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SHORT COMMUNICATIONS

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An Epizootic of Lead Poisoning in Greater Flamingos (*Phoenicopterus ruber roseus*) in Spain

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ABSTRACT: During November 1992 to March 1993, and November 1993 to February 1994, 106 greater flamingos (*Phoenicopterus ruber*) were collected dead or moribund in the wetlands of El Fondo and Salinas de Santa Pola, eastern Spain. Birds still alive were emaciated and had a bile-stained diarrhea. On necropsy, they had liquid in the upper digestive tract and the walls of their gizzards were stained dark green. Fifty-three (93%) of 57 gizzards examined contained lead shot (range one to 277 shot), and fifty-five (96%) of 57 livers contained levels of lead greater than 5 μg/g dry weight (DW) (median = 192.3 μg/g DW, range <2.5 to 992.2 μg/g DW).

Key words: Lead poisoning, lead shot ingestion, liver lead concentration, mortality, greater flamingos, *Phoenicopterus ruber*, Spain.

Lead poisoning by ingestion of lead shot, the commonest metal poisoning in waterfowl, occurs worldwide (Friend, 1989). Spain contains some of the most important bird populations of Europe (Troya and Bernués, 1990), but hunter pressure is also high in some wetlands. A consequence of use of lead shot for hunting has been high lead poisoning prevalences in waterfowl (Guitart et al., 1994).

Lead poisoning is also known as the invisible disease (Friend, 1989), because poisoned birds are usually not observed by people (Pain, 1991). However, in the recent decades, large epizootics have been reported in ducks, geese and swans (Friend, 1989). Nevertheless, reports of lead poisoning in other species are more scarce in the literature.

The first cases of plumbism in the greater flamingo (*Phoenicopterus ruber roseus*) were reported in 1986 near Marseille, France (Bayle et al., 1986). An epizootic involving almost 100 flamingos (*P. ruber ruber*) occurred in 1989 in Yucatan, Mexico (Schmitz et al., 1990), and 22 flamingos died of lead poisoning in 1991 in Doñana National Park, southwestern Spain (Ramo et al., 1992).

In spite of the few cases reported, we suspected that lead poisoning in flamingos was more frequent in Spain than were being reported, a suspicion based on the detection of ingested lead shot in four of 16 carcasses of flamingos killed by illegal shooting at the Ebre Delta Natural Park, northeastern Spain. This prevalence was similar to the prevalence of ingested lead shot for mallards (*Anas platyrhynchos*) in this park (Guitart et al., 1994). However, definitive confirmation of lead poisoning as a disease problem in flamingos came from an epizootic detected in 1992 in wetlands at the province of Alacant, eastern Spain.

Wetlands involved were El Fondo Natural Park (38°16′N, 00°41′W) and Salinas de Santa Pola Natural Park (38°12′N, 00°32′W), both included in the Ramsar List of internationally important wetlands in 1989 (Troya and Bernués, 1990). El Fondo encompasses 2,387 ha and is formed mainly by two reservoirs of 650 and 450 ha, respectively, containing fresh but very eutrophic water, and some small brackish ponds. Santa Pola, with 2,469 ha, is formed by several salt mine lagoons filled with marine waters, with many small brackish ponds around them.

Since the beginning of the epizootic, specialized personnel were assigned to carry out a census of flamingos present in El Fondo, and to recover any dead or sick

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flamingos in both wetlands. Between November 1992 and February 1994, 38 flamingos from El Fondo and 68 from Santa Pola were collected. However, it is estimated that the total number of dead flamingos was higher, as the access to some lagoons or marshes was restricted. Flamingos were necropsied and gizzard and liver were collected and stored at −20 C until analysis.

The gizzard and whole liver were analyzed from 50 individuals; the gizzard alone from seven individuals and the liver alone from seven other individuals also were evaluated. Gizzards were examined following the methods of Guitart et al., (1994), and number and weight of lead shots were noted. Whole livers were homogenized and aliquot parts were dried at 60 C for 72 hr and digested with 25 ml of nitric acid (purity 65%) at 120 C during 12 hr and 10 ml of perchloric acid (70% to 72%) at 220 C during 30 min. Samples were concentrated to 2 ml, and later diluted to 25 ml with deionized water and analyzed by ICP-atomic emission spectrometry, using a Polyscan 61E (Thermo Jarrell Ash, Franklin, Massachusetts, USA). Lead standards were used to determine lead concentrations in samples by linear regression of the SPSS/PC+ Version 4.0 statistical package (SPSS Inc., Chicago, Illinois, USA). Certified bovine liver samples (Promochem, Wesel, Germany) containing known lead levels were analyzed to assure quality of methodology. The limit of quantification was 2.5 μg/g dry weight (DW).

