Use of poisoned baits against wildlife. A retrospective 17-year study in the natural environment of Extremadura (Spain)☆

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ABSTRACT

This study reports the results obtained from toxicological analyses of different types of baits referred to the laboratory of the Toxicology Area (Faculty of Veterinary Medicine, Cáceres, Spain) over a 17-year period (2002–2018). These baits were suspicious materials found in the environment of the region of Extremadura (Western Spain), where such malpractices are a problem to be addressed, as wide livestock farming and hunting activities are combined with a significant wealth of wildlife (especially birds of prey). A total of 246 baits, including 32 commercial chemical products to be used in baits, were analysed. Samples from 183 cases were received and classified according to the material used for their preparation and the toxic substance found. Overall, the most common bait consisted of meat preparations (56.3% of cases) intended to eliminate predators considered ‘annoying’ for livestock and hunting practices, such as carnivores and scavengers. It should be noted that contact baits (as fenthion-impregnated perches) were also detected (7.6%). Regarding the substances detected, anticholinesterase compounds (organophosphates and carbamates) were the most commonly used substances for the preparation of baits (detected in 85.3% of positive baits). Moreover, 8% of the positive baits presented more than one toxic substance in their composition. Due to the types of toxic compounds and the methods used to prepare the baits, this study shows that the malicious use of highly toxic substances in the environment to kill wildlife is a common and current issue and poses a serious risk to different species.

1. Introduction

Malicious poisonings through use of baits in wildlife is a global issue (Berny, 2007; Giorgi and Mengozzi, 2011; Margalida, 2012; Motas-Guzmán et al., 2003; Marquez et al., 2013; Margalida et al., 2014; Ruiz-Suárez et al., 2015; Chiari et al., 2017). Some authors point out humans and predators to be the roots of the conflict (Mateo-Tomás et al., 2012), since both share the same interests for certain limited resources with commercial value, such as wild game or breeding (Cozza et al., 1996; Reynolds and Tapper, 1996; Kaczensky, 1999; Pedersen et al., 1999; Mech et al., 2000; Mazzoli et al., 2002; Graham et al., 2005; Thirgood and Redpath, 2008; Sotherton et al., 2009; Cano et al., 2016). The conflict gains particular relevance when shared resources hold an economic interest and involve predators that are legally protected (Thirgood et al., 2000), leading to unjustified prosecutions that carry severe consequences for these species (Villafuerte et al., 1998). Therefore, predators that feed on small game animals and that may attack livestock have been the main objects of poisoning. It should not be forgotten that this conflict also affects some other activities such as beekeeping and pigeon-breeding; pets are also affected accidentally or intentionally (Bodega-Zugasti, 2014; Cano et al., 2016). This disposal method is massive and non-selective. It seeks to cause death in a short span of time and makes quantification and control over the affected area virtually impossible.

Considering its geographical location and environmental diversity, Spain holds one of the most favourable habitats for wildlife in Europe. However, poisoning is considered one of the main causes of mortality in the wild (Mateo-Tomás et al., 2020; De la Bodega et al., 2020), as in other European countries with similar environmental characteristics (Guitart et al., 2010; Berny et al., 2015; Chiari et al., 2017; De Roma

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et al., 2018; Ntemiri et al., 2018; Di Blasio et al., 2020; Bertero et al., 2020), which is why the presence of poisons as a lethal preparation in the natural environment led us to evaluate their possible ecological impact.

In Spain, the use of poison for hunting and fishing is a criminal offence punishable by law, due to its impact on the species conservation and its massive and non-selective character (Espana, 2007a,b).

All the above-mentioned has given rise to different campaigns against such misconduct have been set in motion involving both private and public sectors in the last decades. These initiatives have enabled us to collect data to assess the magnitude of the issue and the effectiveness of different strategies to cope with it, as well as to plan future measures. At the same time, a highly debatable topic is the use of poisons in a controlled way to deal with pest control. However, it has been proven to be harmful to different species of wildlife (Vituela et al., 2016; Vituela, 2019).

