Spontaneous bleeding in a neonatal calf persistently infected with BVDV1b

Spontane bloedingen bij een pasgeboren kalf met persisterende BVDV1b-infectie

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ABSTRACT

A calf developed skin bleeding on the second day of its life. It was referred to the clinic on suspicion of bovine neonatal pancytopenia (BNP). Hematology showed extreme thrombocytopenia, moderate anemia, but no leukopenia. A PCR test on a heparinized blood sample was bovine viral diarrhea virus (BVDV) positive, as were the two BVDV antigen ELISAs performed three and ten weeks later. Non-cytopathic BVDV type 1b was isolated from the blood. Although the calf recovered from hemorrhagic disease, and continued to be healthy, it was euthanized at eleven weeks of age because of persistent BVDV infection. On the basis of the case history, BNP could be excluded from the differential diagnosis. This case illustrates that hemorrhagic disease is not exclusively associated with BVDV type 2 and that the clinical signs in neonatal calves infected with BVDV1b can be identical to the clinical presentation of BNP.

SAMENVATTING

Een kalf vertoonde op zijn tweede levensdag huidbloedingen en werd doorverwezen naar de kliniek onder verdenking van boviene neonatale pancytopenie (BNP). Hematologisch onderzoek toonde extreme trombocytopenie, matige anemie, maar geen leukopenie aan. Een PCR-test op gehepariniseerd bloed was positief voor het boviene virale diarreevirus (BVDV), net als bij twee BVDV-antigeen ELISA's op bloed die drie en tien weken later genomen werden. Niet-cytopathogeen BVDV-type 1b werd geïsoleerd uit het bloed. Alhoewel het kalf herstelde van de bloedingen en gezond bleef, werd het wegens de persisterende BVDV-infectie geëuthanaseerd op de leeftijd van elf weken. Op basis van de anamnese kon BNP uitgesloten worden uit de differentiaaldiagnose. Deze casus toont aan dat het bloedingssyndroom niet uitsluitend het gevolg is van besmetting met BVDV-type 2 en dat de klinische verschijnselen bij met BVDV1b besmette neonatale kalveren identiek kunnen zijn aan deze bij BNP.

INTRODUCTION

Infection of cattle with bovine viral diarrhea virus (BVDV) may have various clinical presentations, from non-clinical or mild disease to outbreaks of acute, severe disease with high mortality. Hemorrhagic disease (HD) is one of the potential clinical features, characterized by thrombocytopenia and an increased susceptibility to bleeding (Walz et al., 1999). Although severe outbreaks of acute BVDV-infection are commonly termed "hemorrhagic syndrome", hemorrhages were not always among the clinical signs of these outbreaks (Carman et al., 1998; Ridpath and Fulton, 2009). In contrast to American BVDV type 2 strains, European BVDV type 2 strains have rarely been associated with HD (Ridpath, 2005; Vilcek, 2005). Recently, a North American BVDV type 2 strain has been detected in Belgium, but HD was not among the clinical presentations (Letellier et al., 2010). Furthermore, most cases of HD are associated with transient infections, and HD in persistently infected (PI) cattle has rarely been reported (Dabak et al., 2007). In this study by Dabak et al., the calves were older than 1.5 months and died of mucosal disease (MD). In the present case however, spontaneous skin bleeding in a two-day old calf persistently infected with non-

cytopathic BVDV type 1b and not suffering from MD is reported.

CASE REPORT

In June 2011, a two-day old Belgian blue calf developed spontaneous skin bleeding, and was transferred to the Faculty of Veterinary Medicine (UGhent – Merelbeke (Belgium)) on suspicion of bovine neonatal pancytopenia (BNP). The farmer mentioned an increased frequency of neonatal diarrhea and respiratory disease among the calves during the previous months, but no hemorrhages had been noticed among other cattle of the mixed beef and dairy herd. The calf was delivered by caesarian section, had received four liters of colostrum from its own dam, and appeared to be healthy in the first day of life. BVDV vaccination had never been performed in the herd, and the dam was homebred.

On arrival, the calf was depressed, and showed melena and skin bleeding, not only from both ears due to ear tagging, but also on the back and legs. The mucosae were pale and petechiae and submucosal bleeding were found under the tongue and elsewhere on the oral mucosa. The body temperature was 39°C, the pulse rate was 80 per minute, and the respiratory rate was 24 per minute.

