

Pulmonary edema and hydrothorax in swine produced by fumonisin B₁, a toxic metabolite of *Fusarium moniliforme*

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Abstract. Pulmonary edema and hydrothorax were observed in mature swine that died approximately 5 days after consuming corn screenings. These postmortem observations were reproduced in younger swine (16-24 kg) that died within 1 week when fed the corn screenings under experimental conditions. Additionally, pulmonary edema and hydrothorax occurred in a pig (7.1 kg) that died after receiving 4 daily intravenous injections of fumonisin B₁. A fungus was isolated from the corn screenings that is identical to *Fusarium moniliforme* MRC-826 in colony morphology and under microscopic examination.

Simultaneous epizootics occurred on 2 southwest Georgia farms, resulting in the deaths of 34 mature swine. Four cadavers representing both farms were submitted for diagnostic workup. At necropsy, the gross pathologic changes observed were remarkably similar. These changes included extremely marked pulmonary edema and massive hydrothorax. The thoracic cavities were overfilled with golden-yellow liquid. Sections of lung tissue examined microscopically showed edema so severe that individual lobules and pleura were separated from the parenchyma. The alveolar septa were congested but were without hyperplasia or fibroplasia. These pathologic findings indicated the pulmonary edema and hydrothorax were primary concurrent conditions. The absence of epithelial hyperplasia or fibroplasia suggested these conditions were perhaps due to an unusual toxin. Routine diagnostic testing for toxins and infectious agents failed to establish an etiology. These epizootics appeared to represent a heretofore unrecognized disease problem.

Corn screenings from the 1989 crop that had been recently purchased from a local commercial grain dryer were common to both farms. On both farms, death began approximately 5 days after the screenings were first fed and ceased 24 hours after the screenings were removed as the feed source. The corn screenings became the focus of further investigations, and two etiologies for the pulmonary edema/hydrothorax were considered: (1) the rodenticide alpha-naphthyl thiourea (ANTU)^{1,3} and (2) toxic metabolites produced by certain strains of the fungus, *Fusarium moniliforme*.^{5, 6} There are many pulmonary toxicants, however only

ANTU reportedly produces pulmonary edema/hydrothorax without causing epithelial hyperplasia and fibroplasia.³ The corn screenings were assayed for ANTU, but it was not detected. Thus, this study was designed to investigate any possible relationship between the observed swine deaths and the fumonisins.

Materials and methods

Approximately 90 kg of corn screenings were collected from each farm, labeled Feed A and Feed B, and transported to the Veterinary Diagnostic and Investigational Laboratory, Tifton, Georgia.

Fungal culture and mycotoxin assay. Whole corn kernels collected from Feed A and Feed B were surface sterilized by soaking for 1 min in 5.25% sodium hypochlorite. The kernels were rinsed for 1 min in sterile distilled water, placed on potato dextrose agar, and incubated at 25 C for 5 days. A small aliquot from a lyophilized culture of *F. moniliforme* MRC826^a was suspended in 2-3 drops of sterile water, spotted on potato dextrose agar, and incubated at 25 C for 5 days. Approximately 1.5 kg of Feed A and of Feed B were sent to the National Veterinary Services Laboratory, Ames, Iowa, for fumonisin assays.

Experimental feeding study. Seven swine (16-24 kg) were purchased locally, and 2 groups of 3 pigs each were designated Group A and Group B to correspond to the corn screenings they were fed as their only nutrient source. The remaining pig (control) was fed a commercially available grower ration. Body weights were recorded for all pigs initially and at bi-weekly intervals for the 28-day feeding period. The pigs were observed twice daily for any clinical anomaly.

Fumonisin injection study. Fumonisin B₁^b (20 mg) and fumonisin B₂^b (10 mg) were prepared for injection by dissolving the crystalline mycotoxins in injectable saline^c modified to contain 5% ethanol. Four swine (7-8 kg) were purchased locally and used for cranial vena cava injections. One pig (high dose) received daily injections equivalent to 0.4 mg fumonisin B₁/kg body weight for 4 days. Another pig (low

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Table 1. Body weights of swine fed corn screenings.

Pig no.	Body weight (kg)		
	Day 0	Day 14	Day 28
Group A			
34	18.2	15.4	12.2
35	22.2	18.2	16.8
36	15.0	12.7	*
Group B			
37	24.0	21.8	18.2
38	18.6	*	*
39	19.1	*	*
Control			
40	15.4	20.5	31.8

* Deceased or removed from study.

dose) received daily injections of 0.174 mg fumonisin B₁/kg body weight for 7 days. Fumonisin B₂ (0.3 mg/kg) was injected for 5 days, and the control pig was injected for 7 days with 1.0 ml of saline-ethanol (95:5).

