Suspected Fusariomycotoxicosis in Sandhill Cranes (Grus canadensis): Clinical and Pathological Findings

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SUMMARY. In 1985 and 1986, large-scale natural die-offs of sandhill cranes in Texas were attributed to fusariomycotoxicosis. These birds demonstrated a progressive loss of motor control to the neck, wings, and legs. Based on necropsy and/or histopathology of 31 cranes, the most common lesions involved skeletal muscle and included hemorrhages, granulomatous myositis, thrombosis, and vascular degeneration. Serum chemistry results revealed that levels of creatinine kinase, aspartate aminotransferase, and alanine aminotransferase were above published normals. However, only alanine aminotransferase was higher in clinically affected cranes than in normal cranes collected from the same area.

RESUMEN. Sospecha de Fusariomicotoxicosis en grullas (Grus canadensis): Resultados clínicos y patológicos.

La mortalidad en gran escala observada durante 1985 y 1986 en grullas (Grus canadensis) en el estado de Texas fue atribuida a fusariomicotoxicosis. Las grullas mostraron una pérdida progresiva del control del cuello, alas y patas. Basados en la necropsia e histopatología de 31 grullas, las lesiones más comunes consistieron en hemorragias y miosis granulomatosa en los músculos esqueléticos; trombosis y degeneración vascular. Los resultados de la química sanguínea mostraron un aumento en la creatinina kinasa, aminotransferasa aspártica y aminotransferasa alanínica, comparados con los valores publicados para aves normales. Sin embargo, sólo la aminotransferasa alanínica fue mayor en las grullas clínicamente afectadas comparadas con las normales encontradas en la misma área.

During the time period from 1982 to 1987, an estimated 9500 sandhill cranes (Grus canadensis) died during winter die-offs in Gaines County, Texas, and nearby Roosevelt County, New Mexico. The largest single die-off occurred in 1985, when 5000 cranes died in Gaines County. About 200 cranes died in February 1986. Mycotoxicosis was suspected in the 1985 and 1986 epornitics because Gaines County is a peanut-growing area and files from Muleshoe and Aransas National Wildlife Refuges suggested that moldy peanuts may have been responsible for earlier sandhill crane mortalities. Investigations by the National Wildlife Health Research Center led to a diagnosis of mycotoxicosis associated with ingestion of moldy peanuts. The most probable cause of the mortality was trichotheccene mycotoxins produced by Fusarium sp. (17). Evidence to support this conclusion included: 1) Toxigenic Fusarium sp. were recovered from waste peanuts from within the area. Trichotheccenes were also recovered directly from waste peanuts. 2) Peanuts made up the overwhelming majority of the diet of sandhill cranes in this area. 3) Trichotheccenes produced by F. compactum isolated from waste peanuts were toxic to sandhill cranes in experimental studies. 4) No pathogenic bacteria and viruses were isolated from any tissue. 5) Brain cholinesterase analyses eliminated the cholinesterase inhibitor poisons, such as organophosphates or carbamates, as possible etiologic agents. 6) Histopathology was consistent with a toxin acting on the vascular system.

Trichotheccenes form one of the three main groups of mycotoxins produced by Fusarium spp. molds (7). In general, trichotheccenes produce lesions through direct cytotoxicity, which results in a wide range of effects (16). Lesions are variable but generally include epithelionecrosis (12), a chemical stomatitis and necrosis of the gastrointestinal tract (16), and marked hemorrhages throughout the body (18). Pathologic changes, however, are nonspecific and often difficult to differentiate from artifactual or postmortem changes (16). Although Rousseaux
(16) states that epithelial irritation/necrosis and hemorrhages are the best morphological indicators of acute trichothecene toxicosis, in experimental studies birds generally have not been found to produce the hemorrhagic syndrome noted in mammals (2,3,10). Further studies may show hemorrhagic disease in birds, since as recently as 1980 experimental studies failed to demonstrate hemorrhaging in mammals in response to trichothecene intoxication (9). In waterfowl experimentally intoxicated by oral administration of the trichothecene T-2, upper alimentary necrosis, severe lymphoid necrosis, and retarded growth were the prominent findings (10).

