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Lead Poisoning in Sandhill Cranes

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SUMMARY

Seven Florida sandhill cranes (*Grus canadensis pratensis*) and 6 greater sandhill cranes (*Grus canadensis tabida*) were exposed to lead-base paint containing 27% lead.

One bird of each subspecies died enroute to the hospital, with a clinical history of anorexia, weakness, and open-mouth breathing of 36 hours' duration. There were no gross lesions, and microscopic lesions were limited to focal hepatic necrosis and hemosiderosis.

Two of each subspecies of cranes developed clinical signs of lead poisoning, which included anorexia, weakness, green diarrhea, regurgitation, and open-mouth breathing. Diagnosis of lead poisoning was confirmed on the basis of blood lead concentrations ranging from 146 μg/100 ml to 378 μg/100 ml. These 4 cranes were treated successfully with calcium disodium edetate intramuscularly.

Seven of the birds remained clinically normal despite high blood lead levels, especially in the greater sandhill cranes.

LEAD POISONING from ingestion of lead shot has long been recognized as a problem in waterfowl.[2,7,11,26] Recently, lead poisoning has been reported in upland game birds and birds of prey.[3,13,14] Psittacine birds in zoological collections have died of lead poisoning from ingestion of lead-base paint.[27] Though lead shot has been recovered from the stomachs of many members of the order Gruiformes, there is little likelihood of finding lead shot in cranes, due to their feeding habits.[10] A survey of naturally occurring lead concentrations in a variety of wild birds showed a sandhill crane (*Grus canadensis*) to contain 0.7 ppm lead in the liver.[1]

A group of 7 Florida sandhill cranes (*Grus canadensis pratensis*) and 6 greater sandhill cranes (*Grus canadensis tabida*) maintained at the Conservation and Research Center, Front Royal, Va, were moved from their regular winter quarters, because of frozen water pipes, to a room that was later discovered to have lead-base paint on the walls. The initial clinical signs of lead poisoning were noted 19 days after the move.

Materials and Methods

Blood was collected from the vein medial to the tibiametatarsus via a capillary blood collection tube 46/13 EDTA Brown[a] for blood lead determinations by the Delves method of atomic-absorption spectrophotometry.[5] From this same sample, erythrocyte protoporphyrin (EP) concentrations were determined by spectrofluorometry. The EP results were expressed in equivalents of free erythrocyte protoporphyrin (FEP) extracted by the ethyl acetate-acetic acid-HCl method and reported in micrograms per 100 ml whole blood.[6,25] Samples were collected from the 4 cranes with clinical signs of lead poisoning prior to initial treatment, after each regimen of treatment, and weekly until returned to Front Royal. Additional blood samples were taken at approximate monthly intervals for 2 months. The 7 birds in which clinical signs did not develop were tested for blood lead concentrations, for comparison with the cranes with clinical signs. Additional blood samples were taken at 2-, 8-, and 14-week intervals.

Heparinized blood samples were collected from the brachial vein for a complete blood count. Natt and Herrick diluent was used for the white cell counts.[19] Wright's stain was used for differential white cell counts.

Lead determinations in liver and kidney were made by atomic absorption spectrophotometry.

The paint samples were acid leached and placed into an aqueous phase by a double extraction method, as specified by US Public Health Service. The concentration of lead in the paint was compared against a known standard to determine the percentage.

Treatment of clinically affected birds consisted of calcium disodium edetate[b] (35 mg/kg) intramuscularly twice a day for 4 days. This treatment regimen was repeated 4 days later.

Anorectic birds were tube fed pelleted feed that had been soaked with water and blended to a gruel. Amounts fed varied from 70 to 90 ml twice a day, depending on the size of the bird. Duration of tube feeding varied from 6 to 25 days. Baby mice were force fed 3 times a week.

