Liver Disease in Cattle Induced by Consumption of Moldy Hay

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ABSTRACT. Normally innocuous forages are sporadically associated with hepatogenous photosensitization outbreaks at certain times of the year or when grown and harvested during unusual environmental conditions, such as periods of excessive rainfall. Allegations of livestock illness following consumption of such moldy hays are associated with clinical syndromes uncharacteristic of known forage-related diseases, suggesting that unidentified toxin(s) may be responsible. This study was instigated by field observations of hepatogenous photosensitization in cattle fed alfalfa-grass forage. To document the toxic nature of the hay, large bales of hay (450 kg) were fed, ad libitum, to 3 groups of 2 calves each. Elevated serum liver enzymes provided evidence of hepatobiliary disease. Gamma glutamyl transferase activities in serums of the calves sustained at least a 10-fold increase above baseline during the feeding trials. Histologic examination of liver biopsies and postmortem sections revealed mild periportal fibrosis and biliary hyperplasia. Culture material from 12 fungal isolates from the hay failed to induce liver disease in calves.

Liver disease induced by consumption of moldy plant material has been frequently reported in cattle (1-6). Normally acceptable forages, such as alfalfa, red clover and wheat straw, grown and harvested during periods of excessive rainfall, are usually suspected to be at fault. However, the precise environmental conditions and specific mold and forage-mold interactions leading to the onset of these maladies are unknown. Identification of affected herds usually originate from field observations of photosensitization secondary to diffuse liver dysfunction in...
affected cattle. The transient nature, sporadic occurrence and difficulty of experimentally reproducing these conditions has precluded timely investigative procedures needed to elucidate complete understanding of when and why outbreaks occur. Even when liver damage has been reproduced with contaminated forage in controlled feeding trials, the specific hepatotoxins and suspected toxigenic fungi have not been identified.

Mycotoxin contamination of hay and other forages has been shown to adversely affect livestock. Known forage-associated mycotoxins include stachybotryotoxin, aflatoxins, ergot alkaloids, tremorgenins and others (7). However, many descriptions of livestock morbidity and mortality associated with consumption of moldy hay are characterized by clinical syndromes uncharacteristic of known forage-related diseases, implying that unidentified toxins are responsible. Little information is available in the literature to verify the species of toxigenic fungi, the toxic principle(s) involved, or to determine the pathogenesis of liver dysfunction in affected cattle. In all cases, a mycotoxin-related etiology is implied. The toxicology laboratory at the College of Veterinary Medicine, University of Missouri, receives at least a dozen calls annually from Missouri residents concerning suspected toxic forage, but when samples are screened for known mycotoxins, results are consistently negative. Communications with colleagues from other diagnostic laboratories in the midwestern US indicate similar cases exist elsewhere. This syndrome appears to be a unique forage-induced liver disease in cattle.

A TYPICAL CASE

A suspected forage-induced hepatopathy was investigated in April 1991, initially involving photosensitization in 17/28 cows and 1 bull (6). All 28 cows, but not their calves, eventually showed signs and clinical pathology consistent with hepatogenous photosensitization. Besides the usual signs of photosophobia, erythema and edema of nonpigmented skin, the cows lost weight and clinical pathology consistent with hepatogenous photosensitization were selected for comparison with affected udders refused to let their calves nurse. This herd was confined to a 20-acre pasture at the time of illness and was being fed 450-kg bales of second-cutting alfalfa orchard-grass hay harvested and baled during a wet July (14.9 cm rainfall vs 30-y average of 9.3 cm) the previous summer. This hay had been fed for 2 w prior to the owner’s recognition of the problem. Inspection of the interior of one of the bales revealed some whitish mold, although overall the hay appeared to be of average quality. Poisonous plants were not identified. The producer was advised to immediately substitute another forage source.

