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RESEARCH ARTICLE

Lead exposure in consumers of culled invasive alien mammals in El Palmar National Park, Argentina

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Abstract

Consumption of meat from animals hunted with Pb ammunition can cause toxic accumulation with consequent health risks, even if relatively small amounts are consumed in each exposure. In El Palmar National Park, Argentina, invasive alien mammals, wild boar (*Sus scrofa*) and axis deer (*Axis axis*), are culled with Pb ammunition and their meat is consumed. In this study, we evaluated blood Pb concentrations in 58 consumers of culled game and examined Pb exposure risk according to their demographics, duty, and consumption habits. Likewise, the likelihood of exposure was evaluated by quantifying Pb concentrations in meat samples of seven culled axis deer. Twenty-seven participants (46%) had detectable blood Pb levels (limit of detection = 3.3 μg/dL), with an average 4.75 ± 1.35 μg/dL (geometric mean ± geometric S.D.); the average for all participants was 3.25 ± 1.51 μg/dL. Blood Pb concentrations were significantly higher in hunters, in participants who reported consuming game meat more than 5 times per week, and in participants who reported frequently consuming cured game meat (compared to cooked or pickled). Pb concentration varied significantly along the trajectory of the bullet in deer muscle, being highest at mid-point but with detectable Pb levels even in distant tissue samples (control), suggesting potential for dietary intake by consumers. These findings provide evidence of Pb exposure risk in consumers and emphasize the relevance of replacing Pb ammunition with non-toxic alternatives. This change would reduce dietary exposure in frequent consumers and allow the use of game meat as safe food for people whilst eliminating collateral risks to wild animals and the environment.

Keywords Alien species control · Dietary exposure · Food safety · Game meat · Lead ammunition · Public health · Wildlife culling

Introduction

The risks of lead (Pb) exposure from hunting for human, animal, and environmental health have been widely recognized worldwide (Hunt et al. 2006; Green and Pain 2015; Arnemo et al. 2016; Kanstrup et al. 2018). Consumption of meat from animals hunted with Pb ammunition is one of the main sources of Pb for humans and animals (Kosnett 2009; Fachehoun et al. 2015; Hampton et al. 2018; Green and Pain 2019). Many studies have reported that the use of Pb ammunition in big game hunting causes significant contamination of impacted tissues due to the dispersion of bullet fragments, mainly around the wound channel (Dobrowolska and Melosik 2008; Hunt et al. 2009; Tsuji et al. 2009; Gerofke et al. 2018; Menozzi et al. 2019). This route of exposure was often neglected, since it was considered that the bullet could be easily removed after impact. However, radiological studies have shown a massive dispersion of small Pb fragments in game meat, which hinders elimination prior to consumption

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(Hunt et al. 2006; Hunt et al. 2009; Tsuji et al. 2009; Knott et al. 2010; Menozzi et al. 2019).

The consumption of Pb-contaminated game meat over a lifetime may lead to the accumulation of toxic amounts with significant health risks, even if small amounts are ingested in each meal (Iqbal et al. 2009; Kosnett 2009; EFSA 2013). Once ingested, part of the Pb is solubilized by the low pH of the stomach and is then absorbed into the bloodstream in the intestines, while the remaining Pb is excreted in feces and urine (Green and Pain 2019). The acute effects of Pb poisoning are generally seen after brief exposures at high concentrations (WHO 2010). However, chronic toxicity resulting from long-term exposure to small doses is also concerning, since it does not cause obvious symptoms initially but gradually produces disorders affecting multiple organs and tissues (EFSA 2013; WHO 2019). Pb significantly affects the function of the kidneys, liver, heart, and central nervous system, as well as the hematopoietic, immune, endocrine, and reproductive systems (Dietert and Piepenbrink 2006; Flora et al. 2006; Tchounwou et al. 2012; Assi et al. 2016). Children are more vulnerable to the harmful effects of Pb, because very low doses suffice to damage the developing nervous system and can cause permanent cognitive deficits and behavioral changes (Chiodo et al. 2007; Wright et al. 2008; Budtz-Jørgensen et al. 2013).

