BVDV in sheep, diagnosis and control

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Summary
A sheep and beef farm in the Wairarapa experienced an outbreak of bovine viral diarrhoea virus (BVDV) infection in sheep. Infection presented as abortion in two tooth ewes, decreased lamb survival, birth of lambs with congenital tremor and hypertrichosis, decreased growth rate in lambs and a significant economic loss in lamb production. Although this is a rare event in New Zealand, the common practice of co-grazing sheep with cattle, the severity of welfare and economic loss and the risk of perpetuation of the BVDV virus within the sheep flock indicate that sheep producers should ensure that pregnant ewes and valuable rams are not exposed to cattle persistently infected with BVDV virus.

There has been considerable recent effort into educating New Zealand veterinarians about the importance and technical details of BVDV control. This case is yet another example of why this virus requires our urgent and serious attention, and why removal of BVDV from cattle populations in your practice care should be a priority.

Introduction
Pestiviruses are an important cause of reproductive loss in ruminants in New Zealand. Pestiviruses are not necessarily host specific and presentation of the hairy shaker syndrome in sheep is an indication of infection with either border disease virus (BDV) or bovine viral diarrhoea virus (BVDV). Pestivirus infection of cattle in New Zealand is widespread and common, however pestivirus infection of small ruminants is remarkably rare, despite the scarcity of management controls to contain them in sheep and goats. BVDV and BDV are not host specific, BVDV may infect cattle, sheep, swine, goats and other ungulate species (Julia et al. 2009). In New Zealand BVDV has been recorded commonly in cattle with seroprevalence of 58%, in sheep 19%, swine 12%, (Horner et al. 1994), occasionally in deer 9.5% (Motha et al. 2000), goats 4% and very rarely in camelds (Motha and Tham, 1992). The prevalence in sheep may be underestimated because the required diagnostic approach to differentiate BVDV and BDV pestivirus infection in sheep in New Zealand has not been routinely used. Early transmission experiments in sheep for presumed Border disease virus were injections of homogenised neurological tissue so the exact virus being transmitted was not known. Although the seroprevalence in sheep has been reported previously this is the first report of a confirmed BVDV virus infection causing clinical disease in a sheep flock in New Zealand.

In contrast BDV has been confirmed internationally in sheep, swine, cattle and goats (Julia et al. 2009). In New Zealand BDV has been confirmed in cattle once (McFadden et al. 2012) and in pigs (Bingham et al. 2010). Two cases of BDV in cattle have been reported in Australia, and several cases in the United Kingdom (Strong et al. 2010). Fortunately classical swine fever virus (CSFV) seems to be restricted to pigs (Julia et al. 2009)

Aetiology
Bovine viral diarrhoea virus (BVDV) is the most prevalent pestivirus in New Zealand. It is related to Border disease virus and Classical swine fever virus. In New Zealand all strains of BVDV are Type 1 genotype, and the non-cytopathic biotype is the most prevalent. Classical swine fever and type II genotype have not been reported in
New Zealand. Border disease virus is the expected cause of hairy shaker disease, and this disease has been reported in New Zealand since 1957.

Clinical signs in sheep

The reported signs of pestivirus infection in ewes are barren ewes, foetal mummification, abortion, lambs born dead, and lambs born with congenital tremor and fleece changes. In the Wairarapa flock investigated exposure occurred in the days immediately prior to ultrasound scanning. At the June scanning only 3% of the 1400 two-tooth ewes and 1.6% of the 4500 mixed age ewes were non-pregnant. The scanning percentage was 167% and 179% respectively. The farmer noticed abortions and weak lambs. At docking a high proportion – 7% of two tooth ewes were found not to have lambs, as compared to the more typical 4% dry of the mixed age ewes on this farm. In one particular flock of the two tooth ewes the proportion of ewes without lambs was disproportionately large - 20%. The prevalence of lambs with congenital tremor at docking was 60.8%. There were approximately 1500 lambs belonging to two tooth ewes at docking, indicating there were approximately 880 (37%) lambs unaccounted for between scanning and docking in the two-tooths. The proportion of abortions, stillbirths and peri-parturient deaths in these flocks is unknown.

