FORAGE-ASSOCIATED MYCOTOXICOSIS

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INTRODUCTION

Mycotoxin contamination of hay and other forages has been shown to have adverse effects on livestock. Known forage-associated mycotoxins include stachybotryotoxin, slaframine, the ergot alkaloids, tremorgens, and sporidesmin(1). Many allegations of livestock illness and death following consumption of moldy hay are associated with clinical syndromes uncharacteristic of known forage-related diseases, suggesting that unidentified toxin(s) may be responsible. Little information is available in the literature to verify involvement of toxigenic fungi, the toxic principle(s) or the basic biological effects on cattle.

FIELD INVESTIGATION

The importance of a suspected forage-associated mycotoxicosis was convincingly demonstrated in April 1991, when a field investigation revealed photosensitization in 17 cows and one bull out of 28 Simmental-cross cows with calves. Besides the usual signs of photophobia, erythema and edema of nonpigmented skin, the cows lost an estimated 100-150 kilograms of bodyweight and dams with affected udders and teats refused to let their calves nurse. The herd was confined to a 20-acre pasture and was being fed big round bales (approximately 450 kg/bale) of second-cutting alfalfa orchard-grass hay harvested and baled during a wet July (14.9 cm rainfall vs 30-year average of 9.3 cm) the previous summer. This second-cutting alfalfa-grass hay had been fed for about two weeks prior to the onset of the problem. Being early spring, the pasture was short and poisonous plants were unavailable for consumption. The producer was advised to withdraw the hay. Visual inspection of the interior of one of the hay bales revealed the presence of a whitish mold, although overall the hay appeared to be of average quality. Screening of a sample of hay for mycotoxins by thin-layer-chromatography did not detect the presence of aflatoxin, T-2 toxin, diacetoxyscirpenol, ochratoxin A, zearalenone, vomitoxin, citrinin, and sterigmatocystin.

Based on serum biochemistries from affected cows, the photosensitization was classified as hepatogenous. The mean serum gamma glutamyl transpeptidase (GGT) activity from 18 affected cows was 1064 with a range of 66-2509 U/L. The herd was classified and evaluated by group according to the severity of clinical photosensitization (Table 1). Four cows showing no evidence of photosensitization were selected for comparison, however, their serum total bilirubin and aspartate aminotransferase activity

TABLE 1 Serum biochemical changes and clinical classification of cattle exposed to hepatotoxic hay. Date of collection 4-1-91.

Cow No.	T.Bili[1] (mg/dl)	AST[2] (U/L)	SAP[3] (U/L)	GGT[4] (U/L)	LDH[5] (U/L)	Clinical Class
3	1.4	155	50	35	3554	normal
4	1.1	118	37	37	3030	normal
11	1.2	141	56	35	2065	normal
12	1.5	101	69	32	1691	normal
Means	**1.3**	**129**	**53**	**35**	**2585**	
1	2.4	635	141	1478	10759	mild
2	1.5	300	92	929	5537	mild
7	1.5	330	120	893	7262	mild
8	1.2	263	45	66	3992	mild
13	2.1	666	103	1278	7087	mild
19	2.8	434	82	897	6380	mild
Means	**1.9**	**438**	**97**	**924**	**6836**	
18	1.3	314	91	664	5727	moderate
15	1.4	287	60	520	4580	moderate
16	4.3	449	123	1695	6517	moderate
5	1.5	307	113	1246	6111	moderate
Means	**2.1**	**339**	**97**	**1031**	**5734**	
6	3.6	591	94	612	8031	severe
9	1.7	608	156	1709	8145	severe
10	4.7	516	152	1094	5911	severe
14	4.9	545	108	2509	4950	severe
17	4.4	437	113	601	6653	severe
22	1.9	715	106	1163	7522	severe
20	2.6	444	107	488	5777	severe
21	9.0	260	116	1309	4880	severe
Means	**4.1**	**515**	**119**	**1186**	**6484**	

