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Outbreak of post-parturient infectious pustular vulvovaginitis in a New Zealand dairy herd

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ABSTRACT

Case history: In 2023, 160/245 (65%) 2-year-old KiwiCross dairy heifers from a seasonally calving Otago herd developed severe granular vulvovaginitis after calving.
Clinical findings: Affected heifers presented 3–12 days post-calving with tail elevation, vaginal discharge and, in most cases, vulval swelling. Heifers were afebrile although some were inappetent. Vaginal examination revealed severe mucosal inflammation, often featuring erosions and pustules. The heifers responded to treatment with systemic antibiotics and anti-inflammatories. The farm veterinarian became aware that milkers were blowing air through an alkathene pipe inserted into the vagina of some heifers, during milking, to improve milk let-down. The veterinarian advised the farm manager that the practice is prohibited in New Zealand, and it ceased immediately.
Laboratory findings: An initial 10 vaginal swabs from affected heifers gave positive results in PCR assays for bovine herpesvirus type 1 (BoHV-1) and *Ureaplasma diversum*. Subsequent samples requested by the Ministry for Primary Industries from four affected heifers included vaginal swabs and biopsies, and paired blood samples, 4 weeks apart. Of the swabs, 3/4 gave positive results in a BoHV-1 PCR assay, 4/4 gave positive results in a *U. diversum* PCR assay, 2/4 gave positive results in a *Mycoplasma bovigenitalium* PCR assay, and 1/4 gave a positive result in a bovine herpesvirus type 4 (BoHV-4) PCR assay. All gave negative results in a pan-poxvirus PCR assay. Histology revealed severe, chronic, ulcerative and neutrophilic vaginitis with granulation tissue in all biopsies and immunohistochemistry confirmed the presence of BoHV-1 in 2/4 (50%) biopsies. All four heifers had a rising BoHV-1 virus neutralisation test titre. Aerobic culture was unremarkable, while biochemistry found that the mean serum albumin, total protein, calcium, creatinine, sodium, and chloride concentrations were all just below the normal reference range, and haematology found that 3/4 (75%) affected heifers had mildly elevated fibrinogen concentrations.
Diagnosis: Infectious pustular vulvovaginitis, likely involving a mixed infection of BoHV-1, *U. diversum* and *M. bovigenitalium.*
Clinical relevance: Infectious pustular vulvovaginitis can occur post-calving, and mixed infections should be suspected in severe outbreaks. Vaginal insufflation likely contributed to this outbreak, and veterinarians need to be aware of and vigilant for this prohibited practice.

Abbreviations: AHL: Animal Health Laboratory; BoHV-1: Bovine herpesvirus type 1; BoHV-4: Bovine herpesvirus type 4; IBR: Infectious bovine rhinotracheitis; IPV: Infectious pustular vulvovaginitis; MPI: Ministry for Primary Industries; VNT: Virus neutralisation test.

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Cattle; post-parturient; infectious pustular vulvovaginitis; bovine herpesvirus type 1; *Ureaplasma diversum; Mycoplasma bovigenitalium;* vaginal insufflation

Introduction

Bovine herpesvirus type 1 (BoHV-1) causes two distinct, contagious diseases in domestic and wild cattle worldwide: a respiratory disease called infectious bovine rhinotracheitis (IBR), and a genital disease called infectious pustular vulvovaginitis (IPV) (Wang *et al.* 2006; Lawrence 2012). In mild cases, cattle affected with IBR usually present with rhinitis, conjunctivitis, and a slight pyrexia, however in more severe cases they can develop a high fever, become inappetent, depressed, have a profuse nasal discharge which is initially clear progressing to mucopurulent, and a severe cough (Horner 1990; Parkinson *et al.* 2010). Female cattle affected with IPV usually develop clinical signs within a few days of mating, with frequent urination and tail flicking seen in the early stages. The vulva and posterior vagina may be hyperaemic and oedematous, with small red to white ulcers and pustules (0.5–3 mm in diameter)

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developing, referred to as a granular vulvovaginitis. If secondary bacterial infection occurs, a thick, white to yellow, mucopurulent discharge may also be seen (Gu and Kirkland 2008; Parkinson *et al.* 2010). Animals that recover from IBR or IPV are latently infected, probably for life, with virus surviving in the trigeminal ganglia or the sacral ganglia, respectively (Gu and Kirkland 2008).