Although there is a direct relationship between blood Pb concentrations and the severity of observed symptoms and effects, there are no safe levels for Pb exposure (WHO 2019). The blood Pb reference value of 5 μg/dL, which was once thought to be safe, is now known to represent a significant risk to the health especially of children (Budtz-Jørgensen et al. 2013; COEH 2016) and also of adults (Lanphear et al. 2018). In this context, frequent consumers of meat from animals shot with Pb ammunition are at a significant health risk and regulatory frameworks that limit the use of Pb ammunition have been adopted by some countries or jurisdictions worldwide (Avery and Watson 2009; Mateo and Kanstrup 2019; Thomas 2019; Uhart et al. 2019). In regions where there are no regulatory provisions to limit the use of Pb ammunition in wild game hunting, the European Food Safety Organizations recommends that the precautionary principle be applied and the quantity and frequency of meat consumption from animals shot with Pb ammunition should be limited (BfR 2011; FSA 2017; SNFA 2012). Since 2006, El Palmar National Park, in northeastern Argentina, has implemented a program to control invasive alien mammals, namely, wild boar (*Sus scrofa*) and axis deer (*Axis axis*) (Gürtler et al. 2017). The program allows the use of Pb ammunition, and the meat from hunted animals is used for local consumption. However, to date, there has been no analysis of dietary exposure for consumers. The aims of this study were to evaluate blood Pb concentration in game meat consumers from this community and examine its relationship with individual characteristics such as age, gender, duty, and game meat consumption habits. Additionally, we determined Pb concentration in meat samples from axis deer shot with Pb ammunition to assess the potential for dietary intake.

Materials and methods

Study area

El Palmar National Park (EPNP, 31° 51′ 54″ S, 58° 15′ 34″ W) is located in Entre Ríos province, Argentina. Since 2006, a multi-stakeholder management program has been implemented to reduce invasive alien mammal populations, namely, wild boar and axis deer (Gürtler et al. 2017). This program is carried out collaboratively by EPNP personnel and authorized local hunters, through controlled still shooting using firearms with Pb ammunition from watchtowers. The watchtowers are widely distributed within the park, and there is no bag limit for hunting. Hunted animals are slaughtered in situ and a hindquarter of each hunted animal is donated to local schools and community shelters, while the rest of the meat is consumed by the hunters and EPNP personnel. During the program's first 10 years, 1999 wild boar and 2380 axis deer were culled (Gürtler et al. 2017; Gürtler et al. 2018). In 2019, 146 hunters participated in the program, removing 1352 axis deer and 276 wild boar (Sosa unpubl. data).

Collection of samples and data

Game meat consumers: questionnaire and blood sample collection

Participants who consumed meat hunted with Pb ammunition in EPNP and were at least 18 years of age met our criterion for inclusion in this study. Following the standards of the Central Committee for Bioethics in Practice and Biomedical Research of Paraná, Entre Ríos, each volunteer participant gave their written and informed consent (Online Resource 1). The identification data, age, gender, duty in the control program (hunter, park ranger, or other), and information related to game meat consumption of each participant were recorded. A structured questionnaire (Online Resource 2) was carried out, personally and always by the same interviewer. Frequency of game meat consumption was classified as very low (<1 meal per week), low (1–4 meals per week), medium (5–9 meals per week), and high (10–14 meals per week). Information on frequent modes of game meat consumption (cooked, cured, or pickled) and what they did with the bullet-struck portions of the carcass, selective consumption (selectively choosing for consumption the portions that were not affected by bullets), feeding of dogs (body parts affected by bullets were left in a container and then given to dogs), or discarded, was collated. The date of the most recent game meat consumption and its mode of consumption were also recorded. After the questionnaire, blood samples were collected, and participants received information about the risk associated with Pb intake and practical recommendations to reduce dietary exposure. Blood was drawn by venipuncture (5 mL) by the staff of San Benjamín Public Hospital on 26–30 August and 11–15 November 2019. A blood subsample for Pb concentration determination was collected using a capillary immediately after collection, and the remainder was frozen (−20 °C) for future studies.

Hunted deer

Between August and November 2019, samples from select hunted axis deer that presented visible bullet wounds in their forelimbs or hindquarters were obtained, since these sections are usually destined for human consumption. For each animal, three samples of muscle tissue were collected, including the bullet's entry point, the midpoint, and the exit point or end of the path when the bullet was lodged in the piece. In addition, control samples were collected from the furthest possible area, at least 40 cm from the bullet's trajectory (control). Each sample corresponded to 5 cm^2 of muscle, having been collected by the same person, using stainless steel scissors that were washed between samples (Gerofke et al. 2018). When present, macroscopic bullet fragments were removed from the samples in order to simulate the usual behavior of hunters. Samples were stored in sealed plastic bags and frozen (−20 °C) until processing.

Determination of Pb in biological samples

Lead Care® II

Blood samples were processed using the portable anodic extraction voltammetry analyzer, LeadCare® II (LC; ESA Biosciences, Inc., MA, USA) following the manufacturer's instructions (CLIAwaived.com) (Stanton and Fritsch 2007; Ortiz-Ortiz et al. 2017; Cindi et al. 2020). The LC detection range is 3.3 to 65 μg/dL.

