FOOD/FARMED ANIMALS

A winter dysentery (coronavirus infection) outbreak in a dairy herd in Galicia (northwestern Spain)

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Received 20 April 2016
Revised 19 May 2016
Accepted 13 June 2016

SUMMARY

Bovine coronavirus infections in adult cattle are commonly referred as to winter dysentery and present as an epidemic outbreak characterised by the sudden onset of diarrhoea with fresh blood that presents with high morbidity but low mortality. Despite the low mortality, milk production may not return fully to expected levels until several weeks post infection. This case report demonstrates the symptoms, examination and evaluation of a referred outbreak of winter dysentery in adult and youngstock cattle in a dairy herd located in northwestern Spain, highlighting the importance of precise questioning and on-farm assessment for examining the biocontainment practices of a herd.

BACKGROUND

Winter dysentery (WD) is a viral disease resulting in very acute onset of profuse watery diarrhoea with fresh blood in adult cattle, characterised by a highly explosive epidemic nature within a herd, with high morbidity rates. This presentation can result very worrisome and alarming to farmers as in a few days more than 50 per cent of their animals can exhibit bloody diarrhoea. Bovine coronavirus (BCoV) is a common enteropathogen of cattle, usually associated with neonatal scours. However, BCoV can also affect adult animals, resulting in WD. Therefore, clinicians should also remember to include BCoV in their differentials for acute and contagious diarrhoea of adult cattle, even in regions where WD is not frequently encountered, due to the wide spread of BCoV. This case report presents the clinical presentation, investigations, treatment and follow-up of an outbreak of WD in a dairy herd located in a region with no previous reports of WD.

CASE PRESENTATION

Case history

In December 2012, the herd veterinarian requested help with three adult animals showing bloody diarrhoea in a dairy herd. Since the previous visit of the referring veterinarian on the day before, one of the sick animals died and the number of animals with diarrhoea had multiplied. The disease started initially in the pen of the multiparous lactating cows and had spread to the primiparous lactating and youngstock pens, with several animals in each pen showing fresh blood in their faeces. The dead cow was removed by the cadavers' collection truck before the authors' arrival at the farm and, hence, not available for postmortem examination.

A detailed history of the herd management and animal health status was taken before the farm premises were investigated. According to the owner and the herd keeper, all the sick animals started showing signs of respiratory disease (coughing, mucoid nasal discharge and dyspnoea); approximately 12 hours after respiratory signs, their faeces started to lose consistency and, around 24–30 hours after initial respiratory distress, the animals started showing bloody diarrhoea. However, they did not identify any animal with hyperthermia. It was also noted by the farm staff that those affected animals showed a marked reduction in their milk yield, ranging from 7 to 18 l from each cow's previous milking.

Farm details

The labour of the farm consisted of the owner, a new full-time employee and the sporadic help of the retired owners' father. On the day of the visit, the farm was composed of a total of 326 Holstein-Friesian cattle, divided into 192 lactating and 48 dried-off cows, 45 in-calf heifers and one bull plus youngstock. Lactating cattle were kept in two separate barns according to parity: primiparous (n=47) and multiparous (n=145), and milked twice a day in a 2×6 swing-over milking parlour. The bull had his own box, located in the shed of the primiparous cows; newborn calves are kept in individual igloos during the first month of life and afterwards moved into pens bedded on straw in groups of 8–12 animals until the age of six months, when they are moved to the rearing farm, located 20 km away of the main farm. Dry cows are kept in another barn, separated 5 km from the lactating herd. Cows are moved to the pre-calving shed just one week before the expected calving date.

The farm uses artificial insemination with frozen semen from accredited distributors and their own bull for repeat breeders (more than three unsuccessful inseminations). The farm is a close herd and enrolled several years before on a voluntary eradication programme for bovine herpesvirus 1 and bovine viral diarrhoea virus (BVDv), being considered free of those diseases. According to the owner, there was no direct contact among the animals of the different sheds, and the house dogs are kept away from the farm. In addition, no changes in the diet were made in the past months and no new silage clamps were opened recently.

