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EPIDEMIOLOGICAL DATA ON LEAD TISSUE CONCENTRATION IN GAME BIRDS INDUCED BY LEAD PELLETS

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Abstract

The paper presents the level of lead in the liver, gizzard, breast muscles and humerus of game birds, shot during the hunting season of 2016 – 2017 in Bulgaria. In quail gizzards (n=10) radiographic examination showed ingested pellets. In turtle doves (n=10), lead levels in the liver had higher values of 2,501 ± 1,404 mg/kg, compared to the maximum levels of <2 mg/kg. The content of lead in the humerus of partridges (n=10) showed a very high concentrations of 54,241 ± 36,731 mg/kg compared to the base level of 10<20 mg/kg. The high levels of lead in the tissues of the game birds, induced by lead shot exposure, are a significant risk to predators and scavengers.

Keywords: *lead pellets, radiographic, game birds, liver, humerus bones.*

INTRODUCTION

Lead is one of the most toxic metals and its negative effects range from mild biochemical and physiological disorders to serious pathological processes in which major organs and systems may be affected, with following functional and behavioral changes. The probability for a bird to be poisoned is determined by several factors, such as: time of retaining the lead elements, frequency of exposure, nutritional conditions, stress etc. According to some scientists, lead fragments are readily consumed by the animals because of the salty-like taste of their oxidized surfaces, particularly when salt - deficient mammals and birds are concerned (Lewis et al. 2001).

Lead concentrations are highest after direct absorption into the bloodstream, after which the lead accumulates in the kidneys and liver for days or months, and if the process becomes chronic- it is then deposited in the bones with the possibility of a lifetime exposure (De Francisco et al., 2003). If the intoxicated with lead birds are consumed by predators or scavengers, the latter in turn absorb certain amounts of lead, which may result in their intoxication and death (Pain et al., 2009).

MATERIALS AND METHODS

In order to monitor the risk of lead contamination during the hunting season of 2016-2017, through the National Research Station for Wildlife Management Biology and Pathology - Sofia, corpse material of the following game birds was obtained: Colchic pheasant *(Phasianus colchicus)*, partridges (*Perdix perdix*), turtle doves *(Streptopelia turtur),*quail (*Coturnix coturnix,)* and mallards *(Anas platyrhynchos,).* The pheasants and partridges were obtained from hunting farms, while the other species (turtle doves, quail and mallards) were shot during hunting trips.

The fowl game species shot were radiographed in ventrodorsal projection using the Eickemeyer® digitalised Inovet V 125 X-ray machine. The gizzards removed from the abdominal cavity were examined for the presence of incoming gunshot injuries and, if any, were excluded from analysis. The remaining gizzards, intact from lead pellets, were

radiographed in order to find ingested lead fragments.
After the radiographic examinations, the gizzards were sutured and mechanically cleaned from their contents, after which they were washed with distilled water and frozen in sterile containers at -20 ° C.
The survey was conducted in the Laboratory of Ecology and technical tests "Akvateratest" Sofia. The samples were analyzed by the methods of inductively coupled plasma spectrometry. The spectrometers Varian Vista-MPX CCD Simultaneous ICP OES, Varian Australia, and Plasma Quant MS S-NR 105000-AQ032, Analytik Jena AG, Germany were used.
The results obtained were evaluated according to two criteria:

- oral lead exposure due to swallowing of lead pellets, which may lead to a toxic effect in a given individual;
- percutaneous lead exposure for the presence of lead residues in the individuals shot, which implies a potential risk of secondary poisoning of the final consumer.

The analysis and statistical processing of the data were performed by the computer program SPSS 19.0. The data is expressed as mean plus standard error. In this study the assessment is made with guaranteed probability 0.95 (significance level $\alpha = 0,05$), where $p < 0,05$ was adopted as the lowest level of statistical reliability.