Atomic emission spectrometry

Inductively coupled plasma atomic emission spectrometry (ICP-AES) was used to determine the Pb concentration in axis deer muscle samples. ICP-AES was performed using a Shimadzu 9000 system (Shimadzu Corporation, Kyoto, Japan), according to EPA 200.7 standards at the Laboratorio de Análisis Químicos (LANAQUI, CERZOS – CONICET, Universidad Nacional del Sur, Bahía Blanca, Argentina). Pre-treatment was performed with a MARS-5 microwave digester (CEM Corporation, Matthews, NC, USA) using nitric acid, according to EPA standard SW-3052 (400 W and 30 min; max pressure 400 psi; max temperature 160 °C); nitric acid was previously ultra-purified (sub-boiled) with a distillacid BSB-939-IR system (Berghof Products + Instruments, Eningen, Germany) (Damerau et al. 2012; Zowczak et al. 2016). Calibration was performed with the external aqueous method, with certified standards (Chem-Lab NV, Zedelgem, Belgium). Results were expressed in mg/kg of wet weight. The limit of detection (LOD) was 0.01 mg/kg.

Data analysis

Chi-square tests were used to evaluate the association of nominal and ordinal variables to one another (duty, frequency of game meat consumption, consumption of cured game meat, consumption of pickled game meat, use of bullet-struck portions of the carcass, most recent mode of consumption, sex). Because the age and the period since the most recent game meat consumption were not normally distributed (Anderson-Darling test), non-parametric tests (Mann-Whitney and Kruskal-Wallis) were used to evaluate the relationship of these variables to the aforementioned nominal and ordinal variables. Linear regression analysis was used to evaluate the relationship between quantitative individual variables (age and period since the last game meat consumption).

Due to inherent limitations of the LC method, 53.4% of the data was left-censored at the limit of detection (LOD = 3.3 μg/dL). We used the EnvStats R package (Millard 2018) to evaluate whether censored data could be imputed based on a normal, gamma, or lognormal distribution; however, none of these distributions could be applied to our data. We therefore assigned LOD divided by the square root of 2 to the censored values (Tekindal et al. 2017) and used non-parametric tests (Mann-Whitney and Kruskal-Wallis) to compare LC results among different categories of individual variables. Linear regression analysis was used to evaluate the relationship between LC results and quantitative individual variables. The Kruskal-Wallis test was used to compare the Pb concentration between control muscle tissue and bullet entry, middle, and exit point tissue samples of deer carcasses.

Results

Answers to the structured questionnaire and blood samples were obtained from 58 participants, comprising 49 males and 9 females. Average age was 40 years, ranging from 24 to 67 years. Participants comprised hunters (n=31), park rangers (n=14), and "other" duties (n=13). The "other" duty category comprised firefighters (n=10), researchers (n=2), and support staff (n=1). Housewives were grouped in the same duty categories as their husbands since they shared similar game meat consumption habits.

Questionnaire

Table 1 summarizes the questionnaire results and demographic information. The associations between individual variables are summarized in Table 2. Hunters and park rangers reported more frequently to feed dogs with the bullet-struck portions, whereas other participants either selectively consumed or discarded these areas (χ^2=22.38, df=4, P<0.001). Park rangers and hunters were generally older than participants of other duties (H=14.16, df=2, P=0.001). Participants reporting cured meat as a frequent mode of game meat consumption did not overlap with those reporting pickled meat as a frequent mode of consumption (χ^2=6.25, df=1, P=0.012). Participants with a "high" frequency of game meat consumption also reported having consumed cured game meat in their most recent game meat meal (χ^2=14.09, df=6, P=0.029). The most recent game meat consumption was more recent in participants reporting a "medium" (median = 1 day) or "high" frequency of game meat consumption (1.5 days) than in those with a "low" (10 days) or "very low" frequency (7 days) (H=12.01, df=3, P=0.007). Other associations between individual variables were not significant (P>0.05).

Blood Pb levels in game consumers

Blood Pb values of participants in this study are summarized in Table 3. Pb was detected in the blood at a quantifiable level in 27 of the 58 participants (46.6%) (24 males, 3 females, average age 42.0 ± 11.2 years, and range 24–67 years). The remaining 31 samples (53.4%) were below the LOD (3.3 μg/dL). Pb concentrations were significantly different among the participants' duty categories (H=18.25, df=2, P<0.001) (Fig. 1), and they varied significantly depending on the frequency of game meat consumption (H=9.32, df=3, P=0.025). Likewise, individuals who reported frequently consuming cured game meat had significantly higher Pb concentrations than those who did not (W=675.0, P<0.001). In contrast, there were no significant differences in blood Pb concentrations relative to the consumption of pickled game meat (W=193.5, P=0.657), use of bullet-struck portions of the carcass (H=5.58, df=2, P=0.062), and mode of consumption of the most recent game meat meal (H=5.18, df=2, P=0.075). Furthermore, there was no significant correlation between the blood Pb concentration and the age (S=1.807, P=0.561, R^2<0.001) or the

Table 1 Summary of the interview results with demographic details and information related to the consumption of game meat in humans according to their duties within the alien species control program at El Palmar National Park.

