The Failure of Purified T-2 Mycotoxin to Produce Hemorrhaging in Dairy Cattle

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SUMMARY

A Holstein cow was intubated with 182 mg of 97% pure T-2 toxin (0.44 mg/kg of body weight) for 15 days. A dairy ration containing 50 mg/kg (50 ppm) of T-2 toxin was refused. A calf, born four days after onset of maternal treatment, was intubated with 26.2 mg of purified T-2 toxin (0.6 mg/kg of body weight) for seven consecutive days and then on alternate days for a total of 16 days. The calf was severely affected clinically by the T-2 toxin. The T-2 toxin failed to cause bovine hemorrhagic syndrome in either animal. Unspecific gastrointestinal lesions were noted in the cow but none were detected in the calf. In the calf, severe depression, hindquarter ataxia, knuckling of the rear feet, listlessness and anorexia were caused by the T-2 toxin.

RÉSUMÉ

L'incapacité de la mycotoxine T-2 purifiée de provoquer des hémorragies, chez des bovins laitiers

Cette expérience consistait à administrer à une vache Holstein, à l'aide d'un tube oesophagien, pendant 15 jours, 182 mg de toxine T-2 purifiée à 97%, i.e. 0.44 mg/kg de poids vif, parce qu'elle refusait de manger de la ration laitière qui contenait 50 mg/kg, i.e. 50 ppm, de cette toxine. Quatre jours après le début de l'expérience, elle donna naissance à un veau auquel on administra, aussi à l'aide d'un tube oesophagien, 26.2 mg de cette toxine, i.e. 0.6 mg/kg de poids vif, pendant sept jours consécutifs et ensuite à tous les deux jours, pour un total de 16 jours. Le veau manifesta des signes cliniques marqués. Ni lui ni sa mère ne développèrent cependant le syndrome hémorragique bovin attribuable à la toxine T-2. On décela la présence de lésions gastro-intestinales chez la vache, mais non chez le veau. Il manifesta cependant de la dépression marquée, de l'ataxie postérieure, du fléchissement des boulets des membres postérieurs, de l'apathie et de l'anorexie.

INTRODUCTION

The genus *Fusarium* contains numerous species of fungi known to be the cause of plant disease, plant product spoilage, and food-related toxicities in both man and animals.

Several outbreaks (2, 3) and experimental studies (1, 4, 6) of T-2 mycotoxicoses in cattle all describe a hemorrhagic disease. Epistaxis, anorexia, weakness, hemorrhagic diarrhea, extensive serosal, mucosal and subcutaneous hemorrhages are believed to be caused by T-2 toxin-contaminated feedstuffs in cattle (2). Frequent fifth and sixth month abortions of unknown etiology, partial anorexia and extensive serosal hemorrhages are associated with the consumption of T-2 toxin-containing rations in dairy cattle (3). T-2 toxin administered intramuscularly to cattle produces bleeding from the turbinates, petechial to ecchymotic hemorrhages of the epicardium, scattered ecchymotic hemorrhages in the small intestine, massive hemorrhages into the lumen of the large intestine, a few petechial hemorrhages on the rumen and abomasum and hemorrhages in the cervical and mesenteric lymph nodes (4). Experimentally, production of bloody feces, anorexia, dehydration, abomasal ulcers, ruminal ulcers and abomasitis with edema occur in dairy calves given purified T-2 toxin in capsules (6). Dosage (*per os*) of calves with crude and crystalline T-2 toxin, at levels likely to be found in feed contaminated with *Fusarium* sp fails to produce any evidence of clinical or subclinical hemorrhagic disease. Failure continues even when the T-2 toxin dosing is continued for 79 consecutive days and when the total dose of T-2 toxin given to one calf is almost 1.8 g (5).