Postmortem examination. Swine that died during the experimental feeding study or as a result of fumonisin injections were necropsied, and all other swine were euthanized by IV injection of a 26% solution of sodium pentobarbital^d and necropsied. Extensive tissue specimens were collected from all animals, fixed in buffered 10% formalin, embedded in paraffin, sectioned at 5 µm, stained with hematoxylin and eosin (HE), and examined microscopically. Additionally, sections of lung from swine that developed pulmonary edema were stained for fibrin by the Fraser-Lendrum method.⁷

Results

Fungal culture and mycotoxin assay. Fungal growth from Feeds A and B and the lyophilized culture of *F. moniliforme* MRC-826 was examined after 5 days. Visually, each culture contained pinkish-orange colonies with a tannish-brown underside. The isolates were examined microscopically using lactophenol cotton blue stain. The isolates from Feeds A and B were identical to the reference culture of *F. moniliforme* MRC-826 based on colony morphology and microscopic evaluation.

Preliminary data^e indicated the concentration of fumonisin B₁ in Feed A was 105 mg/kg and in Feed B was 155 mg/kg. These analytical values were provided as estimates based on a high-performance liquid chromatographic procedure that is still being developed and modified.

Experimental feeding study. Body weights of the experimental swine were recorded on days 0, 14, and 28, and animals that survived the 4-week feeding period were unable to maintain body weight (Table 1). Regarding animal performance, the nutritional quality of the screenings was obviously poor, and palatability was also a factor because the pigs wasted more feed than they consumed. The screenings contained an ap-

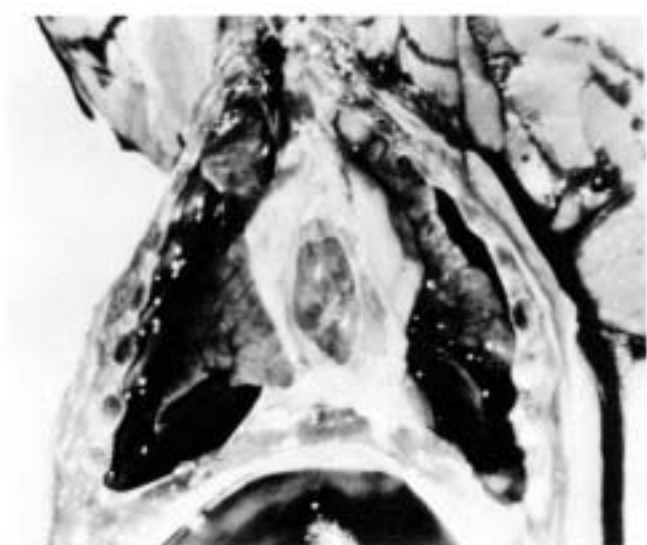


Figure 1. Gross appearance of the open thorax of a pig showing the marked fluid accumulation (hydrothorax). The lungs are displaced (darker area) from the chest wall by the fluid.

preciable quantity of cracked corn kernels, but much of the bulk was composed of glumes, the light chaff material from the corncob.

On day 7 of the feeding study, 1 animal in Group B (pig 38) was found dead, and a severely dyspneic animal (pig 39) was euthanized the same day. At necropsy, both pigs exhibited marked pulmonary edema and hydrothorax previously seen in field cases. The remaining pig in Group B was euthanized and necropsied on day 28 and showed no postmortem indications of pulmonary edema.

On day 14 of the study, a gaunt, severely anorectic animal in Group A (pig 36) was euthanized and necropsied. The remaining 2 pigs in this group were euthanized and necropsied on day 28. None of the swine in Group A developed pulmonary edema/hydrothorax.

Fumonisin injection study. The pig injected daily (cranial vena cava) with 0.4 mg fumonisin B₁/kg body weight was found dead on day 5. This pig received a total of 11.3 mg of the mycotoxin and at necropsy exhibited similar lesions as observed in field and other experimental cases. The remaining solution containing fumonisin B₁ was diluted to a volume of 5 ml with injectable saline containing 5% ethanol. This solution was injected into a pig until the supply was exhausted (7 days). This pig received a total of 8.65 mg of fumonisin B₁ (0.174 mg/kg daily) and was euthanized and necropsied 24 hours after the final injection (day 8). This concentration of the mycotoxin did not induce pulmonary edema. The 10 mg of fumonisin B₂ was injected over a 5-day period (0.3 mg/kg daily), and the pig was euthanized and necropsied 24 hours following the final injection. This pig did not develop pulmonary edema.

