FOOD/FARMED ANIMALS

An outbreak of infectious bovine rhinotracheitis (IBR) in a herd vaccinated with a live glycoprotein E deleted (marker) bovine herpesvirus 1 (BoHV-1) vaccine: lessons to be learned

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Received 9 November 2016
Revised 17 March 2017
Accepted 20 March 2017

SUMMARY

Vaccines are commonly used in the control of bovine respiratory disease; however, the field performance of these vaccines is poorly understood. We describe an outbreak of infectious bovine rhinotracheitis in a 383-animal beef finishing unit in Scotland four months after vaccination with a live glycoprotein E deleted (marker) bovine herpesvirus 1 (BoHV-1) vaccine. Seroconversion to the vaccine was confirmed in acute sera, and seroconversion to field virus was confirmed in convalescent sera. BoHV-1 was also identified in bronchoalveolar lavage fluid and conjunctival swabs using PCR. This outbreak highlights the importance of the reporting of veterinary vaccine suspected lack of expected efficacy events, as well as the paucity of data available to practitioners relating to the field performance of veterinary vaccines.

BACKGROUND

Bovine respiratory disease (BRD) is a major cause of mortality, production loss, antimicrobial use and compromised animal welfare in cattle globally. On feedlots in the USA, production losses and treatment costs alone during a BRD outbreak (not accounting for time and labour) are estimated at approximately $14 per animal on the farm (Snowder and others 2006) or between $23 and $54 in carcase losses per clinically affected animal (Schneider and others 2009). In the UK, daily liveweight gain of cattle with lung lobe consolidation is estimated to be reduced by 72–202 g/day depending on the degree of consolidation, compared with cattle without any evidence of gross lung pathology (Williams and Green 2007). Recent economic analysis of the costs of BRD in the UK is not available; however, Andrews (2000) calculated an average loss per animal within an affected group of £43.26 for dairy and £82.10 for suckler calves. As BRD outbreaks are often complex and multifactorial, disease prevention can often be problematic (Edwards 2010); however, vaccination is a significant component of most prevention strategies in trying to reduce or mitigate economic losses and animal suffering caused by BRD.

Veterinary vaccines are typically developed and licensed using disease challenge models in small groups of animals under carefully controlled conditions. In the UK, field trials are required to demonstrate product safety, however due to difficulties with designing sufficiently powered studies may not demonstrate efficacy. Licensing data are rarely made public, although a detailed scientific discussion based on submitted data is available for a minority of veterinary vaccines available in the UK through the European Medicines Agency. Combined with limited data relating to the field efficacy of vaccines targeting BRD (Taylor and others 2010), practitioners predominantly rely on the Summary of Product Characteristics (SPC), pharmaceutical company representatives and their own experiences when making vaccination decisions (Richens and others 2016).

When investigating a suspected lack of expected efficacy (SLEE) event, it is often difficult for the practitioner to disentangle the performance of the product from the multitude of factors that may contribute to a BRD outbreak. Infectious bovine rhinotracheitis (IBR), caused by bovine herpesvirus 1 (BoHV-1), is a common pathogen involved in BRD in the UK (Graham 2013). Awareness of disease is relatively high within the industry, illustrated by a recent survey of UK beef and dairy herds where BoHV-1 vaccines were used in at least 45 per cent and 60 per cent of herds, respectively (Cresswell and others 2014). The widespread use of glycoprotein E (gE) deleted (marker) BoHV-1 vaccines that allow BoHV-1-naïve, vaccinated and exposed animals to be differentiated has facilitated the practitioner in determining whether BoHV-1 is the causative agent during a BRD outbreak (Ackermann and Engels 2006). Here we describe the diagnosis of an outbreak of IBR in a herd vaccinated with a live gE deleted BoHV-1 vaccine.

