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Case Report - Suspected M1 Aflatoxicoses on a Western Dairy Calf Ranch

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Abstract

An 8,000-unit dairy replacement calf ranch experienced high morbidity and mortality related to exposure of calves to aflatoxin M1 isolated in the milk replacer. During the six-week exposure period, preweaning calf morbidity was 100%, and approximately 3,000 calves died. Aflatoxin M1 is an intermediate metabolite of aflatoxin B1, and is found in milk of dairy cows consuming a contaminated ration. Histopathology demonstrated changes in the centrilobular hepatocytes as well as the biliary tree. Serum chemistry values indicated an ongoing liver disorder. Toxicologic evaluation of the milk replacer revealed the presence of aflatoxin M1. Within three days of removal of the affected milk replacer, morbidity and mortality levels returned to baseline levels for this ranch.

Résumé

Une ferme de 8000 têtes de veaux laitiers de remplacement a été sujette à une forte morbidité et mortalité suite à l'exposition des veaux à de l'aflatoxine M1 trouvée dans le lait de remplacement. Durant les six semaines suivant l'exposition, la morbidité des veaux pré-sevrés était de 100% et près de 3000 veaux sont morts. L'aflatoxine M1 est un produit du métabolisme intermédiaire de l'aflatoxine B1 et se retrouve dans le lait des vaches laitières qui consomment une ration contaminée. L'histopathologie à démontré des changements tant au niveau des hépatocytes centrolobulaires que de l'arbre biliaire. La biochimie du sérum indiquait un désordre hépatique. L'examen toxicologique du lait de remplacement dévoila la présence de l'aflatoxine M1. Trois jours suivant le retrait du lait affecté, les niveaux de morbidité et de mortalité revenaient aux valeurs normales pour cette ferme.

Introduction

This report describes a case of high morbidity and mortality on a western calf ranch presumably related to exposure to aflatoxin M1-contaminated milk replacer.

Aflatoxins are mycotoxins produced by molds of the *Aspergillus* group.[8] Under suitable conditions of moisture and temperature, these molds may grow and produce aflatoxins on cereal grains.[10] The four major aflatoxins are B1, B2, G1 and G2. Aflatoxin M1 is an intermediate metabolite of aflatoxin B1 and is found in milk, urine or other animal tissues.[11] To date, there is neither a reference dose nor a risk-specific dose for a tolerable daily intake for aflatoxin M1 in humans.[1] The U.S. Food and Drug Administration tolerance level of aflatoxin in diets of dairy cows is 20 parts per billion (ppb). In milk intended for human consumption, an action level of 0.5 ppb is also enforced.[6]

Although human literature contains many articles on the health risks of aflatoxin M1 in milk products, there is no literature on this toxin's effect on nursing calves. Cows consuming a ration containing aflatoxin B1 have been reported to secrete 1.7% of that intake as aflatoxin M1 in their milk.[4] Furthermore, humans have experienced a toxic level of aflatoxin M1 after consuming contaminated cheese or other dairy products. Nursing calves are more sensitive than other animals to the toxic effects of aflatoxins.[6] The order of sensitivity to aflatoxins in domestic animals from most- to least-sensitive is: 1) piglets; 2) dogs, calves, foals, horses and feeder pigs; and 3) hogs and lambs.[13]

Important factors in determining the clinical course of aflatoxin exposure are dose of aflatoxin, and age and species of the animal. Less-quantifiable factors, such as environmental stress and nutritional status, can also exacerbate the effects of small doses of aflatoxin. For example, aflatoxin levels that may otherwise cause no obvious clinical signs may cause illness when combined with envi-

ronmental stress and nutritional deficits. The pathophysiology of aflatoxicosis is associated with the liver clearing the ingested toxin. Aflatoxins are inhibitors of nucleic acid synthesis and have been shown to decrease protein synthesis, lipid metabolism and mitochondrial respiration. Hepatocytes are significantly more affected than other cell populations. The net result is inhibition of the critical liver functions involving protein synthesis, carbohydrate and lipid metabolism, and blood clotting.[9]