The purpose of this paper is to attempt to clarify the relationship of T-2 toxin to outbreaks of "moldy corn toxicosis" by administering T-2 toxin to a Holstein cow in late pregnancy and her offspring. Criteria used were: a) clinical signs, b) detectable effect(s) on blood chemistry, peripheral blood, or bone marrow parameters and c) gross and microscopic lesions.

MATERIALS AND METHODS

The 97% pure T-2 mycotoxin used in this study was produced as reported previously (7). The T-2 toxin was mixed with a commercial bovine ration[1] at a concentration of 50 mg/kg (50 ppm). The 50 mg/kg of T-2 toxin was assumed to be palatable and to be sufficient to induce late abortion as parenterally (I.V.) administered T-2 mycotoxin does in swine (9). In the event that the cow refused the 50 mg/kg T-2 toxin ration, the protocol provided that 182 mg of T-2 toxin (in a vehicle of 20 mL of 95% USP ethanol) would be given daily by esophageal intubation to the cow for the remainder of the study. The 182 mg of T-2 toxin represented the amount of mycotoxin in 8 lb (3.6 kg) of ration mixture, the quantity normally consumed by a cow of 409.1 kg in a day.

The healthy, pregnant cow was kept

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[1]Land O'Lakes Feico Dairy, Land O'Lakes, Inc. Fort Dodge, Iowa.

in an isolation facility and was examined daily for any clinical signs. Jugular blood samples were drawn at days 1, 8, 14 and 16 to measure complete blood count (CBC) (EDTA anticoagulant), serum glutamic pyruvic transaminase (SGPT), serum glutamic oxalacetic transaminase (SGOT), alkaline phosphatase, total protein, cholesterol and lactic dehydrogenase (LDH). The CBC included the packed cell volume (PCV), hemoglobin (Hb), total erythrocyte count (RBC), total white blood cell count (WBC) and a 300 cell differential stained with Wright-Giemsa. The PCV was obtained by microcapillary centrifugation[2]. The hemoglobin values were determined using the photometric cyanmethemoglobin method[3]. The RBC and WBC counts were performed using a Coulter counter[4].

Terminal bone marrow samples were taken from the sixth left rib within 20 minutes from the time of euthanasia. The bone marrow was expressed from the rib onto glass slides, smeared, allowed to air dry and then stained with Wright-Giemsa. The cow was euthanatized by an intravenous injection of sodium pentobarbital-sodium nitrate and underwent necropsy. Samples of the following tissues were collected, in 10% buffered formalin solution, for histopathology (hematoxylin-eosin stain): brain, esophagus, reticulum, rumen, omasum, abomasum, duodenum, jejunum, ileum, cecum, colon, rectum, liver, pancreas, thyroid gland, adrenal gland, kidney, ovary, uterus, cervix, placenta (shed at parturition), lung, mesenteric lymph node, spleen, udder and myocardium.

The neonatal bull calf from this cow was given by esophageal intubation (beginning four days postpartum) 0.6 mg/kg of T-2 mycotoxin. This represents one-half of the single intravenous LD_{50} of T-2 toxin in swine (1.21 ± 0.15 mg/kg) (7) and is the quantity of T-2 toxin that should have a less than lethal effect on the calf. All clinical signs were noted. Weekly blood samples from the jugular vein were evaluated for CBC, SGOT, SGPT, alkaline phosphatase, total protein, cholesterol, and LDH. After 16 days of treatment, the calf was euthanized by an intravenous injection of sodium pentobarbital-sodium nitrate and submitted for necropsy. Samples of the same tissues as the mother (except for the reproductive organs) were similarly collected for histopathology.

RESULTS

The cow accepted to consume less than 4 lb (1.82 kg) of the 50 mg/kg T-2 toxin ration and then totally refused the ration mixture and would not even approach the feed box. The palatability of the ration was enhanced by topping it with molasses, ground corn and crumbles but this did not change the animal's aversion to the feed. Even control feed (visually identical to the 50 mg/kg T-2 toxin ration) was avoided by the cow as she remained far from the feed box and consumed only the alfalfa hay (15 kg) given to her daily. This refusal of the 50 mg/kg T-2 toxin ration continued over a five-day period, after which the T-2 mycotoxin was administered by daily esophageal intubation. Three days after the initial intubation of T-2 toxin, the feces of the cow became noticeably loose. After four days of forced administration of mycotoxin (day 9 of the study), the cow gave normal birth to a healthy, apparently full term calf. The cow appeared to be lactating normally and the calf was given free access to nurse.

