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Comparison of lead levels in edible parts of red deer hunted with lead or non-lead ammunition

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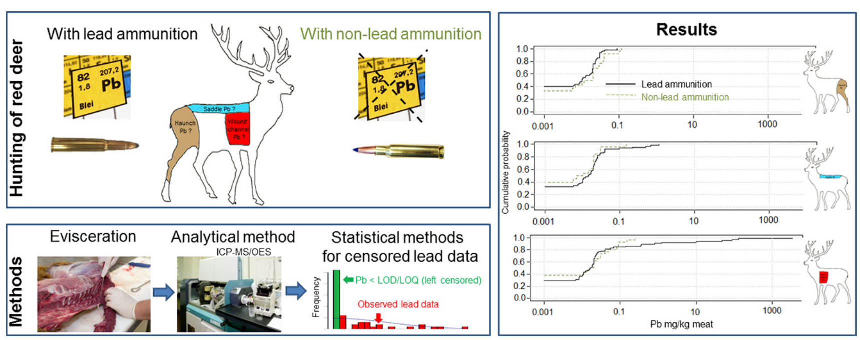
HIGHLIGHTS

- There is an ongoing discussion about the use of lead ammunition in hunting.
- We compared the lead concentration of meat from red deer (*Cervus elaphus*).
- Animals were hunted either with lead or non-lead ammunition.
- We used state of art statistical methods for left-censored data.
- High concentrations of lead in samples of lead shot red deer were observed.

GRAPHICAL ABSTRACT



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ABSTRACT

Game meat may contain elevated concentrations of lead especially if lead-containing ammunition is used for hunting. Then a health risk is possible for consumer groups with high game meat intake.

The lead concentrations in three edible parts (marketable meat from the area close to the wound channel, saddle and haunch) of meat from red deer (*Cervus elaphus)* between animals hunted either with lead or non-lead ammunition were compared. Furthermore, lead levels in game meat of lead-shot red deer were compared with those of lead-shot roe deer and lead-shot wild boar.

Ninety red deer were shot and killed in the context of this study (64 with lead and 26 with non-lead ammunition). Since the lead concentration for a number of the samples was below the limit of detection or the limit of quantification, statistical methods for left-censored data were applied.

The median concentrations of lead in game meat did not differ significantly between lead shot and non-lead shot animals. However, when we analyzed the more elevated lead concentrations, they were significantly higher in edible parts of animals shot with lead ammunition than non-lead ammunition. The highest concentrations were found in samples from edible meat from the area close to the wound channel (max 3442 mg Pb/kg), followed by the saddle (max 1.14 mg Pb/kg) and with the lowest levels in the haunch (max 0.09 mg Pb/kg). A comparison of game species revealed that the lead concentration in haunch and saddle of lead shot red deer was higher than in the corresponding samples of lead shot roe deer.

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Our results have shown that by the use of non-lead ammunition, a significant reduction of the lead concentration especially in edible parts near the wound channel is possible.

1. Introduction

Game meat can exhibit elevated concentrations of lead compared to other foodstuffs (BfR, 2010; EFSA, 2010b). The use of lead ammunition in hunting is one reason for this (BfR, 2014; Gerofke et al., 2018; Knott et al., 2009; Müller-Graf et al., 2017; Pain et al., 2010; Tsuji et al., 2009).

There is no threshold below which lead is not considered harmful to health. The European Commission (2006) in Regulation (EC) No. 1881 (2006) set maximum levels for lead of 0.1 mg/kg wet weight only for meat (excluding offal) of livestock animals such as bovids, sheep, pig, and poultry. Neither red deer meat nor any other game meat is listed in this regulation. One reason for that is the different way of lead contamination. Farm animals may show higher lead levels in different meat tissues depending on feed and water lead contents. Here, the lead contents are due to the intake with the feed or drinking water and are - depending on the tissues - rather uniformly distributed. Game meat from animals hunted with lead-based ammunition is more or less contaminated with small or large fragments of lead depending mostly on the material composition and construction of the bullet and other hunting factors. The lead particles from the bullet are not uniformly distributed in the carcass.

However, it is argued (Lindboe et al., 2012), that meat from big game is such an important meat source for people in certain populations that it would make sense to assign game meat to this foodstuff category. Additionally, other studies utilized a threshold level of 0.1 mg/kg for domestic meat in order to characterize and categorize lead contamination in game meat (Falandysz et al., 2005; Lazarus et al., 2014; Morales et al., 2011; Taggart et al., 2011). It was observed that consumption of meat from big game like white-tailed deer (*Odocoileus virginianus*) and moose (*Alces alces*), killed with lead-containing ammunition once, twice or three times a week, results in an estimated exposure to a lead dose associated with 1 point intelligence quotient (IQ) decrease for 2.9%, 5.8% and 7.7% of children, respectively. Additionally, 1.6%, 2.9% and 4% of adults would be exposed to a dose associated with a 1 mmHg increase in systolic blood pressure (Fachehoun et al., 2015). The bioavailability of lead from ammunition is regarded as one reason for this. A significant increase of blood lead levels has been observed in persons consuming cervid game meat from lead shot game animals compared to non-consumers of game meat (Buenz and Parry, 2017; Hunt et al., 2009; Iqbal et al., 2009; Meltzer et al., 2013). In Norway, 147 hunters and high-level consumers of cervid game meat had 31% higher blood levels than non-consumers (Meltzer et al., 2013). The results indicate that use of lead-based ammunition determines to a large extent the exposure to lead from cervid game, and also other game meat consumption.

In the hunting season 2015/2016, 78,596 red deer were shot in Germany (Deutscher Jagdverband, 2017). In comparison to roe deer (*Capreolus capreolus*) with 1,188,066 shootings and wild boar (*Sus scrofa*) with 610,631 shootings, the shooting quotas are much lower. Many hunters still kill the game with lead ammunition, although there is now well-proven non-lead ammunition on the market that also does not differ from lead bullets in terms of price (Thomas, 2013).

Up to now, there are few studies of lead concentrations in red deer with limited sample sizes in which the lead concentration in various organs (e. g. kidneys, liver) and in the muscle tissue was determined and reported (e.g. Chiari et al., 2015; Falandysz et al., 2005; Jarzynska and Falandysz, 2011; Lazarus et al., 2008). Furthermore, no comparison was made between possible differences in the lead contents in different subsamples (i.e. samples from saddle, haunch and the area close to the wound channel) from animals hunted with lead or non-lead ammunition.

1.1. Aim of research project

The primary goal of the study was to report lead concentration from a larger sample size of red deer and to compare the lead concentration of meat from animals hunted either with lead ammunition or specific non-lead bullets in the Bavarian alpine upland and Tyrol. For this purpose, a supplemental study was initiated from the research project "Food safety of game meat obtained through hunting (LEMISI)". The LEMISI-project was induced by the Federal Ministry of Food and Agriculture (BMEL) and coordinated by BfR.

Moreover, we wanted to find out whether the lead concentrations (median, 75., 90., 95. percentiles) in edible parts (haunch, saddle, marketable meat from the area close to the wound channel) of red deer are higher in animals shot with lead ammunition compared to non-lead ammunition. The study analyzed whether the lead concentration decreases with increasing distance from the wound channel. Furthermore, we looked at the portion of edible parts from red deer with lead concentration exceeding the EFSA Maximum level for lead (Regulation (EU) no. 1881/2006) of 0.1 mg Pb/kg which is valid for some livestock animals to study if it were similar when animals were shot with lead ammunition.

Another question was whether there are differences in spread (expressed by standard deviation) of lead contaminating particles depending on of the fragmentation of the lead bullets.

Finally, we compared the lead concentration of game meat from red deer with that of meat samples from roe deer and wild boar (also from the LEMISI project) according to region of carcass and bullet material.