Two Florida sandhill cranes and 2 greater sandhill cranes developed clinical signs of lead toxicosis. A greater and a Florida sandhill crane died enroute to the hospital at the National Zoological Park within 36 hours of developing clinical signs. The remaining 7 birds remained clinically normal and were removed from the source of the lead

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[a] Walter Sarstedt, Inc, Princeton, NJ.

[b] Calcium Disodium Versenate, Riker Laboratories, Inc, Northridge, Ca.

[c] Flight Maintenance Diet, Ralston Purina Company, St Louis, Mo.

after lead poisoning had been diagnosed in the other cranes. Duration of exposure to the lead was 1 month.

The following case reports describe the variety of clinical signs observed in lead-intoxicated sandhill cranes. The purpose of this report is to show that birds respond to intramuscular injections of calcium disodium edetate, as shown by the more rapid decline in blood lead concentrations than in untreated birds and by the alleviation of clinical signs.

Case Reports

Case 1

The 1st bird hospitalized was a Florida sandhill crane. Clinical signs included anorexia, green diarrhea, pectoral muscle atrophy, and weakness. The bird would lie down for long periods after being handled. Initially, the bird acted as if it was picking at imaginary objects in the air. The normally red plumage on the head was dull and faded.

Within 24 hours of initial signs, the bird began to regurgitate food and water and to extend its neck with open-mouth breathing. At this time, the presence of lead-base paint in the bird's enclosure was unknown. Blood glucose was approximately 90 mg/100 ml, as determined by chemical reagent strip.[d] An infusion of 5% dextrose in saline was started and 10 ml 50% glucose was given as an IV bolus. After 12 hours, the blood glucose concentration increased to 250 mg/100 ml, which was considered normal. No further vomiting occurred.

Lead poisoning was diagnosed on the basis of blood lead determinations 11 days later, after the holding area was found to contain paint with 27% lead, which is not an unusual concentration for pre-World War II paint.[6] The bird was treated with 150 mg calcium disodium edetate (35 mg/kg) intramuscularly twice daily for 4 days. This regimen was repeated 4 days later. The crane lost 0.91 kg of its original 4.32 kg body weight over a 40-day period. A gain of 0.23 kg was made during the last week of hospitalization.

Case 2

The 2nd Florida sandhill crane was hospitalized 12 days after the 1st crane was hospitalized. Clinical signs were minimal and included anorexia, weakness, and slight leg tremors. The red plumage on the head was dull. The bird was treated with 135 mg calcium disodium edetate (35 mg/kg) intramuscularly twice daily. The treatment schedule was identical to that given the first crane. Over a 2-week period, the bird lost 0.34 kg of its original 3.86 kg of body weight.

Case 3

A greater sandhill crane was hospitalized with the 2nd Florida sandhill crane. Clinical signs were more pronounced. In addition to anorexia and weakness, there were wing tremors, a droopy left wing, droopy eyelids, regurgitation, and green diarrhea. This bird also picked at imaginary objects in the air and had faded red plumage on its head. The dosage of calcium disodium edetate (35 mg/kg) was 159 mg intramuscularly twice daily for 4 days, and the entire regimen was repeated 4 days later. The bird remained at 4.77 kg body weight throughout treatment.

Case 4

A 2nd greater sandhill crane was hospitalized 10 days after the hospitalization of cranes 2 and 3. This bird had been screened for blood lead 1 week earlier and at this time was clinically normal. Anorexia, green diarrhea, and faded red plumage were now evident. The neck was held straight out so the bird had a stargazing appearance. Occasionally open-mouth breathing was seen. This bird was highly excitable and its normal trumpet-like call sounded immature and garbled. Two treatment regimens, 4 days apart, of 167 mg calcium disodium edetate (35 mg/kg) intramuscularly twice daily for 4 days were used. This crane initially weighed 4.77 kg and did not lose any weight during treatment.

