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Lead Contamination in Meat and Offal from Game (Ruminants), Destined for Raw Feeding of Dogs and Retailed in Austria

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Abstract: Bullet-derived lead (Pb) is a food safety hazard in meat from hunted wild game. Dogs can be exposed to alimentary Pb when fed with meat from wild game. We studied Pb contamination in 47 commercial "bones-and-raw-food" (BARF) packages from wild game meat and offal sold in Austria. Samples were first tested with a metal detector (calibrated for 2.5 mm diameter objects), then by X-ray, and Pb content was determined in sample areas free from metal-dense particles (as assessed in radiographs). Three samples tested positive by the metal detector (particle sizes ranged from 3 mm × 2 mm × 2 mm to 10 mm × 8 mm × 5 mm), with two particles from lead and one from tombac. Metal-dense objects were found in radiographs of 35/47 samples. In 13/47 samples, particles > 1 mm in diameter, and in 33/47 samples, smaller ones (median of five particles per sample) were noted. Lead content was above 1.5 mg/kg wet weight in 19/47 samples and exceeded the limit for general animal feed of 10 mg/kg wet weight in 5 of these 19 samples. The Pb contents as well as the presence of metal particles indicate that there is a feed safety issue in the samples analyzed. By a metal detector, the presence of large fragments was indicated in three samples, which casts doubt on whether all producers use a metal detector to scan the packages before delivery.

Keywords: lead; particles; wild game; bones-and-raw-food (BARF); contamination

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Citation: Paulsen, P.; Lindinger, S.; Eder-Rohm, K.; Eder, G.; Bauer, S. Lead Contamination in Meat and Offal from Game (Ruminants), Destined for Raw Feeding of Dogs and Retailed in Austria. *Pets* **2024**, *1*, 3–10. https://doi.org/10.3390/pets1010002

Academic Editor: Giacomo Biagi

Received: 3 February 2024
Revised: 12 March 2024
Accepted: 18 March 2024
Published: 20 March 2024

1. Introduction

Lead is an unwanted contaminant not only in foods but also in feed [1]. High lead contents in pet food have triggered alarms in the EU rapid alert system RASFF (e.g., No. 2020.3894; 2022.6707; https://webgate.ec.europa.eu/rasff-window (accessed on 5 February 2024)). Fish and red meat have been identified as a risk factor for higher Pb levels in canned pet food [2]. Lead—and/or bullet-derived—particles in meat from hunted wild game are contaminants relevant for food safety, not necessarily as health hazards, but as contamination rendering the food unfit for human consumption [3]. Assessment of unacceptable Pb levels in game meat is somewhat hampered by the absence of EU-wide limits for Pb in game meat [4], which requires a consumption-based case-by-case evaluation [3,5]. A concise overview of the extent and implications of the use of lead ammunition during hunting and a perspective on replacing lead with other materials is given by Thomas et al. [4].

Several studies have shown that dogs/hunting dogs fed with tissues from hunted wild game are exposed to lead [6–10], with a concomitant rise in blood Pb, which can approach a critical Pb threshold [10]. Lead intoxication in dogs was often associated with proximity to mining areas [11], ingestion of certain paints [12,13] or inadvertent ingestion of lead objects [14], including ammunition [13], at least in the cases with known lead sources. Lead intoxication in dogs often presents as gastroenteric disturbance or central nervous system disorders but also as nephropathy [15]. Hypertension has been reported after experimental low-dose lead administration [16]. A concise summary of effects and applied Pb doses is given in Høgasen et al. [7]. Larger lead particles may reside for a longer period in the gastrointestinal tract and result in a higher amount of lead being released, ultimately causing intoxication [17,18]. Generally, ingested foreign bodies can require surgical intervention [19], and it is known that such foreign bodies could damage mucosal membranes or perforate intestinal walls, but less attention has been paid to the role of bullet fragments as hard and sharp objects.

As regards the exposure of dogs to lead via the ingestion of meat contaminated with lead (bullet) fragments, risk assessment models have been developed [7], taking into account uncertainties about the bioavailability of lead from ingested fragments of various sizes.

Raw feeding of dogs is becoming increasingly popular [20,21], despite well-documented public health issues [22,23]. For hunters' dogs fed trimmings from wild game, alimentary exposure to and uptake of lead has been demonstrated [7,9], and a recent study on lead particles and lead contents in commercial pet food from meat from small game for raw feeding of dogs [24] shows the potential of this pathway for alimentary exposure of dogs to the toxic element lead, taking into account that frequent feeding will result in long-term exposure. In previous studies on raw meat-based pet food, we found that such products from wild game are increasingly commercialized in Austria [25], either from muscle tissues of large wild game or from inner organs or bones.

