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Analysis of number, size and spatial distribution of rifle bullet-derived lead fragments in hunted roe deer using computed tomography

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Abstract
The use of lead-based rifle bullets in hunting poses a risk to human and animal health when bullet fragments remain in the game meat. The objective of this study was to assess, for the first time, the number, size and spatial distribution of bullet fragments in game animals collectively and in three dimensions. Four roe deer (Capreolus capreolus) hunted with lead bullets were radiographed using computed tomography (CT) at each step of meat processing from animal bodies to edible parts. The animal bodies contained 43–199 fragments with a mean volume of 3.71 ± 5.49 mm^3 and a mean distance of 6.5 ± 4.6 cm perpendicular to the wound channel. About 40% of the fragments were in the lower size range of < 1.00 mm^3. Individual fragments were located up to 22.2 cm from the wound channel and were located in the edible parts (ribs, n = 2; haunch, n = 1; shoulder, n = 3) with up to 0.29 g (0.16 ± 0.15 g, mean ± SD) of estimated bullet fragment mass (BFM) deposited in the edible parts of the roe deer. Tissues in a radius of at least 16 cm around the wound channel should have been discarded in order to remove 95% of the BFM from the investigated roe deer. Additionally, around 85.1% of the initial bullet mass corresponding to 9.948 ± 1.040 g BFM were estimated to be introduced into the environment. This study highlights the challenges of ensuring sufficient removal of lead-based rifle bullet fragments in game meat processing for either human consumption purposes or for use as pet food.

1 Introduction

As the consumption of game meat as sustainable food source becomes more popular, efforts to improve the safety and quality of game meat are increasing. Besides the large field of game meat hygiene in terms of biological contaminants, chemical contaminants play an important role in game meat safety. Lead from rifle bullets used in hunting is a frequent

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contaminant in game meat. Lead can cause a variety of toxic effects in humans, including damage to the nervous system in children [1], damage to the renal, reproductive and cardiovascular systems in adults [2] and carcinogenic effects [3]. Infants, children, pregnant women and women at fertile age are particularly susceptible to lead toxicity [3, 4]. According to the European Food Safety Authority (EFSA), no safe level of lead intake can be derived [3]. Therefore, measures should be taken to minimize lead content in food, such as game meat, to minimize health risks for consumers.

Most rifle bullets used in hunting today include lead as the major component. Typical copper-jacketed lead-core bullets contain approximately 70% lead [5, 6]. Most lead bullets expand after impact on the animal and can form hundreds of fragments, as has been shown in several studies [5–9]. Hunt et al. [5] reported transfer of bullet fragments into the edible parts of the deer (*Odocoileus virginianus)*, with one third of the analyzed ground deer meat packages containing metal fragments detectable by fluoroscopy. During meat processing, the goal is to remove all unsafe and low quality tissues while discarding as little meat as possible to get a maximum yield of high quality game meat. In the carcasses, fragment clusters were found to spread up to 45 cm apart [5]. This widely spread contamination of the animal body with bullet fragments thwarts efforts to define a distance from the wound channel that can ensure a minimal risk of lead exposure from the game meat. Compared to game meat of animals hunted with alternative, non-lead-based bullets, meat from lead-hunted game shows significantly elevated lead levels [10]. Consequently, frequent consumption of meat from game hunted with lead bullets has been associated with elevated blood lead levels in humans [11, 12]. This direct correlation to the consumption of game meat is supported by in vivo experiments that revealed increasing blood lead levels in pigs (Yorkshire/Landrace and Berkshire/Duroc cross-bred pigs, and growing Danish Duroc boars) after feeding of lead-shot ground or shopped game meat [5, 13].

Furthermore, hunting with lead-based bullets results in a significant lead contamination of the environment [14]. This contamination is caused specifically by the bullet residues that exit the animals after impact, discarded game viscera when they are left or buried in the forest as is common in hunting practice [14, 15], shot animals that are never retrieved by hunters [8] and missed shots.

The use of lead-based bullets in hunting has been the subject of debate for many years, and the debate is still ongoing. Hunters are used to the ballistic behavior of lead-based bullets and therefore some question the effectiveness and proper handling of alternatives [19]. In addition, many manufacturers oppose the substitution of lead in ammunition because it is a cheap metal with good ballistic properties [20] that has been approved for many years. At the same time, environmental and toxicological researchers advocate the switch to alternatives. Authorities and governments are striving to implement feasible regulations [18, 21–23]. Recently, the use of lead gunshot was banned in and around wetlands to protect wildlife species and the environment [Regulation (EU) 2021/57]. Some regions, such as some Federal States in Germany [22], have also banned the use of lead bullets in hunting. Denmark was the first in the EU to introduce a country-wide ban on lead bullets for hunting; this regulation came into force in July 2022 and will ban lead in rifle ammunition for hunting from April 2024 [24]. An EU-wide regulation on lead in bullets is slowed by the debate.

Previous studies have analyzed whole animal bodies, carcasses and viscera for the number of fragments in different deer species [5, 6, 8, 15], a variety of wild ungulate species [7] and domestic sheep (*Ovis aries*) as surrogates for game animals [8, 16]. Fewer publications concentrated on studying fragments in packages of marketed ground game meat and steaks [5, 25]. However, only few studies addressed the characteristics of the fragments derived from rifle-bullets, such as sizes [26], spatial distribution [5–7] and fragment mass [15].

In the present study for the first time, computed tomography (CT) was used to investigate bullet fragments in game hunted with lead-based rifle bullets. The objective was to assess the food safety of game meat by determining the spatial distribution of bullet fragments in four roe deer (*Capreolus capreolus*) shot under common hunting conditions using common lead-based bullets, thereby determining the number and size of the bullet fragments collectively. It was hypothesized, that fragments remain in the edible parts of the roe deer after processing of the animal bodies. CT scans were conducted over the course of meat processing of the processed parts and the removed tissues of each processing step. The number and size of bullet fragments were investigated as this may have consequences for lead bioavailability in edible game meat. The fragment volumes were used to estimate the mass of bullet fragments in the animal bodies, the edible parts and the mass of fragments that would be expected to be introduced to the environment.

2 Materials and methods

2.1 Hunting conditions, equipment and roe deer data

In November 2017, four roe deer (roe deer 1–4, *Capreolus capreolus*) were shot during hide hunts in hunting grounds administrated by the German Federal Forestry Service in Platkow (Brandenburg, Germany). Hunting was conducted by a trained hunter [according to Regulation (EC) No. 853/2004 and German Tier-LMHV/2004] in accordance with national legal regulations (Federal Hunting Act/1976; Brandenburg Hunting Law/2003) and best hunting practice. Roe deer were shot within the scope of the regular management practices in the German forests regarding population control. Therefore, no further permission for animal experiments was required according to EU legislation.

Hunting conditions for this study were based on the most common conditions in hunting in Germany reported by Gremse and Rieger [27] in a study commissioned by the German Federal Ministry of Food and Agriculture (BMEL). Since this study was focused on lead fragments, a frequently used lead-based bullet type was chosen to represent common lead-based bullets in hunting in terms of their construction, caliber and mass. The type was a soft-point Remington "Core Lokt" projectile in caliber 0.308 Winchester with a weight of 11.7 g, marketed as a controlled expansion bullet, where a tapered copper jacket is coated onto a solid lead-core. The shooting distance was under 100 m and shots were placed at the thoracic cavity of the roe deer. The ammunition was shot from a dedicated hunting rifle, a Blaser R8 Professional Success 0.308 Winchester with a barrel length of 400 mm and an 'ATEC CMM 4' silencer. The gun was zeroed beforehand. The bullet's muzzle velocity (v_0) of 710 m/s was measured using a ProChrono Pal (Competition Electronics, Rockford, USA) shooting speed chronograph. The ProChrono Indoor Lighting System (Competition Electronics, Rockford, USA) was used to illuminate the measuring device. The hunter recorded data of individual hunting parameters and animals (Table 1).