Results

The initial blood lead concentrations ranged from 146 μg/100 ml to 778 μg/100 ml in the clinically ill cranes. With 1 exception (crane 3), the blood lead concentrations declined at a faster rate in the treated birds (Fig 1) than in the birds not treated (Fig 2). In crane 3, the increase in blood lead at 2 weeks after treatment was reversed by another regimen of treatment.

Three of the cranes with clinical signs improved markedly several days after the first 4-day treatment schedule with calcium disodium edetate. The 1st Florida sandhill crane treated did not improve until the beginning of the 2nd treatment regimen, when the open-mouth breathing stopped. The bird was still lying down much of the time at the end of the 2nd treatment reg-

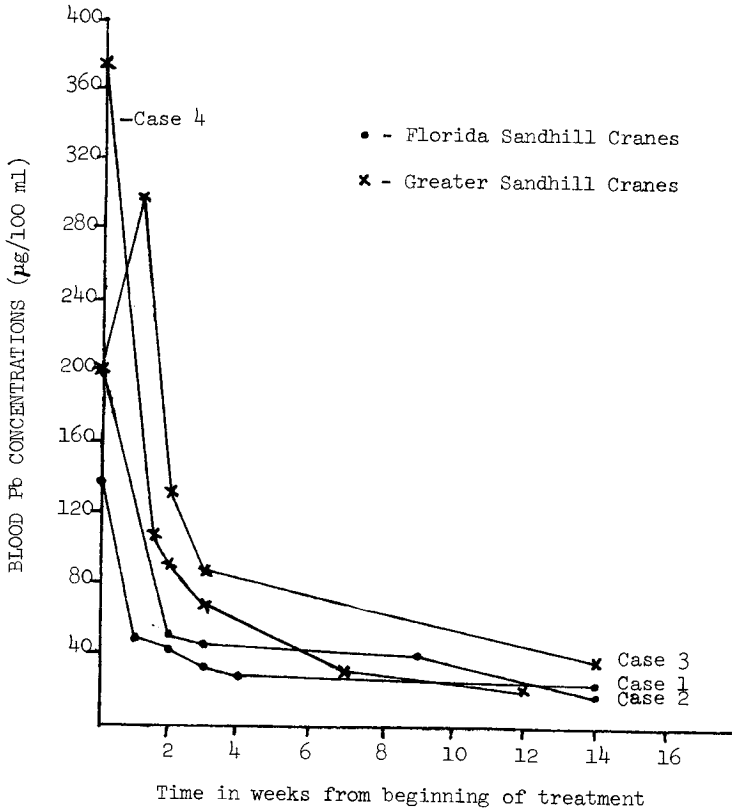

Fig 1—Blood Pb concentrations in relation to time of treatment for cranes with clinical signs of lead poisoning.

[d] Dextrostix, Ames Company, Elkhart, In.

