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Existence of bovine neonatal pancytopenia before the year 2005? Retrospective evaluation of 215 cases of haemorrhagic diathesis in cattle

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ABSTRACT

Haemorrhagic diathesis (HD) in cattle is a relatively rare syndrome that can have many different causes. With the occurrence of bovine neonatal pancytopenia (BNP) in 2007, the number of cases of HD in cattle has increased. This led to an enhanced interest in diseases presenting with bleeding disorders. The possible causes of HD in cattle, the clinical findings, and the course of various diseases are described and evaluated. Furthermore, we determined whether cases of BNP occurred before the introduction of the vaccine Pregsure BVD since its widespread use was associated with the syndrome.

Records of 215 cases of HD in cattle that had been referred to the Clinic for Ruminants with Ambulatory and Herd Health Services at the Centre for Clinical Veterinary Medicine, Ludwig Maximilian University, Munich, between 1982 and 2014 were evaluated. The two most commonly diagnosed diseases were BNP ($n = 95$) and septicaemia ($n = 35$), with fatality rates of 82% and 66%, respectively. In 27 (13%) cases, no clear cause for the HD could be designated. Statistically significant differences were found with regard to the course of the various disorders and the clinical findings. A receiver operating characteristic analysis of thrombocyte counts of affected animals at the time of arrival at the clinic did not provide any predictive information on disease outcome. Two cases of HD occurred before the introduction of Pregsure BVD (1989, 1991). In both cases, clinical, haematological, and pathological findings were identical to BNP. The cause of HD in these two cases could not be determined retrospectively.

Introduction

Haemorrhagic diathesis (HD) occurs because of a disturbance of primary or secondary haemostasis. Generally, disorders of primary haemostasis occur because of vasopathy, thrombocytopenia, or thrombocytopathy. Coagulopathies lead to impairment of secondary haemostasis (Hiller and Riess, 2002). Bell (2011) and Raczynski (2011) have described the causes of HD in cattle. Bleeding disorders in cattle are rare, and the development of accurate diagnostic and treatment protocols is challenging. This is partly due to difficulties in identifying the exact cause of HD and the limited therapeutic options. Because clinical signs of bleeding disorders, such as petechiae, haematochezia, cutaneous haemorrhages and clinical signs of anaemia, are relatively non-specific, an aetiological diagnosis cannot usually be based on clinical findings.

The occurrence of bovine neonatal pancytopenia (BNP) in some European countries in 2007 (Friedrich et al., 2009) led to a sudden surge of cases of bovine HD. High mortality rates and initial uncertainty about the aetiology of BNP resulted in numerous investigations (Bridger et al., 2011; Deutskens et al., 2011; Foucras et al., 2011; Pardon et al., 2011; Kasonta et al., 2012; Lambton et al., 2012; Sauter-Louis et al., 2012; Jones et al., 2013; Benedictus et al., 2014; Bell et al., 2015). The concomitant increase in awareness of HD in cattle both by veterinarians and farmers led to a sharp rise in the number of cattle with bleeding disorders referred to the Clinic for Ruminants with Ambulatory and Herd Health Services at the Centre for Clinical Veterinary Medicine, LMU Munich.

Several epidemiological studies have reported an association between the occurrence of BNP and the use of the bovine viral diarrhoea (BVD) vaccine Pregsure BVD (Pfizer), which had been licensed in 2004 (Paul-Ehrlich-Institut, 2004). Due to a substantial increase in the incidence of HD in calves and the accumulation of evidence associating the vaccine with HD (Carlin, 2011; Reichmann, 2012; Sauter-Louis et al., 2012), the vaccine was withdrawn from the European market in 2010. Additionally, pancytopenia with similar

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clinical and laboratory findings to BNP (pronounced thrombocytopenia, leukopenia, panmyelophthisis) has been described. However, no association with Pregsure BVD could be established. Such cases were categorised as sporadic occurrences, and so far, they have been insufficiently investigated (Ammann et al., 1996; Shimada et al., 2007; Gosselin et al., 2011).

In the present retrospective study, the aetiologies, clinical findings, and outcomes of bovine HD cases from 1982 to 2014 were described and analysed. Associations between clinical findings and aetiologies were evaluated to optimise diagnostic protocols, and receiver operating characteristic (ROC) curves for thrombocyte count were performed.

Materials and methods

Data

Retrospective record analysis was performed for all bovine patients presented to the Clinic for Ruminants with Ambulatory and Herd Health Services at the Centre for Clinical Veterinary Medicine, Ludwig Maximilian University, Munich, admitted between 1982 and 2014. The clinic database was searched for all cattle with HD (search string 'Hämorrhagische Diathese' and 'Blutungsneigung') and the following data were collected: age (age group 1: ≤28 days; age group 2: 1–6 months; age group 3: >6 months), breed, sex, history, date of admission, clinical findings (particularly in relation to HD using standardised searches), body temperature, course of disease, treatment, laboratory findings, results of additional examinations, and post-mortem findings. These parameters were evaluated to assign a specific cause to each case of HD.