Affected lambs may have arthrogyposis, neurological defects including cerebellar hypoplasia and cavitating defects of the adjacent cerebrum, a reduction in the number of myelinated fibres in the cervical spinal cord, and hypertrichosis. The increase in mortality of lambs in the affected flocks is not surprising. The severity of the congenital tremor can diminish with age in some affected lambs, and this was observed in this outbreak. The number of affected lambs in this outbreak, and the economic necessity of raising them to a weight suitable for slaughter meant that some of them were retained on the farm until June the following year. Deaths between docking and weaning were high (5.3%). Between weaning in December and drenching in January approximately 220 lambs (15%) were euthanased due because the presence of pronounced ill-thrift, abnormal head shape and difficulty to muster and drench. In the remaining 1200 lambs approximately half had some congenital tremor but not hypertrichosis. These lambs required more time to move, and had significantly lower growth rates (average 66g/day) than their cohorts averaging more than 145g/day.

Once the aetiology was known, the farmer sought to reduce the number of severely affected lambs to improve their welfare and reduce the number of virus positive animals from the farm before the next mating.

Epidemiology

Pestiviruses are relatively contagious and foetal infection of naïve pregnant animals is the expected outcome, generating the spectrum of reproductive diseases and abnormal lambs described in clinical signs above. The birth of a persistently infected animal – in this case the hairy shaker lamb, poses a future risk to the flock as continual shedding of virus prolongs exposure. Infection of pregnant sheep experimentally with BVDV has been shown to produce both lambs with and without congenital tremor and skin changes. In 1971 Ward demonstrated that infection of ewes with the NADL strain of BVDV resulted in foetal mummification and the birth of lambs with hydrocephalus, cerebellar hypoplasia, and arthrogryposis, but not apparently the tremor, hypomyelinogenesis and hairiness characteristically associated with Border Disease (Barlow et al. 1980) Trials at the Moredun institute in the 1980’s demonstrated that pestivirus naïve Cheviot ewes given NADL strain BVDV virus intraperitoneally at d54 of gestation had the full spectrum of diseases, eight of ten ewes had abnormal progeny, of the six ewes destined to go full term one aborted, one produced a weak hairy lamb with the histopathological changes characteristic of Border disease and three ewes had lambs with no hairiness or tremor but with gross malformations of the central nervous system. These malformations included hydranencephaly, porencephaly and cerebellar hypoplasia and dysplasia. Of particular note here is that the hairy shaker presentation is one of a range of potential presentations of this disease in sheep, other lambs with neurological and physical abnormalities are also possible.

The circumstances leading to exposure in this case were two tooth ewes mustered for scanning were co-grazed with three ill-thrifty heifers that had been purchased as trading cattle earlier that year. The three Friesian heifers were separated from their mob and grazed close to the house where the owner could keep an eye on them, because they were unwell. Ewes mustered for ultrasound scanning were put in paddocks close to the yards, and some of the
two tooth flocks coincidently grazed with these heifers, but only for a few days.

Once the aetiology was confirmed by virus isolation and sequencing, the cattle were screened for BVDV. At the time of investigation the three suspected heifers and the trading cattle were no longer available for testing, however the mixed age cows, their calves, their yearlings and the bulls were present on farm. Polymerase chain reaction (BVDV PCR) tests on sera indicated that all the mixed age cows and bulls were negative, 11 of 62 yearlings (17.7%) were positive, of which ten were determined to be persistently infected by a subsequent test and 2 of 61 calves (3.2%) were persistently infected. There had been no introductions of Angus cows or bulls in the previous year. This indicated that BVD introduction was two years previous, and that the prevalence of persistently infected cattle on farm in the previous joining-scanning period was high. The severity of this outbreak, in the two tooth ewes indicated that this cohort of animals was naïve to this virus.

The reasonable scanning percentage in both mixed age and two-tooth ewes, the absence of dead or mummified foetuses at scanning, and the history of the two tooths not-grazing with the cattle until just before and after scanning indicates that the exposure was most likely in the days around scanning. It was the scanning percentage and knowledge of the ram source properties that lead me to question the provisional diagnosis of hairy shaker disease due to Border disease virus.

