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Pathology of Experimental Poisoning Induced by Lead Shot Pellets in Mallards

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Clinical, radiological, elemental and histopathological analyses were carried out on mallards (*Anas platyrhynchos*) dosed orally with lead shot pellets #3. The clinical signs and pathological changes of mortally poisoned ducks were proportional to the dosage of lead and the length of time the birds were exposed. It was concluded that like the field cases the lead shot poisoned mallards developed severe and fatal ailment, which could be induced even with a single lead pellet in a range of two to five weeks. An important and pathognomonic microscopic finding was the detection of acid-fast intranuclear inclusions within the epithelial cells of the proximal renal tubules of all cases and in the hepatocytes of birds with longer course of intoxication.

Key words: mallard, lead shot, lead poisoning, acid-fast intranuclear inclusions.

Introduction

Lead hunting ammunition sources have been implicated in the deaths of many avian species, best known and documented in waterfowl. All birds are susceptible to the effects of lead but their response shows distinct intraspecific and interspecific differences (2, 4, 5, 9, 11, 14). Lead poisoning is especially problematic for the mallards because their populations are frequently exposed to ingestion of lead pellets in the areas of intensive hunting. Therefore, the abundant epidemiological data in this field was used to develop regulations regarding the use of lead ammunitions in many countries around the world (1, 3, 7, 11). However, studies about the severity of the ailment induced by lead pellets in susceptible birds are insufficient; therefore, the objectives of our study were to assess the severity of the pathology in mallards orally dosed with lead pellets #3.

Materials and Methods

This study was approved by the Animal Care Commission at the Faculty of Veterinary Medicine, University of Forestry – Sofia (permition number: No 80 valid until 2018 to train students and conduct research of Veterinary Medicine) and performed according to the EU and national regulations.

Animals and study design. Sixteen clinically healthy mallards (*Anas platyrhynchos*) 9 to 12 months old were randomly divided into four groups (n = 4/ group) and housed in separate isolators. After a 7-day period of adaptation the birds in group 1 were orally dosed with 3 lead shot pellets #3 (average weight of 0.26746 g), group 2- with 2 lead pellets and group 3- with 1 lead pellet. The last four mallard (group 4) were kept untreated and used as a negative control group. All ducks were monitored daily for the occurrence of clinical signs including growth stunt and diarrhea. Body weight measurings were performed on the 0th, 7th, 14th, 21st, 28th, 35th and 42nd day after administering the pellets.

Radiology. At the beginning and at the end of the experiments each mallard, orally dosed with lead pellets, was examined by native radiography in a ventro-dorsal projection using an Innovet X-ray machine (V 125 Eickemeyer®).

Elemental analysis. Lead concentrations in the livers of all ducks were estimated by atomic absorption spectroscopy (Atomic absorption spectroscopy system "SpectrAA 800").

Histopathology. Tissue samples from the liver, spleen, kidney, heart, cerebrum and cerebellum were fixed in 10% neutral buffered formalin, embedded in paraffin, prepared in 5 to 8 μm sections and stained with hematoxylin and eosin (H&E). Double sections of kidney and liver were stained for acid-fast nuclear inclusions according to the Ziehl-Neelsen.

Results

The surviving period, severity of the clinical signs and pathologic lesions correlated to the lead concentrations in the liver and were proportional to the dosage of the lead pellets and the length of time the birds were exposed (**Table 1**).

One of the earliest symptoms to appear was the lowered food intake. Either the appetite of the affected bird decreased completely or the food consumption decreased to a level below the minimum nutritional requirements, which lead to a progressive weight loss. Passage of characteristic bright green droppings was established as early as the seventh day after the ducks were dosed with commercial #3 shot pellets. Later greenish diarrhoea and greenish staining of the vent were observed in some cases following by signs of weakness and fatigue. Ataxia, convulsions, paralysis of legs or wings, inability to fly, swim or dive and prostration were also a common clinical observations in the diseased birds.