	Hunter	Park ranger	Other	Total
Sample size, *n*	31	14	13	58
Gender, *n*				
Male	29	10	10	49
Female	2	4	3	9
Age, years				
Mean ± S.D.	42.3 ± 11.0	43.8 ± 7.3	32.3 ± 6.8	40.4 ± 10.3
Minimum – Maximum	24–67	35–58	27–50	24–67
Frequency of game meat consumption, *n* (%)				
Very low (<1 meal per week)	3 (10%)	2 (14%)	5 (38%)	10 (17%)
Low (1–4 meals per week)	13 (42%)	8 (57%)	5 (38%)	26 (45%)
Medium (5–9 meals per week)	6 (19%)	1 (7%)	3 (23%)	10 (17%)
High (10–14 times per week)	9 (29%)	3 (21%)	–	12 (21%)
Most frequent modes of game meat consumption, *n* (%) a				
Cooked	31 (100%)	14 (100%)	13 (100%)	58 (100%)
Cured	19 (61%)	6 (47%)	3 (23%)	28 (49%)
Pickled	4 (13%)	1 (7%)	1 (8%)	6 (10%)
Use of bullet-struck portions of the carcass, *n* (%)				
Selective consumption	–	3 (21%)	6 (46%)	9 (16%)
Feeding of dogs	30 (97%)	9 (64%)	4 (31%)	43 (74%)
Discard	1 (3%)	2 (14%)	3 (23%)	6 (10%)
Mode of consumption of the most recent game meat meal, *n* (%)				
Cooked	27 (87%)	12 (86%)	10 (77%)	49 (84%)
Cured	3 (10%)	2 (14%)	2 (15%)	7 (12%)
Pickled	1 (3%)	–	1 (8%)	2 (3%)
Most recent game meat consumption, days				
Mean ± S.D.	11.1 ± 13.2	13.5 ± 14.5	5.5 ± 4.3	10.4 ± 12.3

[a] Variable reported as the frequency of occurrence, i.e., categories are not mutually exclusive

Table 2 Matrix of association between individual variables

Variable	1	2	3	4	5	6	7	8	9
1. Duty (3 categories)		0.123	0.062	0.790	0.001*	0.808	0.114	0.001*	0.589
2. Frequency of game meat consumption (4 categories)	CS		0.059	0.488	0.294	0.029*	0.133	0.595	0.007*
3. Frequent consumer of cured game meat (yes/no)	CS	CS		0.012*	0.405	0.105	0.329	0.975	0.919
4. Frequent consumer of pickled game meat (yes/no)	CS	CS	CS		0.503	0.122	0.268	0.990	0.979
5. Use of bullet-struck portions of the carcass (3 categories)	CS	CS	CS	CS		0.422	0.435	0.327	0.605
6. Mode of consumption of the most recent game meat meal (3 categories)	CS	CS	CS	CS	CS		0.093	0.697	0.766
7. Sex (male/female)	CS	CS	CS	CS	CS	CS		0.471	0.897
8. Age (integer)	KW	KW	MW	MW	KW	KW	MW		0.437
9. Period since most recent game meat consumption (integer)	KW	KW	MW	MW	KW	KW	MW	LR	

Bottom-left cells represent the statistical test and top-right cells represent the *P*-value. *CS* Chi-Square test, *KW* Kruskal-Wallis test, *MW* Mann-Whitney test, *LR* Linear regression analysis. Asterisks indicate significant associations

period since the most recent game meat meal (S=1.766, P=0.090, R^2=0.034).

Pb in deer meat

Twenty-nine muscle samples from seven axis deer were analyzed: seven from the bullet entry point, eight from the middle of the trajectory, seven from the bullet exit point, and seven from the control zone. Pb concentrations were significantly different according to the location of the sample relative to the bullet trajectory (Fig. 2, H=18.03, df=3, P<0.001). Pb was detected in all but one of the samples analyzed (28/29, 96.6%); the only sample without detectable levels was from the control zone. Pb concentrations were lowest in the control samples (geometric mean ± geometric S.D. = 0.107 ± 4.828 mg/kg, median = 0.26, range = 0.007–0.33) followed by the bullet entry point (50.622 ± 17.886 mg/kg, median = 112, range = 0.35–1612), exit point (161.578 ± 17.216 mg/kg, median = 157, range = 6.4–6348), and the bullet path mid-point (1226.879 ± 5.669 mg/kg, median = 695, range = 105–27963).