[1]Total bilirubin--laboratory normal range 0.1-0.6 mg/dl.
[2]Aspartate aminotransferase--laboratory normal range 58-100 U/L.
[3]Alkaline phosphatase--laboratory normal range 41-116 U/L.
[4]Gamma glutamyl transferase--laboratory normal range 22-64 U/L.
[5]Lactate dehydrogenase--laboratory normal range 2666-4293 U/L.

were elevated. Cows with slight erythema of the muzzle and ears and some increase in lacrimation were classified as mildly photosensitized. Cows showing inappetence, weight loss, erythema of exposed, lightly pigmented areas, with some serum leakage at the skin surface, profuse lacrimation and photophobia were classified as moderately affected. Cows showing fissuring of lightly pigmented epidermis with serum exudation and crusting, edematous pendulous ears, kicking at their abdomen, inappetence, severe weight loss, and photophobia, were classified as severely affected.

To monitor the liver damage, a second serum sample was collected from 8 affected cows 10 days later (Table 2). All of these cows were now severely photosensitized. Bilirubin had declined to almost normal concentrations; however, AST and GGT activity were still significantly elevated. Fifteen of the cows required 6 to 8 weeks for recovery and 8 calves were weaned prematurely when their dams refused to let them nurse sore teats. Another trip to the farm in early September revealed hind limb lameness in one cow and the herd bull. Another cow was lame in a front limb. The lameness in all three had persisted for the last 4 to 6 weeks. Examination revealed light-colored hooves on all affected limbs. Hoof overgrowth and transverse cracks were evident. Presumably, this condition was a sequela of the photosensitizing reaction that occurred five months earlier.

TABLE 2 Serum biochemical changes and clinical classification of cattle exposed to hepatotoxic hay. Date of collection 4-10-91.

Cow No.	T. Bili (mg/dl)	AST (U/L)	SAP (U/L)	GGT (U/L)	LDH (U/L)	Clinical Class
6	0.4	443	66	302	4528	severe
10	0.7	351	309	1319	3890	severe
12	0.5	206	98	747	3318	severe
17	0.6	250	93	821	4119	severe
19	0.6	217	99	698	3037	severe
20	0.6	237	101	494	3778	severe
21	1.3	255	128	1040	3471	severe
22	0.4	159	93	709	3058	severe
Means	**0.64**	**265**	**123**	**766**	**3650**	

EXPERIMENTAL EVIDENCE

To document the toxic nature of the alfalfa-grass hay, a large bale from the same hay-cutting was fed *ad libitum* to two 120-kg Holstein steers (calf 1 and calf 5) for 57 days. In addition, two pounds/head of a commercial grain ration was fed daily. The calves were confined to a drylot pen for most of the feeding trial. Disease progression was monitored using a serum biochemical profile of liver function that included albumin, total protein, conjugated

bilirubin, unconjugated bilirubin, total bilirubin, direct bilirubin, AST, SAP, GGT, LDH, and A/G ratio. Baseline values were measured at the start of the experiment, 30 days later, and then every week or so thereafter. A 3-fold increase of serum GGT in one calf and a 24-fold increase in the other calf after 30 days on the hay indicated damage to the biliary tree.

Clinically, the calves were not noticeably affected by the toxic hay during the first 30 days of the feeding trial. Serum samples taken 30 days into the trial, however, revealed GGT values of 90 and 1123 U/L in calves 1 and 5, respectively. In the absence of clinical photosensitization, the calves were permitted to graze green pasture for 5 days to increase the chlorophyll content of the diet. On day-35 of the trial the calves were returned to the drylot with no change in their clinical condition. There was little change in the GGT activity in the serum from the day-30 sample until day-51 when 481 U/L were measured in the serum of calf 1. At this time, calf 5 had a 3-cm-wide strip of desiccated skin spanning the dorsal midline. Liver biopsies taken on day-51 of the experiment revealed prominent periportal fibrosis with moderate biliary hyperplasia. Other signs of mild photosensitization, including crusting and peeling on the dorsum of the nose of both calves, occurred during the next week. On day-57 calf 5, was found unexpectedly in lateral recumbency and could not stand. A serum sample was obtained and the calf was euthanized and necropsied. At the time of euthanasia GGT was 3455 U/L and total bilirubin was 2.6 mg/dl. The skin along the dorsal aspect of the body of calf 5, particularly over the withers, was rugose and thickened with a dry, leathery texture. Yellow, gelatinous fluid distended the subcutis of the ventral jaw. The liver was extraordinarily firm with a very fine reticular pattern throughout. Resistance to incision indicated the presence of excessive fibrous tissue in the liver.