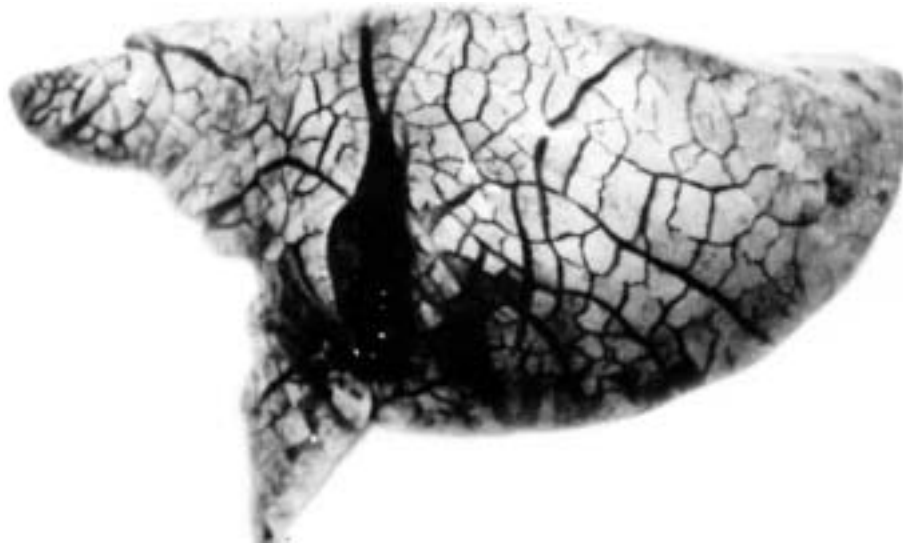


Figure 2. Right lung of a pig showing severe interlobular edema. Individual lobules of all lobes are widely separated because of fluid accumulation.

Pathology. Gross and microscopic lesions observed in swine from the field cases, experimental feeding study, and fumonisin B₁ injections were essentially the same. Thus, although Figs. 1-5 are from the pig that died after receiving injections of fumonisin B₁, these figures show characteristic pathologic changes observed in all affected swine.

The trachea and bronchi contained a clear, foamy liquid. A golden-yellow liquid filled the thoracic cavities (Fig. 1) and clotted after exposure to the atmosphere. These thoracic effusions were modified transudates and had a mean specific gravity of 1.025 and a mean total protein of 3.7 g/dl. Each sample analyzed contained <300 nucleated cells and <30 red blood cells per microliter of transudate.

Interlobular edema was so marked that an obvious reticular pattern was present (Fig. 2), and there was nearly free flow of watery fluid from cut sections of lungs. The interlobular edema was most pronounced in the hilus area; however, the pattern of perilobular edema was evident throughout all lobes (Fig. 3). Lobular atelectasis was also noted.

Microscopically, within the alveoli of the lungs there were few cells, primarily macrophages, and little other material. Focal to diffuse areas of alveolar septal congestion associated with capillary thromboses, indicating thrombostasis, were observed (Fig. 4). Mild alveolar extravasation and accumulation of red blood cells within intralobular lymphatics directly associated with areas of interstitial thrombostasis were noted.