At necropsy a large (2 cm diameter) area of acute mucosal ulceration was found in the anterior, ventral aspect of the rumen. The duodenum appeared normal, but the jejunum had a definite moderate to severe congestion of the mucosa. This congestion increased in severity near the ileo-cecal-colic junction. The jejunum and ileum contained a very fluid fecal material. No other visceral gross lesions were discerned. Histopathology revealed congestion of the blood vessels in the lamina propria of the omasum and rumen. There was submucosal edema of the reticulum. The other microscopic lesions were moderate congestion of the duodenal mucosa with severe, acute congestion of the mucosal villi tips and severe congestion of the vasculature in the lamina propria of the jejunum and ileum. The cecum and colon both had edema of the submucosa, muscular layers, and serosa. There were no microscopic lesions in the placenta shed at parturition. The hematology results were within the normal range (Table I), and the bone marrow was not affected.

Within 20 minutes of receiving the T-2 toxin, the calf developed hindquarter ataxia, knuckling of the rear feet, listlessness and severe depression.

TABLE I
BLOOD VALUES FROM THE COW DAILY INTUBATED WITH 182 MG OF T-2 MYCOTOXIN (N=15)

	Day				Normal
	1	8	14	16	(adult)
PCV (%)	27	30	30	30	24-48
Hb (g/dL)	11.2	11.5	11.5	11.2	8-14
RBC ($10^6/\mu L$)	5.62	5.88	6.00	5.74	5-10
WBC ($10^3/\mu L$)	9.2	3.5	6.7	12.5	4-12
Lymphocytes (%)	35	40	24	15	45-75
Neutrophils (%)	51	40	62	83	15-45
Eosinophils (%)	8	14	7	0	2-20
Basophils (%)	0	1	1	0	0-2
Monocytes (%)	6	5	6	2	2-7
BUN (mg/dL)	25	30	*	18	20-30
SGPT (Frankel Units)	13	10	*	12	8-24
Alkaline Phosphatase (Bodansky units/dL)	3	3	*	3	6-22
SGOT (Frankel Units)	46	53	*	55	42-77
Total Protein (g/dL)	7.3	7.5	*	7.6	7.4-10.2
LDH (I.U./L)	182	174	*	200	119-323
Cholesterol (mg/dL)	85.0	84.5	*	84.0	50-230

*Blood chemistry not performed.

[2]International Equipment Co., Boston, Massachusetts.
[3]Coleman Instrument Inc., Maywood, Illinois.
[4]Coulter Electronics Inc., Hialeah, Florida.

This syndrome of clinical signs lasted for 12 hours per T-2 toxin intubation. Twenty-four hours after mycotoxin administration the calf had recovered and appeared to be clinically normal. After seven daily intubations the effect of the mycotoxin seemed to increase as the calf would not recover within 24 hours, i.e. in time for the next dose of T-2 toxin. At this time the intubation schedule was changed to every other day. Even with this decreased schedule, the calf still showed clinical depression signs 48 hours after an intubation of T-2 mycotoxin. During the second week, the calf became anorectic, would repeatedly submerse his entire head into a water bucket for 15 seconds or more and would loudly grind his teeth. At necropsy there were no gross lesions that we could attribute to the effect of T-2 toxin although the calf had been treated with the mycotoxin for 15 days (11 intubations; cumulative dose of 288.2 mg T-2 toxin). Microscopically there were no lesions that we could attribute to the effect of T-2 toxin in any of the tissues examined. Blood data from the calf was normal except for the total WBC and the PCV. The bone marrow was not affected (Table II).