Microscopic examination of the skin revealed lesions consistent with photosensitization. Liver lesions were characterized by severe fibrosis that bridged periportal areas and in some fields surrounded individual hepatocytes. Associated with these areas was proliferation of small bile ducts. Large portal radicals were similarly surrounded by fibrosis, with budding of medium-sized bile ducts.

Calf 1 was moved to an inside stall on day-57 and the diet was changed to alfalfa hay and the same grain ration. This calf recovered during the following 3-4 weeks and was removed from the trial.

Three fungal species isolated in pure culture from subsamples of the hay were *Scopulariopsis fusca*, *Scopulariopsis candida* and *Eurotium chevalieri*. The *Scopulariopsis* species were the most prevalent molds and members of this genus have never been associated with mycotoxicoses or moldy feed in general. Some members of the *Eurotium* genus are capable of producing the hepatotoxic mycotoxin, sterigmatocystin(2). Mycotoxin screens on two subsamples of the toxic hay did not reveal detectable concentrations of sterigmatocystin.

DISCUSSION

Hepatopathy of uncertain pathogenesis has been extensively reported in livestock(3-7). These cases originated from field observations of hepatogenous photosensitization in pastured cattle in the absence of known hepatotoxic plants or chemicals. Etiologic agents were not demonstrated, and associations between suspect forages and clinical disease were suggested. Normally innocuous forages appear to be sporadically associated with hepatogenous photosensitization outbreaks at certain times of the year or when growing during unusual environmental conditions, such as a period of excessive rainfall. Examples of sporadically toxic forages include oats, wheat and red clover.

Bovine hepatopathy induced by mold-damaged alfalfa hay has been reported previously in the United States(5,8-10). Toxigenic fungal species were not identified. Consistent and characteristic hepatic lesions included bile-duct necrosis, with secondary biliary hyperplasia. Bridging periportal fibrosis with mild bile-duct hyperplasia characterize lesions induced in our cases.

The portal-to-portal bridging fibrosis in the liver together with the bile-duct hyperplasia is consistent with biliary damage corroborated by the extremely elevated serum GGT activity(11). Total bilirubin tended to rise late in the course of the disease. This was due mainly to an increase in conjugated bilirubin implying that hepatocytes were functional while excretion of conjugated bilirubin in the bile was hindered because of damage to the biliary network. One study has suggested that increases in bilirubin dictate a poor prognosis in cattle fed a diet containing *Senecio jacobea*(12). In our field investigation, cows with bilirubin concentrations of 9.0 mg/dl recovered.

Measurement of serum GGT concentration of all exposed cattle in this outbreak was used as an index of liver damage, and in selecting individuals for closer scrutiny. In addition, the rise in serum activity of this enzyme was an early indicator of liver damage in calves fed the toxic hay under controlled conditions. An increase in GGT, but not bilirubin, tended to precede the appearance of clinical photosensitization. In calf 5, the rise in bilirubin on day-57 did not occur until the calf was terminally ill. On day-63, calf 1 had an even higher bilirubin concentration 6 days after removal of the toxic hay, while on day-78 total bilirubin had returned to baseline. This calf recovered with no apparent long-term adverse effects.

Following the toxic injury of bile-duct epithelium, conjugated bilirubin most likely regurgitates through the damaged epithelial cells into the portal tissue space where it drains either into the lymph vessels or directly into the sinusoidal bloodstream (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. It is thought that a similar event may be occurring in the bovine biliary tree exposed to an unknown forage-associated toxicant or its rumen or hepatic metabolite.