Case presentation

A calf fattening unit in the central region of Scotland was populated with 383 weaned springborn calves of various breeds from three markets between October 3, 2014 and November 3, 2014. The cattle were sourced from 96 farms in the Highlands and Islands of Scotland (1–26 calves/farm). On arrival on farm in October, the calves were administered a live gE deleted BoHV-1 vaccine and an inactivated *Mannheimia haemolytica* vaccine. Despite these products not being licensed to be administered concurrently, both vaccines were administered on the same day at different sites by intramuscular injection.

To cite: Tomlinson MS, Hopker A, Corbishley A. *Vet Rec Case Rep* Published Online First: [*please include* Day Month Year]. doi:10.1136/vetreccr-2016-000402

The use of unlicensed vaccine combinations is common in veterinary medicine, and in many systems is the only practical route by which animals can complete a vaccination course before the risk period for disease. While work in veterinary species is limited, there is a strong body of evidence within the human literature to support the simultaneous administration of vaccines, and that there is no increase in either vaccine failure rates or adverse events when vaccines are administered concurrently (CDC 2016). The SPC for the live gE deleted BoHV-1 vaccine used states that 'a decision to use this vaccine before or after any other veterinary medicinal product therefore needs to be decided on a case by case basis'. This was done so in this herd, in conjunction with the market authorisation holder, and therefore the use of the vaccine as described in this case report is compliant with the SPC.

The animals also received a 10 per cent fenbendazole oral drench at 7.5 mg/kg. The animals were then housed for five days and fed a mix of ad libitum silage and straw. The animals were then turned out on to grass/stubble, where they were trained to eat conserved forage with a gradual increased access to ad libitum silage and straw, and trough-fed concentrate mix at 2.5 kg/head. The home-made concentrate mix was approximately 80 per cent barley, 20 per cent brewer's grains and 150 g per head of a general purpose beef finisher mineral.

The animals were housed in December and continued on the same feeding regimen. Three hundred animals were housed in a single airspace in four groups of 75 animals with two pens either side of a central feed trough. The remaining animals were in separate airspaces in groups no larger than 30. On housing, they all received a multivalent live intranasal parainfluenza virus 3 (PI3) and bovine respiratory syncytial virus (BRSV) vaccine. Two weeks later these animals had their backs clipped, pour-on ivermectin administered at 500 μg/kg and a 10 mg/kg subcutaneous injection of nitroxynil.

Investigations

The Farm Animal Practice at the Royal (Dick) School of Veterinary Studies was contacted in early February by the farmer due to a higher than expected incidence of pneumonia. Thirty individual animals in a separate airspace had been noted by the farmer to have poor feed intakes, hypersalivation and a moist cough, with approximately 50 per cent of the animals within the group being pyrexic. The farmer had undertaken metaphylaxis of the group with long-acting oxytetracycline at 20 mg/kg and meloxicam at 0.5 mg/kg. He noted that clinical signs resolved within approximately 48 hours, apart from a few animals with a persistent moist cough.

Approximately one week later the farmer reported a number of animals in a pen of 75 (in the shared airspace) presenting with similar clinical signs as seen previously. At this stage the farmer sought veterinary advice. The farmer provided a history of a similar disease outbreak the previous Christmas. However as the outbreak occurred over Christmas Eve and Christmas Day, a full investigation had not been undertaken and whole-farm metaphylaxis had been implemented.

On examination, the calves in question appeared to be in good body condition and the housing was well ventilated. More than 50 per cent of the animals in the affected group were pyrexic, with a rectal temperature greater than 40°C. Several animals were observed to be hypersalivating, with a mild serous ocular discharge and light cough. A number of animals remained distant from the feed face, and the farmer reported a lack of appetite and reduced feed intakes for the previous 48 hours. One calf examined was extremely dyspneic, exhibiting excessive upper respiratory tract noise and marked respiratory effort.