DISCUSSION

The loud teeth grinding and repeated head submersion in water by the calf are signs indicative of severe head pain, even though there were no gross or microscopic lesions seen in the brain. The calf developed temporary listlessness, depression, hindquarter ataxia and knuckling of the rear feet after receiving the T-2 toxin. This syndrome is similar to the one seen in feeder-swine receiving various I.V. dosages of purified T-2 toxin (7). In T-2 toxin treated swine the duodenum, cecum and colon remain normal. The severity of the intestinal mucosal hyperemia increased with a gradient from the proximal jejunum to the distal ileum and stopped abruptly at the ileo-cecal-colic junction in both species. In sows fed a 12 mg/kg T-2 toxin ration, the same pattern of mucosal hyperemia is seen with the apical portion of the spiral colon also being moderately involved (8).

As the exact breeding date is unknown, we can not say that the calf was truly a full term fetus. T-2 toxin does cause late pregnancy abortions (9) and other reproductive problems in sows (8). If the calf was premature, we do not know if the parturition was induced by the cow's reduced caloric intake when the 50 mg/kg T-2 toxin ration was refused or by the T-2 toxin administered. The cow's strenuous resistance to intubation might have been due to a dislike for the passing of the esophageal tube or to the visceral sensation caused by the T-2 toxin.

The physiological effects of purified T-2 toxin are not yet known, which allows for only partial explanation of the blood parameters data (Tables I and II). We do not know why the WBC total in the cow is decreased on day 8 but the increase on day 16 is due to stress. This interpretation is supported by the decreased percentage of lymphocytes with the concurrent increase in neutrophils on days 14 and 16 (Table I). The elevated total WBC in the calf on day 8 is caused by a respiratory infection (severe dyspnea, moist rales and 39.4° C) which was successfully treated with tylosin[5] (3 mL/100 lb (45 kg), SID, four days). The elevated PCV on days eight and 16 reflect the dehydration of the calf. We offer no explanation for the decreased SGPT value on day 8 or for the decreased alkaline phosphatase, SGOT, or LDH values on day 16. These decreased blood chemistry values may be meaningful once the effect of T-2 toxin on the physiology of cattle is determined.

We did not observe the hemorrhagic disease reported in natural outbreaks (2, 3) (in which T-2 toxin was subsequently recovered from the suspect rations) and in experimental studies (1, 4, 6) when we intubated T-2 toxin (97% pure) into a cow at 0.44 mg/kg (182 mg T-2 toxin per intubation, cumulative dose of 2730 mg T-2 toxin) or into a calf at 0.6 mg/kg (26.2 mg T-2 toxin per intubation; cumulative dose of 288.2 mg T-2 toxin).

Hibbs *et al* do not report the level of T-2 toxin found in the contaminated ration (2), while Hsu *et al* estimate a 2 mg/kg (2 ppm) level of T-2 toxin based on their analysis of the rations involved (3). Grove *et al* administered the T-2 toxin (melting point 146-147.5° C) intramuscularly to their cattle (1), as did Kosuri *et al* (T-2 toxin of unreported purity) (4). These four reports (1, 2, 3, 4) describe a severe hemorrhagic syndrome, whereas Pier *et al* note blood only in the feces of calves receiving as much as 0.64 mg/kg of T-2 toxin (96% pure) by capsules (6). Matthews *et al* report no evidence of hemorrhage in calves dosed with crude and crystalline T-2 toxin for as long as 79 consecutive days when as much as 1.8 g T-2 toxin (cumulative dose) was given to a calf (5).