Discussion

Pb from ammunition is considered an important source of exposure for game meat consumers (Green and Pain 2019; Thomas et al. 2020), and the regularity of this consumption,

Table 3 Blood Pb concentration (determined by LeadCare® II) in humans according to their duties within the alien species control program at El Palmar National Park, their frequency of game meat consumption, and whether they frequently consumed cured game meat

	Hunter	Park ranger	Other	Total
Blood Pb, μg/dL [*n*]				
Geometric mean ± S.D. (all participants)*	3.99 ± 1.53 [31]	2.70 ± 1.34 [14]	2.42 ± 1.14 [13]	3.25 ± 1.51 [58]
Median (all participants)*	4.00 [31]	2.33 [14]	2.33 [13]	2.33 [58]
Geometric mean ± S.D. (participants ≥ LOD)	4.81 ± 1.38 [23]	4.63 ± 1.03 [3]	3.70 [1]	4.75 ± 1.35 [27]
Median (participants ≥ LOD)	4.40 [23]	4.60 [3]	3.70 [1]	4.50 [27]
Maximum	9.50	4.80	3.70	9.50
Blood Pb relative to the frequency of game meat consumption (geometric mean ± S.D., all participants), μg/dL*				
Very low (<1 meal per week)	2.75 ± 1.33 [3]	2.33 ± 1.00 [2]	2.56 ± 1.23 [5]	2.57 ± 1.22 [10]
Low (1–4 meals per week)	3.66 ± 1.45 [13]	2.76 ± 1.36 [8]	2.33 ± 1.00 [5]	3.07 ± 1.43 [26]
Medium (5–9 meals per week)	3.70 ± 1.37 [6]	4.80 [1]	2.33 ± 1.00 [3]	3.31 ± 1.41 [10]
High (10–14 times per week)	5.41 ± 1.58 [9]	2.33 ± 1.00 [3]	–	4.38 ± 1.73 [17]
Blood Pb relative to consumption of cured game meat (geometric mean ± S.D., all participants), μg/dL*				
Frequent consumer of cured meat	4.49 ± 1.47 [19]	2.95 ± 1.44 [6]	2.72 ± 1.30 [3]	3.89 ± 1.52 [28]
Not a frequent consumer of cured meat	3.32 ± 1.54 [12]	2.53 ± 1.26 [8]	2.33 ± 1.00 [10]	2.75 ± 1.39 [30]

*Data were heavily left-censored by the limit of detection (LOD = 3.3 μg/dL). Values below the LOD were imputed as 2.33 μg/dL

Sample size is provided with-in brackets

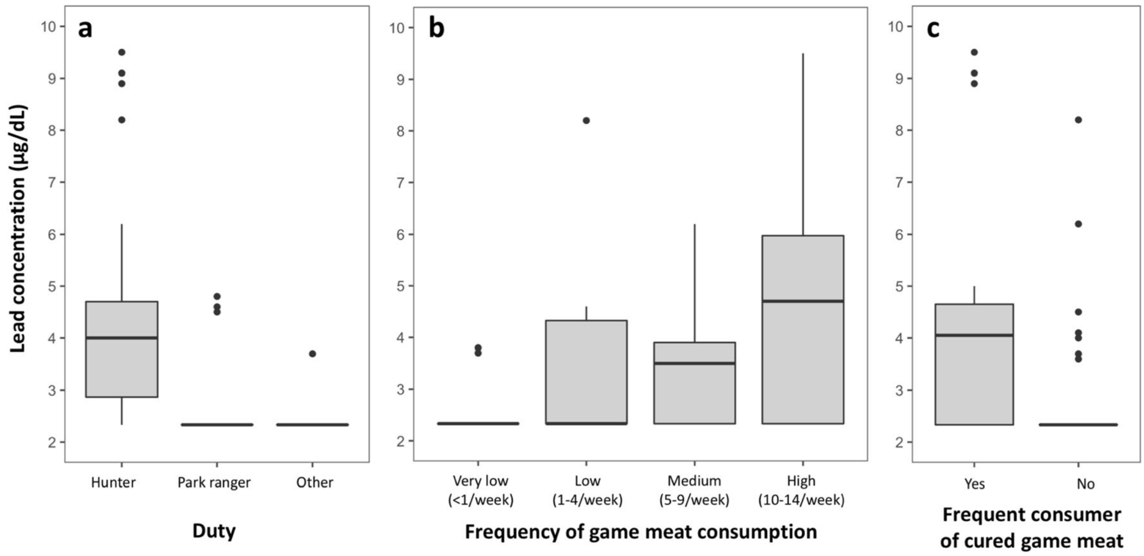

Fig. 1 Boxplot of blood Pb concentrations (determined by LeadCare® II) relative to the individual variables with significant differences

even at low levels, is considered a central risk factor (Kosnett 2009; Knutsen et al. 2015). This is the first study to confirm Pb exposure in people who regularly consume game meat in Argentina. Although the group of consumers investigated was small, the findings suggest that risk is not negligible and probably extends to other areas with similar hunting practices.