2. Material and methods

2.1. Sampling

Data were collected for the research project "Safety of game meat obtained through hunting (LEMISI)" (BfR, 2014; Gerofke et al., 2018; Lahrssen-Wiederholt, 2013; Müller-Graf et al., 2017), a large project initiated by the Federal Ministry of Food and Agriculture. The aim of this project was to determine the concentration of lead in edible parts of meat from roe deer (*Capreolus capreolus*), wild boar (*Sus scrofa*) when using either lead or non-lead ammunition. For the supplementary study "Red deer (*Cervus elaphus*)", which was carried out in Bavaria and Tyrol (n = 3, only), 90 animals were killed, 64 of them with lead-based ammunition and 26 with non-lead ammunition. For each animal shot, hunters had to fill out a standardized sample submission sheet with data concerning the killing of the animal, i.e. hunting method, shooting distance, ammunition used, location of the shot (Martin et al., 2017). After the animal was shot and marked, it was brought to trained game traders who carried out the sampling. Three samples were taken from each animal: meat of the saddle, haunch and marketable meat from the area close to the wound channel. To avoid high lead levels in game meat, all visibly damaged and contaminated tissue surrounding the entry and exit wound was generously removed by trained personnel with knives and shears. But, the area of tissue removed was never quantified in this study. In each case, 100 g of meat samples were packaged and sent for laboratory analysis. Data were then sent to the "University for Sustainable Development" in

Eberswalde, which was responsible for entering the data into the database and the plausibility check. Subsequently, the data were collected and sent to Federal Institute for Risk Assessment (BfR). A further quality control and statistical analysis was carried out at BfR.

2.2. Ethics statement

Licensed hunters killed the animal used in this study during the established hunting season and in accordance with German regulations (German Hunting Act; Bundesjagdgesetz) and best practices. The study did not involve any killing beyond that carried out in regular, German state forest practice on a regular wildlife management basis (population control). Permission was granted from the respective German Federal States and their hunting authorities.

2.3. Analytical method

The samples were transported to one of two laboratories for chemical analysis. Prior to chemical analysis, the samples were homogenized and 0.5 to 1 g of each sample was placed in a high-pressure Teflon container for microwave pressure digestion according to EN 13805:2014 (Nardi et al., 2009).

The concentration of lead in muscle samples was measured either by inductively coupled plasma mass spectrometry (ICP-MS) or inductively coupled plasma emission spectrometry (ICP-OES) (Nardi et al., 2009).

2.4. Description of the variables

The following variables were measured:

- Lead concentration (mg/kg game meat) in three edible parts of red deer
- Edible parts of red deer: haunch, saddle, area close to wound channel
- Bullet material: lead or non-lead
- Bullet type[1]: depending on fragmentation
- Sex of game
- Age of game
- Location of shot placement (entry wound)
- Shooting distance in meter

A detailed description of the variables was already given elsewhere (Martin et al., 2017).

The German hunting encyclopaedia (Deutsches Jagd Lexikon, 2016) defines the bullet types as follows:

- Partial fragmentation bullets: bullets whose front part fragments and the back part remains stable
- Deformation bullets: deform without causing loss of small fragments
- Fragmentation bullets: fragment completely at the target (this bullet type was not used by any hunter in this study)

The distinction between deformation and partial fragmentation bullets is not entirely clear. Bullets with low fragmentation compared to the residual mass are sometimes also listed as deformation bullet (Deutsches Jagd Lexikon, 2016).

Compound bullets belong to the deformation bullets. For these bonded bullets, lead cores do not or only poorly separate from the copper jacket (Zeitler, 2007b).

[1] Different bullet types with different fragmenting effects were used. The bullets commonly used for hunting ungulates deform or partially fragment in the target (Zeitler, 2007a). In this publication bullet types were mostly (whenever possible) classified on the basis of manufacturers' information (bullet type description on websites for marketing).

For statistical analyses we assigned leaded bullets to two different classes according to the manufacturer's specifications:

1. Partial fragmentation bullets
2. Deformation bullets and compound bullets

2.5. Statistical analyses

The Chi-square test (Sachs and Hedderich, 2009) was carried out to determine whether categorical variables are evenly distributed with regard to the bullet material.

The lead concentration of some of the lead shot and non-lead shot subsamples was below the limit of detection (LOD) or the limit of quantification (LOQ); therefore we are dealing with left censored data. There are several statistical methods to evaluate censored data (Helsel, 2012; Lorimer and Kiermeier, 2007), depending on the sample size and the percentage of censored values. A preliminary analysis revealed that the percentage of censored values of our data set (lead in game meat) is between 29.7% and 42.3%, and the sample size of observed values is small ($n < 50$), depend on bullet material and subsamples taken from the edible tissue of red deer. Additionally, in the analyzed data set are multiple censoring levels (detection limits). First, the nonparametric method according to Kaplan-Meier (KM) was used to estimate percentiles of lead concentration for each combination of bullet material (lead or non-lead ammunition) and subsample (haunch, saddle, area close to wound channel). This method estimates the empirical cumulative distribution function (ecdf) for the lead concentration by accounting for the censored observations with a step-function (survival curve) and is suitable for small data sets ($n < 50$) with <50% censoring (Helsel, 2012). In a plot the lead concentrations are plotted as percentiles in which the estimated percentile is the probability of being less or equal to the corresponding lead observation.

Due to large variations of lead concentration in red deer meat, median values are considered suitable measures in this case. However, mean values are also included to enable comparison with other studies that also used means. With the Kaplan-Meier estimator, it is also possible to estimate the mean and the standard deviation of the data. However, when censoring is present, the estimator for the mean is biased (Huston and Juarez-Colunga, 2009). An efficient method to estimate the mean is the Robust Regression on Order Statistics (ROS) (Helsel, 2012). The censored values are not all replaced with the same value as with the simple substitution methods (e.g. upper bound or lower bound approach). The replacement depends on the probability distribution of the observed values. The method is also suitable for smaller data sets ($n < 50$) with <50% censoring. Both, the Kaplan-Meier estimate and the ROS method were performed with the R package NADA and with the function "cenfit" and "cenros", respectively (Helsel, 2012; Huston and Juarez-Colunga, 2009). However, ROS does not give confidence intervals for the parameters estimated. The recommended method to compute confidence intervals for the mean and percentiles is through bootstrap estimation (Helsel, 2012; Huston and Juarez-Colunga, 2009). Bootstrap with replacement consists of taking repeated random samples of the lead in game meat data set. Censored and uncensored observed lead concentrations are equally available for sampling. To select a random sample, the mean is repeated 10,000 times. Confidence limits were then empirically selected from this set of 10,000 means. This bootstrap estimates for censored data was performed with the R package "boot".

To test the significant impact of independent variable (bullet material) on different quantiles (50th, 75th, 90th and 95th) of dependent variable (lead concentration in three subsamples) the non-parametric quantile regression (Koenker, 2010) was used. Due to the strongly right-skewed distributions of the dependent variable and because <50% of the lead concentrations are below the LOD/LOQ we only selected percentiles from the 50th. The high lead levels (approx. From 50th percentile) are not affected by censoring. The method is robust to outliers in lead concentration observations. This test was used with the "rq" function from the R package "quantreg" (Koenker, 2017).

The spread of lead in the meat of red deer between leaded partial fragmentation bullets and leaded deformation bullets was indicated by standard deviation which was also estimated using the Kaplan-Meier method. To show the distribution of lead concentration in the subsamples according to bullet material and bullet type, a pirate plot was used. This plot represents the kernel probability density of the lead concentration at different values (Wickham, 2009). Lead concentrations lower than the LOD or LOQ were replaced by half of the detection (or quantification) limit (middle bound) to produce this plot. The middle bound method can be used when the data are highly skewed (Hornung and Reed, 1990).

To compare the lead concentration in meat samples of lead-shot red deer with those of lead-shot roe deer and lead-shot wild boar separated by subsample and bullet material, the generalized Wilcoxon test was applied. This non-parametric test calculates the differences among the three game species taking into account the multiple detection limits. When comparing the game species, Bonferroni correction for the p values was applied to correct for multiple testing (Abdi, 2007) and hence $p = 0.025$ after applying Bonferroni (0.05/3). All statistical evaluations and graphs were performed with the statistical software R version 3.4.1 (R Core Team, 2015).

3. Results

3.1. Description of study population

Univariate statistics for all variables divided by bullet material are given in Table 1. Overall, a total of 90 red deer were shot. Of the 90 animals, 26 (29%) were killed using non-lead and 64 (71%) using lead ammunition, respectively.