Both conditions are seen in New Zealand, but the clinical signs are usually mild, even sub-clinical, although some more serious outbreaks and deaths in 2-year-old dairy heifers associated with IBR have been reported (Newton *et al.* 2021). This contrasts with the situation abroad, where more severe respiratory signs and death are more often seen with IBR infection (Wang *et al.* 2006).

In addition to respiratory disease, cattle overseas can also abort when infected with BoHV-1. Abortion attributable to BoHV-1 has not been reported in New Zealand and, historically, researchers have been unable to induce abortion using the New Zealand viral strain (Durham *et al.* 1975).

Bovine herpesvirus type 1 can be classified into three subtypes, BoHV-1.1, BoHV-1.2a and BoHV-1.2b, and more recent studies have confirmed that the predominant strain of BoHV-1 present in New Zealand is BoHV-1.2b. There is no evidence that the more virulent BoHV-1.1 and BoHV-1.2a strains, which are associated with severe respiratory infections and abortions overseas (Muylkens *et al.* 2007), are present in New Zealand (Wang *et al.* 2006).

The results of a recent serological survey amongst dairy herds from Canterbury and Otago, which found 100% of herds surveyed to be positive (Holloway 2019), agrees with an earlier study which concluded that BoHV-1 infection is widespread in New Zealand dairy cattle (Motha and Hansen 1998). Interestingly, heifers are often naïve until they enter the dairy herd or, if they have been previously exposed to BoHV-1, have a much lower seroprevalence than older cows in the same herd (Young and Compton 2018; Holloway 2019). Severe outbreaks of post-mating BoHV-1-associated IPV, with up to 70% of heifers affected, have previously been reported in New Zealand (Thompson and Orr 1992; Varney and Gibson 2007; Anonymous 2013). However, severe outbreaks of IPV, also attributed to BoHV-1, have been reported in freshly calved heifers and cows (Hill *et al.* 2000; Anonymous 2010), which are unlikely to be venereally acquired at the time but could be due to recrudescence of infection acquired earlier.

Other major causes of vulvovaginitis in cattle are the bacteria *Ureaplasma diversum*, and *Mycoplasma bovigenitalium* and, rarely, parapoxvirus. Infections with these organisms often cause a granular vulvovaginitis where tiny red nodules are seen on the vestibular mucosa due to hyperaemia and hyperplasia of the lymphoid nodules present in this tissue (Hunter *et al.* 1958). Both *U. diversum* and *M. bovigenitalium* are common commensal organisms that can be associated with a granular vulvovaginitis (Thornton and Wake 1997; Moeller *et al.* 2018; Díaz *et al.* 2019) and can also co-infect with BoHV-1. Severe outbreaks of vaginitis in New Zealand heifers, associated with *U. diversum,* have also been seen (Anonymous 2018, 2019).

Parapoxvirus-associated vulvovaginitis in post-partum cows has been described in California (Moeller *et al.* 2018) and in Sweden (Blomqvist *et al.* 2018). In several animals in the Californian outbreak the small pustules and ulcers that formed initially coalesced, leading to deep eroding ulcers affecting > 50% of the vaginal mucosa, sometimes requiring euthanasia on humane grounds. The Swedish outbreak featured vesicles, erosions, papules and scabs on the vulva and vaginal mucosa. Interestingly, whereas BoHV-1, *U. diversum* and *M. bovigenitalium* usually require venereal transmission (Parkinson *et al.* 2010), in the parapoxvirus-associated outbreaks, mechanical transmission was deemed most likely.