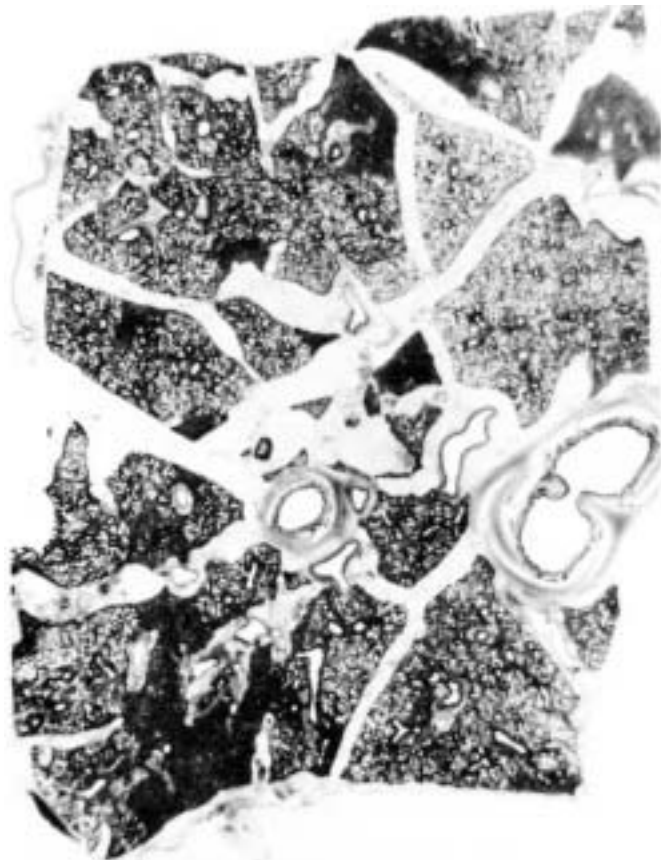


Figure 3. Section of lung of a pig showing the pattern of perilobular and perivascular edema. HE stain.

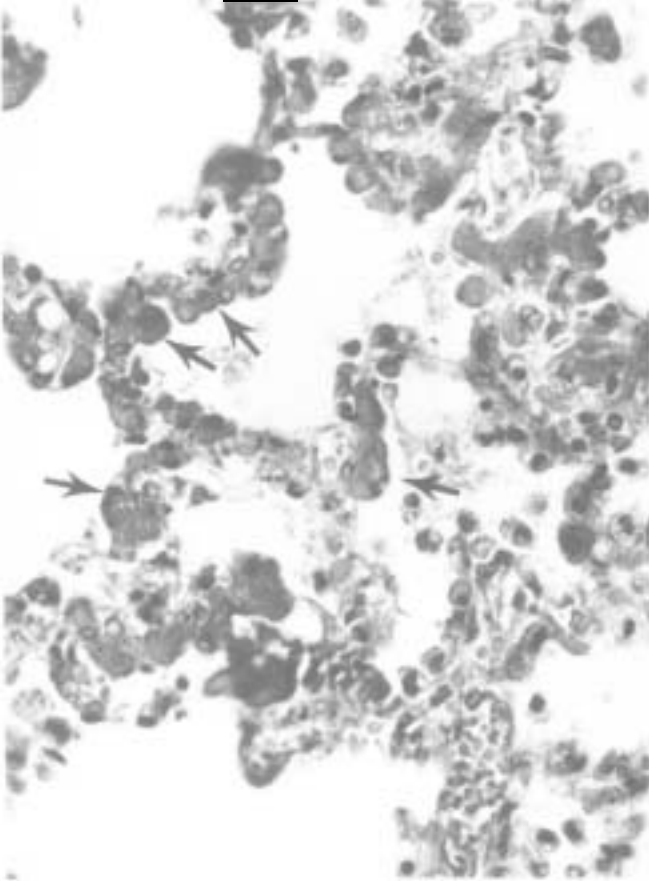


Figure 4. Section of lung of a pig demonstrating alveolar septal vascular distortion caused by capillary thrombostasis. Capillaries contain material that is positive for fibrin (arrows). Fraser-Lendrum stain.

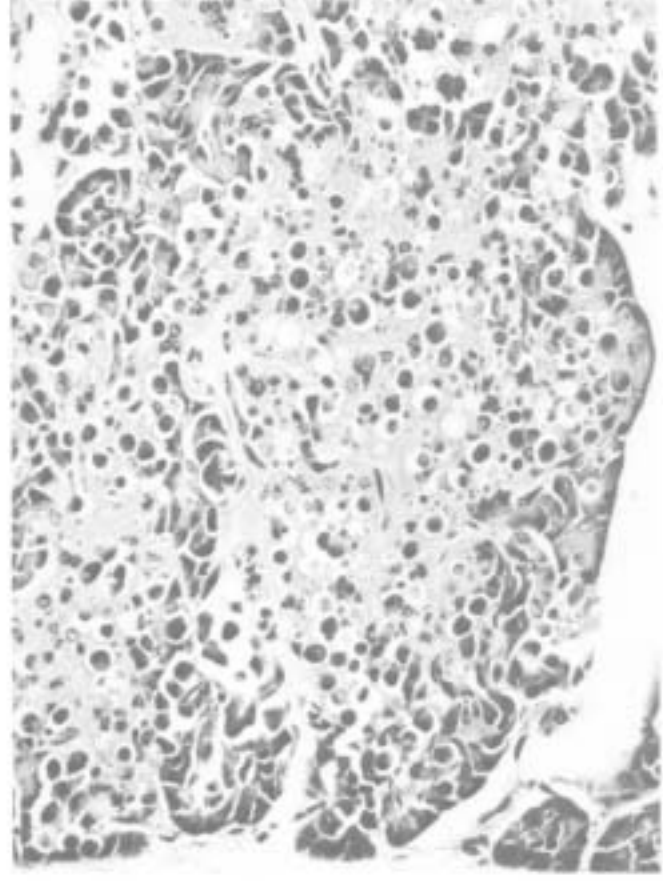


Figure 5. Section of pancreas of a pig demonstrating marked necrosis. Numerous acinar cells are rounded and separated by a clear space from necrotic neighbor cells.