Specifically, Extremadura (the region of Western Spain where this study was conducted) possesses a high environmental value for biodiversity, and the successive events of wildlife poisoning (Soler et al., 1996; Brown et al., 1996; Mineau et al., 1999; Berny, 2007; Berny et al., 2009; Guitart et al., 2010; Caloni et al., 2018; Gupta, 2018) are some examples) but there is a lack of studies on the types of bait used in the environment of Extremadura and the importance of biodiversity in this region of Extremadura where these malpractices are an important issue.

In addition to the legal regulations generated by different governments, the EU Action Plan, 2015 to prevent illegal poisoning of wildlife included a complete list of suggestions to improve the control over legal substances used as poisons and make them less available. Moreover, the COST Action European Raptor Biomonitoring Facility (CA16224), where our toxicology group participates, is promoting to initiate a network of veterinary forensic toxicology laboratories in Europe to improve communication among them to prevent wildlife poisoning (Valverde et al., 2022).

Compilation of data obtained from the toxicological analyses of the different types of baits referred to the Toxicological Diagnostic Laboratory of the Veterinary Faculty of Cáceres (University of Extremadura, Spain) in a 17-year period (2002–2018) is herewith presented. All of them were suspicious materials found in the natural environment of the region of Extremadura where these malpractices are an important issue to be considered, as extensive stockbreeding and hunting activity is performed alongside a varied and rich wildlife (Fig. 1). Although most of the surface of the region is considered hunting area (over 80% consisting of enclosed areas), the Natura 2000 Network of protected areas is made up of 160 spaces that are distributed over 1,264,288 ha, 30.3% of the Extremadura surface (Rengifo and Sánchez, 2016). It must be highlighted that Extremadura is one of the regions that hosts the largest populations of some of the most threatened birds of prey throughout the world, such as the Cinereous vulture–Aegypius monachus, the Egyptian vulture–Neophron percnopterus, the Spanish imperial eagle–Aquila adalberti and the Red kite–Milvus milvus.

There are numerous articles about the epidemiology of poisonings in both domestic animals and wildlife (Antoniou et al., 1996; Franson et al., 1996; Brown et al., 1996; Mineau et al., 1999; Berny, 2007; Berny et al., 2009; Guitart et al., 2010; Caloni et al., 2018; Gupta, 2018 are some examples) but there is a lack of studies on the types of bait used (Giorgi and Mengozzi, 2011; Margalida, 2012; Chiari et al., 2017). Due to this, the毒ological lab has information on the types of poisoned baits used in the environment of Extremadura and the importance of biodiversity in this Spanish region, we collected data for an analysis of the baits received in our laboratory from 2002 to 2018. The main objective was to collect information about the types of baits that have been detected in recent decades to poison fauna, the materials and the substances that have been used to prepare them, and the modalities of application carried out according to the target species. Results will be of interest for the public administration, the scientific community and regulatory agencies because as this malpractice continue to be a threat to wildlife in a natural environment as rich and delicate as the one existing in Extremadura, which may be a reflection of other national and international natural environments.

2. Material and methods

2.1. Study area and sample collection

All the data presented in this work were obtained from our database of potential poisoned baits found in the environment of Extremadura (Spain) (Fig. 1). This region is located in Western Spain, with an area of 41,634 km² and 1,063,987 million people (25.5 people per km²), a low density (if compared with the Spanish average of 94 people/km²) (INE, 2020). As prescribed by law, baits were collected in the field from the whole of Extremadura by Forest Rangers of the regional or national governments during their work of inspecting the natural environment, when wildlife mortality was observed or after complaints by citizens. Subsequently, they were submitted to the ‘Los Hornos’ Wildlife Recovery Center, located in Sierra de Fuentes (Cáceres), where specialised staff evaluated each of the cases. In the event of suspicious evidence of poisoned bait, the sample was sent to our laboratory along with a Record of Delivery in which all details involved were outlined.

A ‘bait’ refers to an individual sample. Chemical substances obtained in the entrance to burrows or beehives, in farms or private buildings, and suspected to be used for the preparation of poisoned baits were also analysed. A ‘case’ is an incident occurring at a certain location and date, and can involve more than one single bait or substance.

2.2. Sample inspection

Once the samples were received at the Toxicology Laboratory, all data related to the case and bait were registered (assigned a register number, type of bait, date and collection point). The different baits were classified according to the material used in their preparation. Every sample was visually examined with a magnifying glass to evaluate the manipulation of the base material (e.g. carcasses, meat, sausages, fish, canned food) and the presence of suspicious chemicals (smell, granular or coloured or unusual substances, for example) on or within the base material.