By means of radiology, it was observed that the shot pellets were stopped in the gizzard and remained there to the end of the experiments. The final radiographs and post mortem findings showed that destruction of the shot in the gizzard was progressive and regular (**Fig. 1**)

In fatal cases, the main macroscopic findings included emaciation, decreasing of body fat, bright green staining of the vent area, mottled bile-stained liver, enlarged and distended with bile gallbladder and presence of lead pellets in the gizzard, as well.

Table 1. Initial and endpoint body weight (BW), the day of death and contents of lead in the liver of experimental mallards

Group	Sex	No of shot per treatment	Initial BW(g)	End point BW(g)	Loss of weight (%)	Day of death	Lead in the liver mg/kg
Group 1	female	3	1120	830	25.8	14	26.2
	male	3	1250	850	32	28	37.6
	male	3	1220	860	29.5	28	37.1
	male	3	1153	740	35.8	21	42.5
Group 2	male	2	1230	900	26.8	35	26.2
	female	2	1180	600	49.2	32	17.5
	male	2	1300	870	33	37	25.3
	male	2	1150	580	49.56	42	73,9
Group 3	female	1	1150	1200	2.61	*	2.5
	male	1	1140	1152		*	1.9
	male	1	1130	1075	4.86	*	8.7
	male	1	1070	525	50.93	35	28.4
Group 4	female		1130	1234		**	0.19
	male		1142	1274		Excluded alive	Not performed
	male		1115	1260		Excluded alive	Not performed
	male		1035	1232		**	0.69

* Euthanized on the 62th day of experiment
** Euthanized on the 75th day of experiment

The pronounced histopathological features were diffuse congestion of the liver, kidney, spleen, heart, cerebrum and cerebellum (**Fig. 2a, b**). Canalicular and intracellular holestasis, hepatic necrosis and hypertrophic nucleoli of the hepatocytes were commonly seen. In the cases with longer course of disease well-developed intranuclear inclusion bodies were detected in many hepatocytes. Focal tubulonephrosis and constantly occurring acid-fast intranuclear inclusion bodies within the epithelium of the proximal tubules were the most specific findings at all. Morphologically, the inclusions could