This case study describes an investigation into a severe outbreak of post-partum vulvovaginitis in a herd of recently calved dairy heifers in the lower South Island and provides strong evidence for the involvement of BoHV-1.

Case history

The outbreak occurred in a 770-head, Friesian–Jersey cross (KiwiCross: LIC, Hamilton, NZ), seasonally calving dairy herd in Otago, which included 245 replacement 2-year-old heifers. Only the 2-year-old heifers appeared to be clinically affected. The heifers had been naturally mated by home-reared Jersey bulls at two dairy support blocks. The planned start of calving for the heifers was 1 August 2023, with the cows starting on 6 August. For calving, the heifers and early calving cows were kept on a woodchip calving pad that had been designed for 150–170 animals. They were fed a diet of straw and rye grass baleage and let out daily onto a paddock for a break of rye grass pasture. It was later realised that the heifers had been mainly eating straw, and the more dominant older mixed-age cows were consuming most of the baleage.

The first case was noticed on about 10 August, and by 15 August, approximately 50/90 (56%) of the recently calved heifers were identified by farm staff as affected. While some heifers had clinical evidence of vulvovaginitis at the time of calving, a case typically presented 3–12 days post-calving, with affected heifers being more restless than expected at milking. The first veterinary visit was on 15 August, with subsequent visits on 22 and 28 August, and 11 and 28 September.

Clinical findings

Fifty-three calved heifers were yarded at the first veterinary visit. Affected heifers presented with tail elevation (sometimes accompanied by tenesmus). There was a vaginal discharge (sometimes foul-smelling) in all cases and swelling of the vulva in most cases. At veterinary examination, where temperature was recorded, no affected heifers were pyrexic, although some heifers were anorexic. Closer inspection of the vaginas of affected heifers showed that most had a severely inflamed vaginal mucosa, often with small lines of erosions and pustules on either side (Figure 1 (A, B)). In some heifers, the vaginal mucosa was so swollen and roughened that it resembled the genital tract of an animal after dystocia, and it was difficult to introduce a gloved and lubricated hand. However, when questioned, the herd manager reported that only about 3% of heifers had required assistance at calving. In some cases, fragments of necrotic material detached from the vaginal wall at examination. Although only 2-year-old heifers appeared to be affected, in delivering a 3-week premature calf from a 3-year-old cow at this visit, the farm veterinarian noted similar vaginal lesions in the cow to those seen in the heifers. Interestingly, one heifer, which was not showing overt clinical signs, also had vaginal lesions on examination.

The farm veterinarian was advised that poor milk let-down was an issue in the first-calved heifers and that milkers had attempted to mitigate this by blowing air through an alkathene pipe inserted into the vagina of these heifers during milking.

Treatment

Prior to the first veterinary visit, the farm manager had been treating affected heifers by administering two intrauterine oxytetracycline pessaries (Oxyfoam Forte containing 1 g oxytetracycline chloride; Virbac New Zealand Ltd., Hamilton, NZ). Two heifers that had shown similar clinical signs before the veterinary visit had died but were not available for post-mortem examination. These two cases were assumed to have developed septicaemia, and parenteral treatment of all new and existing cases commenced. For heifers that had been given intrauterine pessaries, 5 mg/kg oxytetracycline (Engemycin; MSD Animal Health, Upper Hutt, NZ) was given IM once daily for 4 days. As-yet untreated heifers were administered 15 mg/kg of long-acting amoxycillin (Betamox LA, Norbrook NZ Ltd., Auckland, NZ; or Vetrimoxin LA, Ceva Animal Health (NZ) Ltd., Auckland, NZ) IM, with two doses, 48 hours apart. All affected heifers were administered 0.5 mg/kg meloxicam (Rheumocam; Ethical Agents Ltd., Auckland, NZ) SC. Seven heifers were given an intrauterine administration of 500 mg cephapirin (Metricure; MSD Animal Health). The farm veterinarian advised that the use of an alkathene pipe and vaginal insufflation to stimulate milk let-down should cease immediately.