Serum biochemical profiles from affected cows confirmed diffuse hepatic damage. The mean serum gamma glutamyl transferase (GGT) activity from 28 cows was 1064 U/L (range 66-2509 U/L). Four cows not showing clinical photosensitization were selected for comparison of GGT activity; however, their serum total bilirubin and aspartate aminotransferase (AST) activity were elevated also. A second serum sample was collected from 8 affected cows 10 d after removal of the suspect hay. All of these cows were now severely photosensitized. Bilirubin concentrations had declined to nearly normal; however, AST and GGT activity were still elevated. Fifteen of the cows required 6 to 8 w for recovery, and calves, none of which were clinically affected, were weaned prematurely when their dams refused to let them nurse sore teats.

Most feeds are susceptible to invasion by toxigenic fungi during some phase of production, transport or storage. Environmental stress and subsequent reduced vigor, such as occurred during the excessive rainfall period of 1990 in the Midwest, predispose forages to infestation, colonization and contamination by potentially toxigenic fungi. This investigation provides further evidence for the probable existence of a potentially lethal, forage-associated hepatotoxic mycotoxin for cattle.

EXPERIMENTAL EVIDENCE

Trial 1

To substantiate the toxic nature of the alfalfa-grass hay, a large bale from the same hay-cutting was fed ad libitum to a 180-kg Charlois-cross heifer (calf #1) and a 147-kg Limousin-cross bull (calf #2) for 49 d. Disease progression was monitored using serum biochemical profiles of liver function, including albumin, total protein, conjugated and unconjugated bilirubin, AST, alkaline phosphatase, and GGT. Baseline values were established (day -1) and subsequent samples were taken for comparison every few days during and after the feeding trial. Serum samples taken on day +7 of the feeding trial revealed biochemical profiles compatible with liver damage (Table 1).

### Table 1. Serum biochemical changes in calves exposed to hepatotoxins hay

<table>
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<tr>
<th>Day of Exp</th>
<th>Calf No.</th>
<th>T.Bili* (mg/dl)</th>
<th>AST† (IU/L)</th>
<th>SAP* (IU/L)</th>
<th>GGT* (IU/L)</th>
<th>LDH (IU/L)</th>
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*Total bilirubin—laboratory normal range 0.1-0.5 mg/dl.
†Aspartate aminotransferase—laboratory normal range 8-100 IU/L.
‡Alkaline phosphatase—laboratory normal range 41-116 IU/L.
§Gamma glutamyl transferase—laboratory normal range 22-64 IU/L.
&Lactate dehydrogenase—laboratory normal range 266-4293 IU/L.
Because of depression and mild photosensitization, calf #2 was fed nontoxic alfalfa hay from day 18 to day 35.
Clinically, the calves were not noticeably affected by the toxic hay during the first 15 d of the feeding trial. On day +18 the bull calf was depressed, inappetent and slightly photosensitized. Liver biopsies taken on day +18 from both calves revealed mild periportal fibrosis with mild biliary hyperplasia. The bull calf was moved to another pen and fed high quality alfalfa hay for 17 d to allow clinical recovery then returned to the feeding trial on day +35. The white heifer remained clinically normal and continued to eat the toxic hay until it was withdrawn from both calves on day +49.

Trial 2

Both calves recovered during the following 90 d and were used in a second feeding trial of another bale from the same hay-cutting to verify its toxicity. Serum biochemical parameters had returned to nearly normal by the initiation of trial 2 (Table 2). Clinico-pathologic evidence of liver damage was apparent by day +7. By day +19, GGT activity in calf #1 was 12,996 U/L. To our knowledge, GGT activity of this magnitude has not been reported previously. However, the clinical condition of this heifer remained unchanged. Hepatic enzymes of calf #2 also were increased although not so dramatically. The calves were allowed to recover prior to necropsy.

Postmortem liver sections were examined histologically 250 d after withdrawal of the toxic forage. Changes were relatively mild. Mature fibrous tissue containing few fibroblastic or leucocytic nuclei were present in portal regions, with extensions partially connecting the triads. Collagenous tissue was mature in portal regions and mild fibrosis or increased reticulin was noted around central veins, while hepatocytes were histologically normal.