When hunting red deer with non-lead ammunition, 18 hunters used deformation bullets and eight partial fragmentation bullets from a total of 3 manufacturers. In the case of lead ammunition, a total of 36 deformation bullets and 28 partial fragmentation bullets were used from 12 manufacturers. Lead bullets used were more frequently partial fragmentation bullets than the non-lead bullets ($\chi^2 = 4.8$, df = 1, $p < 0.05$). The distribution of all other variables by bullet material was even ($p > 0.05$).

Shooting distances ranged from 30 m to 230 m (median 84 m, mean 95 m) for lead shot animals and from 30 m to 180 m (median 90 m, mean 95 m) for non-lead shot animals ($p = 0.77$).

Table 1
Characteristics of samples from red deer.

	Non-lead (N = 26)		Lead (N = 64)	
	n	%	n	%
Subsamples (edible part of red deer meat)				
Haunch	26	29.0	64	71.0
Saddle	26	29.0	64	71.0
Close to wound channel	26	29.0	64	71.0
Age of game				
Fawns (<1)	14	53.8	25	39.1
Subadult (1–<2)	8	30.8	20	31.3
Adult (from 2)	4	15.4	19	29.7
Sex of game				
Female	14	53.8	41	64.0
Male	12	46.2	22	34.4
Unknown	–	–	1	1.6
Bullet type				
Partial fragmentation bullets	8	30.8	36	56.3*
Deformation bullets/compound bullets	18	69.2	28	43.7
Location of shot placement (entry wound)				
Thorax	23	88.5	59	92.2
Gastrointestinal tract	2	7.7	2	3.1
Neck	1	3.8	2	3.1
Unknown	–	–	1	1.6
Location of shot placement (exit wound)				
Thorax	23	88.5	56	87.5
Gastrointestinal tract	2	7.7	5	7.8
Neck	1	3.8	1	1.6
Unknown	–	–	2	3.1
Hunting ground				
1	13	50	17	26.6
2	9	34.6	11	17.2
3	–	–	3	4.7
Unknown	4	15.4	33	51.6

* $p < 0.05$.

3.2. Description of censored and quantifiable subsamples

The percentage of left-censored values – observations that fall below the LOD/LOQ - ranged from 29.7% (lead ammunition, area close to wound channel) to 42.3% (non-lead ammunition, saddle). The number of observed values varied accordingly and was higher for lead ammunition and considerably lower for non-lead ammunition (Table 2).

Two laboratories analyzed the samples. Both laboratories determined the lead concentration of game meat subsamples from animals shot with lead or non-lead ammunition.

The limit of detection (LOD) was different for both laboratories (0.001 mg/kg and 0.008 mg/kg for laboratory 1 and 2, respectively). The limit of quantification (LOQ) was 0.005 mg/kg and 0.024 mg/kg for laboratory 1 and 2, respectively. Laboratory 2 did not report values below the LOQ.

3.3. Comparison of lead concentration between lead and non-lead shot red deer in three subsamples

3.3.1. Comparison of lead concentrations with measures of location

The first step was to analyze whether median concentrations of lead in meat differed statistically between lead shot and non-lead shot animals. In a next step, it was determined whether very elevated lead concentrations (from the 90th percentile) differ significantly between lead shot and non-lead shot animals. The mean value was strongly influenced by a few elevated values. But for the sake of completeness this measure of location was listed in Table 3. In all combinations (subsample with bullet material) the median was lower than the mean (Table 3), indicating a right-skewed distribution. The analyses were performed for all subsamples and separately for each subsample.

The total 95th percentile (not subdivided into sub-samples) of lead concentration in meat of lead shot red deer was significantly higher (0.492 mg/kg) than in non-lead shot game meat (0.120 mg/kg). No significant differences in lead concentration were found for the lower percentiles (Table 3, Fig. 1).

The lead levels for 64 samples from the areas close to the wound channel of lead shot red deer ranged from less than limit of detection (LOD) to 3442 mg/kg. The 95th percentile for the lead concentration

Table 2
Observed and censored game meat samples.

Subsample	<LOD N (%)	<LOQ N (%)	Quantifiable N (%)	Censored (%)	Total N
Lead					
Haunch	12 (18.7)	14 (21.9%)	38 (59.4)	40.6	64
Saddle	9 (14.1)	12 (18.7%)	43 (67.2)	32.8	64
Close to wound channel	3 (4.7)	16 (25.0%)	45 (70.3%)	29.7	64
Non-lead					
Haunch	4 (15.4)	5 (19.2)	17 (65.4)	34.6	26
Saddle	5 (19.2)	6 (23.1)	15 (57.7)	42.3	26
Close to wound channel	2 (7.7)	8 (30.8)	16 (61.5)	38.5	26

Table 3
Red deer lead concentrations (mg/kg) sampled at haunch, saddle and close to the wound channel classified according to bullet material.

Sub-sample	Bullet material	N	Mean[a,b] (95% CI)	Median[c]	P75[c]	P90[c]	P95[c]	Max
Total	Lead	192	19.42 (0.554; 56.55)	0.0123	0.023	0.040	0.492***	3442
	Non-lead	78	0.0261 (0.0189; 0.037)	0.0124	0.030	0.080	0.120	0.260
Close to wound channel	Lead	64	58.2 (0.970; 168.6)	0.016	0.024	0.820*	48.04***	3442
	Non-lead	26	0.0378 (0.019; 0.063)	0.016	0.040	0.090	0.150	0.260
Saddle	Lead	64	0.0535 (0.0192; 0.1009)	0.014	0.023	0.040	0.220***	1.140
	Non-lead	26	0.0175 (0.0099; 0.0299)	0.007	0.020	0.030	0.030	0.150
Haunch	Lead	64	0.0151 (0.0119; 0.0188)	0.010	0.020	0.030	0.0335*	0.09
	Non-lead	26	0.0232 (0.0137; 0.0355)	0.012	0.030	0.040	0.100	0.12

* $p < 0.05$.
*** $p < 0.001$ (estimated with quantile regression).
a Estimated with ROS statistics.
b Estimates confidence intervals on the mean by implementing ROS using a bootstrapping methodology.
c Kaplan-Meier estimator.

close to wound channel was 48.04 mg/kg for lead ammunition and 0.15 mg/kg for non-lead ammunition ($p < 0.001$), respectively. A significant difference was also observed for the 90th percentile (0.82 mg/kg for lead ammunition and 0.09 mg/kg for non-lead ammunition, $p < 0.05$). For the lower percentiles (<90th) we found no significant differences in lead concentration from subsamples close to the wound channel (Table 3, Figs. 2 and 3).

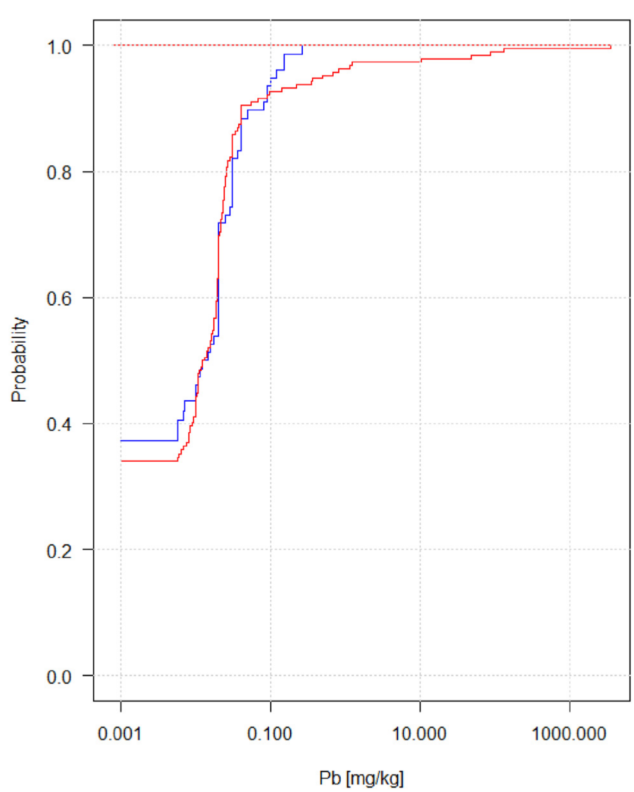

Fig. 1. Comparison of lead concentration in game meat of red deer classified according to bullet material (lead containing ammunition: red, non-lead ammunition: blue). Empirical cumulative distribution function based on the Kaplan-Meier estimation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

The fact that the 95th percentile value (48.04 mg/kg) from the areas close to the wound channel of lead shot red deer is lower than the mean (58.2 mg/kg) with a wide 95% CI (0.97; 168.6 mg/kg) indicates that there was a small number of values that are very elevated. In Fig. 1 it can be seen that the lowest percentile for lead shot and non-lead shot animals is approx. 30% and 38%, respectively. The related lead concentration was 0.008 mg/kg which corresponds to the highest LOD.