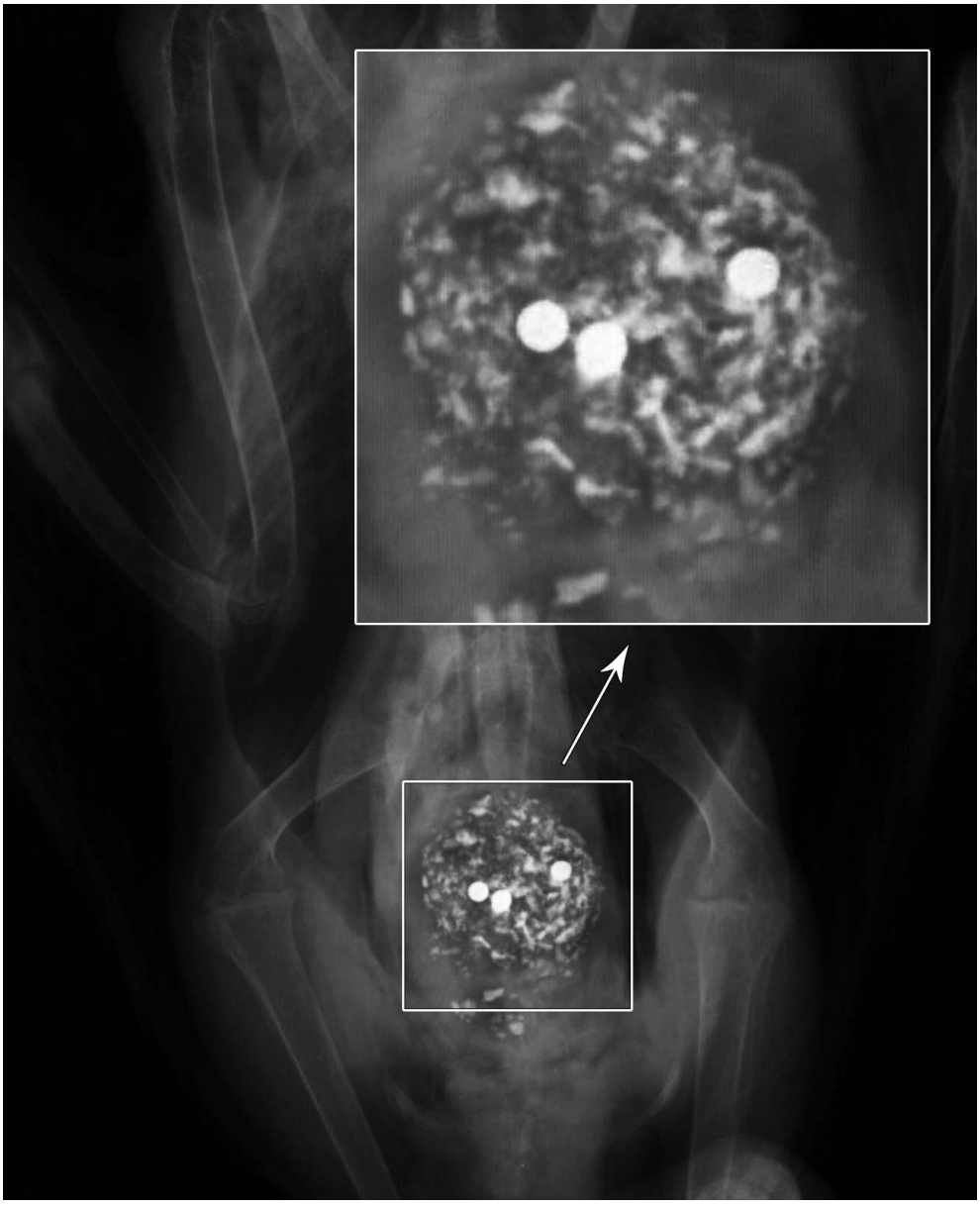

Fig. 1. X-Ray of a dead mallard on the 21st day after it had been orally dosed with three lead pellets #3. The pellets permanently remained in the gizzard decreasing in diameter and eroding asymmetrically

be divided into two types. Inclusions from the first type were observed in birds with acute course of poisoning in a range of a two or three weeks and were found within the epithelial cells of some proximal renal tubule. They were clearly acid-fast appearing as single or granulated small bodies (**Fig. 3a**). Inclusions from the second type were observed in ducks with the chronic cases. They were larger round inclusions in both