The apparent stability of the toxin in this case is confirmed by the fact that hay baled in July 1990 poisoned the cow herd in

April 1991. In addition, cattle feeding trials conducted in March 1992 indicate continued stability of the toxin. To date, we have been unable to demonstrate the toxicity of this hay or extracts of the hay in other species including horses, goats, and mice. This implies the hepatotoxin may be a rumen or liver metabolite unique to cattle.

SUMMARY

Suspected forage-associated mycotoxicosis was investigated during an outbreak of photosensitization in a beef herd. A feeding trial confirmed moldy alfalfa-grass hay as the source. *Scopulariopsis fusca*, *Scopulariopsis candida* and *Eurotium chevalieri* were isolated in pure culture from subsamples of the hay. The *Scopulariopsis* species were the most prevalent molds and members of this genus have never been associated with mycotoxicoses. Serum biochemistries showed elevated levels of GGT and total bilirubin. Histopathological evaluation revealed severe bridging periportal fibrosis and moderate biliary hyperplasia.

ZUSAMMENFASSUNG

Vermutete Futter-bedingte Pilzvergiftung wurde während eines Photosensibilisierungsausbruch in einer Mastherde untersucht. Ein Futterversuch bestätigte die Ursache als verschimmelres Alfalfa Kraulgras. Von Heuproben wurden *Scopulariopsis fusca*, *Scopulariopsis candida* und *Eurotium chevalieri* in Reinkultur isoliert. Die *Scopulariopsis* Spezies waren die häufigsten Schimmelpilze, und Mitglieder von diesem Genus waren bis jetzt nie mit Mykotoxicosen in Zusammenhang gezogen. Blutbiochemische Untersuchungen zeigten erhöhten GGT- und Gesamtbilirubinwerte. Histopathologische Untersuchung wurden fortgeschrittene periportale Fibrose sowie leichte Gallenhyperplasie festgestellt.

SUMARIO

Fueron investigados casos de micotoxicosis relacionados con forrajes posiblemente contaminados durante un brote de fotosensibilización en un hato de ganado de carne. Una muestra del forraje confirmó como la fuente al heno de alfalfa enmohecido. *Scopulariopsis fusca*, *Scopulariopsis candida* y *Eurotium chevalieri* fueron aislados en cultivos puros de muestras del heno. Las especies *Scopulariopsis* fueron los hongos más comunes y miembros de este género nunca antes habían sido asociados con micotoxicosis. Análisis bioquímico del suero demostró niveles elevados de GGT y de bilirrubina. La evaluación histopatológica reveló una severa fibrosis periportal y una hiperplasia biliar moderada.