The lead concentration in samples from the haunch ranged from less than limit of detection (LOD) to 0.09 mg/kg, with a median of 0.01 mg/kg (mean 0.015 mg/kg) for lead shot red deer and from LOD to 0.12 mg/kg, with a median of 0.012 mg/kg (mean 0.023 mg/kg) for non-lead shot red deer, respectively. The 95th percentile of lead concentration from the haunch of lead shot red deer was lower (0.0335 mg/kg) than that of non-lead shot (0.1 mg/kg, $p < 0.05$, Table 3, Figs. 2 and 3). This difference was based only on a sample size of $n = 17$ for non-lead shot red deer. There were no differences between median concentrations of animals shot with lead-based ammunition or non-lead ammunition (approximately 0.01 mg/kg for both bullet materials). Fig. 2 also shows the proportion of the censored values for each subsample and the corresponding bullet material. The lowest percentile of lead concentration for lead shot and non-lead shot animals was approx. 41% and 35%, respectively. The related lead concentration was 0.008 mg/kg which corresponds to the highest LOD observed in this study. It means that 41% and 35% of the samples were below LOD.

In subsamples from the saddle, the lead concentration ranged from less than limit of detection (LOD) to 1.14 mg/kg, with a median of 0.014 mg/kg (mean 0.0535 mg/kg) for lead shot red deer and from LOD to 0.15 mg/kg, with a median of 0.007 mg/kg (mean 0.0175 mg/kg) for non-lead shot red deer, respectively (Table 3). The 95th percentile of lead concentration in the samples from the saddle of non-lead shot red deer was lower (0.03 mg/kg) than that of samples from the saddle of lead shot red deer (0.22 mg/kg, $p < 0.001$, Figs. 2 and 3). For the lower percentile (<95th) no significant differences of lead concentration were found between the two bullet materials (Table 3, Fig. 2). 33% of the samples from lead shot red deer and 42% of the samples from non-lead shot red deer were censored.

The comparison of lead concentrations depending on distance from the wound channel yielded significant differences for lead shot red deer but not for non-lead shot red deer. Samples from the area close to the

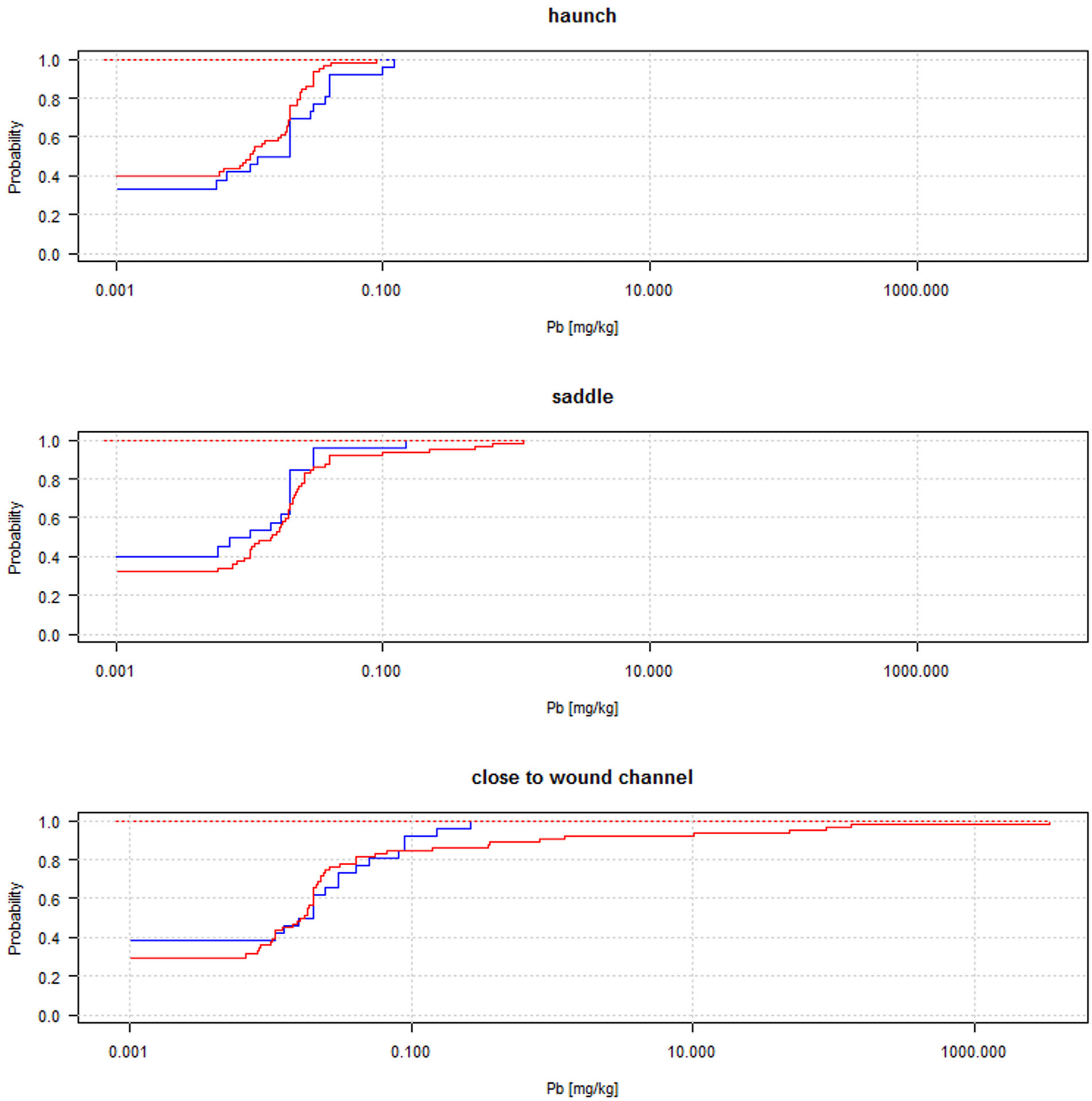

Fig. 2. Comparison of lead concentration between three subsamples (haunch, saddle, area close to the wound channel) classified according to bullet material (lead containing ammunition: red, non-lead ammunition: blue) with multiple detection limits. Empirical cumulative distribution function based on the Kaplan-Meier estimation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

wound channel showed significantly higher lead concentrations (90th and 95th percentile, 95th percentile is shown in Fig. 3) than samples from the haunch and the saddle ($p < 0.001$) when lead-based ammunition was used. No significant differences in median lead concentration were observed between all subsamples for both bullet materials.

3.3.2. Exceedance of 0.1 mg/kg

Comparisons of the lead levels above of 0.1 mg/kg (Maximum level of lead for bovids, sheep, pigs and poultry; Regulation (EC) No. 1881 (2006)) in the haunch, in the saddle and close to wound channel for both bullet materials are shown in Table 4.

When lead ammunition was used, in 10 (15.6%) of the 64 samples from the area close to wound channel lead concentrations exceeded the level of 0.1 mg/kg. In the haunch and the saddle, on the other hand, the lead concentration of 0.1 mg/kg was only exceeded in 0 and 4 (6.3%) of the samples, respectively.