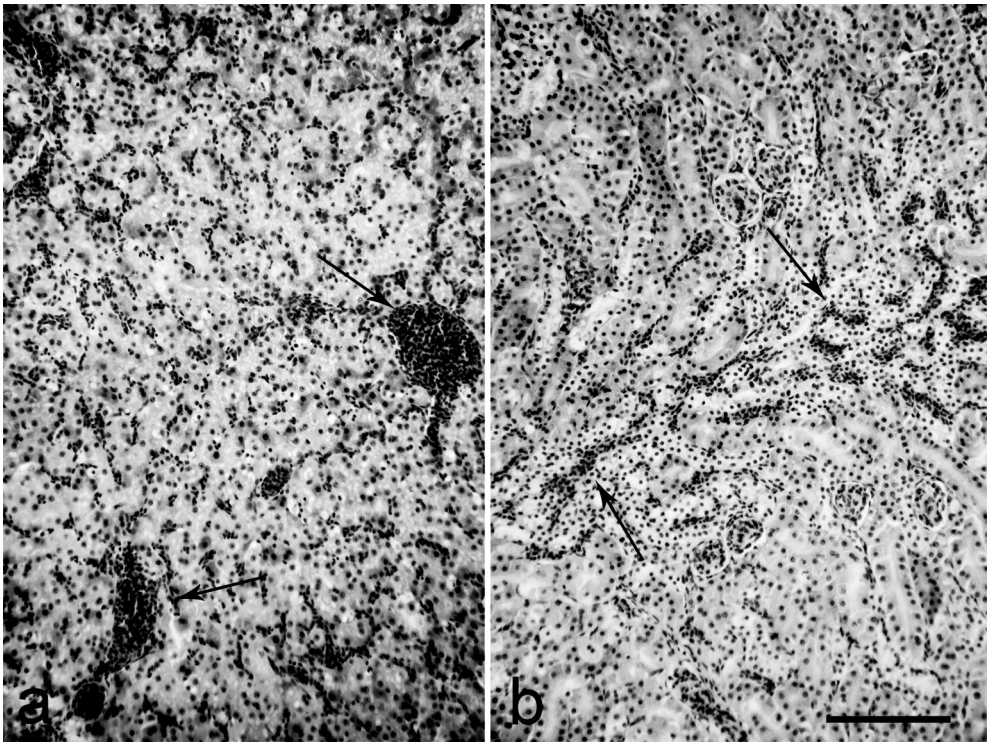

Fig. 2. Histological features of a mallard's liver (a), and kidney (b) – dead on the 28th day after it had been orally dosed with three lead pellets #3. Both organs are severely congested (arrows). Paraffin sections stained with hematoxylin-eosin (H&E). Bar = 100 μm

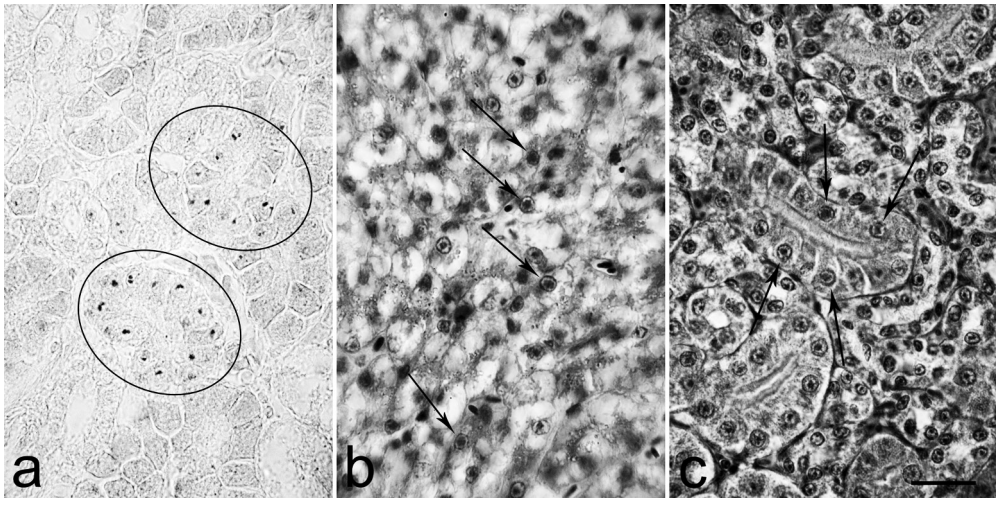

Fig. 3. Acid-fast intranuclear inclusion bodies in the mallard's renal (a and c) and liver (b) cells. Clearly distinguishable acid-fast inclusions (encircled) as single or granulated small bodies within the epithelial cells of two proximal renal tubules of a mallard, dosed with three lead pellets and died on the 28th day (a). Larger nuclear inclusion bodies (arrows) in hepatocytes (b) and renal tubular cells (c) of a mallard that had been dosed with two lead pellets and died on the 37th day. Ziehl-Neelsen stain (a), and H&E (b, c). Bar = 20 μm