Prior to shooting, the four roe deer did not exhibit abnormal behavior, such as limping or confused behavior, which may indicate disease or injury. After shooting, the roe deer bodies were brushed down to remove soil particles and stones from the fur. Whole, untreated animals were required for this study. No further processing of the roe deer, such as evisceration and skinning, was executed. The organs of the animals could not be examined to further classify the marketability of the game meat for human consumption. Each animal received an identification tag and was deep-frozen at – 20 ℃ until further analysis in June 2018. Before the CT investigation, the animal bodies were thawed at room temperature 2 days prior to analysis and subsequently stored at 4 ℃ for further examination.

2.2 Meat processing and preparation for computed tomography imaging

CT scanning of the roe deer (roe deer 1–4) was conducted at the radiological department of the Leibniz Institute for Zoo and Wildlife Research in Berlin, Germany. All steps of the meat processing procedure were executed by the same trained hunter who conducted the hunt according to a developed Standard Operating Procedure (SOP) (Online Resource 1: Standard operating procedure (SOP) for meat processing and CT-Imaging). In brief, this SOP details the meat processing procedure (Fig. 1) and the CT scanning of the animal bodies at each individual processing step. CT scans were obtained of the processed part (from animal body to edible parts) and the removed tissues at each step. CT data are assigned to the previous processing step denoted by the letters A–E. The main path from animal body to edible parts (processed part) is indicated by a 0 after the decimal point (x.0). Removed tissues at each step are indicated by x.1 and x.2, respectively. (A) The animal body is the non-eviscerated roe deer without any further

Table 1 Recorded hunting parameters and data of the four roe deer

Parameter	Roe deer 1	Roe deer 2	Roe deer 3	Roe deer 4
Shooting distance [m][a]	78	42	48	47
Sex	Female	Male	Male	Female
Age group[b]	2	0	2	0
Weight (eviscerated) [kg]	16	12	17	11
Shot entry[c]	Left	Right	Left	Right

[a]The shooting distance was measured using Bushnell Fusion ARC 1600 binoculars (Bushnell, Kansas, USA)

[b]The age groups were classified as age group 0 = < 1 year, age group 1 = 1–2 years, age group 2 = > 2 years

[c]In the dorsal view, the bullet impact site was classified as "left" or "right" side of the body

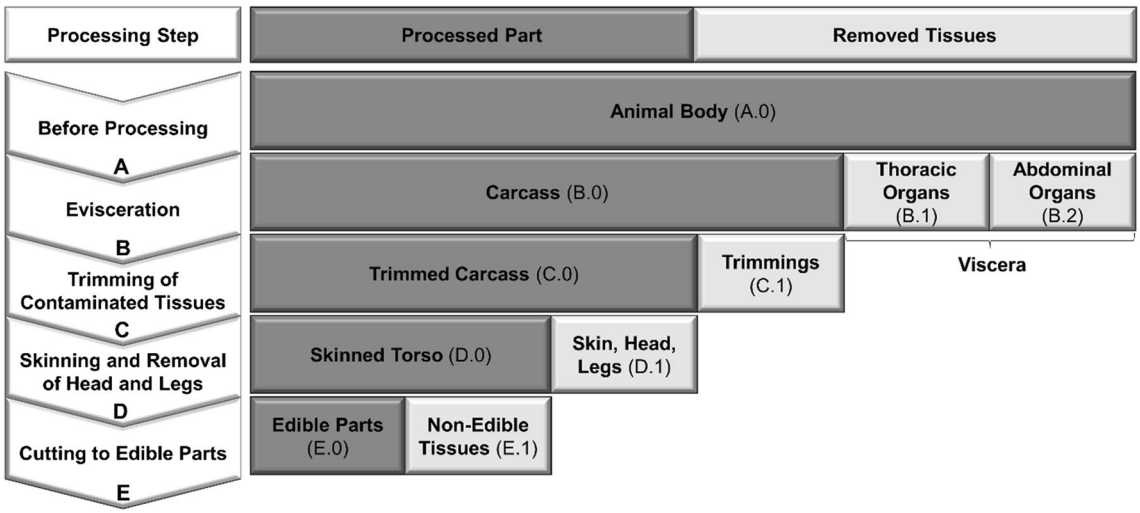

Fig. 1 Overview of the meat processing procedure

meat processing. (B) The carcass was obtained by removing the viscera, including thoracic (lungs, heart, esophagus, trachea, tongue, and diaphragm) and abdominal organs (inner reproductive organs, kidneys, bladder, ureter, urethra, gastrointestinal tract, spleen, and liver). Often, hunters remove all viscera at once. For separate examination, thoracic and abdominal organs were removed successively in this study. (C) The trimmed carcass was obtained by removing all contaminated tissues (trimmings), such as impact-wounded tissue and tissues with visible bullet fragments. (D) The skinned torso resulted from skinning and the removal of head and legs. (E) In the last step, the edible parts (including bone) are obtained: neck, shoulder, ribs, saddle (including rack, loin, and fillet), hind leg (including haunch and shank). Parts that are removed during this step are called non-edible tissues.

2.3 CT imaging and evaluation

The processed parts and tissues of roe deer were positioned as described in the SOP (Online Resource 1) and CT scans were obtained at an energy of 120 kVp and 500 ms exposure time using the dual energy CT device Aquilion ONE (Toshiba Medical Systems GmbH, Neuss, Germany). Datasets were acquired in DICOM format (Digital Imaging in Medicine).

2.3.1 Fragment number, volumes and XYZ-positions from CT datasets

The CT image sequences were saved in DICOM format and processed using the open source software Horos [28]. Reconstruction voxel size was $0.212 \times 0.212 \times 0.25$ or $0.212 \times 0.212 \times 0.5$ mm. Using 3D Volume Viewer, window level and window width were adjusted to remove most soft tissues and bones while retaining the metal fragments. Each image sequence was exported as TIFF files. Remaining bone structures and structures resulting from bright radiation of fragments were removed by setting a threshold and editing using the open source software FIJI [29]. The 3D Object Analyzer tool was applied to calculate fragment properties such as numbers, volumes, and XYZ-coordinates. Volumes were not adjusted for overestimation by blooming artifacts [30]. Handling of roe deer and tissues for CT scans and meat processing steps may have caused some dislocation of fragments from their original location, separation of clustered fragments or clustering of fragments, leading to some variability in fragment numbers and sizes.

2.3.2 Size distribution of fragments in roe deer bodies

Volumes of fragments were collected for each dataset and are given in mm^3. Violin plots for the volumes in the animal bodies were generated using the package "ggplot2" in RStudio [31]. Comparison of fragment sizes between the animals was conducted using Kruskal–Wallis test with Bonferroni posthoc analysis using the package "psych" in RStudio.

2.3.3 Mass balance of the bullet

The volumes of the fragments were used to calculate the bullet fragment mass (BFM) remaining in different parts of the roe deer and the environment. BFM can be calculated from the sum of fragment volumes in the respective part and the density of the fragments according to

$$\text{BFM}\,[\text{g}] = \text{sum of fragment volumes}\,[\text{cm}^3] \times \text{density of fragments}\left[\frac{\text{g}}{\text{cm}^3}\right] \tag{1}$$

Bullet fragments can originate from either lead-core, copper jacket or mixtures. The density of the fragments could be estimated from the proportional composition and the density of the bullet elements, limited to copper and lead as the major components. Using a density of lead of 11.34 g/cm^3 and of copper of 8.96 g/cm^3, the density of fragments can be calculated as

$$\text{density of fragments}\left[\frac{\text{g}}{\text{cm}^3}\right] = 8.96\,\frac{\text{g}}{\text{cm}^3} + 2.38\,\frac{\text{g}}{\text{cm}^3} \times \text{percentage of lead}\,[\%]. \tag{2}$$

As in other radiography-based studies [5, 6, 15], the actual fragment composition could not be determined using CT. As a worst-case scenario in terms of food safety, the composition of the fragments was assumed to be entirely lead for the mass calculation. Other approaches, such as assuming that all mixed particles have the same composition as the original bullet, e.g. 70% lead and 30% copper, would result in a slightly lower BFM (6%) but lower overall contamination with lead. BFM was calculated for the animal bodies (A.0) and the edible parts (E.0).