When suspicious materials were observed in baits (e.g. coloured pellets, grains, paste or microgranules), they were removed whenever possible and separately analysed to facilitate the toxicological analysis. For example, red or blue grains or paste were directly analysed for anticoagulants rodenticides, small blue/green pellets for metaldehyde or black or violet microgranules for aldicarb and carbofuran, respectively. If the specific analysis was negative, then the remaining groups of toxic compounds were investigated.

2.3. Toxicological analysis

The toxicological analysis consisted of a screening that covers the most common toxic substances involved in both intentional and accidental intoxications in animals. Even when visual examination of the bait and associated data did not suggest toxic risk, analysis of anticholinesterase pesticides (carbamates and organophosphates) was routinely carried out with all samples, as they are often involved in intentional poisoning worldwide due to their simplicity of handling, especially as granular samples, and they are the most frequently detected poisons in Extremadura (Soler et al., 2006).

When poisoned baits are analysed, the chemical compounds are in such high concentration that the limits of detection or quantification are not of great importance. In these cases, the detection of large concentrations (>ng/kg) of these substances is sufficient to identify it as a poisoned bait (for the malicious killing of wildlife or companion animals). Due to degradation of some toxic compounds by the physical or
Fig. 1. Geographical distribution of poisoned baits between 2002 and 2018 in this study. Coloured areas allow verify the incidence in Protected Areas that coexist with hunting activity (modified from Rengifo and Sánchez, 2016).
chemical conditions of the environment where the bait is found, the usual rigorous standards of validation of a method in residue analysis cannot be applied, and many residue measurements must be regarded as only semiquantitative (Brown et al., 2005). The main requirement when analysing a poisoned bait is to identify the responsible compound, rather than to measure it accurately. Methods cannot be validated for all sample types and limits of determination/quantitation may vary, but the procedures must be sufficiently robust to cope with most eventualities (Brown et al., 2005).

Analytical chemistry was performed to determine the presence of the following groups of pesticides:

- Carbamates: aldicarb, bendiocarb, carbofuran, carbaryl, methiocarb, methomyl, oxamyl, pirimicarb and propoxur.
- Organophosphates: acephate, azinphos-methyl, cadusafos, chlorfenvinphos, chomemphos, chlorpyrifos-ethyl, chlorpyrifos-methyl, coumaphos, diazinon, dichlorvos, disulfoton, ethion, ethoprophos, fenamiphos, fenitrothion, fenthion, fonophos, heptenophos, isofenphos, malathion, methidathion, methamidophos, mevinphos, monocrotophos, omeothoate, parathion-ethyl, parathion-methyl, phenthoate, phorate, phosalone, phosmet, pirimiphos-methyl, pirimiphos-ethyl, profenofos, sulfotep, terbuthox, tetrachlorvinphos and trichlorfon.
- Organochlorines: aldrin, DDT, dieldrin, endrin, endosulfan, heptachlor, hexachlorobenzene, lindane and methoxychlor.
- Pyrethroids: ametryn, bifenthrin, cyfluthrin, cypermethrin, deltamethrin, fenvalerate, lambda-cyhalothrin, permethrin, phenothrin, tetramethrin.
- Anticoagulant rodenticides: brodifacoum, bromadiolone, clotrazol, dicoflazine, coumatrelol, difacinone, difentraone, floxanum and warfarin.
- Other: strychnine, α-chloralose and metaldehyde.

Pesticide reference standards from Sigma-Aldrich, Dr. Ehrenstorfer and ChemService were used for the identification and quantification of these pesticides as described later.

2.3.1. Sample treatment

As suspected poisoned baits may be composed by a wide range of compounds covering different chemical types, from the nonpolar organochlorines (OCs) through the wide polarity ranges of organophosphates (OPs), carbamates and herbicides, multisriseide analytical methods were selected and applied for the determination of the different groups of pesticides; otherwise for certain individual compounds, such as metaldehyde and strychnine, single qualitative methods were used. Procedures for the extraction, purification, and detection of pesticides, considered to be fit for the purpose of our investigation, were validated in our lab, and they varied over time. The extraction was performed using specific solvents for each group of pesticides.