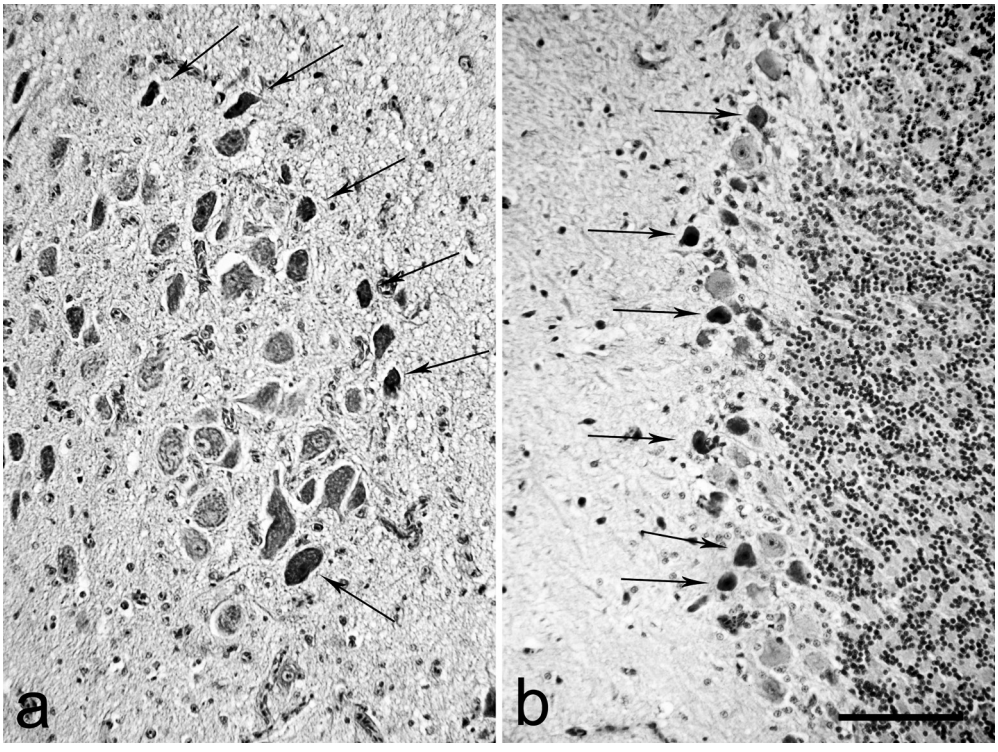

Fig. 4. Medulla oblongata at the obex level (a), and cerebellum (b) from a mallard died on the 37th day after had been dosed orally with two lead shot pellets #3. Necrotic neurons (arrows) within the dorsal vagal nucleus (a), and Purkinje cell layer (b). H&E. Bar = 100 μm

hepatocytes and renal tubular cells. These type inclusions were not strongly acid fast but were well visible in haematoxylin-eosin stained preparations (**Fig. 3b, c**). Marked distention of many blood vessels with swollen and hyperplastic endothelial cells as well as neuronal necrosis were a common findings throughout the brain (**Fig. 4a, b**). Additionally necrotic foci infiltrated with heterophil granulocytes were frequently established within the heart.

Discussion

The data of the present study clearly indicate that the ingestion of one to three lead pellets #3 in a mallard induces acute to subacute fatal ailment with clinical, radiological, toxicological and pathological features, which are in agreement with the data published by other researchers in this field (1, 6, 8, 10, 12, 13, 15). The macroscopic and microscopic findings in liver, spleen, kidneys, heart, cerebrum and cerebellum were not sufficiently different from many other metabolic or toxic changes to make a definitive diagnosis of lead poisoning and were not with significant diagnostic properties. However, the acid-fast inclusion bodies in liver and kidney cells were a constant finding and could be considered as useful diagnostic criteria as it was stated by other researchers as well (1, 6, 7, 13, 16). Therefore, the lead poisoning in this study was confirmed on the basis

of microscopic detection of nuclear acid-fast inclusions in kidney and liver cells and high lead levels estimated by atomic absorption spectroscopy in the liver. Finally, based on the present study and the data of other investigators (6, 9, 17, 18 and 19) we could conclude that the lead shot toxicosis in mallards should be treated as a fatal systemic disease where the hematopoietic system, nervous system, immune system, reproductive system, kidney, liver, bones and heart are general targets of toxic injuries.

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