At the second veterinary visit, 1 week later, the number of new cases had decreased. The original cases had responded, and any new cases were responding, to the parenteral treatment protocol.

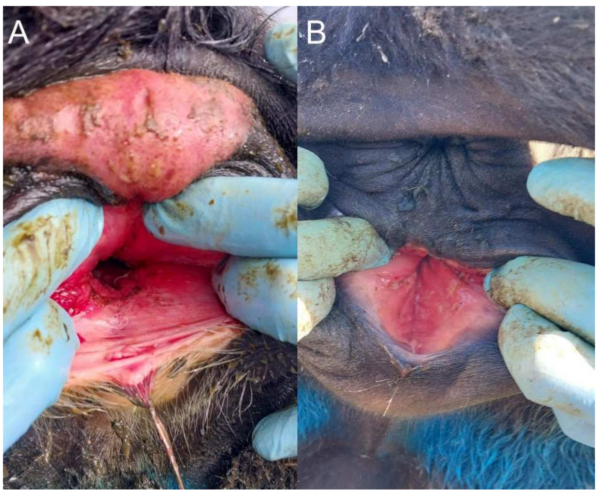

Figure 1. (A, B) Images of the vagina from two affected heifers with infectious pustular vulvovaginitis. The heifer in (A) also had prolapsed the lining of the terminal rectum.

Initial laboratory findings

Based on the clinical signs, the farm veterinarian made a provisional diagnosis of infectious pustular vulvovaginitis. Ten vaginal swabs were taken from treated (n = 5) and as-yet untreated (n = 5) affected heifers on 22 August. These were sent to Awanui Veterinary (Dunedin, NZ), pooled according to whether the heifer had been treated with antibiotics or not and tested for BoHV-1 (Wang *et al.* 2007, 2008) and *U. diversum* (Marques *et al.* 2013) using real-time PCR. Both the treated and untreated pools gave positive results in the PCR assays for BoHV-1 and for *U. diversum.*

Subsequent management

Following confirmation of the involvement of BoHV-1 and concerned that there could be an exotic strain of BoHV-1 involved, the farm veterinarian called the MPI exotic pest and disease hotline and discussed the case with the duty incursion investigator (JPOC). While an exotic strain of BoHV-1 was considered less likely, the incursion investigation team directive includes the investigation of suspect new and emerging diseases. That possibility, coupled with the scale of the outbreak, merited MPI assistance with the investigation.

Sampling protocol

A sampling plan was agreed with MPI and on 28 August, 2 weeks after the initial visit, the farm veterinarian (HRM) took a more complete set of samples. Punch biopsies of the vaginal mucosa, collected into 10% buffered formalin, were taken from four affected heifers, #3, #8, #12 and #635. At the same time blood samples, plain (serum) and EDTA, and three sets of vaginal swabs were also taken from the four affected heifers. One set of vaginal swabs was placed in virus transport medium. In addition, four randomly selected, apparently unaffected mixed-age cows (#778, #48, #118 and #169) were also blood sampled into plain tubes and a contemporaneous vaginal examination was performed on these animals. One (#169) had mild IPV-like intravaginal lesions. A repeat blood sample into plain (serum) tubes was taken from the affected heifers and unaffected cows just over 4 weeks later on 28 September.

Laboratory findings

One set of dry vaginal swabs were sent to Awanui Veterinary Dunedin for PCR testing for *U. diversum,* again using the method by Marques *et al.* (2013), along with the blood samples for haematology and biochemistry and the set of punch biopsies for histopathology. All four vaginal swabs gave positive results in the PCR assay for *U. diversum*. The haematology results were unremarkable except that 3/4 affected heifers had mildly elevated fibrinogen concentrations, and the biochemistry results showed that the mean serum albumin, total protein, calcium, creatinine, sodium, and chloride concentrations were all just below the normal reference range (Table 1).