Overt clinical signs of calves suffering from aflatoxicosis usually occur 2-14 days post-exposure and are consistent with liver compromise. They include hematochezia, steatorrhea and ill thrift. There is also a decrease in the essential serum proteins synthesized in the liver.[13] One feeding trial established that aflatoxins fed to calves at 2.2 ppm in a creep feed for 16 weeks caused death due to liver failure.[12]

Immunosuppression also occurs as the result of aflatoxin's negative impact on T-cell populations and macrophage phagocytic activity.[14]

Aflatoxicosis-associated lesions are principally located in the liver. Hepatocellular degeneration and centrilobular necrosis and vacuolization, with biliary hyperplasia, are the findings of aflatoxicosis in ruminants.[8] Presumptive diagnosis is based on finding aflatoxin in the feed. Further support of that diagnosis can be established through controlled feeding trials with the contaminated feed.[13]

Aflatoxicosis is treated by removal of the contaminated feedstuff. Activated charcoal, combined with 4.55 mg/lb (10 mg/kg) of oxytetracycline for five days, has also been shown to decrease losses if initiated within 24 hours of exposure.[7]

Case History

This case occurred on a calf ranch that receives Holstein calves from several sources and raises them in individual pens to four months of age. Approximately 80 calves a day are received and grouped by age. Calves are fed a milk-product based milk replacer containing greater than 21% crude protein and greater than 18% crude fat. Calves have access to free-choice water at all times. A grain mix is introduced at about two weeks of age, and milk replacer volume is gradually decreased until weaning.

The calf ranch's capacity is approximately 8,000 calves on milk. On arrival, blood samples are taken from a representative number of calves to assess passive transfer. An intranasal, temperature-sensitive infectious bovine rhinotracheitis (IBR) and parainfluenza (PI3) vaccine is administered. Historical annual morbidity rates are approximately 20%, and mortality rates range from 6 to 8%.

Onset of clinical signs coincided with a period of unusually hot weather. Clinical signs became obvious by the third day after arrival on the ranch. The signalment was three- to 14-day-old calves with blood-tinged scours, anorexia and ill thrift. Morbidity was approaching 100% and mortality was approximately 25%. Early in the course of the disease outbreak, 20 fecal specimens were collected. Rotavirus, coronavirus, cryptosporidium and non-enteropathogenic *E. coli* were isolated from these specimens. Since these pathogens are frequent isolates on large calf ranches, the initial plan of action was to improve the colostrum management programs at the source of the calves.

Mortality continued to increase during the next two weeks, and approached 40% in calves 10 days of age. Physical examination of the calves revealed a non-febrile population with rough hair coats, blood-tinged scours and poor body condition. Clinical signs worsened as age increased. Death generally occurred eight to ten days following onset of signs. Differential diagnoses included salmonellosis, colibacillosis and bovine viral diarrhea (BVD). A nutritional/toxin etiology also was considered.

Treatment of affected calves included oral and intravenous fluid replacement and antibiotic therapy. Despite aggressive therapy, mortality continued to increase.

Clinical and Laboratory Findings

Following initiation of the milk replacer diet, blood samples were taken from five calves on Day 1, Day 3, Day 14 and Day 21. The blood was submitted for a food animal chemistry profile as well as serology for BVD, PI3 and IBR. Several necropsies were performed; gross pathology was unremarkable except for presence of a dark brown, fetid fluid in the abomasa. Fresh and formalin-fixed tissues of all major organs from a seven-day-old calf and a 14-day-old calf were sent to the diagnostic laboratory for histopathology, parasitology, viral and bacterial isolation, and toxicology. Milk replacer samples were taken for bacterial isolation, proximate analysis, and toxicology.

Because morbidity remained at 100% and mortality was increasing, the decision was made to discontinue feeding milk replacer to new calves, and feed whole milk instead. No other environmental, water or managerial changes were made at this time.

The morbidity rate of newly arrived milk-fed calves dropped to 18%, and mortality dropped to 6%, within three days. On Day 4, milk replacer was obtained from a new source and morbidity remained at 20% and mortality at 8%.

Laboratory Findings

Histopathology

A hepatopathy, marked by cytoplasmic vacuolization of centrilobular and mid-zonal hepatocytes, was the primary microscopic lesion reported. A mild pleocellular

inflammation of portal tracts and associated bile retention also were present.