RESULTS AND DISCUSSIONS

The radiographic examination of corpse material from different game species showed the presence of contrast-positive oval-shaped objects. In pheasants and partridges, the radiographic images recorded a more concentrated position of the lead pellets, while those in mallards, turtle doves, and quails showed greater dispersion of the pellets.
Following the radiographic examinations performed on one quail from the group, a single positively contrasted spherical image was observed in the region of the gizzard with apparently smaller diameter compared to another positively contrasted object of oval shape that was positioned in the chest area of the same bird (Fig. 1a).
After another radiographic examination of the already removed whole gizzards of the quails, in one of them a positively contrasted object with a spherical shape was observed once again (Fig. 1b).

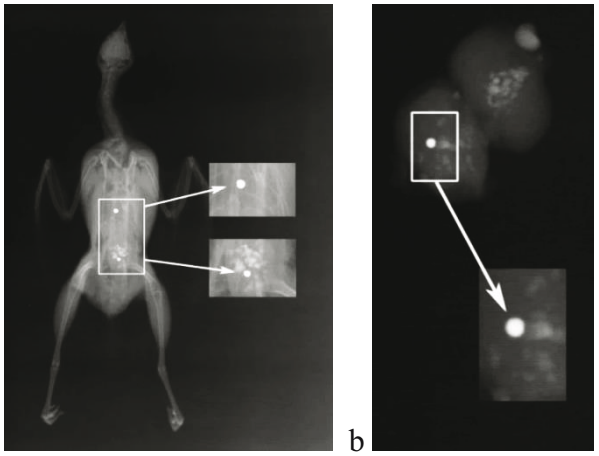

Figure 1. A quail (Coturnix, coturnix, L), shot with lead pellets # 11, ventrodorsal proection. a) two positively contrasted spherical objects can be observed: one in the chest area and one in the ventricular area. The object in the ventricular area is visibly smaller than the one in the chest area b) native radiography of a gizzard of a quail with a presence of a positively contrasted oval object.

After the subsequent sectioning and revision of the gizzard and its contents, we found a graphite-colored lead fragment (defined by XRF) of oval shape and diameter of approximately 1.3 mm and weighing 0.018 g.
The summarized and processed results of a total of 200 biological samples obtained from 50 wild birds that have been hunting objects are presented in Table 8.
Samples showing a lead content of 0.5 mg/kg for liver and gizzard and 0.1 mg/kg for breast muscles served as a control group. For the humerus bones a lead content of 10 mg/kg was taken as a reference.
The quails (n=10) showed mean lead concentrations in the liver of 0.822 ± 0.162 mg/kg, with no statistically significant difference compared to the maximum permissible 0.5 mg/kg (Fig. 2).
The lead concentration in the gizzards of the quails was 1,830 ± 0,528 mg/kg with a statistically significant difference ($p < 0,05$) compared to 0,5 mg/kg.
The lead levels in the breast muscles of the quails was moderately elevated with mean lead levels of 0.968 ± 0.556 mg/kg.

In the humerus bones of the test subjects from the group of quails the lead content of 31,450 ± 16,097 mg/kg exceeded the allowable threshold of tolerance of 10<20 mg/kg.

Table 1: Lead content (mg/kg) in liver, gizzard, breast muscle and humerus bones of game birds, ($\bar{x}$ ± SE).
*Statistically significant difference p < 0,05

Species / Tissue	Quail n=10	Turtle dove n=10	Partride n=10	Colchis pheasat n=10	Mallards n=10
Liver	0,822± 0,162	2,501± 1,404	1,642 ± 0,951	2,828 ± 0,262*	0,534± 0,165
Gizzard	1,830± 0,528*	1,642± 0,476*	0,827 ± 0,198	10,882 ± 10,294	0,591± 0,143
Breast muscle	0,968± 0,556	4,60 ± 2,659*	0,337 ± 0,086	0,351 ± 0,114	6,942 ± 5,164
Humerus	31,45± 16,097	48,25± 21,316	54,241± 36,731	57,225 ± 38,864	28,896 ± 19,388