The histopathology findings were consistent: all four heifers had severe, chronic, ulcerative and neutrophilic vaginitis with granulation tissue. Viral inclusion bodies were observed in one biopsy from heifer #8.

Further slides were also prepared from each punch biopsy for BoHV-1 immunohistochemistry, which was conducted at the University of Guelph (Guelph, ON, Canada), using an anti-BoHV-1 monoclonal antibody. The results showed that tissue samples from heifers #8 and #12 were positive for BoHV-1.

Table 1. Significant serum biochemistry results identified by Awanui Veterinary (Dunedin, NZ) in four 2-year-old dairy heifers affected with severe vulvovaginitis post-calving.

Test	Reference range	Heifer identification number 3	8	12	635	Mean
Fibrinogen (g/L)	2–7	7	7	10	7	7.8
Albumin (g/L)	27–42	25	22	30	26	26
Total protein (g/L)	60–81	52	49	62	53	54
Calcium (mmol/L)	2.00–2.70	1.66	1.81	2.22	1.74	1.86
Creatinine (µmol/L)	39–181	23	25	30	31	27
Sodium (mmol/L)	136–146	104	119	139	106	117
Chloride (mmol/L)	90–104	71	83	97	73	81

The second set of dry vaginal swabs was submitted to the Animal Health Laboratory (AHL; Wallaceville, NZ) for testing for *M. bovigenitalium* by real-time PCR (Parker *et al.* 2017). They were also subjected to a conventional PCR with primers specific for the bacterial class *Mollicutes* (which includes, among others, the genera *Mycoplasma, Ureaplasma* and *Acholeplasma*; Trachtenberg 2005) using a method adapted from van Kuppeveld *et al.* (1992). DNA sequencing of the resulting amplicons was used to identify the species detected. The results of this molecular testing showed that 2/4 heifers were positive for *M. bovigenitalium* and 3/4 were positive for the *Mollicutes* group, with either *Ureaplasma* sp. or *M. bovigenitalium* being identified by DNA sequencing (Table 2).

The second set of dry vaginal swabs was also used for general aerobic culture at AHL with any subsequent bacterial isolates identified using matrix-assisted laser desorption/ionisation (MALDI-TOF) mass spectrometry, in addition to biochemical and motility tests. Aerobic culture yielded light mixed growth for all four heifers, with no significant pathological organisms identified.

The third set of vaginal swabs in virus transport medium were also submitted to AHL for viral PCR testing and virus isolation. A BoHV-1 quantitative PCR was conducted based on the method described by Abril *et al.* (2004), and a pan-poxvirus (Subfamily *Chordopoxvirinae*) conventional PCR using the method described by Li *et al.* (2010). The molecular testing showed that 3/4 heifers gave positive results in the PCR assay for BoHV-1, while the fourth (#635) gave a suspect result, and that all four heifers gave negative results in the pan-poxvirus PCR assay.

Virus isolation consisted of two passages of virus transport medium through bovine embryonic lung (BL12) cells. For samples where virus isolation was successful, DNA extracts were prepared for whole genome sequencing using the Nextera XT library preparation kit (Illumina, San Diego, CA, USA), quality checked, and then sequenced on the Illumina MiSeq system using a 2×251 bp paired-end sequencing design. A bovine herpesvirus-4 (BoHV-4) conventional nested PCR test, as described by Díaz-Cao *et al.* (2020), was then conducted on the viral isolates and the original

Table 2. Results from the Animal Health Laboratory (Wallaceville, NZ) for *Mycoplasma bovigenitalium* and Mollicutes group molecular testing on vaginal swabs from four 2-year-old dairy heifers affected with severe vulvovaginitis post-calving.

