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Source: Journal of Wildlife Diseases, 14(2) : 187-192

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-14.2.187

LEAD POISONING OF RACCOONS IN CONNECTICUT [1]

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Abstract: A wild raccoon *(Procyon lotor)* had clinical signs, histopathologic and ultrastructural lesions indicative of lead intoxication. The diagnosis was confirmed by chemical analyses of liver and kidney tissues which revealed 35 ppm of lead in wet tissues. A survey of hepatic lead concentration in 13 additional raccoons was conducted.

INTRODUCTION

Lead poisoning is well recognized in domestic animals.[18,19] Recent investigations have revealed poisoning and/or high lead concentrations in both captive and free-ranging wild animals as well.[10,13,15,17] Hascheck and Lisk[8] demonstrated toxic lead levels in rodents inhabiting old orchard soils which had been sprayed with a lead arsenate pesticide. Scanlon[16] showed that animals living within 50 m from heavily traveled highways accumulate more lead than those animals living further away. Industrial effluents and waste disposal further add lead to the ecosystem.

Various species of animals may be used as biological markers of environmental contamination. For example, a significant difference in kidney lead concentrations was reported between urban and rural squirrels *(Sciurus carolinensis)* examined in Florida.[13]

The omnivorous feeding habits of raccoons *(Procyon lotor)* and their close association with man qualify this animal as a useful indicator of environmental pollution.[3] The first report of lead levels in free-living raccoons was by Sanderson and Thomas[15] in Illinois. They found an average hepatic lead level of 6.8 ± 1.8 ppm. Twenty-three of 101 animals examined had levels greater than 10 ppm which is regarded to be toxicologically significant.[4,6,12,19] Hoff *et al.*[10] found average renal lead concentrations of 0.47 ± 0.22 ppm for 14 estuarine raccoons in Collier County, Florida. The apparent ability of raccoons to carry a relatively large body burden of lead without showing ill effects makes it a practical biologic marker for lead pollution.

This report presents a case of lead poisoning in a free-ranging raccoon and data on pathologic examination and hepatic lead concentrations in 14 other wild raccoons.

MATERIALS AND METHODS

Raccoons examined in this survey were either trapped in wire box-type live traps or found dead and submitted to the Northeastern Research Center for Wildlife Diseases for pathologic study. Formalin-fixed tissues from one raccoon (Y1275) were submitted for histopathology. Live animals were anesthetized with Ketaset [2] (ketamine hydrochloride).[2] Blood was obtained by cardiac puncture while animals were sedated. Blood smears made from EDTA-treated

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blood were fixed in methanol and stained with Wright's Giemsa stain for microscopic examination. Total red blood cell and white blood cell counts, hematocrits, hemoglobin determinations, and differential counts were determined for all live animals.

After obtaining blood samples, the raccoons were killed by exsanguination. Thorough necropsy examinations were made of all animals. Tissue samples were fixed in 10% phosphate buffered formalin and processed by routine methods for paraffin sectioning. Sections were cut at 6 μm and stained with hematoxylin and eosin. Special stains, including Ziehl-Neelsen, periodic acid-Schiff and Pollack's trichrome, were employed as needed.

Bone marrow was obtained from the femur during necropsy and smears were made using a soft camel hair brush wetted with serum. These slides were fixed in methanol and stained with Wright's Giemsa for microscopic examination. Differential counts based on enumeration of 500 cells were determined for all samples.

Portions of the liver and kidney were frozen for heavy metal determination. Samples were analyzed for lead residues by flameless atomic absorption spectrophotometry at the Connecticut Agricultural Experiment Station in New Haven, Connecticut.

Tissues for electron microscopy were dissected from formalin fixed tissues, washed in 1/15 M phosphate buffer, and post-fixed in 2% osmium tetroxide. Following fixation, specimens were washed in distilled water, dehydrated in graded ethanol solutions, cleared in propylene oxide, and embedded in DER 334. Thin sections were cut on an LKB ultramicrotome III, stained with uranyl acetate and lead citrate, and examined with a Philips EM 300 electron microscope.

RESULTS

Toxicology

The livers of 14 adult raccoons from various areas in Connecticut were analyzed for lead concentration.[7] The results of these analyses are presented in

TABLE 1. Liver concentrations of lead in Connecticut raccoons. (All values in ppm wet weight).