As the separate group of 30 animals on farm had already been successfully treated for pneumonia by the farmer and over 50 per cent of the animals examined were pyrexic, it was recommended that the affected group should be treated metaphylactically for primary/secondary bacterial pneumonia with 20 mg/kg long-acting oxytetracycline by intramuscular injection and 0.5 mg/kg meloxicam by subcutaneous injection, and that the farmer should be prepared to administer the same metaphylactic treatment to any subsequently affected groups if necessary. To minimise the risk of pathogen spread, no movement of stock was to occur between groups in the shared airspace, or of at-risk animals from the affected airspace to other groups on the farm.

Differential diagnosis

Primary respiratory disease was caused by the following:

- BoHV-1
- BRSV
- PI3
- Pasteurella multocida
- Mycoplasma bovis/dispar

Respiratory disease secondary to concurrent immunosuppression was due to the following:

- Bovine viral diarrhoea virus (BVDV)
- Fascioliasis
- Environmental, nutritional or husbandry stressors

Treatment

Further investigation and ancillary testing

Bronchoalveolar lavage (BAL) was performed on three animals and submitted to the local veterinary diagnostic labs that day for viral PCR (BoHV-1, BRSV and PI3) and bacterial culture and sensitivity. Serum and faeces were collected from these three animals, as well as a further three calves. Animals selected for these samples were acutely affected, previously untreated and noticed as not feeding that morning, with a rectal temperature of greater than 40°C and tachypnea, but no nasal discharge.

Faecal worm egg counts and fluke sedimentation were negative when assessed that evening in the practice laboratory. Serum samples were stored in a freezer for the assessment of paired serology three weeks later.

Four days after the initial reported outbreak, one animal from the original affected group died. A field postmortem revealed inflammation of the lungs, larynx and pleural surfaces. The trachea was filled with a necrotic diphtheritic exudate containing caeseous suppurative material. Two conjunctival swabs were taken, one from the dead animal and another from an additional animal presented for clinical examination, and submitted for respiratory virus PCR (BoHV-1, PI3 and BRSV). No other samples were submitted from these two animals. During this visit, the farmer had remarked that the mild clinical signs seen in the initial outbreak had been observed in three of the four groups housed in the affected airspace, and metaphylactic treatment within these groups had been undertaken.

The results from the BAL were available five days after the initial outbreak. All animals were negative for BRSV and PI3. One animal was positive for BoHV-1, and *P multocida* (sensitive to all antibiotics tested except tylosin) was cultured from another animal. The conjunctival swab from the live animal was also found to be positive for BoHV-1. The conjunctival swab from the dead animal was negative for BoHV-1. A presumptive diagnosis of primary IBR was made.

TABLE 1 Paired serology results for six acutely affected animals

	Mycoplasma bovis		IBR gB		IBR gE		BVDV		PI3		BRSV	
Animal	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
1	–	–	+	+	+	+	–	–	+	+	+	+
2	–	–	+	++	–	+	–	–	+	+	+	+
3	–	–	+	++	–	+	–	–	+	+	+	+
4	–	+	+	++	–	+	–	–	+	++	+	+
5	–	+	+	++	+	++	–	–	+	+	+	+
6	–	–	+	+	–	+	–	–	+	+	+	+

The symbols + and ++ denote a positive or rising antibody titre.
BRSV, bovine respiratory syncytial virus; BVDV, bovine viral diarrhoea virus; g, glycoprotein; gB, glycoprotein B; gE, glycoprotein E; IBR, infectious bovine rhinotracheitis; PI3, parainfluenza 3; pre, acute sera; post, convalescent sera.

A live gE deleted BoHV-1 vaccine was administered intranasally to all animals on farm. In total, 280 animals were treated with oxytetracycline and meloxicam. The farmer reported that clinical signs were significantly reduced approximately 48 hours after treatment and that no new cases occurred. Eight animals developed chronic disease and were described as 'persistent coughers' by the farmer. Feed intakes returned to normal approximately two weeks after treatment. Overall one animal death was reported and eight affected animals developed clinical signs consistent with chronic suppurative pneumonia (illthrift, suppurative nasal discharge, persistent cough with excessive abdominal effort and increased respiratory rate). These chronic cases were placed on a four-week course of daily intramuscular procaine penicillin at 10 mg/kg. In total, 1.7 kg of oxytetracycline, 50 g of meloxicam and 600 g of procaine penicillin were used during the outbreak.

