LEAD POISONING IN WATERFOWL

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INTRODUCTION

Lead poisoning due to ingestion and retention of spent lead shots has been recognized as a common disease of wild waterfowl in heavily hunted areas in the United States (Bellrose, 1959). This disease has also been recorded in 21 other countries, and is a serious problem in waterfowl the world over (Pain, 1992). Estimates of 3,000 tons of shots are fired annually by waterfowl hunters in the United States, whereas it is estimated that the annual weight of lead fired into habitats is 300 tons, of which 75 tons are fired into wetlands in all of Japan (Pain, 1992). Lead poisoning in swans was known to have occurred sporadically in Japan during the winters of 1984-1987 (Honda et al., 1990). The first mortality of whooper swans (Cygnus cygnus) in Japan from lead poisoning occurred in 1989 (Ochiai et al., 1992), and some mortality was observed the following year (Ochiai et al., 1993). The objectives of this presentation are to (1) summarize and discuss the pathological findings of lead poisoning in swans and geese and to (2) document the prevalence of lead poisoning of waterfowl in Japan.

Pathomorphology of lead poisoning in whooper swans and white-fronted geese

Whooper swans and white-fronted geese usually migrate between Japan and Siberia. Lake Miyajima in Hokkaido is the northernmost point in Japan where wild ducks, geese and swans arrive in spring and fall every year. We encountered two mortalities of whooper swans and white-fronted geese (Anser albifrons) in the spring of 1989 and 1990 at Lake Miyajima. Thirty-three whooper swans were found dead or in weakened condition between April and May 1989, and 80 white-fronted geese and 18 swans (Cygnus spp.) in the spring of 1990.

Whooper swans

Fifteen whooper swans (eight males and seven females) including six live-traped swans were examined pathologically. All the six live-traped swans (one male and five females) examined clinically showed signs of general weakness, inappetence, green watery feces, and pale conjunctiva. Body weights varied from 3.6 to 7.0 kg, with some birds being emaciated. They finally died after showing signs of dystasia, lethargy, and hypothermia. By hematology, two swans were apparently anemic (hematocrit < 26%), and all six swans revealed an increase of polychromatic erythroblasts with mild-to-moderate anisocytosis and/or poikilocytosis. Radiographically, lead shots (six to 27 pieces per bird) were found in the proventriculus and gizzard.

Common gross findings were greenish, bile-stained livers, distention of the gall bladder, impaction of the proventriculus with feed, green staining and focal hyperkeratosis of the gizzard lining (horny pads). Greenish diarrhea was indicated by staining of the feathers around the vent. The dilated proventriculus was impacted with water plants, mud, and pebbles. The honey pad of the gizzards was generally stained green by bile with mild-to-severe hyperkeratosis in all but one case. Lead shots (five to 30 pieces per bird) up to 3 mm in diameter were recovered from proventriculus-gizzard contents. Most pellets were irregular in shape and in various stages of erosion. There was no apparent correlation between body weight and the amount of lead shot per bird.

Liver discoloration was a remarkable gross finding. The entire liver parenchyma was uniformly colored dark green. Bile-colored viscous fluid oozed from the cut surface of the liver. There was moderate-to-severe distention of the gall bladder. Biliary atresia was not seen in any of the birds.

Histologically, the main lesions were hemosiderosis in the liver and spleen, tubulonephrosis in the kidney, hypoplasia of the bone marrow with an increase in the number of polychromatic erythroblasts, follicular atrophy and lymphoid depletion in the
spleen, and focal myocardial necrosis with fibrosis.

Severe hemosiderosis was observed diffusely in both the hepatocytes and Kupffer cells of the liver. Kupffer cells were activated, and large amounts of hemosiderin and bile pigments were seen in the swollen cytoplasm. Hemosiderin was also deposited in the hepatocytic cytoplasm adjacent to the bile pole, and the central line between the hepatocellular rows was evident. Bile capillaries were dilated, frequently with bile stasis. There was mild granular or fatty degeneration of the hepatocytes, while some cases showed small necrotic foci. Bile thrombi were not recognized in any of the interlobular bile ducts. From these findings, the bile-stained liver was diagnosed as a jaundice mainly caused by hemolysis.