European legislation defines the maximum Pb levels in feed, with 10 mg/kg wet weight as the maximum level in general-use feed [1]. However, pet food business operators are also obliged to identify and assess other chemical as well as physical or microbiological hazards in order to decide if they pose a safety concern and need to be managed under Article 29 of EU Regulation No. 1069/2009 [26], similar to the requirement for food [27].

The aim of this study was to assess the contamination of commercial pet food for raw feeding of dogs by metal particles and to determine the Pb concentration. We compared results from screening with a metal detector and X-ray analysis to Pb contents in regions "free" from particles (based on the radiographs). We assumed that no particles detectable by commonly used metal detectors (i.e., $\geq$2.5 mm diameter) would be present in the packages but expected detectable Pb levels (i.e., >1.5 mg/kg) attributable to/associated with the presence of smaller lead particles.

2. Materials and Methods

2.1. Sample Acquisition

We obtained 34 samples from 11 suppliers, either from retail shops in Vienna or from Austrian online stores. With the exception of one sample (dried sausage), the size was $\geq$500 g. Sample dimensions were 15 cm $\times$ 25 cm $\times$ 3 cm maximum. In addition, 13 samples (7 suppliers) of muscles from game for raw feeding obtained in a previous study [16] were included in this study (150–250 g per sample). Details are given in Table 1. According to the information on the label or given by the retailers, all samples originated from approved pet food plants in Austria or Germany or from registered producers in Austria. Samples were considered "general animal feed", either because this was explicitly specified on the label or because it was evident from the composition of the samples (e.g., rumen or bones with little adherent meat). Samples were obtained in deep-frozen conditions and stored at -20 °C until analysis.

Table 1. Information on the 47 pet food samples for raw feeding of dogs.

Type of Sample	Samples, *n* =	Suppliers, *n* =
Muscle tissue	32	15
Bones	3	2
Liver	4	4
Rumen	2	2
Offal mix	6	3

2.2. Analysis for Foreign Objects and Preparation of Samples for Pb Determination

Samples were brought to 0 °C and scanned by a commercial detector for the food industry (Metron 07 CI; Mesutronic Ltd., Kirchberg im Wald, Germany) which had been calibrated to detect ferrous and non-ferrous objects of 2.5 mm diameter in meat packs of 5 cm in height. Subsequently, all samples were subjected to X-ray analysis (GIERTH HF100; Gierth X-ray International Ltd., Riesa, Germany; settings: 50 kV, 0.9 mAs/0.06 s, with a digital X-ray detector Leonardo DR mini, Oehm & Rehbein, Rostock, Germany; 70 cm distance from focus to detector). A paperclip with 1 mm wire thickness was placed near the sample in a way that the orientation of the sample was defined and, together with the dimensions of the package, the proximate size of radio-dense objects could be assessed. The paperclip acted as a positive control (metal particle). Bones (compacta and spongiosa) were represented by one epiphysis in one sample, and by three samples consisting of smaller pieces of bones with little adherent meat, and their density and contrast were used as negative controls. Distinguishing metal-dense objects from bone fragments was based on brightness (density) and contrast [28].

The radiographs were evaluated visually by three assessors (P.P., S.B. and S.L.) acting independently, and the consensus of the assessments is reported in this article.

Subsequently, we identified the regions of each package in which no metal-dense objects were visible. From these regions, 3–5 samples of 5–10 g were taken with inert ceramic knives (Graef, Arnsberg, Germany), combined, and minced in an inert blender system with a titanium knife (Retsch, Haan, Germany). From the homogenate, a portion of ca. 1 g (0.98–1.07) was taken and placed on the bottom of a nylon tube (CEM Ltd., Kamp-Lintfort, Germany), and 4 mL 65% nitric acid (Rotipuran; Carl Roth, Karlsruhe, Germany) was added. The tubes were placed on a heating plate set to 110 °C and the sample was wet ashed, with the addition of 0.5 mL H_2O_2 35% (Carl Roth, Karlsruhe, Germany) and nitric acid until the liquid was pale and clear. The liquid was allowed to reach room temperature and made up to 10 mL with ultrapure water.

Finally, it was attempted to retrieve larger particles (i.e., 1 mm diameter or more) from the samples. To this end, the regions with metal-dense objects in the radiographs were dissected with toothpicks. Hard, dark-grey objects with a metallic sheen were collected and examined under a stereo microscope (10–20×) by scratching the surface. The emergence of metallic-grey surfaces was indicative of lead. Objects made of copper (alloys) were readily identified by their typical color.