Hematology showed extreme thrombocytopenia (0 platelets/L), a moderate anemia (PCV= 0.23 L/L), but no leukopenia ($9.5x10^9$/L). A PCR test performed on a heparinized blood sample taken on arrival was BVDV positive, as were two antigen ELISAs on whole blood taken three and ten weeks later (IDEXX BVDV Ag/Serum Plus Test, IDEXX Europe, Hoofddorp, the Netherlands). Non-cytopathic BVDV was detected at virus isolation from a whole blood sample taken on day 52. The isolated strain was genotyped as BVDV type1b by RT-PCR and sequencing (Letellier et al., 1999). An EDTA blood sample from the calf's dam was BVDV negative in the same antigen ELISA.

Until day 14, the calf was treated with cefquinome (Cobactan® 2.5% w/v; Intervet) to protect it from secondary infections. From day 2, it was housed in strict isolation. Platelets and PCV normalized after thirteen and twenty days, respectively. The calf had a good appetite and looked healthy until it developed pneumonia with fever peaking at 40°C at 37 days of age. After treatment with gamithromycin (Zactran 150 mg/ml; Merial) and ketoprofen (Ketofen 10%; Merial), it recovered. From this point until euthanasia on day 77, the calf showed no further clinical signs of disease. The only reason for euthanasia was the persistent BVDV-infection. On the farm, hemorrhages have not been recorded in any other stock to date.

DISCUSSION

As the calf had no initial fever, and as there were no other symptoms at the same time as the bleeding syndrome, hemorrhagic septicemia and endotoxemia could be excluded as potential causes of thrombocytopenia. Toxic agents were not suspected of having caused the hemorrhagia in this two-days old calf, because plants, such as field melilot (*Melilotus officinalis*) and bracken fern (*Pteridium aquilinum*), need prolonged intake to cause bleeding syndromes. Moreover, dicumarol, which is present in bracken fern and commercial rodenticides, does not lead to bone marrow depletion (Wang et al., 2007). Furthermore, until now, thrombocytopenia has not been described in cattle affected by hereditary bleeding syndromes (Steficek et al., 1993; Meydan et al., 2009; Shiraishi et al., 2002). In 2008, BNP emerged in Europe as a cause of thrombocytopenia and leukopenia in neonatal calves (Pardon et al., 2010). In this immune-mediated disease, allo-antibodies directed to calf leukocytes and bone marrow precursor cells are transferred to the calf through colostrum (Bastian et al., 2011; Bridger et al., 2011; Pardon et al., 2011). Although it has been demonstrated that the presence of the allo-antibodies in colostrum is associated with vaccination with a particular BVD-vaccine (Sauter-Louis et al., 2012), only a small number of the calves that receive colostrum from vaccinated mothers, develop BNP. It is assumed that this could be contributed to inherited factors (Deutskens et al., 2011). The calf of the present case only received colostrum from its own dam. The cow was born and raised on the farm, and BVD vaccines had never been used in the herd. Moreover, colostrum from other herds had never been administered on this farm. For these reasons, BNP could be excluded, and persistent infection with BVDV type 1b was considered to be responsible for the thrombocytopenia in the newborn calf.

It has been suggested that some cattle may be viremic for a longer period than the generally accepted 14 to 21 days (Collins et al., 2009). Therefore, a second blood sampling for antigen-ELISA was carried out on the calf of the present case ten weeks after the first, to exclude the possibility of prolonged transient infection. Collins et al. found evidence of the presence of BVDV in blood of calves up to three months after infection, but these calves were antigen ELISA negative at that stage. The fact that the calf of the present case was antigen ELISA positive at the second sampling proved that it was PI.

Although persistently infected, the calf showed two of the three predominant symptoms of experimental acute severe BVDV-infection: fever, low white blood cell count and low platelet count (Walz, 1999; Ridpath et al., 2006). Nevertheless, the case was exceptional for several reasons. First of all, the bleeding disorder was associated with a BVDV type 1 strain. To the authors' knowledge, this has only been reported by Dabak et al. (2007) in older PI calves suffering from MD. Secondly, the calf of the present case was much younger than previously reported for HD and the clinical presentation was indistinguishable from the clinical signs of BNP. Thirdly, the thrombocyte count returned to normal in spite of persistent viremia. Therefore, a direct effect of the virus on thrombocytes seemed unlikely. This finding is in line with the re-

sults of a study by Walz et al. (2005), who detected no significant difference in platelet counts between cattle PI with BVDV and control cattle. A hypothesis for the thrombocytopenia might be the removal of virus containing thrombocytes or megakaryocytes after interaction with colostral antibodies comparable to BNP pathogenesis (Deutskens et al., 2011).

CONCLUSION

This case report illustrates that BVDV1b-associated hemorrhages can occur in PI calves younger than one month, not suffering from MD at that stage of infection. As the clinical presentation was the same as for BNP, it is advisable to rule out BVDV-infection in suspected cases of BNP.

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