In the spleen, the cells of the mononuclear phagocyte system in the red pulp were also activated and often contained hemosiderin. Mild to moderate hypoplasia of the bone marrow in the diaphysis of the femurs was seen in all the cases examined. Granuloblasts and myeloid cells declined in number from the extravascular spaces, which were replaced by adipose tissue. There was a moderate-to-severe decline in the number of mature erythrocytes, and in almost all cases early and late polychromatophilic erythroblasts increased in number in the sinuses of the marrows. Hemosiderin-laden macrophages were observed in the extravascular spaces.

Mild-to-moderate granular and fatty degeneration and focal necrosis were seen in the proximal tubules of the kidneys. Pigmentation with hemosiderin was mild and diffusely recognizable in the proximal tubules by Prussian blue stain. Acid-fast intranuclear inclusion bodies were recognized in the epithelium of the proximal tubules of seven cases by hematoxylin and eosin, and acid-fast stains. However, the inclusion bodies in all seven birds were detected with difficulty, even on the sections stained with acid-fast. There were generally one to three small granular inclusion bodies in each of the cells.

The kidneys in two of seven cases with intranuclear inclusion bodies were examined by electron microscopy. The inclusion bodies were easily distinguished from nucleoli by their density and structure. The inclusion bodies were of higher electron density than the nucleoli and could be detected even without staining with uranyl acetate and lead citrate. They had frayed contours and consisted of fine, high-electron-dense granules. The larger inclusion bodies were more homogeneous than the smaller ones.

Lead concentration was determined in the livers of 14 cases and in the blood and/or bone marrow of eight cases by atomic absorption spectroscopy after digestion with nitric acid and perchloric acid. Lead levels in the livers ranged from 5.5 to 44.3 mg/kg wet weight, 1.2 to 12 times higher than levels in blood samples and 4.8 to 16 times higher than levels in the marrow. Results of the chemical analysis did not correlate with the number of lead pellets in the gizzard.

White-fronted geese

Nineteen white-fronted geese (13 males and six females, including 9 immature birds) were pathologically diagnosed with subacute lead poisoning, which was confirmed by demonstrating high lead concentration in the liver. The liver lead concentration ranged from 6.9 to 67.7 mg/kg wet weight. All examined geese had hemosiderosis of mononuclear phagocytic system cells in the liver and spleen, and hypoplasia or edema of the bone marrow with increased numbers of polychromatophilic erythroblasts. All almost of other lesions and their pathogenesis were similar to those of the affected whooper swans. The liver, however, had some differences grossly and microscopically. The livers from geese showed mottled bile-stained discoloration and histologically consisted of Kupffer cell hemosiderosis, large bile plugs in dilated canaliculi, bile pigmentation in hepatocytes, and bile extravasation and associated hepatic necrosis. Seven geese of the remaining 11 birds had also similar necrosis in the liver, whose greenish discoloration was obscure macroscopically. The liver discoloration was pathologically considered a jaundice due to both rapid overproduction of bile from increased breakdown of erythrocytes and intrahepatic impaired excretion of bile.

From the results, lead poisoning resulting from ingestion of spent lead shots was considered as the main cause of the extensive loss of the swans and geese in Hokkaido, 1989 and 1990.

Pathogenetically, the birds were anemic, and successively toxic lesions occurred in the liver, spleen, and bone marrow (Fig.1). Anemia in lead poisoning is considered to result from shortening of erythrocyte survival times; inhibition of heme synthesis, including the interference with delta aminolevulinic acid dehydratase and heme synthetase; and defective erythrocyte production and impaired release of these cells from the bone marrow. Prominent accumulation of hemosiderin in the mononuclear phagocytic system cells of the liver and spleen suggests an increase in erythrocyte destruction by lead, whereas hypoplasia...
of the bone marrow with increased numbers of polychromatic erythroblasts indicates impaired erythropoiesis and a secondary reaction to erythrocyte destruction. The excess breakdown of erythrocytes within the liver resulted in hypercholia followed by discoloration of the gizzard lining, greenish diarrhea, intrahepatic cholestasis and liver discoloration. White-fronted geese revealed hepatic necrosis with bile extravasation probably resulting from severe intrahepatic cholestasis. A bile-stained liver is considered as a characteristic lesion of lead intoxication in whooper swans as well as whistling swans (Rosen and Bankowski, 1960) and Canada geese (Trainer and Hunt, 1965), although the lesion has not been emphasized in previous reports of lead-poisoned ducks.