Although the dynamic storage of Pb in the body limits dose-effect estimates between consumption and Pb blood levels (Buenz et al. 2017), blood Pb reflects recent exposure (~30 days) (EFSA 2013; WHO 2019). In this study, nearly half (46.6%) of the examined people had detectable Pb in their blood, including more than a quarter (26%) of those who reported their last game meal more than 30 days earlier.

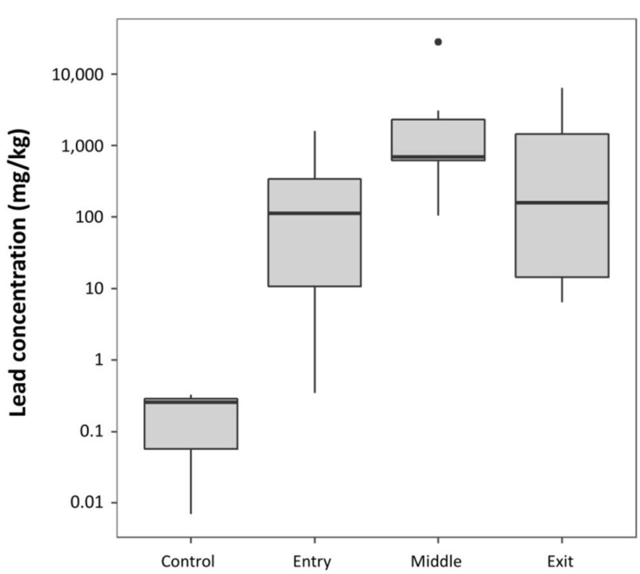

Fig. 2 Boxplot of muscle Pb concentrations (determined by ICP-AES, represented in a $\log_{10}$ scale) in samples of deer carcasses according to location along the bullet path and control

Blood Pb in our study was higher than reported for game meat consumers in Scandinavian countries and the United States (Iqbal et al. 2009; Bjermo et al. 2013; Meltzer et al. 2013), but similar to blood Pb reported for individuals with high game consumption frequency in Norway (Birgisdottir et al. 2013), as summarized in Table 4. This could reflect differences in real intake or be an artifact of the different Pb quantification methods. For reasons of practicality and logistics in this study, we used the LeadCare® II method (LC), but the reference method is inductively coupled plasma mass spectrometry (ICP-MS) (Bonnefoy et al. 2002; Palmer et al. 2006). Nevertheless, high agreement between the results of ICP-MS and LC has been demonstrated (Sobin et al. 2011; Neri et al. 2014; Caldwell et al. 2019), and LC is one of the methods endorsed by the World Health Organization (2020). Furthermore, in our study blood Pb concentrations were lower than those reported for shooting range users measured with LC (Mathee et al. 2017; Naicker et al. 2018), which suggests that LC is sufficiently sensitive to detect varying levels of exposure since shooting ranges are particularly polluted sites (Mathee et al. 2017). Notwithstanding, although ease of use, low cost and minimum volumes required make LC a practical tool for screening (e.g., in children), a limitation of our study was the high proportion (53.4%) of results below the limit of detection. Under similar logistical constraints, the most modern version of the analyzer (LeadCare® System), with an LOD of 1.4 µg/dL (Sobin et al. 2011), would be a suitable option to overcome these limitations.

Despite having eliminated visible bullet fragments as is commonly done by hunters during slaughter, we found high levels of Pb in impacted areas of axis deer muscle, with a maximum Pb concentration of 27,963 mg/kg. This value is far higher than reports in previous studies in large game, such as red deer (*Cervus elaphus*) with Pb levels up to 3442 mg/kg

Table 4 Blood Pb concentrations in game meat consumers: a comparison between previous studies and our study

Reference	Location	Age (years)	Years sampled	n	Median, ug/dL (5–95th percentiles)	Geometric mean, ug/dL (range)	Method	Remarks
Iqbal et al. 2009	United States / North Dakota	2–92	2008	736	–	1.27 (0.18–9.82)	ICP-MS	Recent game meat consumption (<1 month ago) and larger serving size (≥ 56.7 g of wild game per serving) was associated with increased blood Pb concentrations. Ninety-two percent of participants (447/486) reported discarding the meat around the wound channel. Most of the participants reported grinding their venison (57.5%) but not grinding other game meat (57.3%)
Bjermo et al. 2013	Sweden	18–74	2010–2011	273	1.34 (0.58–2.86)	–	ICP-MS	Total meat intake was not related to blood Pb, but when examining categories of meat consumed, frequency of game intake was associated with blood Pb levels
Meltzer et al. 2013	Norway	18–76	2012	147	1.66 (0.75–3.9)	1.70 (0.60–6.93)	ICP-MS	Half of the participants ate game meat once a week or more, which is equivalent to an average consumption of >20 g per day. Blood Pb concentrations were mostly associated with consumption of minced cervid meat, particularly purchased minced meat
Birgisdottir et al. 2013	Norway	-	2003	184	2.45 (0.86–6.51)	–	ICP-MS	Blood Pb concentrations in men were associated with frequent consumption of game meat
This study	Argentina/Entre Ríos	24–67	2019	58	2.33 (2.33–8.31)	3.25 (2.33–9.50)	LC	Blood Pb concentrations were higher in consumers who reported higher frequency of game meat consumption (more than 5 times per week) and in participants who reported frequently consuming cured game meat