INVESTIGATIONS

Initial clinical examination of affected animals

The two initially affected cows were subjected to a thorough clinical exam, where, surprisingly, the only remarkable findings were a hydration status

To cite: Abuelo A, Perez-Santos M. *Vet Rec Case Rep* Published online: [*please include* Day Month Year] doi:10.1136/vetreccr-2016-000328

suggestive of approximately 8 per cent dehydration (eyeballs recession into the sockets of approximately 5 mm and duration of skin tenting on the upper eyelid between 3 and 5 seconds; Roussel 2014) and decreased ruminal fill and motility (rumen fill scores between 1 and 2 according to the system of Zaaijer and Noordhuizen 2003, 0 to 1 ruminal contractions over three minutes), although the cows were eating actively at the feedbunk during the clinical examination. Faeces contained a variable amount of mucus, but no visual presence of blood was observed, although the herd veterinarian assured to have examined the same cows the day before, finding red-coloured faeces with the presence of some blood clots.

Several new cases developed since the last visit of the herd veterinarian. In total, 16 and 6 cows in the multiparous and primiparous lactating sheds, and 4 five- to six-month-old heifers were passing bloody and watery faeces. In conjunction with the herd veterinarian, all the affected animals were examined. Besides haematochezia (Fig 1), the remarkable findings in all the affected animals, regardless of the age, were mucoid nasal discharge, increased respiratory rate and increased respiratory sounds during chest auscultation and a variable degree of dehydration. Despite the loss of blood in the faeces, none of the animals showed pale mucosal membranes. Rectal temperature was only slightly elevated (39.6°C and 39.7°C) in two of the affected animals.

During the clinical examination of the affected animals, it was noted that a total of eight animals in all the affected pens were coughing and had nasal discharge and therefore also subjected to a clinical exam. The only common findings in these animals were increased respiratory sounds on auscultation and the loss of consistency of their faeces in comparison to their apparently healthy counterparts, but no presence of blood in their faeces was observed macroscopically. The majority of these animals had also elevated rectal temperature (39.8–40.1°C). Additionally, no laboratory results, such as packed cell volume, total protein, and so on, were available on the index cows to assess their status.

Isolation of the affected animals from the remainder of the herd was recommended, but unpractical given the premises of the farm and the adverse weather conditions to keep the animals outdoors.

Initial list of problems

- Transient hyperthermia
- Respiratory distress
- Acute diarrhoea
- Haematochezia
- Dehydration
- Rapid spread of the disease
- Drop in milk production

DIFFERENTIAL DIAGNOSIS

Common causes of acute and contagious diarrhoea of adult cattle include

- BCoV
- Salmonellosis
- Coccidiosis
- BVDv

The most likely condition was considered to be BCoV because it could explain both the respiratory and enteric signs, the outbreak presented during winter and was preceded by a sudden decrease in environmental temperature in the previous days (from an average daily temperature of 10–12°C to 3–5°C) and so far had a low mortality. A cow-side test (Rainbow

FIG 1: Haemorrhagic faeces with presence of blood clots in an affected cow

CalfScour 4, Bio-X Diagnostics, Jemelle, Belgium) was employed to detect BCoV in the faeces of two affected animals, yielding a positive result for BCoV. However, BCoV is a ubiquitous agent and their presence alone, without ruling out other conditions, cannot be used to confirm the cause of the outbreak. On the other hand, a BVDv infection was considered the least likely condition as the herd was a closed herd (the bull was also raised on the farm), the biosecurity measures established to reduce contact with animals of unknown status were considered good and the farm was enrolled in an eradication programme several years before, not currently vaccinating animals and regularly monitoring the herd. However, this makes the herd naive against a potential BVDv infection, and considering the ability of this pathogen to disseminate from blood antigen-negative animals (cumulus bulls, Trojan dams, etc) or contaminated fomites, it was considered necessary to rule out this condition.

Samples were taken from the two initially affected cows that seemed to start recovering and from five diarrheic cows/heifers on each of the affected barns. Faecal and ear notch samples were collected and transported to the Official Veterinary Laboratory of the Galician region. Here, faecal samples were subjected to faecal floatation and bacterial culture for the diagnosis of coccidiosis and salmonellosis, respectively; a sandwich ELISA was employed for BCoV detection. Ear notches were analysed with an Ag-ELISA for BVDv detection. Blood samples from these animals were also obtained by coccygeal venipuncture into evacuated tubes without anticoagulants. Serum was harvested, aliquoted and stored at –20°C at the practice in order to have a baseline sample to submit for paired serology samples in the future, if needed.