**prevalence of lead poisoning of waterfowl in Japan**

Current prevalence of lead poisoning in waterfowl in Japan was estimated by the prevalence of ingested shotgun pellets among harvested waterfowl and the frequency of this toxicosis in carcasses of swans and geese collected from various wetlands.

Four hundred nineteen ducks were shot or captured between 1994 and 1997 at hunting grounds in 9 prefectures; Hokkaido, Miyagi, Niigata, Gifu, Aichi, Kyoto, Shimane, Kagawa, Fukuoka. Nine species of harvested birds were the mallard (*Anas platyrhynchos*), green-winged teal (*Anas crecca carolinensis*), pintail (*Anas acuta*), spot-billed duck (*Anas poecilorhyncha*), northern shoveler (*Anas clypeata*), eurasian wigeon (*Anas penelope*), tufted duck (*Aythya fuligula*), pochard (*Aythya ferina*), and greater scaup (*Aythya marila*). The frozen or cooled carcasses were immediately taken to our Laboratory, and necropsied. The proventriculus and gizzard from each bird were opened and the contents flushed into a bowl. Food and other light-weight materials were washed away, and the remaining heavy materials were dried. Lead shots were detected by sight and by X-ray (soft X-ray apparatus Softex CMBW-2; Softex Co., Ltd, Tokyo, Japan) of the proventriculus and gizzard content by the method of Anderson and Havera (1989). Ingested shotgun pellets were found in 15 (3.6%) of 419 harvested waterfowl. None of the birds ingested > 1 shot. The higher prevalence of ingested lead pellets was recognized in Hokkaido (11% [number of birds with a pellet/ total number examined = 4/37]) and Kagawa prefecture (10% [4/40]). Twelve (6.5%) of 186 mallards, 2 of 148 green-winged teal and one (25%) of 4 tufted ducks ingested one shot.

Between 1991 and 1997, 131 waterfowl carcasses other than the harvested waterfowl were collected specifically for investigating lead poisoning from various wetlands in Japan, and necropsied. These birds were found by bird-watchers in areas that are not heavily hunted. We determined whether these birds succumbed to lead poisoning according to the typical gross findings for lead-poisoned waterfowl which included emaciation, distended gall bladder, an impacted proventriculus, a greenish to gray discoloration of intestinal tracts, and greenish diarrhea.

![Diagram](image-url)
oration of the liver and kidney, a moderate to severe enteritis, a greenish to blue-gray slate discoloration of the intestinal tract, and greenish diarrhea which tended to stain the feathers surrounding the vent. Of the 131 carcasses, 32 (24%) birds were pathologically diagnosed as having lead poisoning. Two to 7 birds have been succumbing to this toxicosis each year. Thirty (94%) of these affected birds were swan species. Twenty five (83%) of the swans were found in Hokkaido and 15 (60%) birds found in and around Lake Miyajima, where the first mortality occurred in 1989.

Gravel was distributed into Lake Miyajima as a prophylactic replacement for lead immediately after the first mortality occurred in 1989. But some mortality still occurred the following year. However, this exercise was repeated and mortality has since not been repeated. In addition, hunters have discontinued waterfowl hunting at this lake since the fall hunting season in 1989. Based on the presented results, I believe that the waterfowl, especially swans in Hokkaido are under a higher risk of lead intoxication than other prefectures.

Conclusion
1) Laboratory examination established that lead poisoning was responsible for the majority of the mortalities of swans and geese at Lake Miyajima in Hokkaido during the spring of 1989 and 1990.
2) Histopathologic features in the whooper swans and white-fronted geese were characterized by hemolytic jaundice in the liver, hemosiderosis in the liver and spleen, and hypoplasia of the bone marrow with increased numbers of polychromatic erythroblasts.
3) These morphologic changes were suggested to result from excess breakdown of erythrocytes, hypercholia followed by intrahepatic cholestasis and disrupted erythropoiesis in bone marrow caused by lead.
4) The prevalence of ingested shot among 419 harvested ducks was 3.6%. The higher prevalence was recognized in waterfowl in Hokkaido and Kagawa prefectures. Thirty two of 131 other waterfowl succumbed to lead poisoning between 1991 and 1997. The majority of them were swan species in Hokkaido. These results indicates that lead poisoning may be a threat to waterfowl in Japan, especially to swans in Hokkaido.

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REFERENCES