Fungal Isolations

Hay samples were cultured and 12 fungi were isolated by dilution plating: 3 Eurotium chevalieri (Aspergillus chevalieri), 3 Eurotium amstelodami (Aspergillus amstelodami), 2 Aspergillus versicolor, 2 Scopulariopsis brevicaulis, 1 Aspergillus sydowii, and 1 Scopulariopsis brevicaulis. Aspergillus chevalieri was the most prevalent mold, representing 21% of all colony forming units from the sample. Aspergillus chevalieri, A. amstelodami and A. versicolor are capable of producing the hepatotoxic mycotoxin sterigmatocystin (8), but mycotoxin screens on 2 subsamples of the toxic hay did not reveal detectable concentrations of sterigmatocystin.

Fungal isolates were grown on potato dextrose agar plates for 14 to 21 d at 25 C. Sterilized water was added to the agar plates and the conidia were scraped into a larger volume of sterilized water (100 ml/agar plate). Two hundred quart canning jars containing 100 g of shelled corn and 100 ml water were autoclaved for 30 min. Aliquots (2 ml) of the mycelium-water suspension was added to each jar. The jars were shaken and lids loosened to allow for gas exchange. After 24 h of incubation at 25 C in the dark, the jars were shaken again to ensure complete dispersal of the mycelia. The jars were incubated in the dark for 21 d at 25 C. The cultures were autoclaved at 121 C for 30 min, removed from the jars, and allowed to air-dry overnight in a hood. The culture material was placed in a forced-air oven at 40 C for 48 h and ground to a fine powder, and stored at -10 C until used for feeding trials. We anticipated the stability of the toxin based on our findings from feeding 2-y-old toxic forage stored outside.

Culture material from the 12 fungal isolates failed to induce liver disease in calves when mixed with grain and fed daily for 7 to 10 d.

<table>
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<tr>
<th>Day of Exp.</th>
<th>Calf No.</th>
<th>T. Bilirubin</th>
<th>AST*</th>
<th>ALT*</th>
<th>LDH*</th>
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<td>66</td>
<td>225</td>
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**TABLE 2. Serum biochemical changes in calves exposed to hepatotoxic A. amstelodami and A. versicolor**

**Total bilirubin—laboratory normal range 0.1-0.6 mg/dL.**
**Aspartate aminotransferase—laboratory normal range 5-50 U/L.**
**Alkaline phosphatase—laboratory normal range 41-134 U/L.**
**Gamma glutamyl transpeptidase—laboratory normal range 22-84 U/L.**
**Lactate dehydrogenase—laboratory normal range 266-4293 U/L.**

**DISCUSSION**

Hepatopathy of uncertain etiology is not uncommon in cattle. This case originated from field observations of hepatogenous photosensitization in cattle fed average quality forage in the absence of known hepatotoxic plants or chemicals. A specific etiologic agent was not demonstrated, but the suspect forage did induce the disease experimentally. This normally superior forage appears to be sporadically associated with diffuse liver disease in cattle. Growth and harvesting of normally acceptable forage during times of excessive rainfall presumably supports the growth of a toxigenic fungus or else is conducive to the elaboration of hepatotoxic mycotoxins by normal fungal flora. Another plausible genesis is that viable forage mounted a phytoalexic response to fungal infection, with the phytoalexin being hepatotoxic for cattle.

Bovine hepatopathy induced by mold-damaged alfalfa hay has been reported previously in the US (2,4,9); however, toxigenic fungal species were not identified. Consistent associated hepatic lesions included bile-duct necrosis with secondary biliary hyperplasia. Persistence of fibrosis with mild duct hyperplasia characterized the lesions induced in our case. The degree of mild histopathological change had little correlation with the striking serum biochemical change (GGT 12,996 U/L), which suggested severe damage to the biliary system. In the absence of histopathology supportive of significant
bile-duct necrosis, the induction of GGT synthesis by the hepatotoxin is conceivable.