The initial bullet mass is assumed to be divided into the BFM in the roe deer body and the mass of the exiting bullet residue (mass balance); the latter not being determined but calculated from this mass balance. The environmental burden with bullet metals is assumed to arise from the sum of the exiting bullet mass and BFM in the viscera. Therefore, the environmental burden can be estimated as

$$\text{BFM environment}\left[\text{g}\right] = \text{initial bullet mass}\,[\text{g}] - \text{BFM carcass}\,[\text{g}]. \tag{3}$$

The bullets were supplied as pre-assembled ammunition (i.e. the bullet was fixed in a cartridge, which contains the powder and a primer). The arithmetic mean of 11.69 ± 0.06 g of 17 bullets from one package of disassembled ammunition was used as initial bullet mass. BFM of the carcass was calculated from dataset B.0. BFM in the viscera was calculated from difference of masses in the animal body (A.0) and the carcass (B.0). All BFMs were also calculated in proportion to initial bullet mass.

2.3.4 Spatial distribution—distances of fragments from the wound channel

Perpendicular distances of fragments from the wound channel were calculated using the XYZ-coordinates of the center of mass of the fragments and a reconstruction of the wound channel. The entry and exit points of the bullet were reconstructed from the corresponding lesions, which were clearly visible as destroyed and missing tissues in the thoracic region in dataset A.0. Thereby, entry and exit points were set at the outer edge of the skin (axial view) and centered from a plan view on the wound (sagittal view). A vector across these two points was calculated and set as a reference for the fragment distance to the wound channel. Distances were calculated perpendicularly to this vector for each fragment. In addition, the distance between the center of mass of the fragments and the bullet entry were calculated from the vector directly connecting them. The software Imalytics Preclinical [32], Version 3.0, was used for 3D reconstruction by segmentation of skeleton, fragments, bullet entry, bullet exit and wound channel of roe deer body (A.0). 3D plots of the center of mass of the fragments, bullet entry, bullet exit and the wound channel were generated using the 3D plot feature in Qti-Plot 1.1.2 software (release date 24 November 2022, Copyright 2004–2022 IonVasilief). Correlations between perpendicular distance from the wound channel and size of fragments, and the distance from the bullet entry and the size of fragments were analyzed using Spearman Rank Correlation in default RStudio. Distribution of fragments of the roe deer was compared by Kruskal–Wallis test with Bonferroni post hoc analysis using the package "psych" in RStudio.

3 Results

3.1 Effect of meat processing on fragment number in the different parts

In four roe deer bodies, bullet fragment numbers of 43–199 were observed (Table 2). After evisceration, the carcasses still contained 11–77 bullet fragments.

Fragment numbers decreased with each step of meat processing to the skinned torso (D.0). Most fragments (18–110) were removed at the evisceration of the roe deer; the first step of meat processing. In the viscera (B.1 and B.2), the fragments were predominantly located in the thoracic organs rather than in the abdominal organs. In the last step of meat processing, where all non-edible tissues (E.1) are removed to obtain the final edible parts (E.0), no further fragments were removed. For 3 out of the 4 roe deer, the edible parts (E.0) still contained fragments that were not removed during meat processing. For roe deer 1, two fragments were detected in the ribs and one in the shoulder. The edible parts of roe deer 3 contained a fragment in the shoulder. The two remaining fragments in edible parts of roe deer 4 were located in the shoulder and the haunch.

3.2 Size distribution of fragments in roe deer bodies

The distribution of fragment volumes is plotted as Fig. 2. In roe deer 1, 2, 3 and 4 (A.0), mean volumes of the bullet fragments of 2.79 ± 3.58 mm^3 (range 0.01–23.69 mm^3), 3.47 ± 5.80 mm^3 (range 0.01–41.62 mm^3), 7.34 ± 10.23 mm^3 (range 0.02–50.33 mm^3) and 4.04 ± 5.04 mm^3 (range 0.02–31.18 mm^3) were detected, respectively; 3.71 ± 5.49 mm^3 for all roe deer. Median volumes were notably lower at 1.85 mm^3, 0.89 mm^3, 5.09 mm^3 and 1.85 mm^3 for roe deer 1–4, respectively. Fragment volumes were similar in 3 of the 4 roe deer; volumes differed significantly between roe deer 1 and 3, and between roe deer 2 and 3 (Kruskal–Wallis test with Bonferroni post hoc analysis, $p = 0.001$ and $p = 0.007$). Around 40% of the fragments in all animal bodies (A.0) were in the lower volume range of < 1.00 mm^3. Few individual fragments were of notably large size > 25 mm^3. In the edible parts (E.0) of roe deer 1, fragments with volumes of 1.26 mm^3 and 23.55 mm^3 were located in the ribs; one fragment with a volume of 0.41 mm^3 was located in the shoulder. The shoulder of roe deer 3 contained a fragment with a volume of 5.31 mm^3. Shoulder and haunch of roe deer 4 contained fragments sized 5.63 and 19.57 mm^3, respectively.

3.3 Mass balance of the bullet

The estimated retained BFM was calculated assuming that the fragments were entirely lead and ranged from 3.58 to 6.97 g (31 to 60% of the initial bullet mass of 11.69 ± 0.06 g) in the roe deer bodies (A.0, Table 3). On average, 5.26 ± 1.62 g BFM (45% of the initial bullet mass) was retained in the four roe deer bodies. The fragmentation of the bullet and the meat processing resulted in a mean amount of 0.16 ± 0.15 g BFM (1% of the initial bullet mass) in the edible parts (E.0). For the edible parts of the individual roe deer (E.0), this resulted in a lead mass of 0.28 g in the ribs and 0.01 g in the shoulder

Table 2 Fragment numbers of the processed parts and removed tissues for roe deer 1–4 at each meat processing step

Processing step	Data set		Fragment numbers			
	Processed part	Removed tissues	Roe deer 1	Roe deer 2	Roe deer 3	Roe deer 4
A	Animal body (A.0)		199	107	43	152
B	Carcass (B.0)		77	22	20	11
		Thoracic organs (B.1)	74	66	12	69
		Abdominal organs (B.2)	5	9	6	41
C	Trimmed carcass (C.0)		8	1	14	2
		Trimmings (C.1)	101	27	7	14
D	Skinned torso (D.0)		3	0	1	2
		Skin, head and legs (D.1)	4	0	12	0
E	Edible parts (E.0)		3	0	1	2
		Non-edible tissues (E.1)	0	0	0	0