This paper reports on the clinical and pathological findings in sandhill cranes naturally poisoned by mycotoxins found in peanuts.

MATERIALS AND METHODS

U.S. Fish and Wildlife Service and Texas Parks and Wildlife personnel surveyed areas used by sandhill cranes in Gaines County, Texas, by air and ground. Carcasses and live birds were collected on foot or by using all-terrain vehicles.

In 1985, carcasses of cranes that were found dead or were euthanatized after showing typical signs were sent on ice to the National Wildlife Health Research Center (NWHRC) for necropsy and laboratory analyses. Complete necropsies and histopathological evaluations were done on 11 birds. An additional seven provided gross findings only.

The 1986 field investigation was conducted in February. Cranes observed in various debilitated states were captured and transported 3 to 4 miles to a holding facility on a peanut/cattle farm. Thirteen cranes were maintained in captivity in a wire-mesh enclosure and given free access to water and commercial dog food. Six cranes were decapitated and necropsied after 1 day of observation. Blood samples for clinical chemistry were taken from five of the six. Four more cranes were euthanatized and similarly processed after 2 days of observation. All necropsies were completed less than 1 hour after death. Samples were frozen (toxicologic, serum), fixed (histopathology), or refrigerated (bacteriology, virology) and sent directly to NWHRC for further workup.

Tissue samples from major organs were placed in a large volume of 10% buffered neutral formalin. In 1985, skeletal muscle samples were taken only from areas with gross lesions. In 1986, skeletal muscle samples were taken from the same sites (mid right pectoralis and cranial tibial) on all carcasses for comparative purposes. Tissues were paraffin-embedded, sectioned at 5–6 μm, and stained with hematoxylin and eosin. Samples for toxicologic analysis were wrapped in aluminum foil, placed in sealed plastic bags, and frozen. Livers were analyzed by atomic absorption spectrophotometry for lead residues. Brains were analyzed for cholinesterase activity by ultraviolet-visible spectrophotometry. Blood samples were centrifuged, and serum was decanted and frozen. Serum chemistries were processed on an automated multiple analyzer at the School of Veterinary Medicine, University of Wisconsin. Botulinum type C testing was done using a sample of serum in a mouse toxin neutralization test. Bacterial methodology included routine plating on blood agar and MacConkey’s media. Tissues were screened for viruses in embryonated chicken eggs and duck embryo cell cultures.

Of the remaining three birds collected in 1986, two were observed for 5 days and one was observed for 3 days before euthanasia. Clinical signs were monitored for 30-min periods three to four times daily. Blood samples for serum chemistries were taken daily. The average of these multiple samples is used as a single value for each bird in reporting the results because there was little variation in chemistry during the period of captivity. Skeletal muscle samples collected at necropsy from the right pectoralis and cranial tibial muscles were fixed in 10% buffered neutral formalin.

Blood chemistry samples were taken from two free-flying, apparently unaffected birds from the same area that had been killed by shotgun.

RESULTS

Clinical signs. Affected sandhill cranes exhibited a range of clinical signs in the field. The most prominent was a flaccid paralysis of the neck. Birds would stand with their heads held low near their abdomens (Fig. 1). In the earliest stages, affected birds could be approached in the field but would eventually fly with heads hanging vertically. In later stages, there was loss of flight ability in addition to the cervical paralysis, although birds were alert and could walk with slight ataxia. Several of these cranes had drooping wings. Although they could not fly, the more clinically affected birds tried to defend themselves from capture by extending their wings, hissing, kicking, and flapping. These latter signs were exhibited by all cranes brought into captivity, with only one crane changing in clinical condition during captivity. This crane showed mild ataxia on days 1 and 2, which seemed to improve on day 3, when it was able to partly raise its head and neck (horizontal to the ground) if humans entered the enclosure.
Fig. 1. Sandhill crane found in Gaines County, Texas, in 1986. Note loss of ability to maintain an erect head and neck. Crane is exhibiting defensive posture (wings extended).