2.3. Determination of Pb Concentration

Lead concentration was determined on a Perkin-Elmer AAnalyst 300 system (Perkin-Elmer, Waltham, MA, USA), with the following settings: acetylene 0.8 L/min (AAS grade; Air Liquide, Schwechat, Austria), pressurized air 8.4 L/min., wavelength 217 nm, slit 0.70 mm.

The system was calibrated with Pb standards (ROTI Star; Carl Roth) and ultrapure water as a blank. Reported data are averages from three readings. The limit of detection was 1.5 mg/kg wet weight (w.w.). Per sample, one analysis was conducted.

2.4. Statistical Methods

Results were tabulated in MS Excel (Microsoft, Redmond, WA, USA) worksheets for descriptive statistics. We studied whether the presence of metal-dense objects would constitute a risk factor for higher (i.e., detectable; >1.5 mg/kg w.w.) Pb levels by calculating the odds ratio (OR) and checking if the 95% confidence interval (CI) did not include the value "1". In this case, the presence of metal-dense objects constituted a risk factor for Pb levels > 1.5 mg/kg w.w. with statistical significance ($p < 0.05$) [29].

3. Results

3.1. Presence of Metallic Foreign Bodies According to Metal Detector Testing and Radiological Examination

In 3/47 samples, the metal detector gave a signal indicative of metal fragments. Radiological examination demonstrated the presence of metal-dense particles in 35/47 samples. In 33 samples, at least one dense object < 1 mm diameter was found (median: 5 particles), and in 13 samples, at least one dense particle ≥1 mm minimum dimension was identified. Among the particles ≥ 1 mm, three samples contained one particle each greater than 2 mm, and those were the samples that had tested positive with the metal detector. The number of dense particles per product type is given in Table 2.

Table 2. Presence of metal particles in 47 pet food samples for raw feeding of dogs.

Type of Sample		*n* = [1]	Samples Positive in the Metal Detector	Samples with Metal-Dense Particles (X-ray)	Samples with Particles ≥ 1 mm (Median and Max [2])	Samples with Particles < 1 mm (Median and Max [2])
Muscle tissue		32	2	24	9 (0; 4)	24 (4; 10)
Other tissues:		15	1	11	4 (0; 2)	9 (1; 10)
	Bones	3	1	2	1	1
	Liver	4	0	2	1	1
	Rumen	2	0	1	0	1
	Offal mix	6	0	6	2	6

[1] number of samples per product type; [2] refers to number of objects per sample.

3.2. Lead Content

Since the samples were considered general animal feed, a maximum level of 10 mg/kg w.w. was permitted according to EU legislation [18]. The lead content in the 47 samples is given in Table 3. In 19/47 samples (40%), Pb content was above 1.5 mg/kg w.w. (i.e., the limit of detection of our method). The legally binding maximum level of 10 mg/kg was exceeded in five samples (four muscle; one offal mix) or 11%, with a maximum of 57.1 mg/kg w.w. (in a sample with metal-dense objects, but negative when tested by the metal detector). Since we cannot rule out the presence of very small Pb particles in the homogenized subsamples analyzed, the Pb values are not necessarily representative of the whole package content; however, they are indicative of contamination.

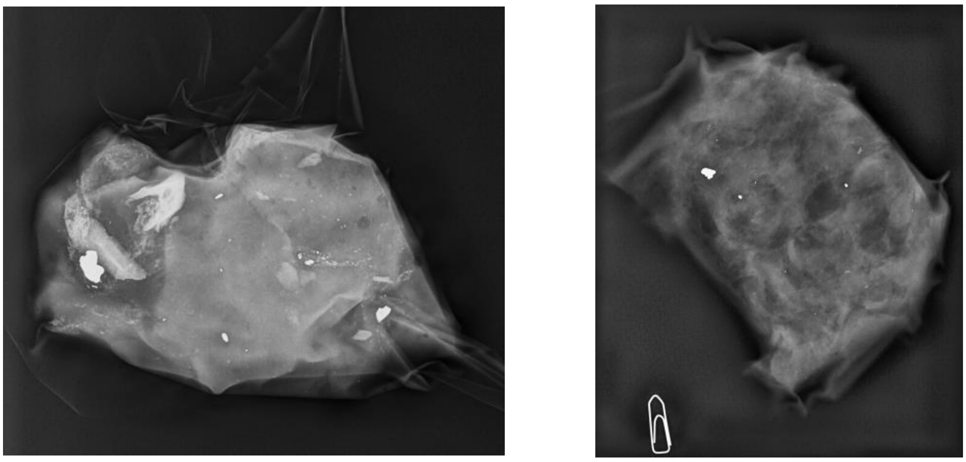
Table 3. Pb content (mg/kg wet weight) in the 47 pet food samples for raw feeding of dogs. Note that only sample parts free from metal-dense objects (assessed by X-ray) were tested.