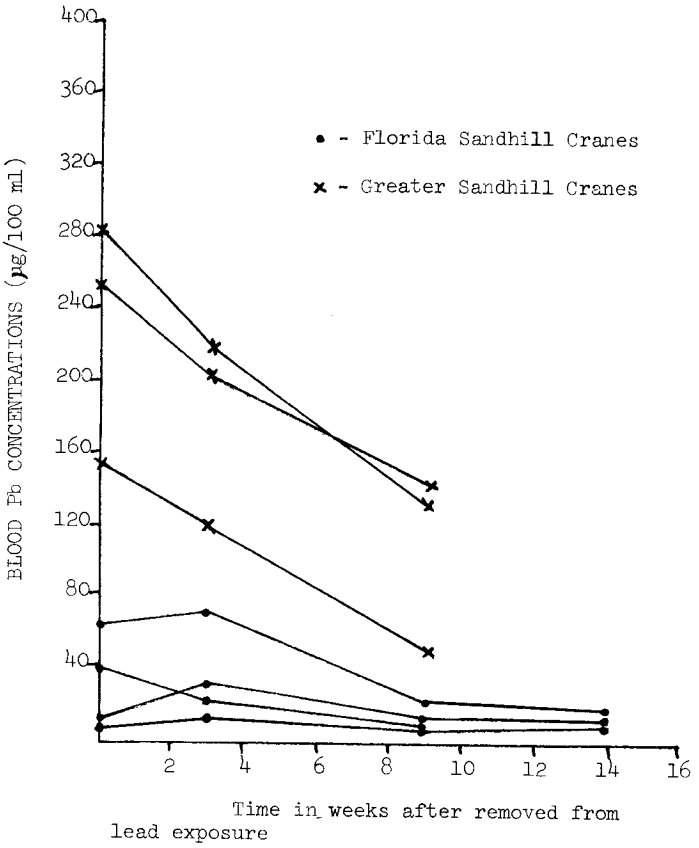

Fig 2—Blood Pb concentrations in relation to time from exposure to lead for clinically normal cranes.

imen, but continued to improve and gain strength over the next week.

Cranes without clinical signs were not anemic, according to hematocrits and number of red blood cells. After the 2nd treatment with calcium disodium edetate, cranes 1, 3, and 4 had red blood cell counts below $2 \times 10^6/mm^3$. The hematocrits did not reflect this decrease. At 1 week after the end of the second treatment regimen, the red cell count returned to normal values of 2.5 to 3.5 $\times 10^6/mm^3$, as determined from accumulated values of previous counts on cranes in the National Zoological Park's collection. Crane 4, which had the least severe clinical signs, never had a decreased red cell count. Basophilic stippling or abnormal red blood cell shapes were not seen. Moderate to slight polychromasia was reported in the 1st blood samples from all but 2 birds. The incidence of polychromasia decreased with repeat bleedings. There was no apparent relationship between blood lead content and number of leukocytes or differential counts.

The 1st bird that died was a Florida sandhill crane, with a clinical history identical to that of crane 1. At necropsy, there were no gross lesions. The only histopathologic finding was extensive areas of focal hepatic necrosis with deposits of hemosiderin. At the time of necropsy, the existence of the lead-base paint was unknown and specimens were not saved for toxicology.

Gross lesions were not observed on necropsy of the greater sandhill crane that died at the time the next 2 cranes were presented for treatment. Microscopically, hemosiderin was seen in liver sections, and a few areas of focal necrosis were seen in the kidney. Acid-fast stains of kidney sections from both birds were negative for intranuclear inclusion bodies. The liver from this bird contained 29 ppm lead, and the kidney contained 18.6 ppm lead. These concentrations are higher than would be expected for background lead concentrations. In another study, concentrations of lead in livers from random sampling of a variety of birds ranged from 0.5 to 7.0 ppm, wet weight.[1] Concentrations of lead in the livers of mallard ducks experimentally poisoned by the ingestion of lead shot ranged from 16 to 76 ppm.[15]

Discussion

Anorexia, weakness, and green diarrhea were clinical signs common to all 4 treated cranes and are indeed common clinical signs in other species of birds. Droopy wings, as seen in crane 3, where the wing hangs from the carpal joint because the extensor muscles are paralyzed, has been reported in waterfowl and is analogous to the "wrist drop" syndrome associated with lead poisoning in man.[4,26] Regurgitation is a clinical sign of lead poisoning frequently seen in dogs, and has been reported to occur in waterfowl.[26] The faded red plumage on the head is characteristically seen in many sick cranes. The most striking clinical sign, however, was the extended neck and open-mouth breathing seen in 2 birds. This sign closely resembles the dyspnea with roaring seen in horses due to pharyngeal paralysis as a result of chronic lead poisoning.[23]

Though hepatic necrosis and hemosiderosis are compatible histopathologic lesions for lead poisoning, they are not diagnostic.[17] Acid-fast intranuclear inclusion bodies in the proximal tubules of the kidney are regarded as strong evidence for lead poisoning in man and birds.[15] Inclusion bodies have been seen in lead poisoning of ducks,[2,15] mourning doves, and an Andean condor.[13,14] Dosage, period of exposure, and diet reportedly affect the occurrence of inclusion bodies.[15] Mallard ducks fed a pelleted ration with lead shot had no acid-fast inclusion bodies at necropsy,[15] which might explain the absence of inclusion bodies in the cranes, inasmuch as they were also on a pelleted diet. However, Canada geese dying from lead poisoning have a low incidence of acid-fast intranuclear inclusions,[16] suggesting a species variation.