3.4. Comparison of lead concentrations depending on bullet type

The spread of lead concentration in the meat of non-lead and lead shot red deer is expressed by the statistical measure of variation, the standard deviation (S.D.). The lead concentration in the muscle tissue of lead-shot red deer showed the greatest spread in edible meat from the area close to the wound channel when using deformation bullets (S.D. 649 mg/kg, Fig. 4), whereas the spread in lead concentrations close to the wound channel in the meat samples of animals shot with partial fragmentation bullets were lower (S.D. 0.15 mg/kg).

This broad spread was not observed in subsamples of haunch (S.D. leaded deformation bullets: 0.017 mg/kg, leaded partial fragmentation bullets: 0.01 mg/kg) and saddle (S.D. leaded deformation bullets: 0.24 mg/kg, leaded partial fragmentation bullets: 0.087 mg/kg) when leaded deformation bullets or leaded partial fragmentation bullets were used.

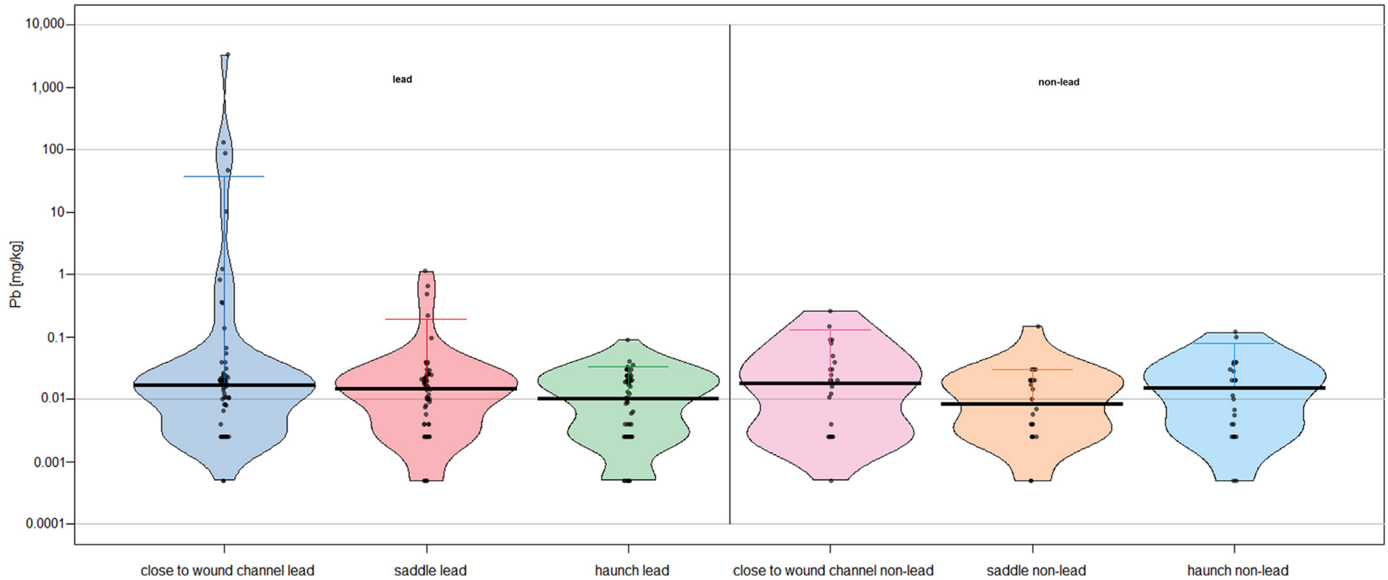

Fig. 3. Comparison of lead concentrations (logarithmic scale) in edible parts of red deer depending on distance from the wound channel according to the bullet material (left side: lead, right side: non-lead ammunition) as pirate plot. The shape of the plot represents the estimated density. The dots represent each data point. The median is indicated by the black solid line. The error bars show the 95th percentile. Lead concentrations lower than the LOD (or LOQ) were replaced by half of the detection (or quantification) limit (middle bound).

A comparison of lead content in meat of non-lead shot red deer between the two bullet types was not possible due to the small number of samples and the different structure and material composition of these bullets.

3.5. Comparison of the lead concentration of lead-shot red deer with those of lead-shot roe deer and lead-shot wild boar classified according to subsample and bullet material

Significant differences in median lead concentration were observed only between lead shot red deer and lead shot roe deer in the haunch and saddle subsamples ($p < 0.01$, respectively) but not in the subsamples close to the wound channel. The median lead concentration in the haunch and the saddle of lead shot red deer was higher than that of lead shot roe deer. The comparison of the lead concentration of the three subsamples from red deer and wild boar showed no significant differences when lead ammunition was used (Table 5, Fig. 5).

4. Discussion

4.1. Statistical methods for dealing with censored data

To estimate the mean and median lead concentration in meat from red deer we used statistical methods for the analysis of censored data because lead concentrations below the level of determination (LOD) and level of quantification (LOQ) occurred. This is a novel approach which was possible because of the development of appropriate computer tools. Traditionally, other authors used substitution methods like the upper bound, and/or lower bound approach or the middle bound approach (Danieli et al., 2012; EFSA, 2010b; Fachehoun et al., 2015; Hunt et al., 2009; Lazarus et al., 2014). The first method substitutes a censored value by LOD/LOQ if the censored value is less than LOD or LOQ. The second method substitutes the LOD/LOQ by zero. With the middle bound method the values of all data points falling below than the LOD were set to LOD/2 or LOQ/2.

The average lead concentration (mg lead/kg) of 2521 samples (59.4% < LOD) of game meat was 3.137 mg/kg (median 0 mg/kg) for the lower bound approach and 3.153 mg/kg (median 0.02 mg/kg) for the upper bound approach, respectively (EFSA, 2010b). However, that this approach has no theoretical basis because it is very unlikely that all values below the detection limit will have the same value. This approach has been shown to lead to distorted estimators (Lorimer and Kiermeier, 2007; Shorten et al., 2006). The lower bound approach consistently underestimates the mean whereas the middle and the upper bound overestimate the mean (EFSA, 2010b).

However, lower bound, middle bound and upper bound approach are quite often used to depict the concentration in figures.

The consideration of non-detects is important because substances with very low concentrations can also be an important part of the data. For a larger number of values below the detection limit, simple substitution methods are not recommended (Helsel, 2012). The middle bound approach was used to estimate the lead concentration in meat from wild boar (Danieli et al., 2012). Here, the values of all data points falling below than the LOD were set to LOD/2, whereas those data points falling between the LOD and LOQ were numerically reported. The average lead concentration was 0.124 mg/kg (median 0.119 mg/kg).

The lead concentration in White-tailed deer (mean 0.283 mg/kg, median 0.004 mg/kg) and moose (mean 0.17 mg/kg, median 0.003 mg/kg meats) killed by lead ammunition was also estimated with the middle bound approach (Fachehoun et al., 2015).

These substitution methods are considered as simple but possibly biased (Fu and Wang, 2012).

The results of a simulation study (EFSA, 2010a) showed that the number of samples had little effect on the accuracy and precision of estimates for the various methods, but that the degree of censoring had a large effect. The substitution with zero or LOD showed larger bias than all the other considered models (e.g. maximum likelihood estimation

Table 4
Number of samples with lead concentrations (mg/kg) above threshold of 0.1 mg/kg grouped by bullet material and subsamples.