On days 4 and 5, ataxia was absent, but the crane made no attempt to raise its head and neck unless it was frightened.

Clinical pathology. Eighteen serum chemistry parameters, including six electrolytes, were evaluated (Table 1). Only alanine aminotransferase (ALT) in affected birds (ranging from 367 to >500 IU/liter) was markedly different from the clinically normal birds that were collected by shooting (108 IU/liter) and normal values reported by Gee et al. (6) (49.0 IU/liter). Although aspartate aminotransferase (AST) exceeded 500 IU/liter in affected birds, clinically normal cranes also had values greater than 500 IU/liter. Gee et al. (6) reported 169 IU/liter as normal. Creatinine kinase (CK) exceeded 800 IU/liter in clinically affected and unaffected birds collected in 1986. CK was not measured in 1985 or by Gee et al. (6).

Anatomic pathology. All 28 (18 from 1985 and 10 from 1986) carcasses examined were in good body condition with moderate to abundant fat stores. The cranes had recently been feeding, and peanuts were found in 27 gizzards (96.4%). Subcutaneous edema of the head, particularly over the calvarium in the unfeathered area, was present in 19 birds, two of which had only mild edema. Multiple muscular hemorrhages or focal pale areas in skeletal muscle occurred in 65% of the cranes (20/31 total birds, which includes the three birds in 1986 from which muscle samples were collected but necropsies were not done). One additional crane had multiple muscle petechiae. Most affected were the dorsal neck and the proximal cranial tibial musculature, but lesions were also found in the pectoralis muscles. Hemorrhage in the dorsal cervical musculature was often severe and occupied most of the muscle.

Skeletal muscle damage was the most commonly found histologic lesion, occurring in 53% (8/15) of samples examined. The initial lesion
Table 1. Serum chemistry data collected from clinically ill and clinically unaffected sandhill cranes in Gaines County, Texas, in 1985 and 1986. Normal data of Gee et al. (6) are provided for comparison.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1985 (N = 6)</th>
<th>1986 (N = 12)</th>
<th>Unaffected (N = 2)</th>
<th>Normal</th>
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<tr>
<td>Sodium</td>
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<td>145.0</td>
<td>149.0</td>
<td>147.0</td>
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<tr>
<td>Potassium</td>
<td>— b</td>
<td>6.8</td>
<td>6.7</td>
<td>3.5</td>
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<td>Chloride</td>
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<td>199.9</td>
<td>110.0</td>
<td>107.0</td>
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<tr>
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<td>10.3</td>
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<td>96.0</td>
</tr>
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<td>2.5</td>
<td>2.9</td>
<td>—</td>
</tr>
<tr>
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<td>5.9</td>
<td>6.1</td>
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</tr>
<tr>
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<td>3.8</td>
<td>3.8</td>
<td>3.9</td>
</tr>
<tr>
<td>Albumin</td>
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<td>1.6</td>
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<td>&gt;800.0</td>
<td>—</td>
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<td>Alanine aminotransferase</td>
<td>367.0</td>
<td>&gt;500.0</td>
<td>108.0</td>
<td>49.0</td>
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<td>Aspartate aminotransferase</td>
<td>&gt;1000.0</td>
<td>&gt;500.0</td>
<td>&gt;500.0</td>
<td>169.0</td>
</tr>
</tbody>
</table>

aClinically unaffected cranes killed by gunshot in the same area as affected cranes, 1986.
bNot determined.

appeared to be a vascular insult that resulted in thrombosis followed later by granulomatous inflammation. Typical lesions were characterized by infiltration of large foamy macrophages and fibroblasts forming numerous invasive tongues between the myofibers and through adipose tissue and blood vessels. The boundary of these changes was generally abrupt and bordered finely vacuolated myofibers lacking cross striations (Zenker’s necrosis). Within the infiltrated area, marked vascular damage was characterized by medial/intimal necrosis and vascular thrombosis (Fig. 2). Thrombosis was not found in all sections with granulomatous myositis; however, blood-vessel damage was apparent. In areas with grossly apparent hemorrhage, Zenker’s necrosis and hemorrhage were noted histologically.