Additionally, nasal swabs and transtracheal wash samples from two animals in each pen (multiparous, primiparous and five- to six month-old heifers) showing only respiratory signs were collected and transported to the Regional Official Veterinary Laboratory for viral and bacterial isolation to establish whether the respiratory conditions were associated with the haemorrhagic diarrhoea. Serum samples from these animals were also collected.

TREATMENT

After sample collection, affected animals were treated symptomatically when needed: dehydrated animals were drenched with a commercial electrolyte product (Rehidratante oral-Iven. Laboratorios e Industrias IVEN SA, Madrid, Spain) dissolved in 30 l of warm water, pyrexic cows were treated with flunixin

meglumine (Flunixcen 50 mg/ml. Cenvisa SL, Tarragona, Spain) intravenously (2.2 mg/kg bodyweight), and cows with haematochezia were treated with etamsylate (Hemo 141. Laboratorios Esteve SL, Barcelona, Spain) intravenously/intramuscularly (7.5 mg/kg bodyweight every 12 hours) until the disappearance of blood in the faeces. Fresh water and feed were always available to sick animals.

OUTCOME AND FOLLOW-UP

Second and third visits to the farm

In the evening of the same day, the farmer reported that more animals showed now bloody and watery faeces and therefore the farm was visited again. The visit took place during milking time, and it was noted that even though the farmer said that the different pens had no contact, primiparous and multiparous cows could establish direct contact over a fence in the collecting yard of the milking parlour.

Among others, those animals that in the morning were only showing respiratory signs were all now passing haemorrhagic faeces; and six new cows were now showing respiratory distress and mucoid nasal discharge. The farmer reported an overall decrease in milk production in that milking of approximately 14 per cent in comparison with the average from days previous to the outbreak. Newly affected animals were treated symptomatically as mentioned earlier.

In the morning of the following day, another visit to the farm was undertaken. Those cows with haemorrhagic diarrhoea in the previous morning were now showing signs of improvement (increased appetite, increased consistency of faeces and no visual presence of blood in faeces, respiratory rate within limits), and in those animals that started in the afternoon/evening, some clots of blood were still present in their faeces, but the faeces were no longer of watery consistency and not as profuse as the day before. The cows that the previous evening had only respiratory signs were now showing the enteric process developed by their counterparts and only two new cows were identified with respiratory signs. While examining the heifers, it was noted that in addition to their hay and concentrate they were also being fed with the total mixed ration refusals from the lactating cows.

In the following days, no new cases developed. In total, 12 out of 47 (25.5 per cent) primiparous and 30 out of 145 (20.7 per cent) multiparous cows were affected enterically during the outbreak (21.9 per cent of adult cattle[i]). The incidence of the enteric process in the five- to six-month-old heifers during the three days of the outbreak was lower, and only 5 out of 38 (13.2 per cent) animals were affected. On the other hand, the mortality rate of the process was 0.43 per cent (1/230).

Laboratory results and interpretation

Laboratory results arrived after the disappearance of the clinical signs. Box 1 summarises the results obtained from the lab.

All sampled cows were negative for BVDv antigen, thereby implying that they were not undergoing an infection with this virus. Also, *Salmonella* species was not isolated from any of the faecal samples. Coccidian oocysts were identified in some of the samples from affected youngstock, but not in any of the samples from the adult animals. In addition, some of the samples positive for *Eimeria* species oocysts were also positive for BCoV. Hence, coccidiosis was considered a concurrent condition in the heifers as no sample from adult cows tested positive for Coccidia.

The faecal samples from the first two cows developing bloody diarrhoea were negative for all the tests; however, when the samples were taken, no visual presence of blood was detected on their faeces and the cows were starting to show signs of improvement (increased appetite and faeces consistency, respiratory rate within reference interval). Indeed, from the representative sample of animals with haematochezia at sample collection, 11 out of 15 animals tested positive for BCoV in their faeces. Even though this finding alone does not necessarily support the causative role of BCoV in the enteric process, as animals with a respiratory BCoV infection can shed virus in the faeces (Thomas and others 2006), the exclusion of other causes of acute and contagious diarrhoea of adult cattle, the fulfilment of the epidemiological characteristics of an WD outbreak—history of acute onset of diarrhoea and dysentery affecting at least 15 per cent of the adult cattle, rapid spread causing a drop in milk production of 10 per cent or more and less than 2 per cent of fatalities (Smith and others 1998)—combined with the spontaneous recovery over a few days is highly suggestive of an enteric BCoV infection (Boileau and Kapil 2010).