OUTCOME AND FOLLOW-UP

Definitive diagnosis

Paired serology was completed after obtaining a second serum sample three weeks after the initial outbreak. The results (Table 1) demonstrate that all of the animals were seropositive to BoHV-1 glycoprotein B (gB), while two of the animals were seropositive to BoHV-1 gE before the outbreak, hence indicating that four of the animals were naïve to field virus but had been vaccinated. Five of the six animals seroconverted to BoHV-1 gE during the outbreak, hence demonstrating an immune response to the field virus.

All of the animals were seronegative to BVDV and seropositive to PI3 and RSV before the outbreak, which is consistent with vaccination and/or natural exposure. No animals demonstrated a rising titre to BRSV, while only one animal demonstrated a rising titre to PI3. Two of the six animals seroconverted to *M bovis* during the outbreak. Experimental studies have shown that BoHV can exacerbate respiratory disease due to *M bovis* (Prysliak 2011). A diagnosis of a primary breakdown of IBR in a live gE deleted BoHV-1 vaccinated herd was made.

The farmer was advised to alter his vaccination regimen in future years as follows: intranasal administration using a live gE deleted BoHV-1 vaccine upon arrival in October and a second intramuscular administration of the same vaccine at housing in December. This protocol is advised by the SPC for use of the vaccine in animals 'at immediate risk of IBR' and was implemented in 2015. No respiratory disease has since been observed or reported by the farmer, while total mortality in the 2015/2016 housing period was 1 per cent. It is worth noting that the single dose vaccination protocol used before the outbreak was in accordance with the SPC's advice on vaccine administration to calves over three months of age.

DISCUSSION

A suspected adverse reaction (SAR) to a veterinary pharmaceutical product is any observation in animals that is unfavourable and unintended and that occurs after any (label or off-label) use of a veterinary medicine. This includes SLEE events or reactions in human beings (ANON 2007). Of the 399 Veterinary Medicines Directorate (VMD) recorded adverse events in UK cattle during 2014, 168 (42 per cent) were SLEE events and 141 (84 per cent) were related to vaccines (ANON 2016). Unfortunately, the VMD does not report the name of the products involved or the sales volumes of each product.

To the authors' knowledge, the annual pharmacovigilance review by the VMD (ANON 2016) is the only data describing vaccine SARs or SLEE events in the UK. These limited data are broken down by species and then by product groups only, with a brief description of predominant clinical signs and a few comments describing general trends. No details of suspected predisposing factors for SLEE events or confirmed case related data are available. The currently available data provide little guidance for a practitioner dealing with cases on their clients' farms. The data relating to these SARs must be recorded as they are reported to the competent authority (the VMD in the case of the UK) and the marketing authorisation holder. Specific data related to SARs and SLEE events will also be held by product manufacturers obtained during field trials conducted when a product is licensed. Until this information is made publicly available for all products in the market, practitioners will not possess the necessary information to make informed decisions regarding the use of veterinary vaccines.

Due to the differences in veterinary vaccines used in the USA and the EU, case-based data relating to SSLE events from the USA are of limited relevance to practitioners within the EU. There has been some discussion in the literature regarding the appropriate investigation of SLEE events related to BoHV-1 vaccination. Allcock and others (2010) have reported two SLEE events in dairy herds vaccinated using a live marker BoHV-1 vaccine. These cases were diagnosed on the basis of clinical signs, response to booster vaccination and fluorescent antibody testing (FAT) of conjunctival swabs. Penny (2013) noted that BoHV-1 FAT testing has a poor specificity, and outlined the importance of investigating, diagnosing and reporting SLEE events correctly, specifically that confirmation of active BoHV-1 circulation requires serological testing for BoHV-1 gE and gB titres, as well as the use of PCR from either BAL fluid, nasopharyngeal swabs