Our results coincide in part with the findings of Pier *et al* (6), reporting that T-2 toxin produces anorexia, abom-

TABLE II
BLOOD VALUES FROM THE CALF INTUBATED 11 TIMES WITH T-2 MYCOTOXIN OVER A 16 DAY PERIOD

	Day			Normal
	1	8	16	(calf)
PCV (%)	29	32	36	26-41
Hb (g/dL)	11.4	11.2	12.5	9.4-14.4
RBC ($10^6/\mu L$)	7.34	7.38	8.12	7.08-11.7
WBC ($10^3/\mu L$)	11.2	18.4	13.7	4.95-15.9
Lymphocytes (%)	46	41	47	40.5-85.2
Neutrophils (%)	48	54	50	10.7-52.4
Eosinophils (%)	0	1	0	2.6-6.9
Basophils (%)	1	1	0	1.1-2.0
Monocytes (%)	5	3	3	2.3-14.4
BUN (mg/dL)	10	10	10	4.6-16.3
SGPT (Frankel Units)	12	2	10	4-22
Alkaline Phosphatase (Bodansky units/dL)	22	22	6	5-21
SGOT (Frankel Units)	46	37	28	37-64
Total Protein (g/dL)	4.6	5.2	4.9	5.2-6.7
LDH (I.U./dL)	138	180	99	134-293
Cholesterol (mg/dL)	88.0	78.5	82.5	42-210

[5]Tylan-50 Elanco, Indianapolis, Indiana.

asal ulcers and abomasitis with edema in dairy calves. We found that T-2 toxin caused anorexia in a dairy calf, whereas in a pregnant dairy cow only a ruminal ulcer, edema of the submucosa of the reticulum, cecum and colon occurred. Our results support those of Matthews *et al* (5) in that no evidence of clinical or subclinical hemorrhagic disease is seen in the cow (cumulative dose of 2.73 g T-2 toxin) or calf (cumulative dose of 0.2882 g T-2 toxin). Future investigation into the effect of purified T-2 mycotoxin on cattle should include examination of coagulation parameters such as platelets, clotting time, etc.

We conclude that T-2 mycotoxin is not the etiology of "moldy corn toxicosis" in dairy cattle. Though previous reports (2, 3) infer that T-2 toxin causes a severe hemorrhagic disease in dairy cattle under field conditions, other (unidentified) mycotoxins in those mold-contaminated rations were probably responsible for the hemorrhagic diathesis.

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LETTER TO THE EDITOR

Pet Foods in Canada

DEAR SIR:

I wish to comment on the fine article in the Canadian Veterinary Journal's January 1980 issue, "Toxic Myopathy in a Dog Associated with the Presence of Monensin in Dry Food" by Dr. J.S. Wilson. Ten years ago, my colleagues and I similarly reported a different calamity associated with feeding commercial pet foods (1). Several other articles in the recent veterinary literature attest to disease in dogs and cats directly resulting from dietary inadequacies or contaminants in commercial products (2-4).

In the decade 1970-1980, the Canadian Veterinary Medical Association's pet food certification program has been brought into existence in the continued absence of regulation of pet foods' nutritional adequacy in Canada. It is a matter of public record that the CVMA (as well as the Canadian Feed Manufacturers Association, Consumers Association of Canada and the Department of Consumer and Corporate Affairs) pressed in the winter of 1974-1975 for inclusion of pet foods under amendments to the Feeds Act (5). On the other hand, the Pet Food Manufacturers Association argued against the inclusion of pet foods under that Act, and the Act was not amended to include them. Amazingly, even commercial fish and rabbit feeds are now regulated under the Feeds Act (along with livestock feeds); Dr. Wilson was simply fortunate, as he pointed out in his paper, to have obtained the analytical services of the Plant Products Division of Agriculture Canada, to help solve his mystery.

Until there is meaningful new legislation, or regulation under existing legislation (as in the Feeds Act), the CVMA's voluntary pet food certification program will continue to be the *only* independent program assuring nutritional adequacy of a pet food in Canada. I urge practicing veterinarians to submit case reports of the type published by Dr. Wilson to continue to document the risks of a nutritionally unregulated marketplace for Canada's 10 million dogs and cats.

Sincerely yours,

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