In studies of lead poisoning in mallard ducks, decreased erythrocyte counts were related to the dosage of ingested lead. Mallard ducks and Japanese quail fed lead nitrate or acetate at sublethal dosages did not develop anemia.[9,18] The treated crane that had the least severe clinical signs of lead poisoning (crane 2) never had a decreased red cell count, which would suggest that less lead was ingested.

White cell count variation without a consistent pattern of change has been reported in mallard ducks fed lead nitrate.[4] This observation corresponds with the lack of pattern of leukocyte counts to blood lead seen in the cranes.

Lead is known to inhibit many steps in the biosynthesis of hemoglobin in mammals. The inhibition of heme synthetase causes increased production of protoporphyrin in the mitochondria of erythroid precursors. After the red blood cell matures and loses its nucleus, it

is no longer capable of synthesizing hemoglobin. Thus, the increased amount of protoporphyrin in circulating red blood cells reflects primarily the toxic effects of lead on erythroid cells in the bone marrow some 1 to 3 months earlier.

In children, a good correlation coefficient exists between blood lead values and the logarithms of blood protoporphyrin values.[22] The correlation coefficient for these values in the cranes was found to be 0.55, which is not considered significant. The usefulness of protoporphyrin data is, therefore, questionable in this case. Such considerations as the shorter life span of avian red cells[23] and their ability to produce hemoglobin as mature cells because they retain their nuclei[12] add variables that do not exist when evaluating EP concentrations for man and other mammals. Also, the excitation wavelength of the spectrofluorometer is calibrated for protoporphyrin IX, which does not exist in birds. Thus, the emission wavelength will be incorrect. To evaluate the effect of these variables on EP concentration and establish base-line values are beyond the scope of this report. In 1 study, protoporphyrin concentrations in mallard ducks were proportional to the quantity of lead ingested.[20] In other studies in mallard ducks, investigators have measured δ-aminolevulinic acid dehydratase (ALAD) activity, an earlier site of heme inhibition by lead, and found an inverse correlation with lead in blood.[8,9] The ALAD activity was considered a sensitive, indirect measure of lead concentrations; yet another investigator using the same method to determine ALAD activity considered the test insensitive, with poor reproducibility.[24] Clearly more work is needed in this area.

Response to intramuscular injections of calcium disodium edetate was good. Attempts to treat swans with subcutaneous injections of calcium disodium edetate for lead poisoning were unsuccessful. Only intravenous injections would obtain a clinical response in these cases.[21]

The blood lead concentrations in clinically normal greater sandhill cranes averaged more than 5 times the concentrations in clinically normal Florida sandhill cranes and 1½ times the concentrations in clinically ill Florida sandhill cranes (Fig 1 and 2). These concentrations were equivalent to the blood lead concentrations of the 2 greater sandhill cranes that had clinical signs. These birds were not treated, despite the high lead concentrations, because they never developed clinical signs.

The higher blood lead concentrations in clinically normal greater sandhill cranes, compared with Florida sandhill cranes or even with clinically ill cranes, could not be explained. Perhaps there is subspecies resistance to lead poisoning, considering neither of the 2 clinically ill greater sandhill cranes lost any weight, though all 4 treated birds were anorectic. All birds are well currently, 6 months after treatment. Four of the 5 greater sandhill cranes have been shipped out to other zoological parks and can no longer be evaluated.

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