Pancreatic lesions were present in all pigs that developed pulmonary edema/hydrothorax. These lesions consisted of focal to massive necrosis, acinar cell dissociation, and rounded individual acinar cells (Fig. 5). Additionally, liver changes were observed in all pigs from the experimental feeding study. These changes were characterized by centrilobular and random hepatocellular cytoplasmic vacuolar change, hepatocellular cytomegaly, disorganized hepatic cords, and early perilobular fibrosis. These lesions were most obvious in pigs that survived the 28-day feeding period and were mild to equivocal in pigs that developed pulmonary edema. Liver damage was not detected in the pigs injected with fumonisin B₁ or B₂. No pulmonary, pancreatic, or liver pathology was observed in control pigs.

Discussion

Animal and human health problems related to food products contaminated with toxic metabolites produced by fungal growth have long been recognized.

Two such problems are associated with the growth of *Aspergillus flavus* (aflatoxins) and *F. moniliforme* (fumonisins) on corn. In all species studied, the aflatoxins are expressed as potent hepatotoxins,¹⁰ but the fumonisins are not directed toward a single target organ.⁵ However, both aflatoxins and fumonisins are hepatocarcinogenic in rats^{2,4}

Frequently, fungus-elaborated toxins are highly strain specific, and their production is further restricted by temperature, humidity, and nutrient availability. It is unclear why corn screenings from the 1989 crop provided a suitable environment for elaboration of the fumonisins.

The chemical structure of the fumonisins was recently elucidated, and fumonisin B₁ was demonstrated as a cause of leukoencephalomalacia in horses.^{5,8} In our laboratory, leukoencephalomalacia (moldy corn poisoning) in horses is a common pathologic diagnosis, but pulmonary edema/hydrothorax in pigs consuming moldy corn is rare. Both these clinical syndromes have now been reproduced by intravenous injections of fumonisin B₁. From the limited data available, it appears that the quantity of fumonisin B₁ that will induce leu-

koencephalomalacia in horses is much less than that required to produce pulmonary edema in pigs.

Not all affected animals on both farms died during the epizootics; several dyspneic swine (primarily mature sows) returned to clinical normalcy 24-48 hours after the corn screenings were removed. Additionally, abortions occurred on both farms, and this problem also ceased when the screenings were removed.

Regarding differential diagnosis, the lesions induced by fumonisin B₁ are remarkably distinctive and should not generally be confused with other conditions that induce pulmonary and/or thoracic effusion. For example, the gastrointestinal tract edema and subcutaneous edema of the head and eyelids commonly associated with edema disease⁹ were not observed in cases of acute fumonisin B₁ toxicosis. Likewise, the pericardial effusion and myocardial hemorrhage that characterize mulberry heart disease¹¹ were not observed in the field or experimental fumonisin cases.

In cases of suspected fumonisin B₁ toxicosis, examination of the pancreas should provide valuable diagnostic information. The pancreas and lung appear to be similarly sensitive to fumonisin B₁; pancreatic lesions were present in all 3 cases of experimentally induced acutely fatal fumonisin B₁ toxicosis.

Liver lesions were seen only in the swine experimentally fed corn screenings. This material was obviously nutritionally deficient for young growing swine, and the liver damage observed may have been related to nutrient availability. Additional research is needed before fumonisin B₁ could be considered a hepatotoxin for swine.

Acknowledgements

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Sources and manufacturers

- a. Fusarium Research Center, Pennsylvania State University, University Park, PA 16802.
- b. Fumonisin B₁ and B₂ (98% purity), National Chemical Research Laboratory, Council for Scientific and Industrial Research, Pretoria 0001, South Africa.
- c. Travenol Laboratories, Inc., Deerfield, IL 60015.
- d. Fort Dodge Laboratories, Inc., Ft. Dodge, IA 50501.
- e. Provided by the National Veterinary Service Laboratory, Ames, IA 50010.

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