Type of Sample		*n* = [1]	Median	Maximum	>1.5 mg/kg to 10 mg/kg; *n* =	>10 mg/kg, *n* =
Muscle tissue		32	<1.5	57.1	9	4
Other tissues:		15	<1.5	32.5	5	1
	Bones	3	<1.5	<1.5	0	0
	Liver	4	1.5 *	2.3	2	0
	Rumen	2	<1.5	<1.5	0	0
	Offal mix	6	1.9	32.5	3	1

[1] number of samples per product type; * since two results were <1.5 and two were >1.5 mg/kg, the median is displayed as 1.5 mg/kg.

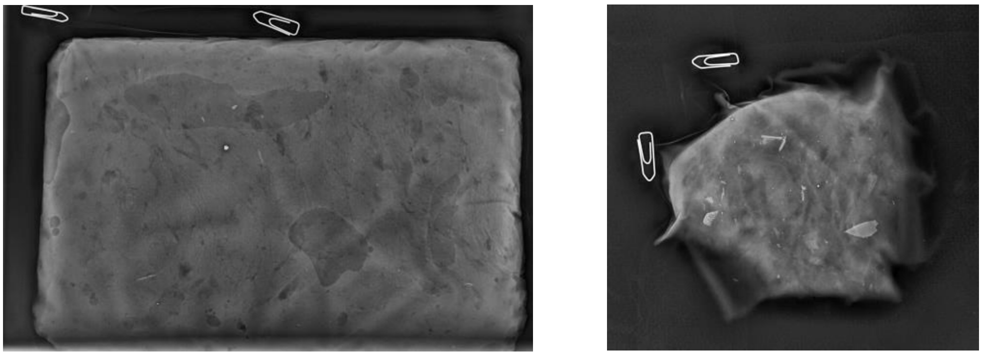
3.3. Distribution of Dense Particles on the Radiographs and Relation of the Presence of Metal-Dense Fragments and Pb Content

As regards the distribution of small metal-dense objects, both clustering as well as distribution throughout the packages were observed (Figures 1 and 2). Since during production, all samples had undergone cutting and most samples had undergone mincing before packaging, it is conceivable that the size and distribution of the particles were not only influenced by fragment distribution in the body of wild game (i.e., after shooting) but also by the subsequent processing steps, e.g., mincing. We could retrieve metal fragments of various dimensions, from ca. 0.1 mm to >5 mm in size, from 16 samples. The dimensions of the largest fragments were 10 mm × 8 mm × 5 mm and 7 mm × 5 mm × 3 mm.



(**a**) (**b**)

Figure 1. X-ray of packages containing minced meat from wild game and that had tested positive for metal parts by the metal detector. (**a**) To the left, a large metal-dense object (bright white) is seen below the bone fragments; additional larger dense objects are seen in the bottom (middle and right) section, together with a group of smaller objects near the center (Pb content of the sample was 49.5 mg/kg); (**b**) one large object >2 mm, and two objects around 1 mm in diameter (Pb content = 22.4 mg/kg).



(**a**) (**b**)

Figure 2. X-ray of packages containing minced meat from wild game that had tested negative for metal parts by the metal detector. (**a**) Near to the center, a metal-dense object of ca. 1.5 mm diameter (Pb content < 1.5 mg/kg); (**b**) one object of <1 mm diameter, smaller dense objects (Pb content = 57.1 mg/kg) and larger bone fragments.

Notably, not all dense particles identified in the radiographs were necessarily lead. Indeed, the third largest fragment we retrieved (3 mm × 2 mm × 2 mm) was from tombac and part of a bullet jacket.

We studied whether there was a relation between the presence of metal-dense objects and the Pb contents (Table 4). In the group of samples with metal-dense objects (n = 35), the median Pb content was 1.6 mg/kg, and the 75th percentile was 6.75 mg/kg. In the group with no visible dense objects (n = 12), the Pb median and 75th percentile were <1.5/<1.5 mg/kg. The odds ratio for Pb contents > 1.5 mg/kg given that metal-dense particles had been detected was 11.6 [95% CI: 1.4–100.1; p = 0.024]; see Table 4. This means that, although we tried to analyze non-contaminated regions, there was an indication that very small and thus undetected particles had been in the samples.