With regard to the samples obtained from the respiratory tract, in addition to four out of six BCoV-positive samples, different pathogens commonly involved in the bovine respiratory

BOX 1: Summary of the laboratory results from the submitted samples

Faecal samples

Faecal floatation

- Three samples positive for *Eimeria* species (≥1 oocyst)

The number of oocysts excreted by an animal is not strictly related to the degree of clinical disease (Daugschies and Najdrowski 2005).

Bacterial culture

- No *Salmonella* species detected in any of the samples.

BCoV Ag-ELISA

- Two cows initially affected: negative.
- Fifteen cows/heifers acutely affected: 11 samples positive.

Ear notches

Bovine viral diarrhoea virus Ag-ELISA

- All negative.

Nasal swabs

Virus antigen identification (bovine herpesvirus 1, bovine respiratory syncytial virus (BRSV))

- Two samples positive for BRSV.

Bacterial culture and identification

- One sample was inconclusive: *Mannheimia haemolytica* and *Pasteurella multocida* isolated from three different samples.

Transtracheal wash

Virus identification (RT-PCR)

- Four samples positive for BCoV (one primiparous, two multiparous, one heifer).
- Other respiratory viruses also isolated in some samples: PI-3 (one heifer), BRSV (one primiparous).

Bacterial culture

- In two different samples, growth of different bacteria was observed: *M. haemolytica, P. multocida* and *Ureaplasma diversum*.

[i]Dry cows were not included in the calculation because they were located 5 km away from the lactating herd and hence not considered 'at-risk' animals.

disease complex (*Mannheimia haemolytica, Pasteurella multocida*, parainfluenza virus type 3 and bovine respiratory syncytial virus) were isolated. However, these pathogens are commensal inhabitants of the upper respiratory tract in cattle, producing secondary bacterial pneumonia after initial virus-associated lesions in the respiratory mucosa (Hodgins and others 2002). BCoV is a pneumoenteric virus that replicates in the epithelium of the upper respiratory tract, and the literature supports that enteric and respiratory BCoV may be the same virus at different stages of its infectious cycle (Reynolds and others 1985, Cho and others 2001, Thomas and others 2006), being initiated in the respiratory track (oropharynx) and spreading subsequently to the gastrointestinal tract (Saif and Smith 1985, Thomas and others 2006). Indeed, this supports the fact that affected animals started showing signs of respiratory distress followed by dysentery, as previously reported in other outbreaks (El-Kanawati and others 1996, Guard and Fecteau 2009). Considering this, the isolation of the other respiratory pathogens (*M. haemolytica*, *P. multocida* and *Ureaplasma diversum*) could be attributed to a secondary infection.

DISCUSSION

WD is common in North America and has been reported in other European countries such as the UK, France, Belgium or Italy (Boileau and Kapil 2010), but the authors did not find a report in the scientific literature about this epizootic disease in Spain. This might be, therefore, the first reported case where they identified BCoV in a dysentery outbreak of adult cattle. However, BCoV is widespread in the cattle population, but the clinical manifestation of the syndrome is not solely related to the virus itself but also to host and environmental factors (Tsunemitsu and Saif 1995). BCoV persist in adult cattle as subclinical infections (Crouch and others 1985, Collins and others 1987), and under stressful conditions adult cattle shed BCoV in faeces and nasal secretions (Crouch and others 1985). Indeed, stressors such as inclement weather (Decaro and others 2008a) or shipping are relevant contributing factors that may exacerbate disease from BCoV infections (Boileau and Kapil 2010). WD occurs usually during the colder months of the year (Saif 1990). As previously described, the epizootic characteristics of the outbreak are also in line with what is commonly observed in cases of WD, with the period of illness in an individual being brief and the outbreak within a herd lasting for less than two weeks (Boileau and Kapil 2010).