Heifer identification number	*M. bovigenitalium* PCR	Mollicutes group PCR and sequencing
3	Negative	*Ureaplasma* sp.
8	Positive	*M. bovigenitalium*
12	Positive	*M. bovigenitalium*
635	Negative	Inconclusive

dry vaginal swabs to check whether BoHV-4 was present as a mixed infection with BoHV-1. Viral isolation gave positive results for samples taken from heifers #8, #12 and #635, and whole genome sequencing identified that heifers #8 and #12 were infected with BoHV-1.2b and that heifer #635 was infected with BoHV-4. The BoHV-4 nested PCR on the viral isolates confirmed that heifer #635 was infected with BoHV-4 and that heifers #8 and #12 were not co-infected with BoHV-4. BoHV-4 nested PCR testing on the original vaginal swabs confirmed this finding.

Antibody measurements for BoHV-1 on the paired serum samples were also conducted at AHL using a BoHV-1 virus neutralisation test (VNT) accredited by International Accreditation New Zealand. Sera were double-diluted from 1:1 in a 96-well microplate format and incubated overnight (16 hours) at 37°C with 100 $TCID_{50}$ of BoHV-1 isolate 75.0114. Bovine lung cells (BL12) were then added to serum wells and cultures were incubated at 37°C in 5% CO_2 for 3 days. VNT titres were determined using light microscopy by absence of cytopathic effect in cell monolayers. All procedures, quality controls and analysis of results were based on recommendations in WOAH (2024).

The serology results showed a rising BoHV-1 VNT titre, from first to second sampling, for all of the affected heifers and for the mixed-age cow (#169) that had suspicious IPV-like lesions, whereas for the unaffected mixed-age cows, the VNT titre fell from the first to second sampling (Table 3).

MPI incursion investigator visit

On 11 September, 4 weeks after the initial visit, two MPI incursion investigators (HT, JPOC) and the farm veterinarian (HRM) attended the morning milking. Visual inspection of the vulva and vagina of a sample of 30 mixed-age milking cows and 33 milking heifers was conducted, and vaginal lesions were observed in 9/30 (30%) mixed-age cows and in 26/33 (79%) heifers, respectively. In most cases, lesions consisted of scars in the dorsal and lateral vagina. Pustules and erosions of the vagina were observed in a smaller proportion of the more recently calved heifers. Although 160/225 (71%) heifers calved by 11 September had been recorded as cases, there had been no new cases since 4 September, with only 20 heifers left to calve.

Table 3. Antibody titres for bovine herpesvirus type 1 measured by virus neutralisation test[a], in paired serum samples collected 4 weeks apart from four heifers affected with severe infectious pustular vulvovaginitis (IPV) post-calving, and four mixed-age cows.

Animal identification number	First sample	Second sample	Lesions consistent with IPV
Heifers			
3	32	64	Yes
8	< 2	32	Yes
12	32	64	Yes
635	192	768	Yes
Mixed-age cows			
778	128	96	No
48	384	96	No
118	384	192	No
169	< 1	8	Suspect

[a]Test performed at the Animal Health Laboratory (Wallaceville, NZ).

Discussion

A severe outbreak of IPV in post-parturient dairy heifers has been described. The results of the extensive molecular testing, as part of the MPI investigations, present strong evidence that BoHV-1 was involved in the outbreak, with the virus found by PCR in swabs from treated and untreated heifers. Furthermore, 2/4 immunohistochemistry tests conducted at the University of Guelph were positive for BoHV-1 and a rising antibody titre from the first to second blood sample was shown for all affected heifers. However, a mixed infection could not be ruled out, with strong evidence that *U. diversum* was also involved and some evidence for the involvement of *M. bovigenitalium*. As stated in the introduction, these three infections are usually transmitted venereally and yet these heifers had only recently calved. Importantly, there was no evidence that *Parapoxvirus,* which does present post-partum, was involved in this outbreak.