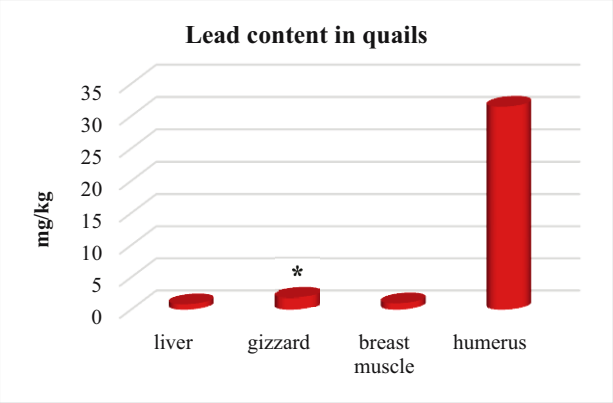

Figure 2. Lead content in tissue samples from quails (Coturnix coturnix, L).
*Statistically significant difference p < 0,05 compared to 0,5 mg/kg

In turtle doves (n=10) the levels of lead in the liver showed high values of 2.501 ± 1.404 mg/kg without statistically significant differences compared to the acceptable 0.5 mg/kg (Fig. 3)
In the gizzards of the turtle doves a high lead content of 1,642 ± 0,476 mg/kg, with a statistically significant difference (p < 0,05), compared to the permissible 0.5 mg/kg, was recorded. The content of lead in the breast muscles of the turtle doves was on average 4,60 ± 2,659 mg/kg with a statistically significant difference (p < 0,05) compared to the permissible 0,5 mg/kg.

The content of lead in the humerus bones of the turtle doves was increased, with mean values of 48,250 ± 21,316 mg/kg.

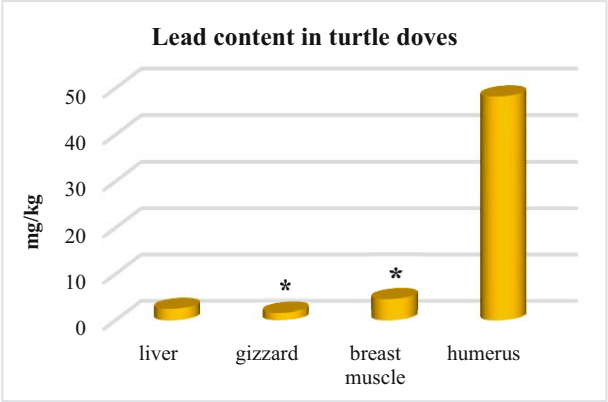

Figure 3. Lead content in tissue samples from turtle doves (Streptopelia turtur, L).
*Statistically significant p < 0,05 compared to 0,5 mg/kg lead content in liver and gizzard and 0,1 mg/kg in breast muscle.

The lead content in the livers of the partridges (n=10) was 1,642 ± 0,951 mg/kg, and of the gizzard 0,827 ± 0,198 mg/kg, with no statistically significant difference compared to the permissible 0,5 mg/kg (Fig. 4).
Unlike the higher concentrations measured in the previous two species (quail and turtle doves), the amount of lead in the breast muscles (n = 10) was lower with mean values of 0.337 ± 0.086 mg/kg (Fig. 4).
The lead content recorded in the humerus bones of the partridges was high 54,241 ± 36,731 mg/kg, compared to the maximum permissible values of 10<20 mg/kg (Fig. 4).

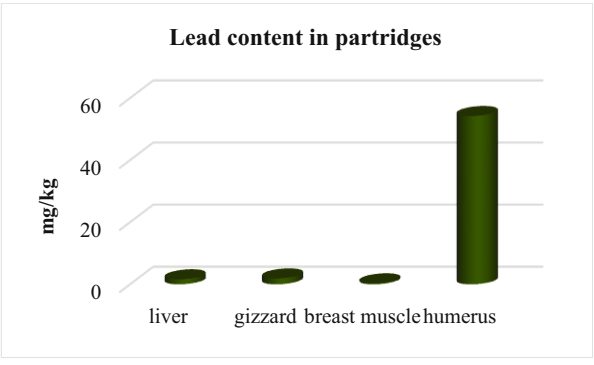

Figure 4. Lead content in tissue samples from partridges (Perdix, perdix, L).