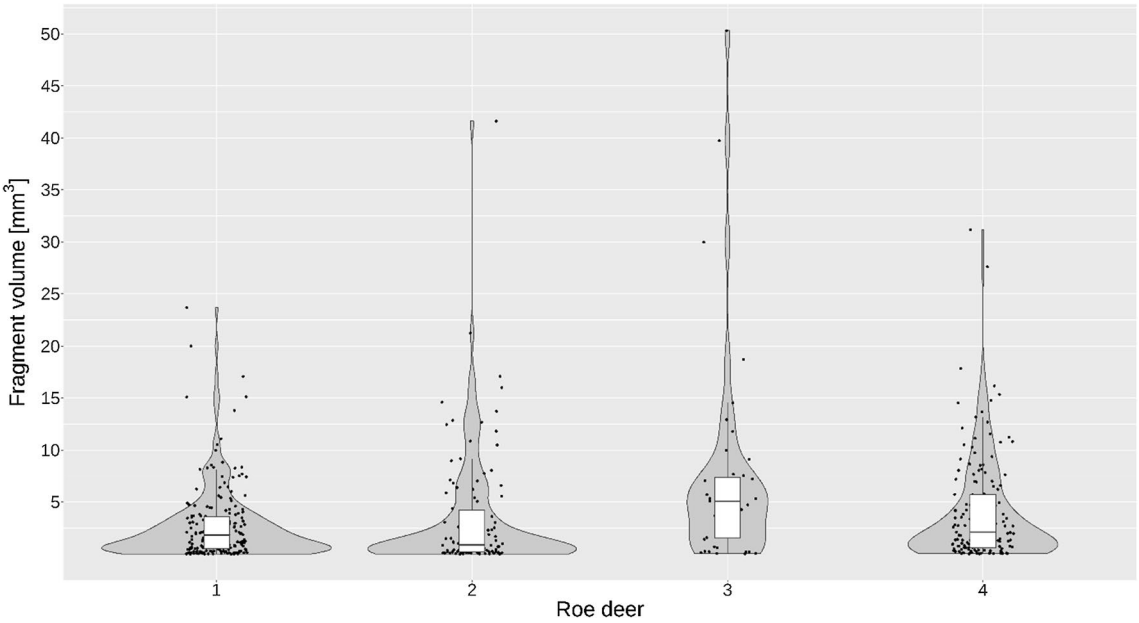

Fig. 2 Violin plots with embedded box-plots of fragment volumes in the bodies of the four roe deer (A.0) with volumes of the individual fragments indicated as black dots

Table 3 Results of estimated retained bullet fragment mass (BFM) in the roe deer bodies (A.0), the edible parts (E.0), the carcasses (B.0), the mass of exiting bullet residue (initial bullet mass–BFM animal body), fragment masses in the viscera (A.0–B.0) and the fragment mass expected to be introduced to the environment (BFM environment)

BFM [g (%)]	Roe deer 1	Roe deer 2	Roe deer 3	Roe deer 4
Animal body	6.30 (54)	4.21 (36)	3.58 (31)	6.97 (60)
Edible parts	0.29 (2)	0.00 (0)	0.06 (1)	0.29 (2)
Carcass	2.90 (25)	2.34 (20)	0.96 (8)	0.78 (7)
Exiting bullet residue	5.39 (46)	7.48 (64)	8.11 (69)	6.43 (40)
Viscera	3.39 (29)	1.87 (16)	2.63 (23)	6.18 (53)
Environment	8.79 (75)	9.35 (80)	10.74 (92)	10.91 (93)

Relation of each mass to initial bullet mass of 11.69 ± 0.06 g is given in brackets

of roe deer 1; 0.06 g in the shoulder of roe deer 3; and 0.06 g in the shoulder and 0.22 g in the haunch of roe deer 4. In the viscera, 3.52 ± 1.88 g BFM (30% of the initial bullet mass) was found. The environmental burden with bullet metals for the individual hunting situations was assumed to be the sum of exiting bullet mass plus the mass of fragments that is expected to remain in the field due to practice of burying viscera in the forest soil. Therefore, 85% of the initial bullet mass corresponding to 9.95 ± 1.04 g is assumed to be introduced to the forest.

3.4 Spatial distribution of fragments in the animal bodies

The lengths of the wound channel were 18.1, 20.3, 20.9 and 15.2 cm for roe deer 1–4, respectively. Fragments were widely distributed across the roe deer bodies (A.0) (Figs. 3, 4 and Online Resource 2: Rotational view of bullet fragments in the body of roe deer 1). Distribution of fragments around the wound channel differed significantly among all roe deer (Kruskal–Wallis test with Bonferroni post hoc analysis, $p = 4.43 \times 10^{-4}$). Fragments in roe deer 1 and 2 were evenly distributed along the wound channel and mostly concentrated close to the wound channel. In roe deer 3, fragments were predominantly located near the bullet exit. In roe deer 4, fragments were not evenly distributed around the wound channel but more concentrated on one side in direction of the hind legs, resulting in a higher mean distance. Mean distance of fragments from the wound channel for roe deer 1–4 were 4.7 ± 3.6 cm, 3.4 ± 2.6 cm, 7.9 ± 4.1 cm and

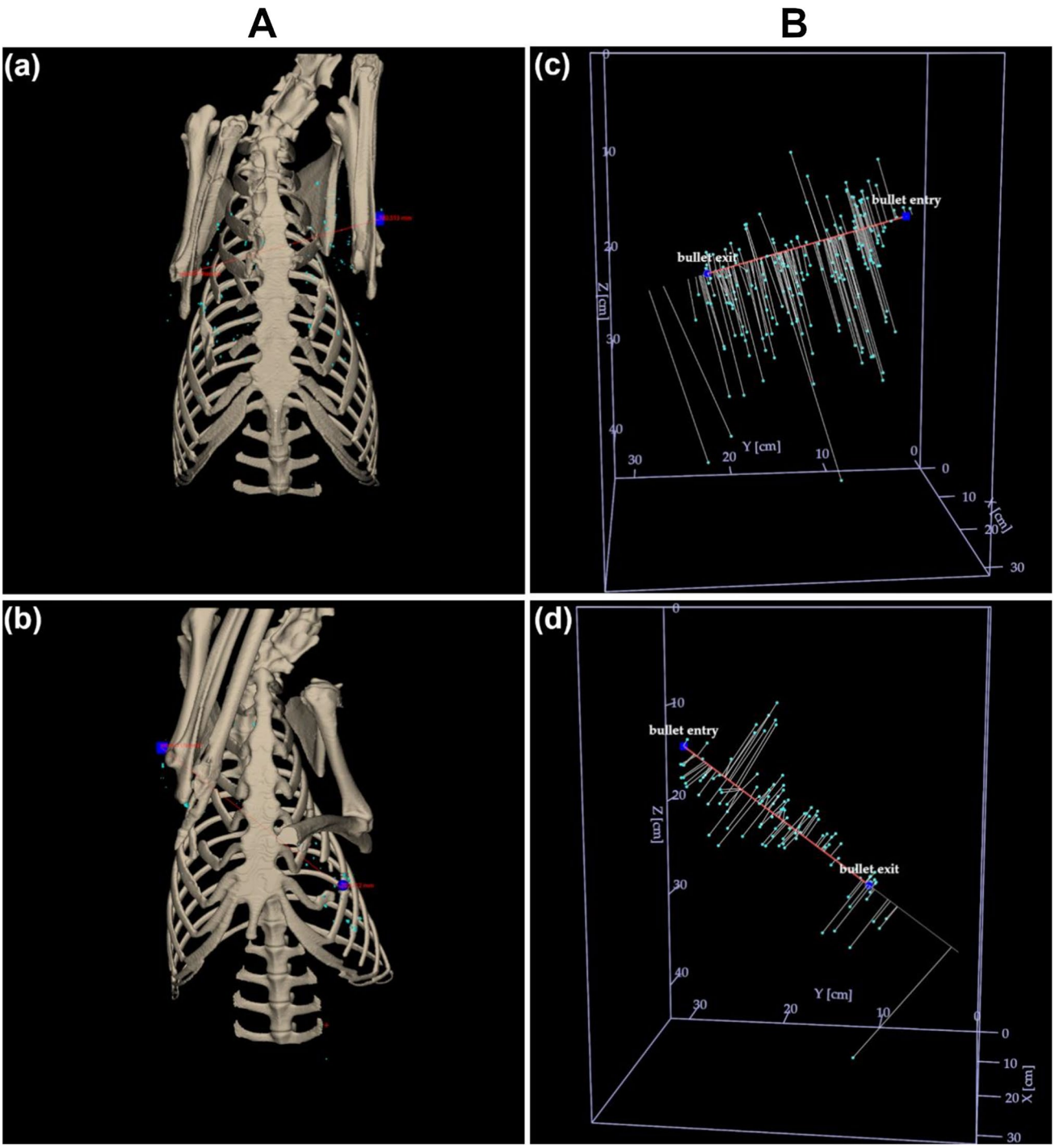

Fig. 3 3D-reconstruction of bullet fragments from CT datasets: column **A** reconstruction of skeleton (thorax, spine and front legs), bullet fragments, bullet entry, bullet exit and wound channel; column **B** reconstruction of bullet entry, bullet exit, wound channel, center of masses of fragments and perpendicular vectors from wound channel to center of mass of fragments of **a**, **c** roe deer 1 and **b**, **d** roe deer 2. Bullet fragments and center of mass are displayed in turquoise; bullet entry is indicated as dark blue box; bullet exit as dark blue sphere, wound channel as a red line and perpendicular vectors as white lines; images were positioned to a front view on the sternum of the roe deer