This exposure period of pregnant ewes to persistently infected (PI) cattle can be relatively short, Carlsson reported that ewes in one of his experimental groups had been separated from all sheep and cattle during gestation except for 11 days when they were in contact with a non-cytopathic BVDV persistently infected heifer. In this study five pregnant ewes were in contact, with gestation periods from d39-d78, all five ewes seroconverted, of the 12 lambs, two were stillborn, and three were born with congenital tremor, and seven were normal yet all ten live lambs were virus positive at birth (Carlsson 1991). The absence of clinical signs in lambs does not mean you can assume they are virus negative in a cohort where hairy shaker disease is apparent. Interestingly Carlsson also reported the amelioration in clinical signs of tremor as lambs matured.

In a separate natural infection event in a small Swedish flock, in the lambs born to 28 ewes, 19 (39.5%) had congenital tremor, six (12.5%) were born weak, one was malformed, two were stillborn, and 20 (42%) were apparently healthy (Carlsson 1991).

**Diagnosis of disease**

Fetuses infected with BVDV and border disease virus (BDV) provide strong antigenic stimulation to their dams, so antibody detection by virus neutralisation titres are potentially a tool by which ewes can be assessed as to their previous exposure status. Antibody detection by ELISA can also be used but will not distinguish between antibodies to BVDV or BDV.

In contrast to cattle – persistently infected sheep have much lower levels of virus, particularly in serum. For this reason PCR testing of sheep should be used to find animals where testing of individual valuable animals (rams) is required. BVDV antigen ELISA tests may lack the sensitivity to determine the BDV virus status of sheep or goats (Kittelberger and Pigott 2008) but should be able to detect BVDV infections. If antigen ELISA tests are being used whole blood is the preferred specimen.

As a result of a previous case of BDV virus recovered from cattle, the routine PCR protocols at Gribbles Pathology New Zealand have now been changed to detect BDV, BVDV 1, and BVDV 2 but not distinguish between them (McFadden 2012). For practical purposes in New Zealand, testing the serum of rams with the Gribbles BVD/HSD multiplex PCR will find both BVDV and BDV and any pestivirus positive rams should be culled.

For the clinician investigating hairy shaker syndrome, the most accurate approach is virus isolation followed by sequencing of the genome of the virus isolated. Accuracy is important if the control program needed involves potentially both cattle and sheep, and particularly if the presentation is abortion and abnormal fetuses. Virus isolation is achieved by sterile removal of the spleen of a hairy shaker lamb, followed by submission of that spleen promptly to Wallaceville. Notification of the laboratory of an impending submission is necessary to ensure the best outcome and turnaround.
Although it is cheaper and faster to do pestivirus PCR test and sequence the PCR fragments, it is less sensitive, and may miss one of the differential diagnoses. Virus isolation is preferred because it preserves the option of isolation of other viruses, which could potentially cause injury to neurological tissue, abnormal lambs and congenital tremor – e.g. Akabane virus.

In this manner the diagnostic process does not miss the non-pestivirus viral causes of congenital tremor, and sequencing the virus will clearly identify it.

**Control**

The control of pestiviruses is known – reduce transmission, remove persistently infected animals as soon as practical, and protect both potential sires and pregnant animals using vaccines that are safe and effective. In this case the farmer was unable to source alternative grazing for the less severely affected but potentially virus positive survivors, and they were still on farm so despite my concerns about vaccination we decided to vaccinate the two tooth ewes and rams in use that season.

The removal of persistently infected cattle as soon as practical is the first priority. Reducing the risk of transmission from cattle to sheep, because although pestiviruses can be maintained in sheep populations, they are a very low risk of transmission to cattle, horizontal transmission in sheep is a lower risk, their prevalence within flock is typically lower than cattle and they shed less virus than cattle.

The second and more difficult priority is the removal of persistently infected sheep. The cost of testing and risk of retaining persistently infected animals with the possibility of false negative diagnosis in sheep is not low. It is safer to treat that age cohort of lambs as all potentially persistently infected and treat them as trading lambs. Removal from the farm before tupping starts may be difficult as their growth rates are lower, and even though the risk of transmission from sheep is low, it is not zero and their status should be disclosed to any grazer, which could limit grazing options.

