 16 | 0 | 0 | 0 | 0 |
| Galway | 10 | 160 | 4 | 8 | 40 | 5 |
| Kerry | 7 | 112 | 3 | 4 | 43 | 4 |
| Kildare | 3 | 40 | 3 | 3 | 100 | 8 |
| Kilkenny | 3 | 48 | 1 | 1 | 33 | 2 |
| Laois | 3 | 48 | 0 | 0 | 0 | 0 |
| Leitrim | 2 | 32 | 0 | 0 | 0 | 0 |
| Limerick | 1 | 16 | 0 | 0 | 0 | 0 |
| Longford | 2 | 32 | 1 | 2 | 50 | 6 |
| Louth | 2 | 32 | 1 | 1 | 50 | 3 |
| Mayo | 7 | 112 | 0 | 0 | 0 | 0 |
| Meath | 3 | 48 | 3 | 11 | 100 | 23 |
| Monaghan | 2 | 32 | 1 | 1 | 50 | 19 |
| Offaly | 3 | 48 | 3 | 11 | 100 | 23 |
| Roscommon | 4 | 64 | 3 | 4 | 75 | 6 |
| Sligo | 2 | 32 | 0 | 0 | 0 | 0 |
| Tipperary | 4 | 64 | 2 | 3 | 50 | 5 |
| Waterford | 2 | 32 | 1 | 2 | 50 | 6 |
| Westmeath | 2 | 32 | 1 | 1 | 50 | 3 |
| Wexford | 4 | 64 | 1 | 1 | 25 | 2 |
| Wicklow | 5 | 80 | 2 | 3 | 40 | 4 |
| **Totals** | **91** | **1448** | **42** | **81** | |
with the 137/4 BDV strain were performed using secondary foetal bovine kidney (FBK) cell cultures. Sera were tested over a dilution range of 1/8 to 1/1,024. After incubation for three days, the monolayers were fixed at 80°C, overlaid with porcine polyclonal pestivirus antiserum followed by a commercial HRPO-conjugated rabbit anti-pig immunoglobulin. Virus growth was indicated by the presence of reddish brown intra-cytoplasmic staining on microscopic examination (Jensen, 1981).

Results

A total of 81 (5.6%, CI 95 ± 1.2%) of the 1,448 sheep were positive for pestivirus antibody. Positive sheep were detected in 42 (46%, CI 95 ± 10.2%) of the 91 sheep flocks tested (Table 2). The average pestivirus antibody prevalence among the positive-only flocks was 11.4%, with a maximum of 30.0% and a minimum 6.3%. The mean COD of the positive sera was 0.59, with a minimum of 0.25 and a maximum of 1.39.

The second focused phase of testing concentrated on 139 samples taken from eight nonadjacent flocks identified as having high seroprevalence. Twenty-eight (20.1%) of these second phase samples proved positive. The mean overall intraflock antibody prevalence among this specific group of eight flocks was 23.9% (CI 95 ± 5.1%) with a range of 15.7% to 30.0%; the mean positive COD among this subset of samples remained virtually unchanged at 0.61.

The per-county geographic pattern of seroprevalence among individual animals and among individual flocks is illustrated in Figures 1 and 2.

The results of testing 42 pestivirus-antibody ELISA-positive sera by comparative neutralisation tests are shown in Table 3. Thirty-nine sera contained neutralising antibody to the BVDV

| Virus strain (pestivirus designation) | No. of positive sera | Reciprocal geometric mean antibody titre |
|--------------------------------------|----------------------|------------------------------------------|
| NADL (BVDV)                          | 39                   | 136                                      |
| Morehen (BDV)                        | 39                   | 92                                       |
| 137/4 (BDV)                          | 36                   | 21                                       |

**FIGURE 1:** Percentage of individual sheep that were seropositive to pestivirus in each county in the Republic of Ireland. NS: not sampled.

**FIGURE 2:** Percentage of sheep flocks that were seropositive to pestivirus in each county in the Republic of Ireland. NS: not sampled.
strain, NADL and the BDV strain, Moredun, while 36 sera contained neutralising antibody to BDV strain 137/4. Three ELISA-positive sera (mean COD = 0.29) had neutralising titres less than 1/8 in all SNTs. The highest geometric mean antibody titres were obtained against the NADL strain of BVDV followed by the Moredun strain, with strain 137/4 having lowest geometric mean antibody titres. Among those that tested positive to the NADL strain, the reciprocal geometric mean titre was 136, with a minimum of 16 and a maximum of 512. For the other SNTs, Moredun reciprocal antibody titres ranged between 8 and 1,024, while strain 137/4 antibody titres were limited to between 8 and 64. Thirty-four (81%) sera had four-fold or greater (≥ 2 dilutions) antibody titres to BVDV than to BDV strain 137/4, while 16 (38%) sera had four-fold or greater antibody titres to BVDV than to BDV strain Moredun. Higher antibody titres to BDV strain 137/4 compared to BVDV were not detected, although three sera had four-fold or greater antibody titres to BDV strain Moredun than to BVDV.