Insufflation, also known as cow blowing or kuhblasen, is the blowing of air, either directly (by the mouth) or indirectly (using a tube), into a cow's vagina to stimulate oxytocin release and milk let-down during milking (Sierksma 1963). This technique has been used for thousands of years, being recorded on ancient Saharan rock drawings (Le Quellec 2011), and its use has continued into modern times (Bilal *et al.* 1997). As stated earlier, the farm veterinarian became aware that an alkathene pipe was being used on the farm to insufflate the vagina and stimulate milk let-down in the heifers. Rapid and extensive spread of IPV could have been facilitated by this alkathene pipe, which would likely have caused sufficient abrasions to inoculate the infectious organisms directly into the vaginal mucosa.

It is unlikely that the alkathene pipe was solely responsible for the scale of the outbreak; the farm veterinarian observed that IPV was affecting some heifers prior to calving, and that not all heifers affected post-calving had been subjected to insufflation. Furthermore, some mixed-age cows were also subclinically affected, and these had not received the milk let-down intervention. In spring 2024, on the same farm, approximately 15 heifers developed IPV post-calving, even though the pipe was long retired, so it is likely that the milk let-down intervention was not the only factor involved in the 2023 outbreak. We hypothesise that peri-parturient stress, caused by suboptimal nutrition and overcrowding on the calving pad in 2023, led to recrudescence of latent BoHV-1 infection (Raaperi *et al.* 2014) in a small number of heifers, possibly acquired at natural mating, which was then spread to other heifers by the alkathene pipe intervention. Any vaginal abrasions incurred using the pipe then allowed for mixed infections to develop.

In New Zealand, under Regulation 6, Prohibited methods of milk stimulation in cattle, of the *Code of Welfare: Dairy Cattle* (MPI 2019), a person must not stimulate milk let-down in a cow by inserting anything into the cow's vagina. A person who fails to comply with this regulation commits an offence and is liable, on conviction, to a fine not exceeding $900. The offence is an infringement offence and incurs a $300 infringement fee. In this case, the farm veterinarian informed the farmer that this procedure is prohibited under New Zealand animal welfare regulations, resulting in the farmer immediately ceasing the practice, meaning further action was not taken. In New Zealand, when a veterinarian observes an animal welfare issue that may be causing unreasonable or unnecessary pain or distress, or that may breach animal welfare legislation, they must act immediately to provide advice on resolving the problem. If this advice is not followed and the welfare concern remains, the veterinarian should seek a second opinion or refer the matter to an animal welfare inspector (MPI 2022).

How common the practice of vaginal insufflation is in New Zealand is unknown. One of the authors (JPOC) observed it on a Bay of Plenty farm in 2005. Another (KEL) has heard of the use of insufflation, or warm water hoses placed in the vagina, being used to stimulate milk let-down on other New Zealand dairy farms. The authors and MPI expect that publishing this case will increase awareness of and compliance with the above regulation – and should the practice of vaginal insufflation be more common than thought – reduce the incidence and severity of post-partum outbreaks of IPV.

The isolation of BoHV-4 from one of the affected heifers was also interesting, although it was the opinion of an experienced AHL virologist (DJP) that the cow had a mixed infection of BoHV-1 and BoHV-4, but only the BoHV-4 was found at virus isolation. BoHV-4 has been associated with post-partum metritis in dairy cows (Bilge Dağalp *et al.* 2010) and, had the same isolation been made from all four samples, a possible aetiological role for BoHV-4 in this outbreak may have been inferred.

There was good agreement between the serological testing and the clinical signs, with all four affected heifers and one suspect mixed-aged cow showing a rising BoHV-1 VNT titre from first to second sampling. Usually, a three-to-four-fold rise in antibody titres from first to second sampling is associated with a recent active infection, although this can depend on when in the course of the disease the samples are taken. In this case, it is likely the first samples were taken a little later than optimum.

In conclusion, severe outbreaks of IPV associated with BoHV-1 can present post-partum. In this outbreak, it is likely that a mixed infection with *U. diversum* and possibly *M. bovigenitalium* increased the severity of the clinical signs. The practice of vaginal insufflation, involving the use of an alkathene pipe to stimulate milk let-down, contributed to the scale of the outbreak.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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