There are no BVDV or BDV vaccines currently registered for sheep in New Zealand, however Bovilis® BVD has been used in sheep in France. In 2009, an epizootic of pestivirus occurred in sheep breeding in the central region of France, including the Aveyron department, where the first occurrence of the disease was notified in France in 1984. In 2010, the prevalence and incidence of pestiviruses were estimated to 5.8% and 1.9% in dairy units and 21.7% and 8.5% in meat units respectively. No specific vaccines are commercialized against BDV. Since BVDV and BDV share some common antigens, vaccines against BVDV were used in the field to control pestivirus infections in ovines. Usually sheep are immunized with half or the quarter of the dose used to vaccine cattle.

Meyer measured the serum neutralising antibody produced by three groups of six ewes, by serial serum collection following the administration of two doses Bovilis-BVD vaccine administered 28 days apart, compared to a non-vaccinated control group. Group 1 was unvaccinated, Group 2 received a 1ml dose of vaccine (half dose) and Group 3 received a half ml dose (one quarter of the cattle dose) at each interval. The results are shown in Figures 1 and 2.

The six black lines plotted refer to the individual serum neutralisation (SN) titre of all six ewes in each treatment groups displayed with a logarithmic scale on the y axis. The mean of each treatment group is plotted in red. The top pair in Figure 1 are displaying a measure of antibody production following vaccination. The bottom pair in Figure 2 are displaying how well that antibody performs (neutralizes) against three pestiviruses. The mean SN titre of interest here is the blue line against a BVDV virus. Note that the confidence intervals here are for an effective dose 50 (ED50) not a ED95.
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Figure 1. NA antibodies against BVBD-I NADL strain, expressed as the effective dose 50% (ED50) calculated by the Spearman-Kaber method. Individual (black) and mean (red) neutralising antibodies (NA) titres in sheep vaccinated with half (A, Group 2) or the quarter (B, Group 3) dose of the Bovilis BVD vaccine. No NA were detected in the non vaccinated Group 1.

Figure 2. Mean NA titres against BDV (Aveyronite-2, 10F405, 10F6390) or BVD (NADL) strains, expressed as the effective dose 50% (ED50) calculated by the Spearman-Karber method. Sheep were vaccinated with half (Group 2) or the quarter (Group 3) dose of Bovilis BVD vaccine. No NA antibodies were detected in the non vaccinated Group 1. NA titres against 10F10405 and 10F6390 BVD isolates were similar.

Predictably this vaccine is likely to be more effective against BVDV virus in sheep than BDV virus, and day 28 titres are significantly lower in sheep receiving a lower dose of vaccine. Of note is that in the group receiving a ¼ dose, in 2 out of 6 sheep the antibody response was transient and declined rapidly after 42 days (Figure 1 – right graph). The effect of this on the ED 50 is obvious, particularly on the border disease isolates On the basis of this data it would seem that the right dose to choose in sheep is the half cattle dose, or 1ml of Bovilis-BVD vaccine in sheep. The timing of vaccination needs to be similar in principle to that used in cattle although in practice a longer lead in time is more beneficial to prevent the effect of yarding on ovulation in females and in males to ensure protection is robust at the time of joining. Looking at the range of ED50 in Group 2, even at day 168 there appears little risk of rapidly declining SN titres and therefore the vaccine is likely to be effective against BVDV well beyond the window of high risk in rams and for the 152 - 4 days gestation in ewes.

In our Wairarapa region, at-risk rams should be vaccinated in January and February in order that they are well protected by March and the risk of them obtaining an infection that could limit reproductive performance is reduced. Given the cost of this vaccine it’s use as routine prophylaxis is limited. I have used this vaccine in sheep safely with no adverse events but it is off-label use and therefore should be restricted to those cases with clear evidence of ovine pestivirus infection.

There were no adverse events associated with vaccination. Scanning, lambing, and docking percentage returned to normal the following season, and my client was extremely happy that the apparent prevalence had reduced so quickly. It is difficult to assess the efficacy under New Zealand conditions because with removal of persistently
infected cattle, and only a surviving group of lambs, the worst affected animals have died or have been culled, there is likely to be a much lower residual challenge on farm and we don’t have a measure of that. I would certainly not hesitate to use the vaccine again if faced with the same situation but whether or not this vaccine does protect pregnant ewes remains a question, that was not answered by this case study. A challenge study using PI cattle and vaccinated vs unvaccinated pregnant ewes would be more informative.

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