Animals usually start shedding BCoV after stressful situations. During the week preceding the outbreak, there was a significant drop in environmental temperature, and also a new employee started working in the farm, who had no experience working with cattle and the farm's owner acknowledged him being 'brusque' when handling animals, thereby potentially increasing the stress suffered by the animals. BCoV infection is primarily transmitted via faecal-oral route. The incubation period for WD ranges from 2 to 8 days. It seemed that the dysentery cases appeared first in the multiparous pen and spread from here to the other pens. Direct contact between primiparous and multiparous animals was possible in the collecting yard, and the spread to youngstock can be explained via refusals contaminated with nasal secretions, because the shedding of BCoV in nasal secretions starts around three days before the virus is detected in faeces (Thomas and others 2006). Also, the farmer reported that his dogs did not have access to the pens. However, during one of the visits, it was noted that his father was walking the dogs unleashed close to the farm. Therefore, the dogs as passenger of BCoV (Kaneshima and others 2007) cannot be excluded.

As mentioned earlier, isolation and segregation of affected animals in quarantine was not an option because of the lack of space and adverse weather conditions. However, it was recommended to the farmer to disinfect with a quaternary ammonium compound any equipment and footwear when moving between sheds to limit the spread of the disease. Also, as BCoV can remain infectious for up to three days in soil, faeces and bedding materials (Boileau and Kapil 2010), it was stressed to the farmer the importance of maintaining a basic hygiene routine, including manure removal and preventing the contamination of feed or water sources with manure, as well as not using manure-handling equipment for handling feed unless it is thoroughly disinfected before handling feed.

BCoV-associated clinical syndromes have been recently reviewed (Boileau and Kapil 2010). BCoV is spread worldwide in the cattle population, causing respiratory and digestive diseases in both calves and adult cattle. There has been, however, some debate about whether BCoV strains isolated from the respiratory and digestive tracts are the same virus or are dissimilar in biological, antigenic and/or genetic terms, with research reports supporting both similarities (Reynolds 1983, Zhang and others 1994) and differences (Hasoksuz and others 1999, Gelinas and others 2001) among respiratory and digestive strains. The authors have not subjected the respiratory and faecal samples to BCoV-strain differentiation; however, the progression of the disease in this case is compatible with the hypothesis that enteric and respiratory BCoV may be the same virus detected at different stages of its infectious life cycle (Reynolds and others 1985, Saif and others 1986, Cho and others 2001, Thomas and others 2006), with the infection starting in the respiratory tract and the viral particles reaching the intestines through ingestion of respiratory secretions.

Cross-infection between different species has been documented as BCoV are related to other coronaviruses in the same antigenic group (Boileau and Kapil 2010). Species that could facilitate cross-over transmission of coronavirus include other domestic animals, such as water buffalos (Decaro and others 2008b), horses, or New World camelids (Genova and others 2008), and also wildlife such as deer and elk (Majhdi and others 1997). However, although the importance of biosecurity measures needs to be highlighted in dairy farms to prevent the introduction of new diseases, in the case of BCoV-associated diseases, the role of reservoirs is probably more limited than for other diseases as most cattle become exposed to BCoV in their lifetime and usually the infection is exacerbated by different stressors (Crouch and others 1985). Hence, limiting the exposure of cattle to stressing events is helpful in reducing the susceptibility of adult cattle to BCoV infections. The economic impact of BCoV infections is usually associated with the involvement of the virus in the bovine respiratory disease complex in cattle and calves, as well as the neonatal diarrhoea complex in young calves, causing decreased performance, mortality and expenses of medication and labour to treat sick animals (Cho and others 2001, Hasoksuz and others 2002). In cases of WD, however, although there is a significant decrease in milk production during the outbreak, this is usually of short duration. Additionally, immunity after a WD outbreak usually lasts for 3–5 years (Guard and Fecteau 2009) and therefore no vaccination is required to prevent new WD outbreaks in the same herd in the upcoming years. Immunisation against coronavirus in cattle is just targeted to increase the level of passively acquired immunity in neonatal calves through vaccination of pregnant cows (Clark 1993), as current commercially available vaccines for bovine respiratory disease do not include BCoV antigens.

Acknowledgements This case was part of the credentials approval of AA for the certifying exam of the European College of Bovine Health Management (ECBHM).

Contributors AA led the herd investigation, the diagnostic and treatment plans, assisted by MP-S. AA wrote the case report, which was also critically revised by MP-S. Both authors approved the final version of the manuscript.

Competing interests None declared.

Provenance and peer review Not commissioned; externally peer reviewed.

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