Histologic lesions occurred in 11 of 21 (52%) livers examined. Six livers had primarily inflammation most prominent around blood vessels in portal areas. Earliest changes included infiltration of lymphocytes around arterioles. Many of the more severe changes were characterized by macrophage infiltration and necrosis. Degenerative changes, characterized by diffuse vacuolation of hepatocellular cytoplasm, occurred in four livers. Inflammatory and degenerative changes generally did not occur in the same bird, suggesting that the lesions were independent. One liver section contained thrombi.

Vasculitis and thrombosis observed in two spleens were characterized by multiple small veins that contained partial occlusive thrombi or emboli and no associated lesions in the blood-vessel wall. One bird had severe vasculitis of a large splenic artery characterized by marked medial degeneration (irregular, pale, swollen myocytes with fragmented nuclei), necrosis, and moderate infiltration of mononuclear cells. This bird also had several thrombi located in the small blood vessels of the brain. One additional bird had multiple myocardial infarcts. No thrombosis was found in other tissue sections. No significant results were obtained from bacteriological, virological, and toxicological tests. Botulism tests were negative.

**DISCUSSION**

The clinical syndrome affecting the cranes was a progressive flaccid paralysis reminiscent of botulinum intoxication, although numerous
tests showed no presence of botulinum toxin. There were also several clinical differences from botulism. There was no paralysis of the third eyelid or muscular weakness usually seen in botulism. Generalized neuromuscular weakness would be expected for any toxin that causes neuromuscular blockade. The observed cervical paralysis was not associated with generalized central nervous system dysfunction, because mental acuity apparently was unimpaired. Cranes were still alert when approached and attempted to evade capture, although they were ataxic. Neurologic signs associated with the trichothecene T-2 have been reported to consist of seizures, dropped wings, and loss of righting reflexes (10,19). Geese consuming T-2-contaminated barley were depressed and ataxic before death (7,8). These observations suggest that at least one trichothecene (T-2) has some effect, either directly or indirectly, on the brain. The inability of cranes in the present study to raise their heads and necks may have been related more to the skeletal muscle damage than to neural dysfunction. This head-down posture was probably at least partly responsible for edema of the head, although leakage from damaged blood vessels may also have been involved.

Clinical signs exhibited by sandhill cranes were not seen in other species that fed from the peanut fields (Chihuahuan ravens and crows) and watering lakes (ducks, coots, and various shorebirds).

Except for ALT, we were unable to demonstrate any differences in serum chemistry between affected and unaffected birds. Although CK has been suggested as the best indicator of muscle damage in five avian species (5), CK and AST were elevated in clinically normal birds as well as in affected birds. AST is also used as an indicator of skeletal and cardiac muscle damage, although it is nonspecific and occurs in other organs. AST was considerably lower in normal sandhill cranes reported by Gee et al. (6) than in our clinically unaffected birds. CK was not reported in Gee et al.

There are two possible explanations for the elevation of CK and AST: either apparently normal birds were subclinically affected, or CK and AST were affected by our method of collection (gunshot). Blood was taken from birds within 30 to 90 seconds after they were shot. A rapid
rise in enzyme levels in general circulation seems unlikely, although there may have been leakage of enzyme from areas of trauma both in birds that were shot and in birds that were decapitated.

If apparently unaffected birds were subclinically affected (causing abnormally high CK and AST), these enzymes could serve as an early indicator of intoxication. Dosing studies with associated serum chemistry are needed to determine the effect of trichothecene toxicosis on CK and AST levels.