Serology

Four of the 20 calves had no antibody titer to IBR, BVD or PI3 viruses. The lack of titers to all three antigens, combined with a mean total protein of 4.3 g/dl, signified that these calves were failure-of-passive-transfer (FPT) animals. The 20% incidence of FPT in the sample population was similar to the ranch's historical rate.

Serum Chemistry

The chemistry profile of selected calves are shown in Table 1. The mean total protein for the group tested at Day 1 was 5.4 g/dl, which was above the 5.2 g/dl cutoff point for FTP.[6] However, by Day 14 the total protein level dropped to 4.8, and was 5.0 g/dl at Day 21. While a drop in total protein at this age could reflect a nutritional deficit of protein or an alteration in protein metabolism, standard practice on this ranch was to tube feed any animal that had not ingested its milk replacer within 1-2 hours. The high quality of the milk-based protein in the milk replacer, and the practice of tubing the calves, suggested that nutritional protein deprivation was unlikely.

The mean albumin value was initially low and remained relatively unchanged for the three-week period. Hypoalbuminemia can result from (1) deficient protein intake; (2) deficient synthesis; (3) excessive protein breakdown; or (4) loss of albumin.[19] Hypoalbuminemia on Day 1 could be the result of inadequate ingestion, but further intake beyond that point would adjust the deficit. Deficient protein intake was previously ruled out. Albumin could be lost through the kidneys *via* an acute nephritis, but acute renal disease is inconsistent with the normal blood urea nitrogen (BUN) and creatinine levels recorded in the chemistry panel. The third possible cause of hypoalbuminemia– protein breakdown– can occur with prolonged fever, disorders such as diabetes mellitus or trauma. These differential diagnoses did not fit the signalment of the case. Most likely cause of the hypoalbuminemia was deficient synthesis by the liver.

Globulin, the third protein fraction evaluated, initially was within the normal range. This was a credible indicator of adequate colostrum ingestion in our sample population. By Day 14, however, globulin levels fell below reference values. When all other body systems are working, globulin levels should rise in response to a bacterial or viral antigenic stimulus. Thus, decreasing globulin levels made a diagnosis of a primary infectious agent less likely. Plausible solutions for the lack of globulins were T-cell impairment or destruction, and/or impaired globulin synthesis in the liver.

Liver enzymes evaluated were gamma glutamyl transferase (GGT), alkaline phosphatase, aspartate amino transferase (AST) and serum dehydrogenase (SDH). Elevations in GGT and alkaline phosphatase were present early in the study. Colostrum is rich in GGT and can be used as an indicator for passive transfer.[2] Also, it is not uncommon to have alkaline phosphatase levels slightly outside of the normal range in young animals.[16] This could explain the initial high values of GGT and alkaline phosphatase. However, a rising and prolonged discrepancy from the reference values indicates a pathologic cholestatic disorder.[2]

Table 1. Serum chemistry results*

Date	Biochemical test	Mean lab value: Day 1	Mean lab value: Day 3	Mean lab value: Day 14	Mean lab value: Day 21	Normal reference
10/9/00	Creatinine	1.4	1.2	0.8	0.8	0.9-1.3 mg/dl
10/9/00	Urea nitrogen	18.6	21.0	7.5	10.5	8.0-23.0 mg/dl
10/9/00	Glucose	**24.0**	**16.0**	**23.0**	**27.5**	33-66 mg/dl
10/9/00	Total protein	**5.4**	**6.4**	**4.8**	**5.0**	6.8-8.6 g/dl
10/9/00	Albumin	**2.3**	**2.4**	**2.1**	**2.5**	3.0-4.3 g/dl
10/9/00	Globulin	**3.1**	**3.9**	**2.6**	**2.5**	3.0-4.9 g/dl
10/9/00	AST	53	45	56	53	43-127 IU/l
10/9/00	Alkaline phosphatase	**149.7**	**207.0**	**306.0**	**173.0**	27-107 IU/l
10/9/00	Gamma GT	**135.7**	**282**	**106.0**	**57.5**	15-39 IU/l
10/9/00	SDH-37	8.7	8.7	10.0	8.0	12-53 IU/l
	Bilirubin total	**0.5**	**0.5**	**0.4**	**0.4**	0-0.1 mg/dl

*Laboratory values listed are the mean value of five randomly selected calves taken on the same day after arrival on the ranch.