**Discussion**

The level of seroprevalence for pestivirus antibodies found in the initial phase of this survey approximated well with the 5.3% found in a study of the Northern Ireland (NI) flock (Graham et al., 2001). As all sampled sheep were of breeding age, it was assumed that maternal antibodies no longer persisted and that antibody indicated direct exposure to pestivirus. The detected seroprevalence may underestimate pestivirus activity in infected flocks. While the antibody has persisted for up to 485 days in ewes, infected when pregnant (Huck et al., 1975), it may become undetectable earlier in other sheep (Sawyer et al., 1986). Out of the 91 flocks tested, 42 (46%) were found to contain sheep that were seropositive for pestivirus. This flock seroprevalence level was considerably higher than the 30.4% reported in the NI study by Graham et al. (2001) and the 32.6% flock prevalence for indigenous sheep reported in an earlier NI study (Adair et al., 1984). This disparity may be because a distinction was not made in the present survey for flock-seroconversion. Substantial regional variations in the use of particular sheep breeds and variable breed susceptibility to pestivirus may confound these management effects (Schaller et al., 2000).

All flock disease problems were considered significant during the state of high alert of the foot-and-mouth disease crisis, so it may be assumed that the vast majority of seropositive sheep did not have clinical signs of oral/digital lesions, pyrexia, or depression. Similarly, abortion storms were reportable events during this period, and none was reported in the 91 flocks sampled.

In the current study, the sample collection period was early to mid-summer, some months after the normal lambing season. As pestiviruses are commonly released in foetal fluids at lambing, high seroprevalence levels would be expected when sampling at this time (Nettleton et al., 1998). Beyond the lambing season, the virus seems to spread rather slowly among sheep at grass. Only four out of 22 sheep seroconverted after three months of mixing with known persistently-infected animals (Bonniwell et al., 1987). Trough-feeding, housing and other intensive farm management features are considered to increase the pestivirus transmission rates among sheep (Nettleton et al., 1998). More targeted testing of antibody-positive flocks was undertaken to evaluate disease dynamics within such flocks. None of these seropositive flocks had levels of infection above 30%, and the mean level was less than 25%. Cattle were present on all of these higher incidence farms. Levels of seroprevalence of this order would suggest medium-level pestivirus transmission, probably due to the presence of persistently-infected animals within these flocks or transmission from cattle to sheep on these farms.

Persistently-infected animals can show quite marked temporal changes in levels of pestivirus antibodies (Roeder et al., 1987). There has been little research as to the probability of one or more animals, persistently infected with border disease virus, acting as the source of above-average infection rates in...
particular sheep flocks, although a comparable situation exists with BVDV in cattle (Carlsson and Belak, 1994). Examination of the ELISA-positive sera by comparative serum neutralisation tests found that the highest antibody titres were to the NADL strain of BVDV. Similar findings were obtained in the NI sheep and it is suggested that interspecies pestiviral transmission in Ireland may be predominantly from cattle to sheep, mainly due to BVDV (Graham et al., 2001). This is supported by the detection of four-fold or greater antibody titres to BVDV than to BDV strain 137/4, in over 80% of samples tested and, similarly, fourfold or greater antibody titres to BVDV than to BDV strain Moredun, in almost 40% of samples tested.

Cattle were present on a very high proportion (92%) of the farms tested, so this study proved unsuitable to test fully the relationship between the concurrent presence of cattle and levels of ovine pestivirus serocconversion. Interestingly, only 2.7% of samples collected from sheep farms where cattle were not present, were pestivirus antibody-positive, in contrast to 5.8% seropositive on farms where cattle were present. Transmission of BVDV from cattle to sheep has been reported before (Carlsson, 1991) but pestiviruses are not thought to spread commonly from sheep to cattle (Paton et al., 1999; Pratelli et al., 2001).

The high serum neutralising antibody titres to the Moredun strain of BDV detected in the present study could be due to cross reactions with BVDV but may also indicate the local occurrence of BDV strains related to subtype B (Vilcek et al., 1997). Three sera had four-fold or greater antibody titres to BDV strain Moredun than to BVDV. Low antibody titres to BDV strain 137/4 in both the Republic of Ireland and Northern Ireland (Graham et al., 2001) suggest that BDV strains of subtype A may not be present in Ireland. While infection of pigs in Ireland with ruminant pestiviruses is uncommon (O’Connor et al., 1991; Graham et al., 2001), the strains circulating in sheep have the potential to cross-react in serological surveillance and diagnostics for CSFV in pigs in Ireland, as well as mimicking chronic or pre-natal CSFV infection clinically (Terpstra and Wensoort, 1988; Paton and Donc, 1994).

This study highlights the apparently uneven distribution of pestivirus infection among Irish sheep, with some flocks apparently naïve to the viruses involved and, as a result, highly vulnerable to outbreaks of pestivirus-related disease.

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