ALT was the only blood chemistry parameter that differed appreciably between clinically normal and affected cranes. Assuming subclinical intoxication in cranes that were shot, ALT would apparently indicate a more severe or longer-lasting intoxication because it was elevated only in clinically affected birds. The importance of this enzyme to pathologic conditions is unknown. The level of ALT in tissues varies with the species. Some species, such as turkeys, have high ALT activity in skeletal muscle (11), whereas other species have the highest activities in kidney, such as mallard ducks (5), or liver. The significance of ALT in sandhill cranes is unknown; in the present study, however, the most conspicuous lesions occurred in skeletal muscle. Further investigation on the tissue levels and specificity of ALT in sandhill cranes is needed before more conclusions can be made.

The most prominent pathological change observed in the different organs of the cranes was associated with vascular damage resulting in marked hemorrhaging and myositis. Vascular toxicity and subsequent hemorrhaging is a well-known effect of T-2 intoxication. T-2 causes hemorrhages through increased capillary permeability and interference with the blood-clotting mechanism (4,13,14), although Hayes and Wobeser (10) have pointed out that the hemorrhagic syndrome has not been reproduced in experimental studies on poultry. Certain characteristics (notably the numerous thrombi) of the pathology suggest that disseminated intravascular coagulation (DIC) may be involved in the pathogenesis of this intoxication. Most notably, those cases with numerous splenic and cerebral thrombi are consistent with DIC. In most cases, however, thrombi were restricted to skeletal muscle and were observed only in areas with significant pathology to blood-vessel walls. Petechiae and ecchymoses were not observed. Also, the earliest vascular changes in the liver appear to be inflammatory. Experimental studies should explore whether DIC is an intermediary mechanism in the pathogenesis of this intoxicating process.

T-2 toxin in birds has been associated with severe necrosis of the oral cavity, skin, intestine, liver, and kidney (2,15,18,19). The severe epithelial necrosis typical of trichothecenes (including T-2, fusarenon-X, deoxynivalenol [vomitoxin], and diacetoxyscirpenol [DAS]) was not found in any of the sandhill cranes examined, although mild reddening of the oral mucosa was noted in a few cranes in 1985. No morphologic lesions were observed in the gastrointestinal tract, a site commonly affected by trichothecenes. Trichothecenes are thought to act at sites of active cell proliferation (1); however, lesions can be subtle and are probably dose dependent. In an investigation into the cause of mortality in geese that fed on T-2-contaminated barley, the only gross lesion observed was necrosis of the mucosal surfaces of the esophagus, proventriculus, and gizzard (7). Trichothecenes isolated from waste peanuts collected during the 1986 epornitic caused death in experimentally dosed sandhill cranes. These cranes lacked any gross lesions except small submucosal and/or muscular hemorrhages in the gizzard. Mucosal necrosis was absent.

The significance of the hepatic changes is uncertain. Although aggregations of mononuclear cells are common in portal areas of chickens, waterfowl, and wading birds, the changes observed in some of the affected cranes appear to be more directly associated with blood vessels and included macrophages and necrosis. The vacuolating degenerative changes are not typical of normal avian livers. However, both the inflammatory and degenerative changes were mild.

The most probable cause of the sandhill crane mortality was trichothecene mycotoxins produced by Fusarium sp. This conclusion is based on evidence reported by Windingstad et al. (17) and includes the isolation of toxigenic Fusarium and recovery of trichothecenes directly from waste peanuts found where the birds died. These trichothecenes were later shown experimentally to be toxic to cranes. The identity of the specific causative trichothecenes is unknown, although the pathogenesis of intoxication for the agent appears to be vascular damage (and pos-
sibly coagulopathy) resulting in widespread hemorrhaging primarily in skeletal muscle. Early detection of intoxication may be possible through elevations in CK and AST with subsequent elevation of ALT in clinically affected birds.

REFERENCES


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