Raccoon	Accession No.	Sex	Lead	Submission
1	Y 1275	?	35	Fixed tissue
2	Y 2134	F	20	Dead animal
3	Y 2645	M	15	Live animal
4	Y 2646	F	10	Live animal
5	Y 2280	M	7	Dead animal
6	Y 3132	M	6	Live animal
7	Y 2776	M	5	Live animal
8	Y 3507	F	4	Live animal
9	Y 2281	M	4	Live animal
10	Y 2490	M	3	Dead animal
11	Y 3177	M	3	Dead animal
12	Y 2803	F	2	Live animal
13	Y 2802	M	1	Live animal
14	Y 2804	M	<1	Live animal
Mean*			6.2	
S.D.			5.4	

*Case No. 1 was not computed into determination of the mean of standard deviation.

Table 1. All values are in ppm wet weight. Concentrations ranged from less than 1 ppm to 35 ppm. The mean value was 6.2 ppm and standard deviation 5.4. Kidney tissues were not analyzed routinely since concentrations vary between cortical and medullary areas.[18]

Pathology

Case 1 was the only animal in which morphologic lesions of lead intoxication were observed. The chemical analysis recorded lead concentrations at 35 ppm in liver and kidney. The raccoon had been seen during daylight hours wandering around a suburban neighborhood and was subsequently attacked and killed by a dog. It was reported that the animal behaved in an abnormal fashion. A field necropsy was performed and formalin-fixed tissues submitted to the Northeastern Research Center for Wildlife Diseases. Blood and bone marrow samples were not available.

Histopathologic examination disclosed moderate to severe pulmonary edema. The spleen was characterized by relatively few germinal centers frequently containing an amorphous pink-staining material and by nucleated red blood cells in the sinusoid spaces. Small multifocal areas of hepatic degeneration were seen. Many of the hepatocytes contained large brightly eosinophilic intranuclear inclusion bodies (Fig. 1). Degeneration of the epithelium in the proximal tubules and eosinophilic intranuclear inclusions in the degenerating cells were the major lesions observed in the kidneys (Fig. 2). Marked karyomegaly was seen in the liver and kidney. Mild endothelial hyperplasia and scattered neuronophagia were subtle pathologic changes found in the brain.

The hepatic and renal inclusions were round and often located in the center of the nucleus. The inclusions varied in size and morphology from small basophilic granular bodies to large structures composed of an inner homogeneous eosinophilic portion and surrounding baso-

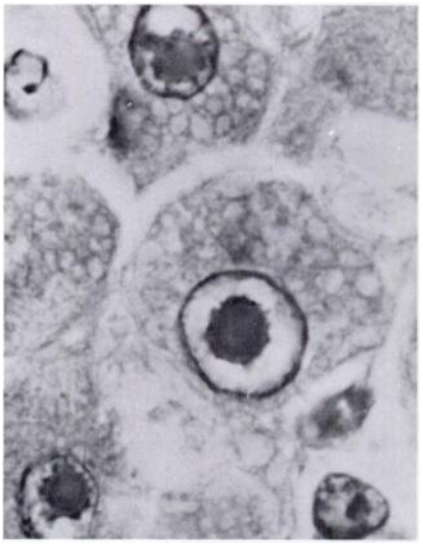

FIGURE 1. Hepatocyte containing an acidophilic intranuclear inclusion body. Note the two zones of the inclusion. H & E; ×1400.

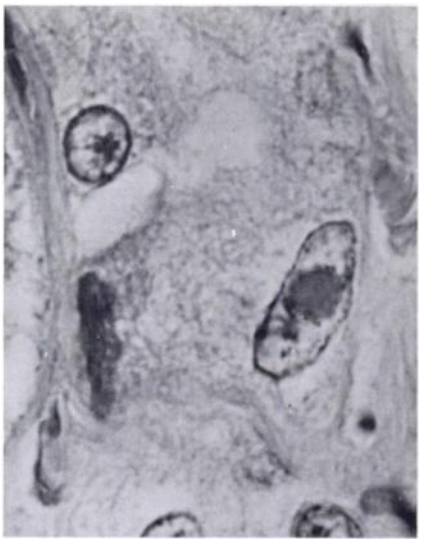

FIGURE 2. Epithelial cell of the proximal renal tubules depicting tubular degeneration and an inclusion body. H & E; ×1400.

philic layer. Nucleoli were displaced toward the nuclear membrane. Chromatin fragments were marginated. The number of inclusion bodies per nucleus varied from 1 to 3; however, single inclusions were most prevalent.

The inclusion bodies in the liver and kidney were found to be acid-fast when stained by the Ziehl-Neelsen technique. Examination of the inclusions after staining with Pollack's trichrome stain and periodic acid-Schiff procedures revealed the inclusions to be composed of a central core of red-orange staining material. Surrounding the inner core, an outer fringe of bluish-stained delicate granular material was seen.