(Martin et al. 2019) and wild boar up to 8704 mg/kg (Menozzi et al. 2019). When considering Pb levels along the bullet's path, Pb levels in our study ranged from a geometric mean of 50.6 ± 17.9 mg/kg at the entry point to 1226.9 ± 5.7 mg/kg at the middle of the bullet trajectory. These results are remarkably higher than those reported by Müller-Graf et al. (2017), who detected 14.0 mg/kg and 14.3 mg/kg near the bullet path in roe deer and wild boar, respectively. In contrast, Dobrowolska and Melosik (2008) reported 233.3 mg/kg and 149.9 mg/kg Pb mean values at bullet entry point and 78.9 mg/kg and 178.3 mg/kg at the exit point in red deer and wild boar, respectively, which resembles our findings. Variations between studies may be due to ammunition fragmentation, which is conditioned by various factors such as the caliber, the bullet type and mass, the resistance of impacted tissue, and the distance to the prey (Kollander et al. 2017; Martin et al. 2019; Broadway et al. 2020). At PNEP, the most widely used calibers are .308, 30.06 and .270 with high velocity rifle bullets ranging from 15 to 18 *g* (Sosa, unpubl. data) for which risk of fragmentation has been reported (Hunt et al. 2006; Hunt et al. 2009; Grund et al. 2010; Stewart and Veverka 2011; Broadway et al. 2020). Our results coincide with other studies in that Pb concentrations varied along the bullet trajectory in the game meat and were lower in the most distant areas (e.g., control zone) (Dobrowolska and Melosik 2008; Martin et al. 2019; Menozzi et al. 2019). Dobrowolska and Melosik (2008) found higher Pb levels in tissues where the maximum expansion of the bullet occurred, which could involve the entry or exit path zone, depending on the resistance of the tissue in adult or young animals. This could explain the similarities in Pb levels found at both ends of the bullet path in our study. It remains unclear why Pb was higher in the middle of the bullet trajectory.

We simulated the maximum intake risk by sampling the complete bullet trajectory in game meat. However, it is likely that under domestic conditions, consumers would have removed some or all affected sections, e.g., to feed their dogs (Menozzi et al. 2019, Fernandez et al. unpubl. data), although the findings of lead in consumers' blood suggests that some contaminated parts are being consumed. In fact, only the pieces without visible bullet wounds are donated to community and school canteens, while hunters are the ones who receive the damaged pieces (Sosa, unpubl. data). However, our aim is to demonstrate that there are serious risks to consumers' health since the incomplete elimination of these tissues would result in the ingestion of significant amounts of Pb (Dobrowolska and Melosik 2008; Hunt et al. 2009; Tsuji et al. 2009). Furthermore, although the highest Pb levels were found in the wound channel, toxic concentrations of Pb have been detected in meat taken 15–25 cm from the bullet's trajectory (Menozzi et al. 2019) and dispersion of Pb fragments up to 45 cm has been reported (Hunt et al. 2009; Grund et al. 2010). This is consistent with our finding of low but detectable Pb values in control samples. Likewise, the authors have observed that after slaughter, EPNP hunters often rinse the meat with water and some participants even reported leaving it to soak overnight. It has been documented that washing does not remove Pb from game meat, but rather disperses it to other areas (Grund et al. 2010).

Many studies have reported elevated Pb levels in the blood of frequent game meat consumers (Iqbal et al. 2009; Birgisdottir et al. 2013; Bjermo et al. 2013; Meltzer et al. 2013; Wennberg et al. 2015). In the present study, blood Pb concentrations varied according to the frequency of game meat consumption, being higher in people who reported a higher intake frequency. Likewise, Pb levels were higher among hunters, who consume game meat more frequently. However, game meat is often shared with family and friends, so the potential health impacts of Pb could extend beyond the hunters themselves (Sevillano Morales et al. 2018; Gerofke et al. 2019). This is relevant because pregnant women and children are highly vulnerable due to their greater sensitivity to the neurotoxic effects of Pb (Gerofke et al. 2019). Although the people who participated in this study were adults, 37 of them (68.5%) reported having children in their household with whom they potentially shared game meat meals (Tammone unpubl. data).