Subsample	Bullet material	>0.1 mg/kg n (%)	N
Haunch	Non-lead	1 (3.8)	26
	Lead	0 (0)	64
Saddle	Non-lead	1 (3.8)	26
	Lead	4 (6.3)	64
Close to wound channel	Non-lead	2 (7.7)	26
	Lead	10 (15.6)	64

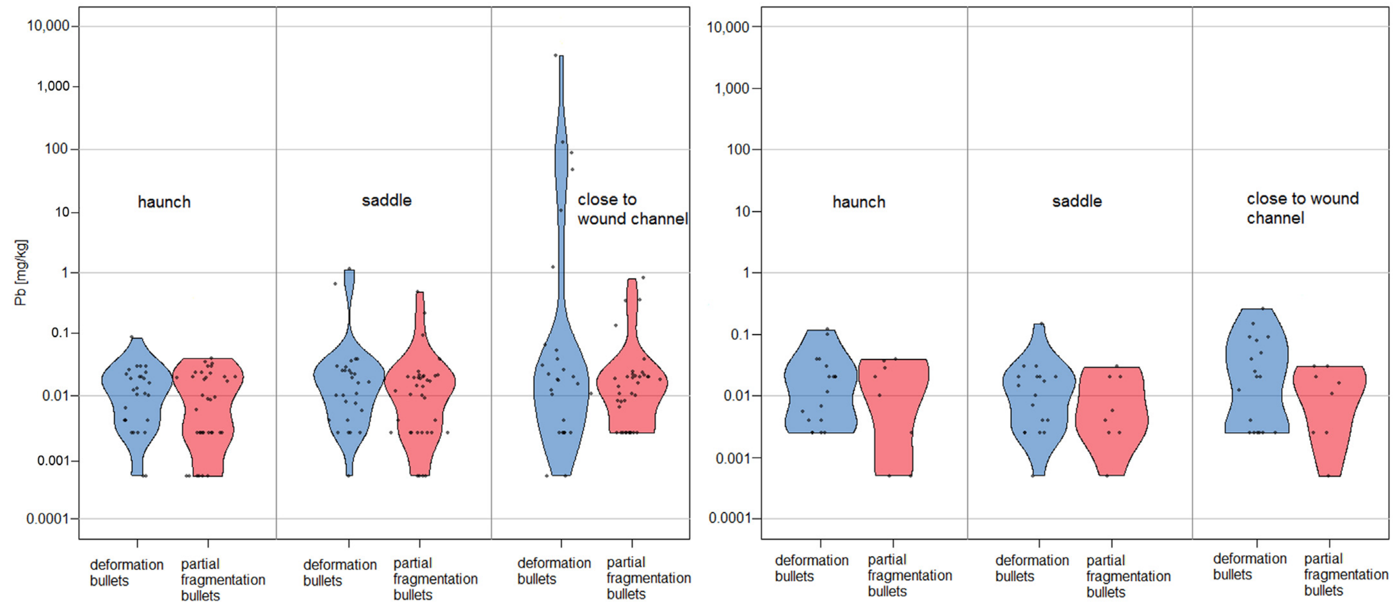

Fig. 4. Distribution of lead concentration (logarithmic scale) in edible parts of red deer according to the bullet type (deformation bullets or partial fragmentation bullets) and bullet material (left: lead, right: non-lead) as pirate plot. Lead concentrations lower than the LOD (or LOQ) were replaced by half of the detection (or quantification) limit (middle bound). The shape of the plot represents the estimated density. The dots represent each data point.

method, KM-method, Bayesian methods) when estimating the mean of the distribution (EFSA, 2010a).

But there are also studies using special statistical method for the analysis of censored lead concentrations in game meat (Lindboe et al., 2012; Müller-Graf et al., 2017). In the first study the mean of lead in meat from moose was estimated using Monte Carlo simulations (Lindboe et al., 2012). In the second study, for the determination of the geometric mean with 95% confidence intervals of lead concentration in meat samples from roe deer and wild boar, a Tobit regression for censored data was used (Müller-Graf et al., 2017). It is argued that the percentage of censored values was almost always over 50% and the sample size of observed values was always high ($n > 50$). If >50% of the data are censored in a sample larger than 50, methods with Maximum Likelihood estimation (here Tobit regression) is recommended for the analysis (Helsel, 2004, 2012; Lorimer and Kiermeier, 2007). For our study exactly the opposite is true, i.e. sample size $n < 50$ and lower percentage of censored values <50%. For this reason Kaplan-Meier method (KM), Robust Regression on Order Statistics (ROS) and the generalized Wilcoxon were used for statistical analysis.

Table 5
Comparison of the lead concentration of red deer with lead concentrations (mg/kg) of roe deer and wild boar sampled at haunch, saddle, and close to the wound channel (only lead shot animals).

Subsample	Bullet material	Game species	N	Median	p[a]
Haunch	Lead	Red deer (reference)	64	0.010[b]	Ref.
		Roe deer	745	0.0028[c]	<0.01
		Wild boar	514	0.00398[c]	n.s.
Saddle	Lead	Red deer (reference)	64	0.014[b]	Ref.
		Roe deer	745	0.00399[c]	<0.01
		Wild boar	514	0.0073[c]	n.s.
Close to wound channel	Lead	Red deer (reference)	64	0.016[c]	Ref.
		Roe deer	745	0.01299[c]	n.s.
		Wild boar	514	0.019[c]	n.s.

[a] Bonferroni-adjusted significance level ($\alpha = 0.025$), n.s. non significant.
[b] Kaplan-Meier estimator.
[c] Maximum likelihood estimation (MLE).

It is advisable to have a policy on the handling of censored data to ensure comparability of the data.

4.2. Differences in lead concentrations in game meat between lead and non-lead shot red deer

Differences between median lead concentrations in meat from red deer shot with lead ammunition or shot with non-lead ammunition (in all three subsamples and total samples) were not statistically significant. This is in contrast to other results of the LEMISI research project (BfR, 2014; Gerofke et al., 2018; Müller-Graf et al., 2017). These previous results clearly showed that lead-based hunting ammunition significantly increased the geometric mean lead concentration in the meat from roe deer and wild boar compared to animals shot with non-lead ammunition (Müller-Graf et al., 2017). In the context of this previous study the lead concentration of 1254 samples from roe deer and 854 samples from wild boar in three subsamples (haunch, saddle, close to wound channel) shot with lead or non-lead ammunition were analyzed (Müller-Graf et al., 2017). Similar effects were expected for red deer. However, due to the small sample size of shot red deer animals ($n = 90$) differences in the median lead concentration may not have been as significant. When we analyzed the high lead concentrations using quantile regression (90th and 95th percentile), lead concentrations close to the wound channel and the saddle were significantly higher when lead ammunition has been used instead of non-lead ammunition. There are various explanations for this, which should take into account the bullet construction as well as the properties of the bullets used. Significant differences between lead-containing and non-lead bullets exist in the material characteristics of lead and copper, since lead is more fragmented lighter than copper.

However, we also found individual elevated lead levels in muscle tissue of non-lead shot red deer. This may be due to the fact that non-lead bullets may contain traces of lead (Göttlein et al., 2013).

Also a high lead concentration of 477 mg/kg was measured in the entry wound of a meat sample from lead shot red deer (Dobrowolska and Melosik, 2008). Other authors also found high levels of lead in the muscle tissue of red deer (Bilandzic et al., 2009; Chiari et al., 2015; Gasparik et al., 2004; Morales et al., 2011; Taggart et al., 2011), with a

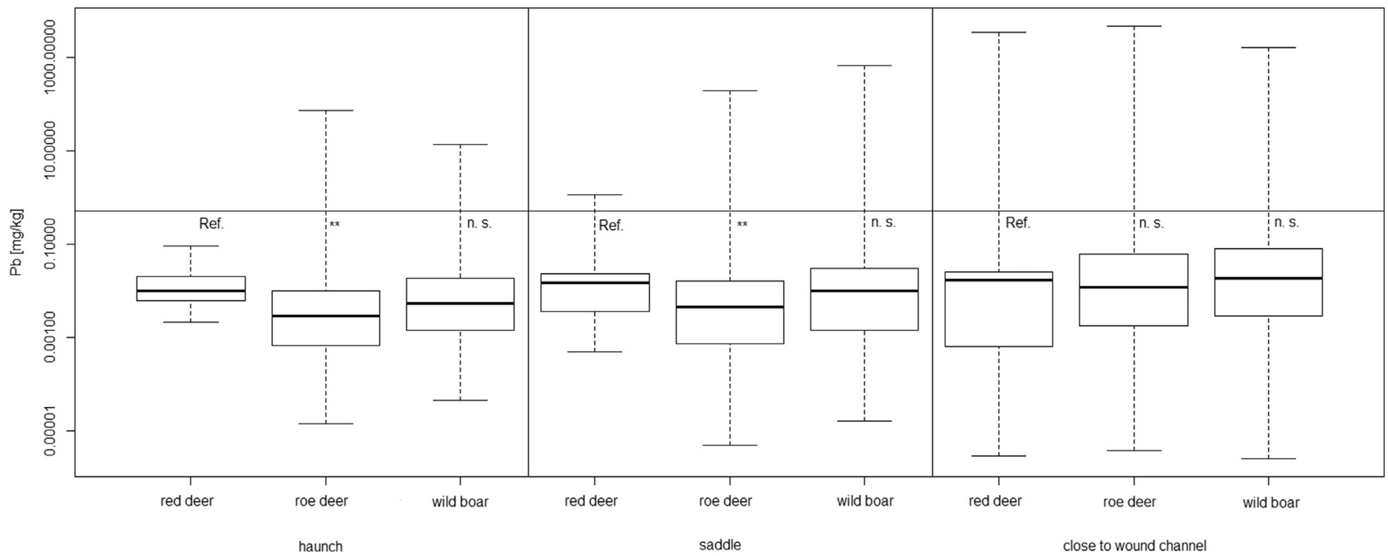

Fig. 5. Censored boxplot: Lead concentration (logarithmic scale) in edible parts of red deer in comparison with lead concentration in roe deer and wild boar (lead ammunition). The horizontal line is drawn at the highest detection limit in the data set.