Suspected bait materials usually have the poison on their outer surface and the amount present is usually very large (according to residue analysis standards). When the suspicious material (microgranules, powder, formulations …) was easily removed from the bait without interfering materials, a small amount was dissolved in a suitable solvent and diluted with the same solvent before its analysis without further clean-up. If the result of the analysis was negative, a concentration 2 orders of magnitude higher was tried (Brown et al., 2005).

In general, for the analysis of the vast majority of baits a clean-up step of the extracts was required after solvent extraction. Different purification techniques were used depending on the types of toxic compounds and the time of analysis. Initially, clean-up was carried out using solid phase extraction (SPE, Florisil and C18 cartridges) according to Stahr (1991). Later, the methodology proposed by Brown et al. (1996, 2005) using gel permeation chromatography (GPC) with glass columns filled with Bio-Beads S-X3 as the stationary phase and n-hexane/ethyl acetate (1:1, v/v) as the mobile phase (pumped by a Merck-Hitachi HPLC Pump L-7100 and collected in a Waters Fraction Collector) was applied. During the last 3 years of the study the methodology proposed by Luzardo et al. (2014) comprised of a general solid-liquid extraction followed by purification steps (freezing centrifugation, alone or followed by GPC with Bio-Beads S-X3 in very dirty samples) has been applied in the extraction and purification process.

2.3.2. Instrumental analysis

Extract analysis was carried out in our laboratory using diverse chromatographic techniques as analytical equipment changed throughout the investigational period.

During the first years, qualitative thin-layer chromatography (TLC) was used as the initial step (screening) of the analysis of anticholinesterase pesticide. Extracts and pesticide standards were applied on silica-gel G60 plates using a semi-automatic sampler (Camag Linomat IV) and subsequently revealed by spraying acetylcholinesterase onto the plate (Zoun and Spierenburg, 1989). Results were confirmed by more sophisticated chromatographic techniques.

For the semiquantitative determination of toxics in the extracts, the following chromatographic techniques were used:

- high performance liquid chromatography (HPLC; Shimadzu UFLC System composed by a LC 20AD pump and a SIL-20AHT autosampler) coupled to a diode array detector (HPLC–DAD; Shimadzu SPD-M20A) and a fluorescence detector (HPLC–FL; Shimadzu RF-10A XL).
- high performance liquid chromatography coupled to a mass detector (ion trap) (HPLC-MS; UHPLC 3000 Ultimate Bruker Daltonics System with Thermo autosampler and an Ion Trap Amazon SL MS detector).
- capillary gas chromatography (GC; Agilent 6890N with 7683G2614A autosampler) coupled to a nitrogen phosphorus detector (GC-NPD) and an electron capture detector (GC–ECD).
- capillary gas chromatography coupled to electron impact mass spectrometry (GC–MS; Shimadzu GC–2010 coupled to MS-QP2010 Plus detector and AQC-20i autosampler) in full scan mode.

The use of GC-MS allowed the search for unknown compounds when a qualitative study of the peaks (peak by peak) was done. The comparison of the peak mass spectrum with a library of mass spectra (Shimadzu XL), and its subsequent quantification with an appropriate standard. This allowed the identification of new pesticides (atrazine, ethoxyquin, imidacloprid, metalaxyl, thiram and triflumuron) and drugs (phenobarbital) that were not previously included in the analysis.

By using MS detectors, compound identification was carried out using retention time and full-scan mass spectrum data for each compound.

The chromatographic techniques used in the analysis of the extracts for each group of pesticides were the following: TLC for strychnine and anticholinesterase pesticides; GC–ECD and GC–MS for organochlorines and pyrethroids, GC–NPD and GC–MS for organophosphates; HPLC–DAD and HPLC–MS for carbamates; HPLC–DAD/FL and HPLC–MS for anticoagulant rodenticides; GC–MS for metaldehyde and α-chloralose; HPLC–MS for strychnine.

Colorimetric methods were also used when necessary. So, the method described by Saldana et al. (1981) was applied for the analysis of strychnine. In the case of metaldehyde, chloroform extraction (Jones and Charlton, 1999) was followed by colorimetric detection (Smith, 1987).