The classification was based on the definitions or detection methods given in Table 1. If the cause of disease was unable to be determined, the case was assigned to the group 'cases of unknown aetiology'.

Statistical analyses

Data analysis was performed using commercially available software (MS Excel 2007, Microsoft; SPSS, IBM, version 21). Animals were euthanased if their prognosis was considered grave. No animal was euthanased for economic reasons. Associations between outcome (fatal or not fatal) and the cause of HD and between age groups were assessed using chi-square tests and Fisher's exact tests for pairwise comparisons.

For the two most frequent causes of HD (BNP and septicaemia), receiver operating characteristic (ROC) curves were constructed using disease outcome (fatal or not fatal) and thrombocyte count on the day of admission to the clinic. A multivariable stepwise backwards binary logistic regression model was used to identify clinical findings associated with the most common diagnoses, BNP and septicaemia (inclusion into the model, if Wald $P < 0.05$). For this reason, all animals that did not have BNP or septicaemia were used as a reference group.

Table 1
Conditions and diagnostic methods used in bovine haemorrhagic diathesis in cattle ($n = 215$).

Condition	Diagnostic method
Bovine neonatal pancytopenia	≤28 days of age, thrombocytes $<200 \times 10^3/\mu L$ and leucocytes $<4 \times 10^3/\mu L$ and/or panmyelophthisis in necropsy, negative BVD virus antigen test
Septicaemia	Typical clinical findings and thrombocyte count $<200 \times 10^3/\mu L$
Cases of unknown aetiology	No classification possible
Drug intoxications chloramphenicol, furazolidone	History: Use of the drug in conjunction with the occurrence of clinical findings
Bovine virus diarrhoea virus infections	Positive antigen test
Intoxications rodenticides, mycotoxins, toxic plants, trichloroethylene	History and/or toxicological analysis
Thrombocytopathy Simmental hereditary thrombopathy	Platelet function analyser and/or genetic analysis
Other diagnoses leukosis, haemangioma, parafilariasis	Clinical findings, detection of parasites or microfilariae in case of suspicion of parafilariasis

Table 2
Conditions associated with bovine haemorrhagic diathesis ($n = 215$) and fatality rates.

Condition	Cases (n)	Fatalities (%)
Bovine neonatal pancytopenia	95	82
Septicaemia	35	66
Cases of unknown aetiology	27	44
Drug intoxications chloramphenicol, furazolidone	18	78
Bovine virus diarrhoea virus infections	13	69
Intoxications rodenticides, mycotoxins, plants, trichloroethylene	10	40
Thrombocytopathy Simmental hereditary thrombopathy	9	11
Other diagnoses leukosis, haemangioma, parafilariasis	8	63

Results

HD case classification according to cause/disease

Table 2 presents the conditions associated with bovine HD. Cases of leukosis, haemangioma, and parafilariasis (not a cause of HD but a cause of spontaneous cutaneous haemorrhage) were combined into one category of 'other diagnoses' because of low case numbers. All cases that could not be definitively assigned to a specific diagnosis were designated cases of unknown aetiology. Case fatality rates were associated with the cause/diagnosis of HD ($P < 0.001$), indicating that some diseases were more frequently fatal than others. The case fatality for BNP was 82%, while case fatality among the cases of unknown aetiology was 44%, case fatality due to intoxications 40%, and case fatality due to thrombocytopathy (TP) was 11%.

Distribution and frequency of cases across the time period evaluated

Fig. 1 shows the distribution of cases over the 33-year study period and the most frequent diagnoses. Until 2007, fewer than 10 cases per year were reported. Septicaemia causing HD, drug intoxications, and cases of unknown aetiology were the most frequent diagnoses. An increase in the number of cases of BNP was recorded in 2007, reaching a peak in 2009 and has declined since. Besides BNP, the inherited platelet dysfunction TP in the German Simmental breed and cases of unknown aetiology occurred relatively frequently. Septicaemia with HD, which was recorded 26 times from 1982 to 2004, occurred nine times in the subsequent time period (2005–2014).

Two cases clinically indistinguishable from BNP occurred in 1989 and 1991. The calves exhibited all of the defining characteristics of BNP. They were <28 days old and had the typical clinical signs of HD, pronounced thrombocytopenia and leukocytopenia (Table 1), and non-regenerative anaemia. Despite aggressive symptomatic treatment including blood transfusions, the calves deteriorated and were euthanased. Necropsies revealed the typical signs of HD, including panmyelophthisis. The aetiology of the panmyelophthisis was not determined despite extensive virological, bacteriological, and histological investigation. The vaccination status of the dams of these two calves was unknown. One of the dams had given birth to a total of eight calves, of which only one survived.

Breed, sex, age, signs of haemorrhage, and body temperature and causes/diagnoses of HD

The 215 cattle evaluated consisted of 195 German Fleckvieh, seven Holstein Friesian, and 13 animals of other breeds. The sex distribution was nearly equal, with 112 females and 103 males. In total, there were 136 animals affected in age group 1 (≤28 days), 42 in age group 2 (1–6 months), and 37 in age group 3 (>6 months). There was an association between the proportion of affected animals per