Typically, early cases of liver disease are clinically inapparent, but are detectable by elevations of liver enzymes in serum. The first serum sample taken after initiation of the feeding trial (day +7) provided evidence of hepatobiliary disease in both calves. Measurement of serum GGT activity proved a sensitive and long-lived indicator of liver insult, as most serum GGT is derived from canaliculal membranes of hepatocytes. Like alkaline phosphatase, GGT appears in the serum as a result of increased synthesis rather than from cell leakage (10). Gamma glutamyl transferase activity in serum of both calves sustained at least a 10-fold increase above baseline for the duration of the feeding trials. On day +28, calf #1 had more than a 350-fold increase in GGT activity compared to baseline (Table 1). In trial 2 calf #1 had a serum GGT activity 433 times baseline. The unidentified hepatotoxin appeared a potent stimulus for GGT synthesis. By contrast, the other liver parameters returned to near or below baseline concentrations at some point during the trial in 1 or both calves. The large rise in bilirubin in calf #2 on day +12 preceded clinical recognition of illness on day +18, at which time the bull calf was inapparent, depressed, and mildly photosensitized. Intrahepatic cholestasis seemed a characteristic feature of this syndrome. The clinical condition of affected cattle was directly related to serum bilirubin concentration, not serum GGT activity. The calf was removed from the study for 17 d to allow clinical recovery, after which a serum sample revealed near normal bilirubin, but a sustained elevation of GGT activity reflected both its continued synthesis and longer half-life in serum.

The portal fibrosis and bile-duct hyperplasia are consistent with insult to the biliary system as corroborated by the extreme elevation in serum GGT activity (11). Compared to GGT, the increase in total bilirubin tended to occur later in the course of the disease in contrast with a deteriorating clinical condition. The increase in total bilirubin was due mainly to an increase in conjugated bilirubin, implying that hepatocytes were still functional in this capacity while excretion of conjugated bilirubin in the bile was hindered. With bile blockage or intrahepatic cholestasis, the direct-to-total bilirubin ratio is greater than 1 (12), as was the case with the calf in our study. Following toxic injury of bile-duct epithelium, conjugated bilirubin most likely reaccumulates in bile and is refluxed through the damaged epithelial cells into the lymph vessels or directly into the sinusoidal blood (13). This finding was demonstrated in rats dosed with a nitrosourea compound (CCNU). Bile ducts were shown to accumulate CCNU more than any other area of the liver. In our case, it is thought that a similar event may be occurring in the bovine biliary system exposed to a forage-associated mycotoxin or more likely its rumen or hepatic metabolite.

Failure to reproduce liver disease in calves using the isolated fungal strains suggests alternative methods are needed to identify the toxigenic species. Corn maize may not be the appropriate substrate for hepatotoxic production. Future efforts will grow the fungal isolates on forage and omit autoclaving of culture material. The feeding trials will be extended to a minimum of 14 d.

The apparent stability of the toxin was confirmed by the fact that hay baled in July 1990 poisoned the cow herd in April 1991. In addition, cattle feeding trials conducted in June 1992 corroborate the persistence of this toxin in forage. We have been unable to demonstrate the toxicity of this hay in other species, including horses, goats, and sheep, fed dosed cattle with extracts. This implies the proximate hepatotoxin may be a rumen or liver metabolite unique to cattle.

REFERENCE


"THE BIOSCIENTIST AS AN EXPERT WITNESS"

The personal experiences and conclusions about serving as an expert witness are recorded by Farrel R Robinson DVM, PhD in his recently published monograph, "The Biologist as an Expert Witness". His informative insights are available as Supplemental Issue of Veterinary and Human Toxicology. The 40-page treatise may be purchased for $15 (prepaid) from the Publication Office, Comparative Toxicology Laboratories, Kansas State University, Manhattan, KS 66506-5606. Payment should be made to "Veterinary and Human Toxicology", and sent with the order. Postage and delivery charges will be added to orders not accompanied by payment.

Vet Human Toxicol 37 (3) June 1995 251