Aspartate aminotransferase (AST) and sorbitol dehydrogenase (SDH), linked to hepatocellular damage,[3] were within the reference ranges. Although SDH has a short life and is not stable, an elevation could have provided specific evidence of active hepatocellular necrosis. Aspartate aminotransferase is associated with heptatocellular damage, but also can be elevated if muscle, heart or red blood cells are damaged.

The final liver function test was the measurement of serum bilirubin. Elevated serum bilirubin levels can be seen in the bovine species with severe hepatic disease or biliary obstruction. Average bilirubin levels were markedly elevated at each test.

The sample population was also hypoglycemic on all days tested. Hypoglycemia, a common finding in aflatoxicosis, is associated with liver impairment of glucose metabolism. Electrolyte levels were all within normal values, indicating normal hydration.

Bacteriology / Virus Isolation

A coronavirus and group B salmonella were isolated from the intestines of the 14-day-old necropsy specimen. Salmonella was not isolated from any of the previous 20 fecal samples or any other necropsy specimen. This organism was determined to be a secondary invader on an individual calf, and not representative of the population. The milk replacer sample cultured negative for salmonella, clostridia and coliforms.

Based on clinical presentation, laboratory findings, histopathology and response to removal of the milk replacer, diagnostic efforts focused on possible problems in the milk replacer. Aflatoxin M1 was identified in two separate milk replacer samples sent for toxicological evaluation. Aflatoxin was detected using a qualitative high-performance liquid chromatography (HPLC) test. The aflatoxin M1 level was 0.05 ppb, prompting a presumptive diagnosis of aflatoxicoses due to aflatoxin M1.

Discussion

This is a unique case of potential aflatoxin M1 hepatotoxicity on a Holstein calf ranch. Acute aflatoxicosis presents with signs primarily associated with liver dysfunction, with histological lesions in the centrilobular hepatocytes and elevations in cholestatic and hepatocellular enzymes. Other etiologies of hepatotoxicity were ruled out based on a singular food source and lack of evidence to support an infectious disease. The final presumptive diagnosis was based on isolation of aflatoxin M1 in the primary food source of the calves.

Salmonellosis cannot be totally ruled out in this case. The lone calf with a positive isolate, despite several animals being cultured, did not appear to represent the population. Moreover, mean serum chemistries taken of the cohort do not support an infectious agent or salmonellosis. If salmonella was the etiology for the population, removal of the salmonella-negative milk replacer would have had no effect on disease incidence. Since the only management practice changed was the milk replacer, it is probable that this calf was an isolated case.

Two factors motivated the decision to remove the milk replacer on the first day of the investigation. First, all calves were non-febrile, yet morbidity was 100%. Absence of fevers made an infectious agent less likely. Also, the 100% morbidity rate necessitated a critical evaluation of the nutrition of the population. Second, previous practice experience had shown calf scours to improve when whole milk was substituted for milk replacer. Removal of the milk replacer was the crucial step in stopping the problem and then directing the diagnostic plan. A feeding trial of the milk replacer would have added further support to the diagnosis.

Questions remain as to how the aflatoxin got into the milk replacer. Since aflatoxin M1 is a metabolite of aflatoxin B1, the source must have been animal in origin. Presumably it was a contaminated milk source. Aflatoxin M1 reportedly is excreted in the milk of cows consuming aflatoxin-contaminated feed. Furthermore, questions remain regarding what constitutes a safe feeding level for neonatal calves under the environmental stresses of a modern calf ranch. The aflatoxin M1 action level of 0.5 ppb for milk intended for human consumption has not been tested in pre-ruminant calves. Additional research is necessary to determine if a safe level of aflatoxin can be established for a large confinement calf ranch.

Conclusions

A case of high morbidity and mortality on a calf ranch was given a presumptive diagnosis of aflatoxicosis from aflatoxin M1 exposure. Histopathology, liver enzymes and serum protein values supported the diagnosis. Source of the aflatoxin was determined to be a milk-based milk replacer. Removal of the aflatoxin-contaminated milk replacer resulted in immediate abatement of clinical signs.

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