To study lead shot concentrations and availability of grit in sediments of El Fondo, 150 samples from one reservoir and 40 samples from a pond were taken at a depth up to 20 cm. Samples were collected every 25 m in 14 transects; each sample was separated by 35 m and we used a core of 119 mm in diameter. Presence of shot were determined by sieving samples through 1 mm mesh size. An aliquot of five samples from the reservoir were granulometrically analyzed by sieving through different mesh sizes (4, 2, 1 and 0.5 mm).

Sediment of El Fondo had low weight percentages of grit particles: 0.5 to 1 mm = 0.60%, 1 to 2 mm = 0.24%, 2 to 4 mm = 0.01%, and >4 mm = 0.007%. On the other hand, sediments of the reservoir and the pond were rich in lead shot: 1,630,398 and 1,236,285 pellets/ha, respectively.

Flamingos were captured by hand while still alive. They were found isolated from the other birds. They were emaciated, had bile-stained diarrhea, were unable to fly, and died few hours after capture. Some of the carcasses of dead flamingos were partially consumed by raptors or dogs. Mean ± SD weight in fully preserved carcasses was 2,578 ± 366 g for 27 males and 2,249 ± 564 g for 22 females. On necropsy, they had some liquid in the upper digestive tract; the content and walls of the gizzards often were stained dark green, with parasites resembling *Tretrameres* sp. in the membranes of some proventriculi.

Fifty-three (93%) of 57 gizzards contained lead shot, with a median of 37 shot per gizzard (range 1 to 277 shot). The median weight of lead was 0.94 g (range 0.05 to 13.67 g). The mean ± SD weight of the nonlead shot grit ingested was determined in 29 individuals, and it showed a broad range of size selection by flamingos, including grit similar in size to shots (1 to 3 mm): <0.5 mm = 0.13 ± 0.29 g, 0.5 to 1 mm = 1.04 ± 1.91 g, 1 to 1.5 mm = 0.74 ± 0.50 g, 1.5 to 2 mm = 0.97 ± 0.57 g, 2 to 3 mm = 0.68 ± 0.48 g, 3 to 4 mm = 0.82 ± 0.66 g, and >4 mm = 0.18 ± 0.70 g. There were not significant differences (ANOVA test) in the amount and proportion of grit selection when compared by the sex or age of the flamingos, or the presence of ingested shot in the gizzard (SPSS/PC+ Version 4.0 statistical package, SPSS Inc.).

Fifty-five (96%) of 57 livers contained lead levels >5 μg/g DW, considered diagnostic of abnormal lead exposure (equivalent to 1.5 μg/g wet weight) (Guitart et al., 1994). Median of determinations was

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192.3 μ/g DW, and the range was <2.5 to 992.2 μg/g DW. Liver lead levels were correlated with the number of lead pellets found in gizzards and with the weight of these shots (both with $r = 0.78$, $P < 0.001$).

In 57 of the 64 flamingos found dead, the minimum number of lead shot ingested was eight and the minimum lead liver concentration was 77.2 μg/g DW; we believe that most or all of them died by lead poisoning. For this group, logarithm transformed data of number of pellets in the gizzard and lead liver concentration fitted a normal distribution (Kolmogorov-Smirnov test, SPSS/PC+ Version 4.0 statistical package, SPSS Inc.) that can reflect their mean (95% confidence interval) lethal values for flamingos: 40.3 (34.2 to 47.6) pellets and 212.2 (186.4 to 241.5) μg/g DW, respectively. In the other seven flamingos, no more than two shot were found and the maximum liver concentration was 7.0 μg/g DW, thus they may have died from other causes.

The high lead levels in the livers of poisoned flamingos were consistent with those previously reported for the Yucatan (Schmitz et al., 1990) and Doñana (Ramo et al., 1992) epizootics.

The 1992 to 1993 and 1993 to 1994 epizootics of flamingos detected in El Fondo and Santa Pola may have been related to the extreme low rainfall and reduced water levels during this period. In normal years, flamingos concentrated in Santa Pola (Fernández-Cruz et al., 1988). Historically the number of hunters were less than half of those present in El Fondo and therefore we assume less lead was deposited. However, adverse weather conditions in 1992 to 1994 reduced the water levels in El Fondo, allowing the flamingos to feed in a zone normally too deep for them. Due to this, the number of flamingos in El Fondo increased markedly; many flamingos were seen moving from Santa Pola to El Fondo each morning, and returning to Santa Pola in the evening. They were probably attracted by the rice and other crops provided by hunters in El Fondo to capture ducks. In addition to the increased potential for exposure to lead, this granivorous diet in flamingos may have forced them to consume more grit than normal.

We believe that most flamingos in this study died from lead poisoning. Thus, lead poisoning in this species may be more frequent in Spain than previously assumed, as this is the second epizootic confirmed in flamingos in this country. Moreover, mortalities involving five to 15 individuals have occurred before in El Fondo and other Spanish wetlands, and although the cause of death was not established, it is reasonable to suspect lead poisoning. These facts support the urgency to ban lead shot for hunting waterfowl in Spain, a measure already undertaken by other European countries and the United States.

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