In colchic pheasants (n=10), the registered lead content in the liver averaged 2.828 ± 0.262 mg/kg with a statistical confidence level of ≤

0.05 compared to the permissible 0.5 mg/kg (Fig. 5).
In the gizzards of the pheasants, a relative increase of 10,882 ± 10,294 mg/kg of the lead content was measured, without any statistical significance. Lead content in the breast muscles was low 0.351 ± 0.114 mg/kg.
Similarly with the partridges, the content of lead in the humerus bones of the pheasants also showed high mean values of 57,225 ± 38,864 mg/kg, compared to the maximum permissible threshold of 10<20 mg/kg (Fig. 5).

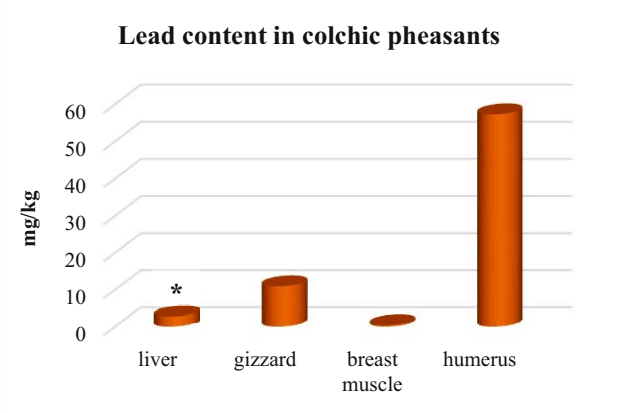

Figure 5. Lead content in tissue samples from Colchic pheasant (Phasianus colchicus, L).
*Statistically significant difference p < 0,05 compared to 0,5mg/kg lead content in the liver.

The lead content in the liver and gizzard of the mallards (n = 10) was low, with mean values of 0.534 ± 0.165 mg/kg and 0.591 ± 0.143 mg/kg (Fig. 4).
In contrast to the low content in the internal organs, the lead content in the samples obtained from the breast muscles of the mallards exceeded the maximum permissible norms, with high mean values of 6.942 ± 5.164 mg/kg. The lead content in the humerus bones was 28,896 ± 19,388 mg/kg (Fig. 4).
The lead content in the soft tissues of the quail group is variable and the liver values obtained were comparable to those reported by other authors (Kendall and Scanlon, 1981). The presence of lead ammunition in the gizzard was consistent with other similar cases reported, according to which the accelerated metabolism and acidity in the stomach of the quail are a prerequisite for the faster absorbtion of the lead (Kendall and Scanlon, 1981, Lewis and Schweitzer, 2000).

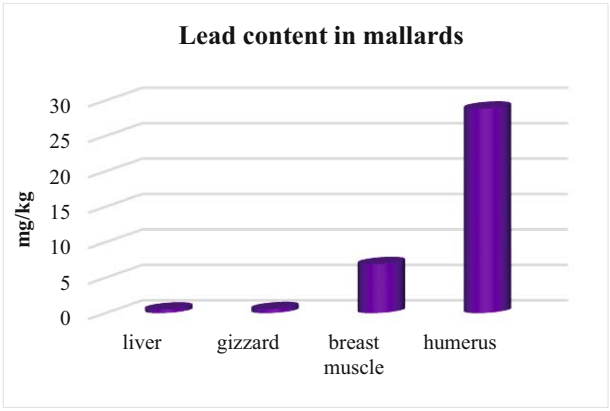

Figure 6. Lead content in tissue samples from mallards (Anas platyrhynchos, L).