maximum of 6.69 mg/kg, 7 mg/kg, 104.9 mg/kg, 4.6 mg/kg and 23.7 mg/kg, respectively. In all these studies, the lead concentration of muscle tissue samples was measured, but exact information on the bullet material used and the hit in muscle tissue are unfortunately not available. Therefore, a comparison with this study or a meta-analysis of data is difficult. Also in other studies the comparison of mean or median lead concentrations in meat of red deer is complicated by incomplete information about the ammunition used and the location of the hit in muscle tissue (Falandysz et al., 2005; Jarzynska and Falandysz, 2011; Lazarus et al., 2008; Skibniewski et al., 2015; Srebocan et al., 2012). In future studies this information should be collected and published.

4.3. Influence of distance from the wound channel on lead concentration

The highest lead concentrations were found in samples from the area close to the wound channel (max. 3442 mg/kg) when lead containing ammunition (partial fragmentation bullets) was used and when the animal was shot in the neck. Only a thorax shot with rapid killing effect and minimal destruction of the game meat, low microbial burdens and a short escape distance following the shot is recommended (Drees et al., 2008; TVT, 2011). Of the 64 animals shot with lead bullets, 59 (92.2%) were hit in the thorax (see Table 1, Location of shot placement (entry wound)). One explanation is that the haunch is furthest away from the wound channel and thus also furthest from the thorax and has the lowest lead concentration. Lead bullets are the secondary source of Pb in all tissues of hunted big game, especially in the immediate vicinity of the wound, where the lead concentration can be very high when lead shot is used (Jarzynska and Falandysz, 2011). The environment is regarded as the one source for the lead contamination of game meat, but there has already been a significant decrease of lead in the environment and contamination with this heavy metal, for example by splinters from the killing projectile, is becoming more and more clearer (Hecht, 2000).

Several studies have found elevated lead concentrations and lead fragments at different distances from the wound channel in meat from lead shot game animals (Dobrowolska and Melosik, 2008; Grund et al., 2010; Müller-Graf et al., 2017). As expected, the lead concentrations were the highest at the entry and/or exit wounds in these studies. A decrease in the lead concentrations with increasing distance from the wound channel was reported in other species (Müller-Graf et al., 2017). This finding is also supported in a study (Dobrowolska and Melosik, 2008), who analyzed muscle and soft tissue of red deer for lead concentration immediately after being killed at different distances (5, 15, 25 and 30 cm) from wound channel. Lead particles at a distance of 30 cm from the entry wound were detected in each sample. The highest lead concentration was 477 mg/kg near the wound channel and 1.1 mg/kg 30 cm away from the entry wound. All animals were killed with lead-based ammunition. Another study (Grund et al., 2010) analyzed the lead contamination levels in muscle tissue samples from white-tailed deer and sheep at different distances from wound channel (5, 25 and 45 cm). Detectable lead levels were found at all distances, and the highest concentrations were found closest to the wound channel. Hecht (2000) detected lead fragments up to 30 cm transversely to the wound channel, depending on the position of the shot and the projectile parameters, and Stewart and Veverka (2011) recommended removing muscle up to 40 cm around the wound channel before consumption in cases when white-tailed deer was shot in lower neck by sharpshooting.

Also other authors advise discarding the tissue surrounding the wound channel, because it may contain lead bullet fragments (Jarzynska and Falandysz, 2011; Tsuji et al., 2009). The meat from the wound channel with presumably highest lead concentration is normally cut away and discarded, but bullet fragments may remain, and the area of tissue around the wound channel which is removed varies among hunters (Meltzer et al., 2013).

Due to the small size of the lead fragments and the large area (much larger than the immediate area around the wound channel) contaminated with lead particles it may be impossible to remove all fragments from the carcass tissue.

4.4. Characteristics of samples exceeding 0.1 mg Pb/kg

There is no maximum level for lead in game meat. For lead in some livestock meat a maximum level of 0.1 mg Pb/kg (from European Commission (2006) in Regulation (EC) No. 1881 (2006)) applies. This maximum value has been applied as a comparative measure in other studies (Falandysz et al., 2005; Lazarus et al., 2014; Lindboe et al., 2012; Morales et al., 2011; Taggart et al., 2011) and was also used in this study for a better description of distribution of lead levels in game meat.

The comparison of the lead levels exceeding of 0.1 mg/kg showed that only samples close to the wound channel (for lead shot red deer) were more frequently (approx. 16%) above this maximum value.

Although this maximum level for lead of 0.1 mg/kg is only valid for meat of bovids, sheep, pig and poultry it is often used to compare lead levels. Studies from Poland, Norway and Spain have shown that this value was exceeded frequently in meat of red deer and moose (Falandysz et al., 2005; Lindboe et al., 2012; Morales et al., 2011; Taggart et al., 2011).

In 31 (60%) of the 52 batches sampled from moose in a study (Lindboe et al., 2012), lead concentrations exceeded the maximum level of 0.1 mg/kg. All moose were killed with lead-containing ammunition.

In southern Spain it was demonstrated that big game meat from red deer and wild boar is a significant source of lead (Morales et al., 2011). Eighty four point four percent and 28% of the muscle samples from wild boar and red deer, respectively, exceeded the maximum level for lead of 0.1 mg/kg (Regulation (EU) no. 1881/2006).

The highest proportion (89%) of samples exceeded the maximum level of lead were found from fawn muscle tissue meat of red deer (Falandysz et al., 2005).

Due to the high proportion of self-marketing and direct consumption by hunters and their families, the introduction of maximum levels would, however, have virtually no effect on the exposure of the average consumer and would provide insufficient protection for consumers from hunter's households (BfR, 2010).

4.5. Influence of bullet type on the lead concentration and consideration of bioavailability

We looked at whether the bullet type can influence the lead concentration in the game meat. Here, the proportion of samples with elevated lead concentrations is higher in meat samples from the area close to wound channel shot with leaded deformation bullets than in meat samples shot with leaded partial fragmentation bullets of the same meat area. This can be explained by the fact that deformation bullets mushroom in the wound channel and larger lead fragments remain mostly in the wound channel or in the adjacent tissues (Ulbig, 2013). In contrast, partial fragmentation bullets fragment to a higher extent in very small particles and with a smaller residual body, whereby the fragments are also released into more distant tissues. For these reasons, animals shot with deformation bullets often have elevated lead levels in the near close to wound channel than animals shot with partial fragmentation bullets.

Similar results were obtained of the LEMISI research project (Müller-Graf et al., 2017). Here, bonded bullets and non-bonded bullets were compared. This classification is similar to the classification of deformation bullets and partial fragmentation bullets in this study. In this study it was shown that there was a tendency of higher lead concentrations in the saddle ($p < 0.001$) and around the wound channel ($p < 0.05$) of roe deer when using deformation bullets.

In our study we have no information on the size of lead particles. The size of the fragments in white-tailed deer and mule deer, hunted with standard, center-fire, breach-loading rifles, ranged from a few >5 mm to tiny fragments beyond the limit of unsupported vision, which was estimated at about 0.5 mm (Hunt et al., 2006).