or postmortem samples. Due to epithelial destruction as the disease progresses, BoHV-1 is often not isolated from animals that have died during an IBR outbreak, with histopathology of the respiratory tract also often unrewarding. This highlights the importance of sampling animals early in the disease course and underpinned the rationale behind performing BALs on carefully selected animals in the acute stages of infection in this outbreak. To improve the chances of a satisfactory diagnosis, the authors would recommend that postmortem examinations are undertaken at a recognised veterinary investigation centre; however, this was not feasible in this outbreak. A definitive aetiological diagnosis for the animal that died cannot therefore be made; however, the gross postmortem findings and testing of other animals within the same management group support a presumptive diagnosis of IBR. To the authors' knowledge, this is the only published case report of an SLEE in a BoHV-1-vaccinated herd to use both PCR and serology to confirm circulating BoHV-1 as the primary pathogen related to the clinical signs seen. This highlights the need to increase the reporting of SLEE investigations using appropriate diagnostic tests. Only then can the predisposing factors leading to SLEE events be thoroughly investigated and the field performance of veterinary vaccines understood.

In this case, a presumptive diagnosis was achieved within five days by PCR following BAL and conjunctival swabs, which informed targeted herd management decisions. The BoHV-1 viral PCR used is unable to distinguish between field and vaccine virus (F Howie SAC, personal communication), hence the importance of serology in confirming the active cycling of field virus. More rapid diagnosis would have allowed these decisions to be made earlier and would have reduced the amount of antimicrobials used in this outbreak. This illustrates the need for rapid diagnostic tests to avoid inappropriate antimicrobial use. We also note that only one of the three BAL samples was BoHV-1 virus-positive, hence highlighting the need to select an appropriate sample size and the importance of serological surveillance.

The use of a gE deleted vaccine allowed a more granular analysis of the serological data by differentiating between vaccination and field virus exposure, hence confirming that field virus was actively cycling and infecting naïve animals. This highlights the necessity of using marker vaccines in the control and surveillance of BoHV-1 and that if vaccines are available that allow differentiation between infected and vaccinated individuals that these should be used preferentially.

Two of the six animals involved in the serological testing converted to *M bovis* during the outbreak. The role of *M bovis* as a primary or secondary pathogen in this outbreak warrants discussion. Prysliak and others (2011) described how six-month to eight-month-old calves were more likely to develop clinical disease related to *M bovis* after exposure to BoHV-1. Given that only two of the six animals tested seroconverted to *M bovis* compared with five of the six seroconverting to BoHV-1, *M bovis* is more likely to have been a secondary pathogen in this outbreak.

The SPC for the vaccine used before this outbreak notes that 'After a single dose vaccination, a significant reduction of virus shedding duration has been demonstrated upon challenge for six months. After two doses of vaccine, the intensity and duration of clinical symptoms as well as the titre and duration of virus shedding are significantly reduced following infection'. This outbreak occurred approximately four months after a single injection; therefore, it could be argued that the vaccine was performing according to the expectations of the SPC by reducing viral shedding but not necessarily the intensity and duration of clinical signs. That said, the vaccine did not perform according to the client's and prescribing veterinary surgeon's expectations. This was reported to the market authorisation holder who supported the investigation of this outbreak, provided additional vaccine free of charge and reported the event to the VMD.

Immunosuppression either at the time of vaccination or the time of the outbreak could have been a contributory factor to this outbreak. While the acute sera demonstrated seroconversion to the vaccine, only a small proportion of the herd was sampled, while serology gives no indication as to the avidity of the antibody response or magnitude of the T cell response following vaccination. The possibility of a 'poor quality' response following initial vaccination due to concurrent disease or immunosuppression cannot therefore be excluded.