The risk of exposure to Pb is also related to the manner in which the meat is prepared for consumption (Mateo et al. 2007). In the present study, the most frequent game meat consumers also reported consuming cured meat regularly. These individuals also showed a tendency to present higher blood Pb values. This could be associated with the use of meat from areas close to the wound channel to produce sausages, as these sections of the carcass are considered of lower quality for other preparations (Falandysz et al. 2005; Tsuji et al. 2009; Stokke et al. 2017). Hunters reported consuming cured and pickled meat more frequently, again highlighting the increased risk of Pb exposure in this group. Although an association with blood Pb levels could not be established in those people who reported consuming pickled meat, it is known that the use of vinegar, wine, lemon juice, or other acidic substances increases the conversion of inorganic to organic Pb, enhancing its absorption into the bloodstream (Mateo et al. 2011; Menozzi et al. 2019). Regardless of the mode of preparation and consumption, however, the large dispersion of Pb fragments (visible and microscopic) in game meat and the difficulty in eliminating them are likely leading to significant Pb intake in regular game meat consumers (Tsuji et al. 2009; Lindboe et al. 2012; Fachehoun et al. 2015; Green and Pain 2019). For this reason, although discarding meat portions near the bullet trajectory may be helpful, it is not sufficient, and the adoption of non-toxic ammunition is warranted. Buenz and Parry (2017) recorded a rapid decrease in blood Pb levels in consumers immediately following replacement by meat from animals hunted without Pb ammunition.

In Argentina, the program to control invasive alien mammals at EPNP is considered a successful model (Gürtler et al. 2017; Ballari et al. 2019) that contributes to the conservation of native biodiversity. However, environmental contamination with Pb as a result of shots that miss the target species has been identified as an undesirable collateral effect of the program (Gürtler et al. 2017). The Pb levels found in hunted deer muscle in our study suggest that there is also a high potential for environmental contamination due to unrecovered shot animals. During 2015, 197 wild boar and 513 axis deer were reported hunted, of which 9 (4.6%) wild boar and 16 (3%) axis deer escaped injured (Gürtler et al. 2017; Gürtler et al. 2018). It is thus probable that Pb from missed shots and Pb contained in carcasses of injured animals are both noteworthy sources of environmental contamination, as mentioned in other studies (García-Fernandez et al. 2008; Romano et al. 2016). This is particularly concerning for scavengers, for whom secondary Pb exposure can become a significant conservation problem (Lambertucci et al. 2011; Legagneux et al. 2014; Wiemeyer et al. 2017; Arrondo et al. 2020; Slabe et al. 2020).

Pb exposure from hunting constitutes a global environmental health problem (Hampton et al. 2018), and growing scientific evidence reinforces the urgent need to replace Pb ammunition with non-toxic alternatives (Fachehoun et al. 2015; Arnemo et al. 2016; Kanstrup et al. 2018; Thomas et al. 2020). Recognizing the risk of Pb dietary intake and its consequences for game meat consumers and for the environment is a necessary first step in collectively committing to transition to non-toxic alternatives (Broadway et al. 2020). Our work therefore provides further evidence that while it is important to support invasive species control programs in protected areas, it is also necessary to adapt practices to reduce or eliminate exposure to Pb in consumers and in the environment. Although regulatory frameworks restricting the use of Pb ammunition are incipient in Argentina, recent progress is encouraging (Plaza et al. 2018; Uhart et al. 2019). In 2019, the National Parks Administration established a period of 2 years for the gradual replacement of Pb bullets for the control of alien species at EPNP (RES. HD N° 289-2019) and in other protected areas (RES. HD N° 417-2019). This is a prominent indicator of success and could position the EPNP control program as a pioneer and model for the country and South America. Notwithstanding, availability of non-lead bullets is yet to be resolved.

In conclusion, the results from the current study reveal Pb dietary intake risk and emphasize the relevance of replacing Pb ammunition with non-toxic alternatives to reduce exposure and promote sustainable hunting practices. In our research area as in similar contexts, this change would allow local people to consume game meat safely even if the consumption is frequent, without posing collateral risks for wild animals and the environment.

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Availability of data and materials Supporting data and materials will be made available online upon acceptance at https://zenodo.org

Author contribution All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by AEC, AT, WEC, VF, RETV, and MMU. The first draft of the manuscript was written by MMU, AT, and VF, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Declarations

Ethics approval and consent to participate All procedures performed in this study involving human participants were in accordance with the ethical standards of the Argentinean National Research Committee (Central Committee of Bioethics in Practice and Biomedical Research of the city of Paraná, Entre Ríos province, reference number 2312401) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

Consent for publication Informed consent was obtained from all individual participants included in the study.

Competing interests The authors declare no competing interests.

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