The bullet type affected the likelihood of bullet fragments being retained in carcasses (Pauli and Buskirk, 2007). In this study it was found that 87% of prairie dogs shot with expanding (fragmentation) bullets contained bullet fragments. In contrast, only 7% of the cadavers shot with non-expanding bullets (non-fragmentation) contained bullet fragments. The lead fragments in the carcasses shot with fragmentation bullets were small. 73% of the total lead mass in each carcass consisted of fragments weighing <25 mg each. These small fragments can be easily absorbed into the gastrointestinal tract by secondary consumers (Pauli and Buskirk, 2007).

Increased lead levels (when fragmentation bullets used) are most likely caused by lead dispersion into the animal body after bullet fragmentation (Danieli et al., 2012; Hunt et al., 2009).

Non-lead bullets are usually offered as deformation bullets (Ulbig, 2013) but can also contain small quantities of lead (Göttlein et al., 2013). This also corresponds with the results of our study, 69.2% of the non-lead shot animals were killed with deformation bullets but only 30.8% of the non-lead shot animals were hunted with partial fragmentation bullets. When comparing the lead concentration it seems that both non-lead bullets tend to spread only few fragments in the carcasses. Due to the brittleness of copper, there is no comparable "splinter cloud" in non-lead partial fragmentation copper bullets (Ulbig, 2013).

An average of 356 metal fragments was visible on radiographs of the carcass from 10 red deer and two roe deer after being shot with a copper-jacketed lead-cored bullet (Knott et al., 2010). The authors did not distinguish between copper and lead fragments. They assumed that the large fragments come from the bullet's copper jacket because the copper component of bullets is less frangible than the lead component. Therefore it is less likely that the copper component can form small fragments. The vast majority of fragments however, especially the small size categories, were likely composed of lead.

Analyzing the effect of bullet construction is notoriously difficult, since there are a myriad of different types of bullets. Even the classification of deformation and fragmentation is difficult and may lead to contradictory results when classified by different experts. In future these data need to be conscientiously collected to allow better conclusions regarding the effect of the bullet type.

However, the analyzed lead concentration allows only limited conclusions of the bioavailability of the lead. The size of the fragments may exert an impact on the bioavailability of the lead. It is assumed that while the bioavailability of larger particles is low, it increases significantly with smaller particles (Fromme, 2013). Compared to larger particles, small particles have larger surfaces and thus increase the surface area for chemical reactions (Hecht, 2000).

There may also be an effect of different bullet sizes on lead content of red deer meat from animals of different size. We can use the size of caliber as an indicator of bullet size. However, the study design did not specify the caliber to be used and the hunters shot with their own familiar weapons. German hunting legislation allows the use of calibers below 6.5 mm for roe deer only. Wild boar and red deer may only be shot with bullet diameters of 6.5 mm and above and 2000 Joules bullet kinetic energy at 100 m. The available data show that hunters for roe deer do not differentiate to smaller calibers but rather use red deer and wild boar legal calibers for hunting any ungulates in Germany.

Eighty percent of the red deer hunters (shot with lead ammunition) used caliber 0.308 Winchester or 30.06 Springfield with the same diameter of 7.82 mm and bullet weights from 10 to 12 g. With these both calibers different-sized animals were shot (juvenile, subadult and adult red deer). Twenty percent of the lead shot animals were killed with smaller (diameter from 6.5 to 7.06 mm and bullet weight from 8 to 9 g) or larger (diameter from 8.2 to 9.3 mm and bullet weights from 13 to 15 g) calibers. Calibers with a smaller diameter were used almost exclusively for hunting of animals less than one year old (juvenile). In the case of non-lead shot animals, 81% of the hunters used the calibers 0.308 Winchester or 30.06 Springfield with a diameter of 7.82 mm and bullet weights from 9 to 11 g. The distribution of calibers is similar to the lead bullets. With both calibers animals of different size were shot. Only one animal under one year of age was killed with a smaller caliber (diameter 7.06 mm and bullet weight 8.4 g). Three animals (2 adults and 1 subadult) were killed with larger bullets caliber (diameter 9.3 mm and bullet weight 11.9 g). Due to unevenly distribution of caliber using a more in-depth statistical analysis of the influence of the caliber on the lead content of game meat from animals of different size had to be omitted in this paper. This possible influencing factor should be analyzed in future studies.

4.6. Game species influences on the lead concentration of edible parts

Differences between lead concentration in all three edible parts of lead shot red deer and lead shot roe deer were noted. The higher lead concentration in subsamples of red deer, especially in those body regions away from the thorax seem counter intuitive with regard to the body weight and size difference of red deer, wild boar and roe deer. The distance from the area close to the wound channel (most frequent and recommended hit position is the thorax) to the saddle is approximately 50 cm for red deer, but approximately only 20 cm for roe deer (BfR, unpublished own data). Considering the distance from the area close to the wound channel to the haunch, this is approximately 100 cm for deer and approximately 40 cm for roe deer (BfR, unpublished data).

With regard to the small sample size available for red deer, further research may be warranted. The weight of red deer was not available in the present study, but the age and sex of animals (Table 1) could be used to predict the weight. Using sex and age (Golze, year not published) an average weight of approx. 50 kg was estimated for red deer. The average weight of the 745 lead shot roe deer was about 13 kg.

The comparison of the lead concentration in the subsamples of red deer with wild boar (mean body weight wild boar 37 kg) showed no significant differences when lead-based ammunition was used. Similar results were also shown by Chiari et al. (2015). Here, the mean lead concentrations in muscle tissue were 2.6 ± 3.27 mg/kg and 2.04 ± 3.32 mg/kg for wild boar and red deer, respectively.

It could be speculated, that due to the tougher skin and stronger muscle tissue and the higher bone hardness in red deer and wild boar, compared to roe deer, the highest lead concentration occurs in the surrounding tissue of the wound channel. In the area close to the wound channel, however, this was not borne out by the data. There were no significant differences in the lead concentrations in these subsamples between red deer and roe deer.

Another explanation for the different lead contents could be that the three animal species were shot at different shooting distances during hunting (red deer: median 84 m, roe deer: median 60 m, wild boar: median 50 m). With increasing shooting distance the impact velocity of the projectile decreases and thus its readiness to fragment upon impact. New scientific information has become available on the importance of the impact velocity for bullet fragmentation (DEVA, 2011; Gremse and Rieger, 2012; Kneubuehl, 2013), which contributes to the discussion of bullet rating. However, the focus is on the killing effect of the bullets. Investigations into the fragmentation of the bullets under standardized conditions are still pending.

On the basis of the available data, we cannot conclude, why different levels of lead have been found in meat of red deer, wild boar and roe deer. Other factors may also play a role, such as the different calibers and mass of bullets used to kill game animals of different body size. Another important factor is whether the bullets struck bone or only soft tissue upon entry. Bone strikes cause great bullet fragmentation.

5. Conclusion

Our results show that use of lead ammunition may lead to elevated concentrations of red deer meat. However, in this study the median differences in lead concentrations between lead and non-lead shot animals were not significant due to the considerable variation in lead concentration in animals shot with lead-based ammunition. There is however an indication that observed differences could be significant with a larger sample size. Lead ammunition may thus pose a health risk to humans with very high consumption rates of red deer meat. Individual game meat samples showed elevated lead contents especially in edible meat from the area close to the wound channel.

In the long term, it is desirable to kill game intended only for human consumption with ammunition that lowers the release of lead in accordance with the “ALARA-Principle (As Low As Reasonably Achievable)” into food.

Several studies have shown that non-lead ammunition is suitable for practical hunting in accordance with animal welfare regulations (Hackländer et al., 2015; Kanstrup et al., 2016; Knott et al., 2009; Martin et al., 2017; Trinogga et al., 2013). Large game such as red deer and wild boar can also be killed just as effectively with non-lead ammunition at 300 m - if appropriate bullets are used (Gremse and Rieger, 2012). The availability and effectiveness of non-lead hunting ammunition has been extensively investigated and is given (Thomas, 2013; Thomas et al., 2016). Newer non-lead bullet types, such as full copper bullets, plastic-tip copper bullets and coated metal powder bullets, are becoming increasingly popular (Caudell et al., 2012).

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