10.6 ± 4.0 cm, respectively. The mean distance of fragments for all animal bodies was 6.5 ± 4.6 cm from the wound channel. Single individual fragments were detected very distant and up to 22.2 cm away from the wound channel (roe deer 1). Out of the four roe deer bodies, the least scattering of fragments around the wound channel was found in roe deer 3, where the most distant fragment was found at 17.3 cm. From the CT datasets, bone hits are likely in roe deer 2 and 3.

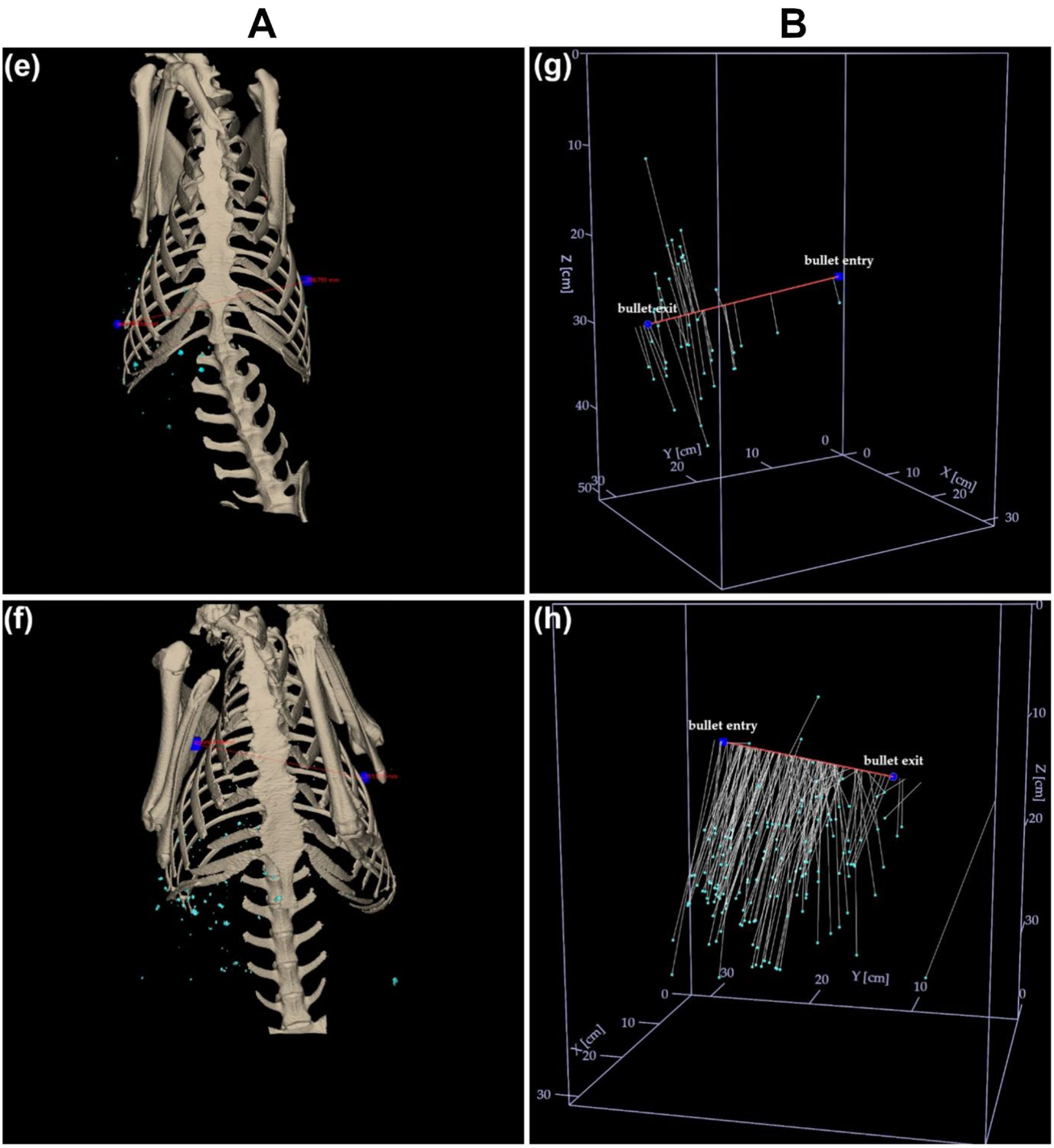

Fig. 4 3D-reconstruction of bullet fragments from CT datasets: column **A** reconstruction of skeleton (thorax, spine and front legs), bullet fragments, bullet entry, bullet exit and wound channel; column **B** reconstruction of bullet entry, bullet exit, wound channel, center of masses of fragments and perpendicular vectors from wound channel to center of mass of fragments of **e**, **g** roe deer 3 and **f**, **h** roe deer 4. Bullet fragments and center of mass are displayed in turquoise; bullet entry is indicated as dark blue box; bullet exit as dark blue sphere, wound channel as a red line and perpendicular vectors as white lines; images were positioned to a front view on the sternum of the roe deer

Size of detected fragments was correlated to the distance to the bullet entry only for roe deer 2 (Spearman Rank Correlation: roe deer 1 $p = 0.070$, roe deer 2 $r_{sp} = 0.292$ and $p = 0.002$, roe deer 3 $p = 0.151$, roe deer 4 $p = 0.429$) but not correlated with the perpendicular distance from the wound channel for any roe deer (Spearman Rank Correlations for roe deer 1–4 of $p = 0.539$, 0.806, 0.754 and 0.262, respectively). The cumulative mass of bullet fragments that cumulates

along the perpendicular distance of the wound channel and in distance to the bullet entry is displayed in Fig. 5 for the four roe deer. Maximum perpendicular distance of fragments from the wound channel reached up to 22.2 cm as discussed above. For roe deer 1, most of the fragment mass is covered in a 5 cm radius around the wound channel. For roe deer 2 and 4, the mass is distributed more evenly with increasing perpendicular distance to the wound channel. In roe deer 4 fragment mass is barely located within a 5 cm radius and then gradually increases with distance to the wound channel. A radius of 11.5, 9.0, 13.8 and 17.2 cm around the wound channel covered 95% of the mass of the bullet fragments in the bodies for roe deer 1–4, respectively; 15.9 cm for all roe deer. For roe deer 1, 2 and 4 fragment mass cumulated evenly