Table 4. Association of the presence of metal-dense particles (X-ray) and Pb contents > 1.5 mg/kg wet weight.

	Pb > 1.5 mg/kg, n =	Pb $\leq$ 1.5 mg/kg, n =	Total, n =
metal-dense objects detected	18	17	35
no metal-dense objects detected	1	11	12

Odds ratio $_{\text{(Pb > 1.5 mg/kg | metal-dense objects present)}}$ = 11.6 [95% CI: 1.4–100.1; p = 0.024].

4. Discussion

Metal particles in pet food can present physical hazards to dogs [19], although the relevance will depend on their size and shape. However, since this hazard has been identified in pet foods ([24] and this study), pet food business operators need to assess this hazard under Article 29 of EU Regulation No. 1069/2009 [26], similar to the requirements for food set out in Article 15 of EU Regulation No. 178/2002 [27], and to adapt their safety management systems accordingly. It can be argued that at least the use of a metal detector should be a standard procedure, although in our study, this was obviously not observed by all producers. However, this will not ensure low Pb levels, as demonstrated in our results, where a sample negative in the metal detector contained 57.1 mg Pb/kg wet weight.

As regards the assessment of Pb concentration, it is questionable if "representative" results can be obtained, since the presence of numerous, irregularly dispersed, very small particles can be expected in wild game meat when lead-based bullets have been used [30,31], eventually forming a "lead cloud" [5]. Assessment of effects after ingestion would depend on the bioavailability of Pb and is influenced, inter alia, by particle number and size [5], and thus, it is not easy to perform [7]. Most samples (42/47) did not exceed the maximum level according to EU legislation [1], but it has been questioned if this level should be revised [7].

Apart from a toxicological assessment, the key point is that bullet fragments represent physical and/or chemical, but in principle avoidable, hazards for safe pet food production. Safety can be accomplished when pet food producers process game meat from animals killed with non-lead bullets [24]. Other options might include the selection of animals with shot wounds distant from skeletal muscles (e.g., head shots), which could easily be accomplished when processing meat from farmed game. Admittedly, the use of by-products from the processing of hunted wild game meat in pet food generates added value and contributes to a sustainable use of natural resources.

The general use of ammunition with non-lead bullets with a deforming rather than fragmenting pattern in hunting large game would generate a few large fragments, which can more reliably be detected in commercial metal detectors.

The caveats of this study are that the results should not be taken as prevalence data or indications of actual alimentary exposure of dogs to Pb, all the more so as the Pb contents reported refer to the feed portions free from radio-dense objects. Without this, Pb contents would most likely have been higher. While we tried to obtain samples from all online suppliers we could identify in Austria and retailers in Vienna, we did not study batch-to-batch or intra-batch variations. Although the latter might be useful information for pet food business operators, the presence of metal particles generally indicates that the production process is not under control.

5. Conclusions

Pet food from game meat can contain fragments of bullets or shots, with various dimensions and numbers and often composed of lead. In order to avoid the presence of larger particles constituting sharp and hard objects (physical hazards), scanning raw material or finished meat packs with a metal detector is recommended. However, this procedure will not ensure compliance with the maximum Pb level for feed set out in EU legislation (10 mg/kg), due to the presence of smaller Pb particles in the raw material. In accordance with the literature, processing meat from hunted wild game that has been killed by non-lead bullets is recommended to ensure low Pb levels in pet food. Other options, e.g., processing meat from game killed with head shots, are more realistic in the utilization of farmed rather than wild-hunted game.

Author Contributions: Conceptualization, P.P.; methodology, P.P., S.L. and G.E.; formal analysis, P.P.; investigation, S.B., P.P., S.L., K.E.-R. and G.E.; resources, P.P.; data curation, P.P.; writing—original draft preparation, P.P. and S.L.; writing—review and editing, P.P., S.L. and K.E.-R.; project administration, P.P.; funding acquisition, P.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are available under https://phaidra.vetmeduni.ac.at/ (accessed on 1 February 2024).

Acknowledgments: The assistance of Brigitte Pilz in extracting lead particles from the samples is acknowledged. Thanks are due to PANNATURA GmbH, Austria, for providing the scans of samples with a metal detector. The method settings for Pb determination on the AAS were provided by M. Sager. This article is based on work from the COST Action Safety in the Game Meat Chain (SafeGameMeat), CA22166, supported by COST (European Cooperation in Science and Technology).

Conflicts of Interest: The authors declare no conflicts of interest.

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