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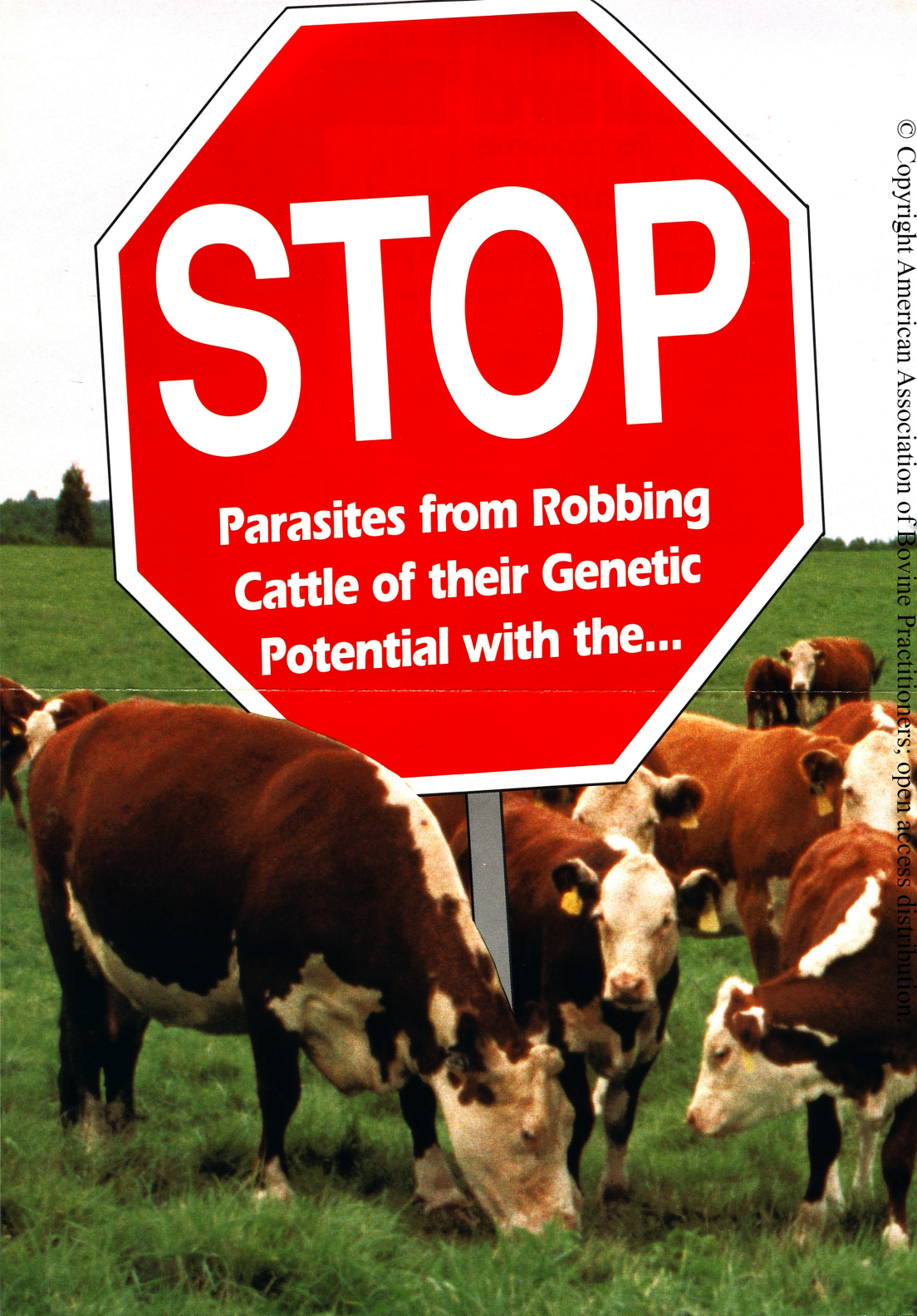

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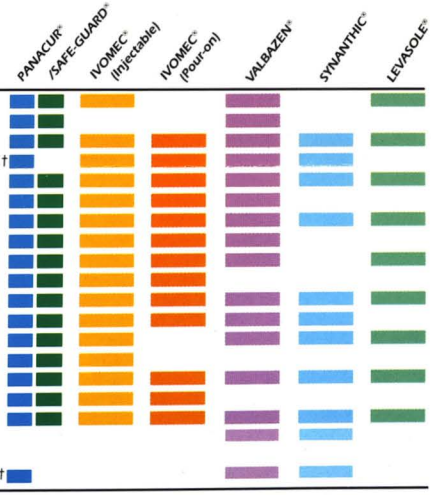

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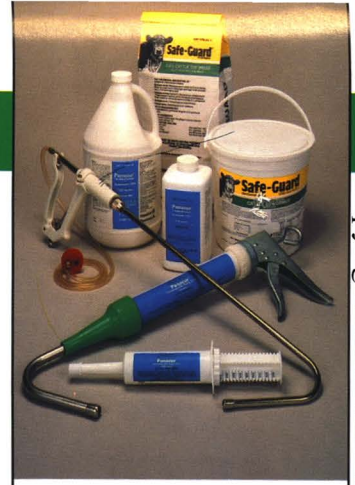

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Intestinal worm (adults & 4th stage larvae): Bunostomum phlebotomum (hookworm) Nematodirus helvetianus (thread-necked intestinal worm) Cooperia punctata and C. oncophora (small intestinal worm) Trichostrongylus colubriformis (bankrupt worm) Oesophagostomum radiatum (nodular worm)
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