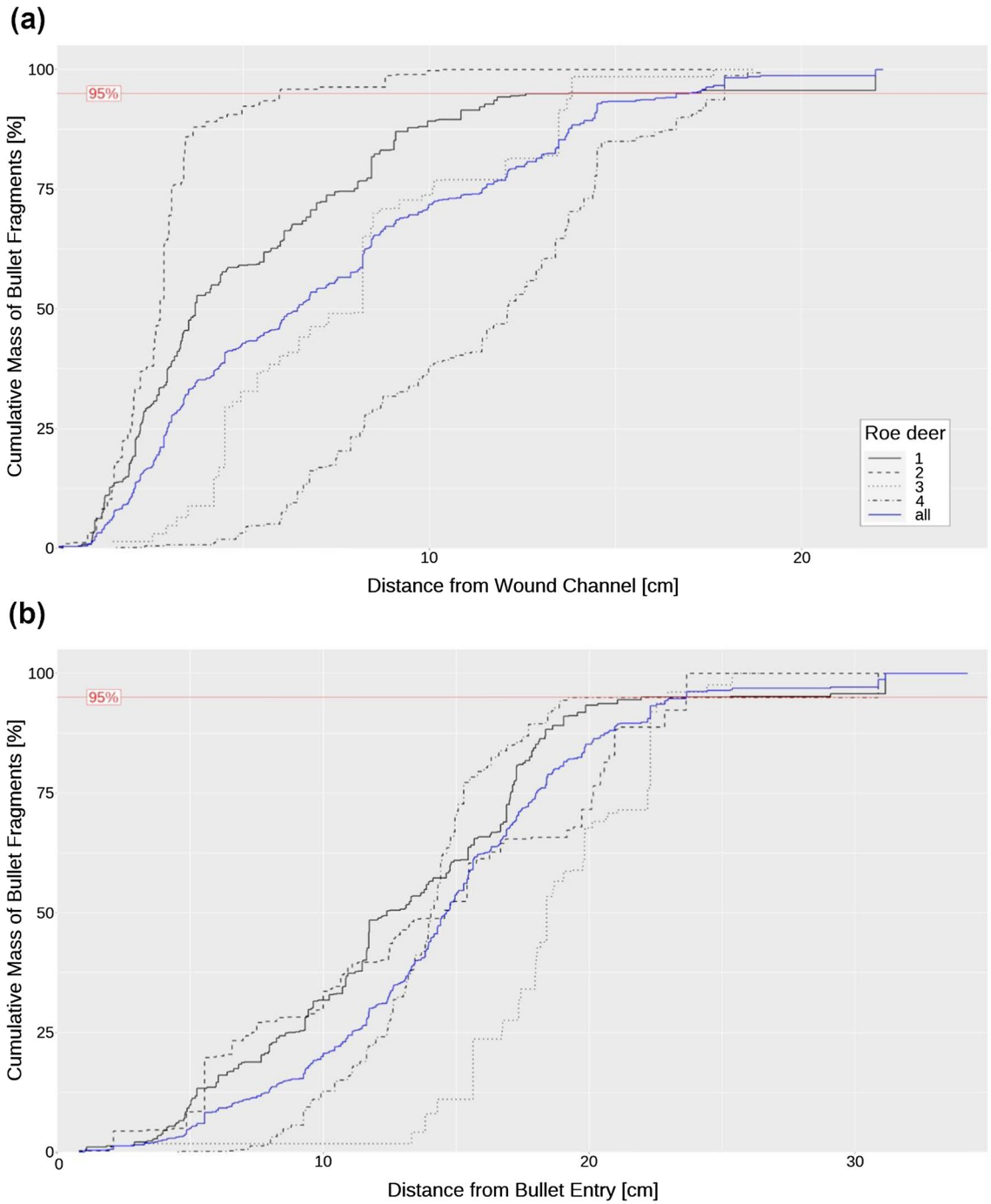

Fig. 5 Cumulative bullet fragment mass (BFM) in relation to the **a** perpendicular distance (radius) from the wound channel and **b** in distance to the bullet entry for the four roe deer bodies (A.0)

with distance to the bullet entry up to around 20 cm. A small amount of the fragment mass is spread up to 34.2 cm from the bullet entry.

4 Discussion

Previously, CT has been used to detect rifle bullet fragments in ground game meat packages [25] and ballistic soap [19], as well as gunshot fragments in game bird carcasses [30]. In this study, CT was used for the first time to collect 3-dimensional information on the spatial distribution and volumes of bullet fragments in whole animal bodies to assess food safety of game meat. Additionally, the number of rifle bullet fragments in animal bodies and the successive meat processing steps was recorded (Fig. 1). The volumes of bullet fragments were used to estimate their mass, assuming that the fragments are composed entirely of lead as a worst-case scenario for food safety.

Under standard hunting conditions, the controlled expansion rifle bullet Remington Core Lokt soft-point (0.308 Winchester, 11.7 g) fragmented into 43–199 pieces in the four roe deer bodies. The conditions of this study were similar to those of Grund et al. [10], using the same bullet of the same caliber, but using sheep as a game surrogate. Comparable fragment numbers were found in carcasses of 28–138 in their study and 11–77 in the present study. Table 4 shows the number of fragments reported in different studies using similar types of bullets.

In all studies, the detected number ranged from a few to hundreds of fragments. These fragment numbers demonstrate that fragments can always be found in animals shot with lead-containing rifle bullets and that the amount of fragmentation varies greatly dependent of several factors. This is also the case under standardized shooting conditions, such as the experiments of Grund et al. [8] with sheep in a contraption. However, numbers in more dynamic real hunting situations are even more variable. Explanations for this high variance in fragmentation have been discussed by Cruz-Martinez et al. [16] and Dobrowolska and Melosik [34]. In brief, bullet behavior and therefore fragment number varies greatly depending on several influencing factors. These include the type of bullet and factors of individual shooting situation. The type of bullet includes the projectile, i.e. rifle bullets, shotgun or muzzleloader projectiles, the last two being rarely or not used in hunting in Germany; construction and composition of the bullet used [7, 8, 15, 18]. The individual shooting situation includes the distance and angle of target and hunter, the bullet velocity and the properties of the impacted tissue, as well as, age-dependent characteristics of the shot animal [8, 16, 19, 34, 35]. Bone hits are not necessary to produce a large number of fragments [7, 33] as can be seen from the data of the present study. The two roe deer, where bone hits were unlikely, displayed the highest fragment numbers in the animal body. Bullet constructions are often categorized as dimensionally stable, deforming, partially fragmenting and fragmenting [7, 19] with increasing loss of mass after impact on the target [10]. Others classify controlled expanding and rapidly expanding bullets, and describe that controlled expanding bullets are designed to resist fragmentation while rapidly expanding bullets produce fragments [8, 16]. The bullet in caliber 0.308 Winchester used in this study is advertised as a controlled expansion bullet with weight retention of the bullet residue of 50% [8], which coincides with the order of magnitude of our findings. About 45% (5.26 ± 1.62 g) of the initial bullet mass was estimated to be retained in the animal bodies. Therefore, about 55% of the weight would be retained in the exiting bullet residue. For the same bullet, Cruz-Martinez et al. [16] found a weight retention of 48.4% when shooting sheep as surrogates for game animals. When using a smaller caliber 0.270 (8.39 g) Norma bullet, Knott et al. [15] estimated about 17% (1.2 g) of bullet mass to remain in the carcass. In addition, some studies found that some bullets designed to resist fragmentation produced high numbers of bullet fragments in carcasses [8, 16]. Gremse and Rieger [27] described that there is a velocity threshold at which bullet behavior changes between deforming and fragmenting. Therefore, fragmentation is more likely when the animal is hit with higher velocity, e.g. when the shooting distance is short. Furthermore, no legal definitions of construction types have been established, especially in terms of fragmentation. An approach has been proposed by Lahrssen-Wiederholt et al. [36] to define bullet fragmentation classes in standardized experiments. According to their classification system, the bullet of this study is in fragmentation class III with > 40–60% of delivery of bullet material into the game meat as partial disintegrator with defined lower residual body.