Investigations at the time of the outbreak failed to identify any other concurrent diseases or potential causes of immunosuppression. The growth rate and body condition score of the calves before the outbreak were appropriate as was the ration and minerals on offer. Furthermore, abattoir reports showed that active liver fluke was present in less than 2 per cent of animals at slaughter, while faecal worm egg count and fluke sedimentation tests indicated that concurrent immunosuppression caused by parasitism was unlikely. Metabolic profiling was not undertaken and may have identified negative energy balance at the time of the outbreak, but given the lowered feed intakes due to respiratory disease, it would not have been possible to determine whether any negative energy balance was primary or secondary to the clinical outbreak.

The stocking density, air quality and ventilation were assessed and deemed to be satisfactory for the main shed housing 300 animals. Poor ventilation and air quality could have been a contributory factor to the disease observed in the separate airspace housing the remaining 83 animals. The farmer reported going on holiday before the outbreak starting and was concerned that a change in management and routine may have occurred during this period. Nothing unusual was reported by the farm staff, and it is the authors' opinion that it is unlikely that this precipitated the outbreak.

The prevention of BoHV-1 circulation within a herd should ideally be achieved by appropriate biosecurity measures and protection of stock from pathogen exposure. Where possible, herds should be 'closed' and bought-in stock should be from a herd known to be negative for BoHV-1. Where the status of the herd of origin is unknown, bought-in animals should be isolated and tested for BoHV-1 antibodies and then segregated depending on risk (Van Winden and others 2005). With this in mind, vertical integration of farming systems may help to improve biosecurity and mitigate disease risk (Kahan 2013). That said, the business model of the farm in this case report relies on purchasing calves from a large number of crofters in the north-west of Scotland. These units invariably do not know their disease status and there is a strong tradition of selling calves through markets, where they may be exposed to a variety of pathogens. Within this context, discussions relating to biosecurity have not been tractable and the use of vaccines has become the mainstay of BoHV-1 control.

The economic impact of this outbreak, excluding labour, is summarised in Table 2. The reduced liveweight gain is calculated as a result of the overall reduced feed intakes for 383 animals over a two-week period. As no animals were weighed during the outbreak and animals were only weighed at the start and end of the housing period (as is common practice), a conservative estimate reduction in daily liveweight gain of 0.5 kg/day and the 2015 average market value of approximately £1.80 per kg of liveweight have been used.

TABLE 2 Approximate costs incurred during the disease outbreak.

Initial vaccine costs	£1271
Total treatment spend	£6966
Oxytetracycline	£2856
Procaine penicillin	£360
Meloxicam	£3750
Repeat vaccination	£1271
Total POM-V spend	£9502
Reduced liveweight	£5040
Death of one animal	£1000
Vet fees	£278
Diagnostics	£344
Total cost of this IBR outbreak	£16 164

IBR, infectious bovine rhinotracheitis; POM-V, Pescription Only Medicine-Veterinarian.

Had the revised vaccination programme been implemented before the outbreak in December 2014, the farm would have saved £13 662, assuming effective vaccine efficacy.

CONCLUSION

When investigating an SLEE event, it is often difficult for the practitioner to disentangle the performance of the product from the multitude of factors that may contribute to a BRD outbreak. Penny (2013) noted the importance of investigating, diagnosing and reporting SLEE events correctly. The currently available data provide little guidance for practitioners dealing with cases on their clients' farms, and limit decision making and appropriate herd health planning. This can ultimately impact animal welfare and farm profitability when such disease breakdowns do occur. This case report not only reviews the impact of one such breakdown, but also highlights the need for more data surrounding the subject to be made available to the general practitioner.

Correction notice This paper has been amended since it was published Online First. Owing to a scripting error, some of the publisher names in the references were replaced with 'BMJ Publishing Group'. This only affected the full text version, not the PDF. We have since corrected these errors and the correct publishers have been inserted into the references.

Competing interests None declared.

Provenance and peer review Not commissioned; externally peer reviewed.

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Vet Rec Case Rep 2017 5:
doi: 10.1136/vetreccr-2016-000402