Ultrastructurally, the hepatic inclusions were composed of an intensely osmophilic material (Fig. 3). The inclusions varied in size and density. The smallest bodies were composed of a few intertwining strands of microfibrillary structures. The larger, dense inclusion bodies had a round to oval compact central core which was surrounded by a granular mycelia-like envelope. The peripheral filamentous area is believed to correspond to the stippled area of basophilia which was observed at the light microscopic level.[1] Distinct separation of these zones was not apparent. The area around the large inclusions was devoid of nuclear chromatin.

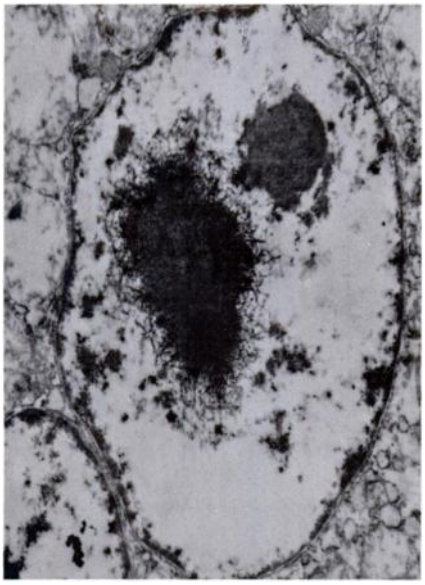

FIGURE 3. A lead induced intranuclear inclusion body in an hepatocyte. Note the filamentous periphery and compact core. ×12,000.

Cases 2, 3, and 4 had liver lead concentrations greater than 10 ppm (Table 1). Microscopic lesions which are thought to be associated with plumbism consisted of edema of the brain characterized by an enlarged Virchow-Robins space and endothelial hyperplasia.[11] Inclusion bodies characteristic of lead poisoning were not seen. Basophilic stippling of erythrocytes, regarded as a sensitive indicator of plumbism in dog and man, was not evident in any of the raccoons.[5,18] Other blood parameters were within normal ranges.[9]

DISCUSSION

The lead residue levels of this study indicate that raccoons in Connecticut are exposed to considerable environmental lead. These data support the work of Sanderson and Thomas[15] and suggest that the raccoon is relatively resistant to the toxic effects of lead. The lead burdens varied greatly between the surveys in Florida and those in Connecticut and Illinois. Lead concentrations reported by Hoff *et al.*[10] in raccoons in Florida are consistent with those of unexposed animals.[6,19] The high standard deviation of mean liver lead concentrations obtained in this report indicates that animals are not uniformly exposed to lead contamination.

There are many sources of lead available to wild raccoons.[4,8,15,16] Raccoons frequently are scavengers of garbage cans, which may be a source of lead contamination. Additional investigations emphasizing the epidemiologic

aspects of plumbism in free-ranging raccoons would be desirable.

The levels of lead believed to represent a toxic dose have been determined for many domestic animals and man. In most species, values of liver lead between 5 and 10 ppm are regarded as suspicious or toxic, and concentrations greater than 10 ppm are diagnostic for plumbism.[1,11,19] In this study, 4 animals were found to have liver lead burdens of 10 ppm or greater. One raccoon with 35 ppm lead in the liver and kidney had morphologic lesions of lead poisoning. The only other reported case of lead poisoning in the raccoon is in a captive South American crab-eating raccoon *(Procyon cancrivorous)*.[19]

Although the raccoon is able to tolerate a large burden of lead without developing the lesions of plumbism, is is not known how this metal might affect the stressed animal. Cantarow and Trumper[4] point out that the quantity of lead required to produce signs and lesions of intoxication varies greatly. The toxic level required is related to age, sex, chronicity, absorption, and route of exposure. Stress, such as disease and an inadequate diet — particularly a diet low in calcium — may lower the required toxic dose.[4,14]

Often the signs and lesions of lead poisoning are subtle and its clinical course insidious. It is possible that a content of lead in the body insufficient to cause obvious disease may give rise to slowly evolving and long-lasting effects. Lead can cross the placental barrier giving rise to a wide spectrum of fetal changes, ranging from abortions and premature births to weak offspring.

Acknowledgements

The authors wish to extend their appreciation to Dr. Lamia Khairallah for her advice and technical skill in electron microscopy, to Dr. John Anderson for assistance in capturing live specimens, and to Ms. Lita Strolle for her help with the hematology.

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Received for publication 23 August 1977