Previously, the spatial distribution of bullet fragments was measured in radiographs as distances between fragments (scattering) [5], distances from fragments to the center of the wound channel [7] and dispersion of fragments around the wound channel to a reference point [6] (Table 5). From CT data, the actual distance of the fragment to the wound channel could be determined. The wound channel was assumed to be a straight line (vector) from bullet entry to bullet exit [7]. The distances of fragments to the wound channel were determined as the perpendicular distance between the fragment and the wound channel vector. This does not necessarily reflect the actual trajectory of the fragments, but could be used as a tangible parameter in food safety. Additionally, distances of fragments to the bullet entry have been calculated.

Table 4 Numbers of bullet fragments with reported information on the detection method, bullet parameters and investigated animals in original research articles compared to the results of this study

Reference	Detection method	Size detection range	Bullet type	Bullet brand and name	Bullet caliber	Animal species	n	Processing status	Fragment number
Trinogga et al. [7]	Radiography	n.a.	Simple Semi-jacketed lead-core bullets	Several	Most frequently 0.30–06 springfield, 9.3 × 62, 8 × 57 IS, 0.308 winchester	Various ungulates[a]	297	Animal bodies	2–410
Krone et al. [33]	Radiography	< 1–10 mm	Semi-jacketed lead-core bullets	n.a.	n.a.	Various ungulates[b]	315 (77[c])	Animal bodies	25–500
Hunt et al. [6]	Radiography	n.a.	Copper-jacketed lead-core bullets	Several	7 Different calibers	Deer[d]	5	Animal bodies	416–783
							9	Carcasses	38–544
							20	Viscera	0–521
Grund et al. [8]	Radiography	n.a.	Rapid expansion lead-core bullets	Remington core lokt	.308 Winchester	Sheep[e]	10	Carcasses[f]	28–138
Hunt et al. [5]	Radiography	n.a.	Copper-jacketed lead-core bullets	n.a	7-mm Remington magnum	Deer[g]	30	Carcasses[h]	15–409
Knott et al. [15]	Radiography	n.a.	Copper-jacketed lead-core bullets	Norma	0.270	Red deer, roe deer	10, 2	Carcasses[i]	166–621
							12	Viscera[i]	82–321
Cruz-Martinez et al. [16]	Radiography	> 1 mm	Rapid expansion lead-core bullets	Remington core lokt	0.308 Winchester	Sheep[g]	10	Viscera	280 ± 52.1
This Study	Computed tomography	> 0.01 mm^3	Rapid expansion copper-jacketed lead-core bullets	Remington core lokt	0.308 Winchester	Roe Deer	4	Animal bodies	43–199
							4	Carcasses	11–77
							4	Viscera	18–110

Parameter values are given as either range or mean ± SD

n.a. = data not available

[a] 5 chamois (*Rupicabra rupicabra*), 87 fallow deer (*Cervus dama*), 23 red deer (*Cervus elaphus*), 103 roe deer (*Capreolus capreolus*), 79 wild boars (*Sus scrofa*)

[b] 2 Chamois (*Rupicapra rupicapra*), 103 Fallow Deer (*Cervus dama*), 3 Red Deer (*Cervus elaphus*), 110 Roe Deer (*Capreolus capreolus*), 97 Wild Boar (*Sus scrofa*)

[c] Fragment numbers were not displayed for all 315 animals, but for 77 animals shot with lead-core bullets

[d] *Odocoileus virginianus* and *O. hemionus*

[e] Sheep as surrogates for game animals

[f] The skin was also removed

[g] *Odocoileus virginianus*

[h] Skin and head were also removed

[i] Only abdominal organs were removed and radiographed separately

Table 5 Spatial distribution of bullet fragments with reported information on the detection method, bullet parameters and investigated animals in original research articles compared to the results of this study

	Detection method	Size detection range	Bullet type	Bullet brand and name	Bullet caliber	Animal species	n	Processing status	Distance [cm]
Trinogga et al. [7]	Radiography (distance to center of wound channel)	n.a.	Simple Semi-jacketed lead-core bullets	Several	Most frequently 0.30–06 springfield, 9.3 × 62, 8 × 57 IS, 0.308 Winchester	Various ungulates[a]	297	Animal bodies	11.7 ± 5.2 max. 28
Hunt et al. [6]	Radiography (dispersion of fragments around a reference point in the wound channel)	n.a.	Copper-jacketed lead-core bullets	Several	7 Different calibers	Deer[b]	5	Carcasses[c]	7 ± 3 max. 15
Hunt et al. [5]	Radiography (maximum distance between fragments)	n.a.	Copper-jacketed lead-core bullets	n.a.	7-mm Remington magnum	Deer[d]	30	Carcasses[e]	24 ± 9 max. 45
This study	Computed tomography (distance perpendicular to the wound channel)	> 0.01 mm^3	Rapid expansion copper-jacketed lead-core bullets	Remington core lokt	0.308 Winchester	Roe deer	4	Animal bodies	6.5 ± 4.6 max. 22.2

Parameter values are given as mean ± SD and the maximum distance in that respective measuring method. The defined distance in each study is described in brackets in the detection method column

n.a. = data not available

[a]5 chamois (*Rupicabra rupicabra*), 87 fallow deer (*Cervus dama*), 23 red deer (*Cervus elaphus*), 103 roe deer (*Capreolus capreolus*), 79 wild boars (*Sus scrofa*)

[b]Fragment numbers were not displayed for all 315 animals, but for 77 animals shot with lead-core bullets

[c]The skin was also removed.

[d]*Odocoileus virginianus* and *O. hemionus*

[e]Skin and head were also removed

As in other studies (Table 5), scattering of small fragments could be observed along the wound channel; single individual fragments showed large displacements up to 22.2 cm perpendicular to the wound channel in the animal bodies and fragments could be found in all types of tissues [7]. The measured distribution of fragments was similar between the studies considering the different methods of distance calculation. In this study, as expected fragments were found in the typically marketed tissues of the haunch (n = 1), shoulder (n = 3) and ribs (n = 2). Among the four roe deer, a minimal scattering of fragments around the wound channel was found in roe deer 3, where a radius of 17.3 cm covered all fragments. The longest distance between a fragment and the bullet entry was 34.2 cm. Hunters are advised to generously remove tissue around the bullet entry and exit [4, 37]. Even if hunters are particularly conscientious and careful, this may might not guarantee sufficient removal of lead contamination in game meat [4] due to the size of the fragments, as discussed below. In this study, we determined that a radius of at least 16 cm around the wound channel should have been discarded to remove 95% of fragment mass from the investigated roe deer. However, it is unlikely that main edible parts of the roe deer that can also contain bullet fragments, such as haunch, shoulder and saddle will be discarded. Therefore, lead contamination can never be precluded when consuming game meat shot with lead-based bullets.

Volumes (V) of rifle bullet fragments in roe deer bodies were detected in a range of 0.01–50.33 mm^3. The majority of the particles were in a lower volume range; 40% were < 1.00 mm^3. This corresponds to a diameter of < 1.2 mm by assuming spherical shape with diameter = $(6\ V/\pi)^{1/3}$. Green et al. [30] showed that sizes of metal particles are overestimated when using CT because of blooming artifacts. Under their conditions, the diameter of lead spheres was overestimated by 27.6%. The extent of overestimation of volumes was not determined in this study, but particles are proposed to be smaller than the calculated volume. In previous studies using medical radiography, aided and unaided visible fragments were detected but sizes were not determined. Detected particles were approximately in a range of 0.5 mm to 10 mm [5, 33]. Small fragments have been reported to be < 2 or < 1 mm in diameter and to appear perfused or clustered as "dust", "clouds" or "snowstorm" [6, 7, 16]. Knott et al. [15] categorized sizes of fragments in weight classes by comparison to sizes of reference fragments with known mass in radiographs of deer shot with a caliber 0.270 (8.39 g) Norma bullet. The mass of fragments with smaller estimated weight than 0.01 g accounted for 34% of the total found in red deer and roe deer carcasses with thoracic organs. In our study, as well as, in Knott et al. [15], the large amount of smaller fragments suggests that even smaller particles might be present in the animal bodies, which could not be detected using radiography. Kollander et al. [26] revealed the presence of millions of nanometer-sized fragments in game meat near the wound channel using single particle ICP-MS. Leontowich et al. [9] confirmed that bullets fragment into low micrometer-sized fragments (< 10 µm diameter) when shooting on ballistic gelatin blocks using synchrotron radiation. It might be proposed that larger fragments travel larger distances [19] due to their higher energy density. This hypothesis could not be tested from the CT data because the actual trajectory of the fragments is uncertain. However, fragments of all sizes were scattered over all distances from the wound channel and the sizes of detected fragments were not correlated with their perpendicular distance from the wound channel or to the distance to the bullet entry. Thus, even smaller fragments below the detection limit of CT might be spread all over the body.