Therefore, given the results obtained, it is possible that other individuals in the quail group have also absorbed single lead pellets which, due to their small size, can easily be excreted (Yamamoto et al., 1993). The moderately high levels of lead measured in the breast muscles of the quails, may have been due to fragmented to dispersed lead micro particles as a result of percutaneous (intradermal) exposure. However, due to elevated levels of lead in the liver and gizzard in some of the quail group individuals, it was possible to refer to the residues as resulting from oral lead exposure. The reported mean levels of lead in the humerus bones of the quails exceeded the maximum permissive background levels of 10<20 mg/kg. According to Kendall and Scanlon (1981) similar high levels are due both to the cumulative action of lead after oral lead intake and to the possible transcostal inoculation of fragmented lead particles after a shooting.
Of the fowl game species studied, turtle doves showed high concentrations of lead in the liver, with measured mean values exceeding the maximum permissible toxicity threshold of 2 mg/kg (Ohi et al., 1974; Schulz et al., 2002). The elevated lead concentrations in the liver corresponded to the mean levels measured in the gizzard, which, according to Buerger et al. (1986), may be due to oral lead exposure. The obtained high levels of lead in the breast muscles in our case may have been due to percutaneous exposure due to the shooting, but according to experimental studies based on oral administration of lead ammunition, similar levels of lead in turtle doves were recorded (Buerger et al. 1986; Kendall et al., 1996).

The high average lead content in the humerus bones of the turtle doves according to Franson and Pain (2011) is an indication of lead accumulation as a result of recurrent lead exposure.

The reported high mean levels of lead in the gizzards of the pheasant group, according to other studies, are related to the characteristics of its habitats and the nature of its nutritional needs (Clausen and Wolstrup, 1979; Fisher et al. 2006; Kanstrup, 2012). A similar lead content in the gizzard is characteristic of pheasants inhabiting the territory of pheasant hunting farms which soil layers contain significant amounts of lead ammunition (Butler et al., 2005; Kanstrup, 2012; Runia and Solem, 2014) which pheasants can swallow while eating. The high lead levels found in the humerus bones of the studied colchic pheasants, according to Butler et al. (2005) is indicative of a likely subclinical course or chronification of the process with a potential manifestation of a toxic effect.

Partridges are terrestrial birds, often sharing the same habitats with pheasants on hunting grounds with high-intensity shootings. The lead content in the liver and gizzard of the partridges was comparable to the threshold toxicity level to 2 mg/kg (Franson and Pain, 2011). The obtained high levels of lead in the humerus bones were consistent with other similar studies, which identified the partridge as a specific bioindicator for increased lead content (Clausen and Wolstrup, 1979; Keymer and Stebbings, 1987; Kendall et al., 1996).

In contrast to our previous studies, in experimental mallards treated orally with lead pellets (Stamberov et al., 2017), in the current study only in two individuals from the mallard group (n = 10) the lead content in the liver exceeds 1.5 mg/kg which according to other studies may be grounds for the presence of chronic lead exposure (Bellrose, 1959, Guitart et al., 1994). The mallards were a pooled sample from different wetlands rather than a particular habitat. This further explained the obtained low liver and gizzard results. The high lead content in the breast muscles was probably due to the fragmentation of lead ammunition after the contact with dense bone tissue as a result of the shooting. This implies a potential risk for the final consumer, such as some protected predatory bird species that will be secondarily exposed to lead by feeding on wildfowl shot with lead ammunition (Frank, 1986, Mateo, 2009).

CONCLUSIONS

Based on the results in the studies conducted in wildfowl, we could summarize that:

Lead content in wildfowl is highest in humerus bones of the pheasants and the partridges inhabiting areas with intensive hunting activities and landfilling of lead ammunition which suggests chronic oral exposure.

The presence of a lead pellet in the gizzard of quail and the high lead level in the liver of turtle doves are indicative of the possibility of oral exposure for these two migratory species.

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