Using a standard meat processing routine in this study, fragment numbers decreased at each step. The viscera contained most of the fragments; numbers were in the range of the findings of other studies using similar bullet types (Table 4). In this study, 30% (3.52 ± 1.88 g) of initial bullet mass (11.69 g) was located in the viscera. Using a smaller caliber 0.270 (8.39 g) Norma bullet, Knott et al. [15] found that about 0.21 g or 2.5% of initial bullet mass (8.39 g), were located in the viscera [15]. As common practice, viscera are left in the forest or buried in the forest soil [14, 15]. In addition to the bullet mass in the viscera, the environmental burden includes the mass of the exiting bullet residue. In total, 85% of the initial bullet mass, corresponding to 9.95 ± 1.04 g of lead in the worst-case assumption for fragment composition, could be assumed to be introduced to the forest from the roe deer in this study. Here, bullet fragments are available for wildlife and considered a major source of lead contamination in scavengers, many other wild species and the environment [6, 7, 15–17, 38]. This has been broadly discussed by Pokras and Kneeland [38]. Additionally, viscera and trimmed parts of carcasses are often used as or in dog feed, causing acute and chronic lead poisoning [39, 40]. The growing awareness of this type of lead exposure in animals is reflected in the increasing number of notifications in the European Rapid Alert System for Food and Feed (RASFF) on elevated lead levels in animal feed (e.g. https://webgate.ec.europa.eu/rasff-window/screen/notification/599200 and https://webgate.ec.europa.eu/rasff-window/screen/notification/598903).

After all meat processing steps, the final edible parts of 3 out of the 4 roe deer still contained up to 3 detectable fragments. In a comparable experiment, Hunt et al. [5] tracked the path of bullet fragments in deer carcasses into the resulting ground meat packages. Visible fragments were detected in ground meat packages of 24 out of 30 deer. 32% of the 234 total meat packages contained at least one fragment; some of them contained up to 8 fragments. They found no relationship between the number of metal fragments in carcasses and in the meat packages from the same deer, as

well as inhomogeneous distribution of the fragments in the resulting meat packages [5]. This inhomogeneity can lead to sporadically high levels of lead, as found in fluorescence-guided biopsies of fragment-containing samples by Cornatzer et al. [25]. In the present study, on average 1% (0.16 ± 0.15 g) of the initial bullet mass was estimated to remain in the edible parts using mass estimations from measured fragment volumes. For a worst-case scenario for human health, fragments were assumed to be entirely composed of lead. All of the edible parts that contained fragments were suspected to exceed the set EU Maximum Level for lead of 0.1 mg Pb/kg in livestock meat (Regulation (EC) No 1881/2006), which is not applied to game meat. Although the median level for lead in game meat obtained from lead-shot game was found to be relatively low, game meat samples can sporadically contain extremely high levels of lead [10]. As shown by Gerofke et al. [10] high lead levels are not limited to edible parts close to the wound channel, but can also be found in parts further away, such as the haunch. However, setting a maximum level for lead in game meat is difficult to apply due to the inhomogeneous distribution of fragments in the meat. Representative sampling of game meat for monitoring is virtually impossible. Lead levels in game meat have been shown to positively correlate with the number of shotgun pellets, as well as, the number of small metal fragments in cooked game bird meat [41]. Consequently, a positive correlation between the number of rifle bullet fragments and lead levels in the game meat may be expected. The lead from lead-hunted game meat has been shown to be bioavailable in studies with exposed animals [5, 13] and frequent consumption of game meat obtained with lead-based bullets has been associated with elevated blood lead levels in humans [11, 12]. The bioavailability of lead from bullet fragments is influenced by marinating practices during cooking and has been suggested to relate to the size of the fragments. Smaller fragments may dissolve more readily due to their higher surface-to-volume ratio [13]. Therefore, the mere presence of fragments is already problematic because of the dissolution in meat during storage and cooking [13, 26, 42–44]. In Germany, the average adult consumer eats 1 to 2 meals of game meat a year and is therefore generally at low risk for high lead exposure from this source. The risk for high lead exposure increases with higher frequency of consumption of game meat obtained with lead bullets (e.g. 10 or more game meals a year) [10]. However, sporadically high lead levels in game meat might be concerning even at low consumption frequencies in light of the fact that EFSA found no safe level of lead intake [10]. Consequently, children, pregnant women and women of childbearing age are generally advised to abstain from eating game meat harvested with lead bullets due to the neurotoxic effects of lead [10]. From the data discussed above, it can be concluded that all meat from animals hunted with lead bullets has the potential to contain lead, in agreement with Grund et al. [8]. In terms of food safety, it is extremely difficult to give precise recommendations on how to handle the carcasses, particularly for trimming around the wound channel, to ensure minimal risk of lead exposure. However, as hunting becomes more popular and as long as the use of lead ammunition remains permitted, different approaches to minimize human exposure to lead should be considered. An approach could involve extensive characterization of bullets by the manufacturers as suggested by Lahrssen-Wiederholt et al. [36]. Furthermore, the switch to alternative, non-lead bullets is supported as these bullets have proven to be just as effective as lead-based bullets [19, 27].

5 Conclusions

The objective of this study was to determine the spatial distribution of bullet fragments in four roe deer shot with standard semi-jacketed lead bullets and to investigate fragment number and size using CT. It was hypothesized that not all fragments are removed during meat processing of the roe deer, leaving fragments in the edible parts. All of the animal bodies contained fragments with high variability in their spatial distribution, number and size. Fragments were widely distributed in the animal bodies and moved into the edible parts. The inhomogeneous distribution of bullet fragments in game meat may expose consumers to sporadically high levels of lead, potentially causing adverse health effects. Although meat processing involves the generous removal of contaminated tissues (trimming), complete removal of all fragments cannot be ensured. In particular, fragments that are located in the main edible parts, such as haunch, shoulder and saddle, are unlikely to be removed because these parts are unlikely to be discarded. In addition, bullet fragment containing viscera that are left or buried in the forest, as well as exiting bullet residues expose wildlife and the environment to high levels of lead. Therefore, measures should be considered in the early stages of game harvesting to minimize the overall risk of lead exposure from hunting.

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Author contributions Conceptualization: MS, CG and ML-W; data curation: AH; formal analysis: AH; funding acquisition: ML-W; investigation: MS, CG, BK and GF; methodology: AH, MS, CG, BK, TH, GF and ML-W; project administration: CG and ML-W; resources: ML-W; software: AH; supervision: AM, JS-W and RP; validation: AH; visualization: AH, AM, JN, J-LM and JS-W; writing—original draft: AH, AM, BK and HJ; writing—review & editing: AH, MS, CG, AM, BK, HJ, TH, GF, JN, J-LM, JS-W, ML-W and RP.

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Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Code availability Not applicable.

Declarations

Competing interests The authors have no conflicts of interest to declare that are relevant to the content of this article.

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