2.3.3. Data quality controls

All of the procedures described above were tested as they were developed. As previously indicated, methods cannot be validated for all sample types and limits of determination/quantitation may vary and are not of great importance in our case, but the procedures must be sufficiently robust to cope with most eventualities (Brown et al., 2005).
In order to monitor instrumental output and column performances in the chromatographic analysis, work mix standard solutions were checked at the beginning of the analytical session. In every analytical batch a negative control (blank) was analysed and in every sample triphenyl phosphate (TPP) was added before extraction as internal standard (IS). The mean recovery of pesticides in the different semiquantitative methods was in the range of 65–85% and the limits of quantification were in the range of 0.01–0.5 mg/kg.

3. Results

3.1. Types of baits

Between 2002 and 2018, 246 samples were analysed, of which 197 were positive (Figs. 2 and 3). Table 1 shows the number of cases in which each type of bait appears, and it was noteworthy that 12 cases/16 baits included more than one type of bait (8.1/8.5% of the cases/baits). The average number of total baits per case was 1.34. The case with the largest number of samples presented 10 commercial baits with the anticoagulant rodenticide brodifacoum located on the ground, numerically followed by one case with six pieces of fresh meat. In relation to the type of bait, the table shows that most poisoned baits were constituted by meat (56.3% of the baits), in different formats: a) 30.9% of the baits were pieces of fresh meat joined to bones and/or skin; b) 14.2% were cured dried meats and sausages (typical Spanish products consisting of cured and spiced fatty meat, which remain long unaltered in the environment) (Fig. 4); c) 4.6% of the baits consisted of viscera of different species and the placentas of recent births; d) 6.6% were complete animal carcasses covered or filled with poison (the animals used were small, such as rabbits, pigeons, quail and new-born lambs). Commercial chemical products or substances, i.e., substances that are not sold to be used as baits (mainly commercial forms of agricultural pesticides as microgranules, concentrates solutions or powdered), but are used maliciously with that purpose, made up 16.2% of the samples. These powdered and granular substances were placed directly in the environment (at the entrances to burrows or beehives) without any food support or were found in farms or private buildings and suspected to be used for the preparation of poisoned baits.

Most of the baits were designed to poison by ingestion, but 7.6% of the samples consisted of objects used as perches and impregnated with a pesticide (fenthion) to be toxic by contact. These were objects such as ropes, tree branches and metal sticks (Fig. 5).

Of the samples, 10.1% were commercial baits containing anticoagulant rodenticides formulated and designed to be used as baits, attracting mammals and killing them by ingestion, and 5.6% of the baits were made with cooked human food such as croquettes, patties and cooked meat. Fish fillet (1 bait) and pieces of lobster (2 baits) were also found (1.5%). Three samples (1.5%) were covered seeds designed to be buried but placed on the surface as baits. Two samples (1%) consisted of impregnated vegetable remains (grass and straw).

3.2. Substances detected

Table 2 shows the substances detected in the positive baits and the number of baits containing each substance, as well as the number of cases in which they appear. The 19.9% of the baits (49 of 246) were negative (any substance was identified). The anticholinesterase compounds aldicarb and carbofuran (pesticides belonging to the carbamate group) were the substances most used to prepare baits. Of the positive samples (baits and commercial microgranules), 64.5% (127 out of 197) contained at least one of these pesticides: 43 baits (21.8%) contained aldicarb, 25 (12.7%) carbofuran (not as commercial microgranules), 38 (19.3%) presented microgranules of aldicarb on their surface (Fig. 4), 14 (7.1%) presented microgranules of carbofuran on their surface, 5 (2.5%) contained both substances and two were made with aldicarb mixed with another pesticide (the organochlorine lindane in one case and the organophosphate fenamiphos in the other). Organophosphate pesticides were detected in 38 samples (33 samples with only one pesticide and 5 samples mixed with other compounds). Fenthion was the most detected organophosphate insecticide (15 samples) being always related to impregnated objects used as perches to kill Bee-eaters-Merops apiaster. Other organophosphorus compounds were occasionally detected, such as chlorpyrifos (10 samples), fenamiphos (8 samples), malathion (4 samples) and monocrotophos and chlorfenvinphos (one sample each). The carbamates methomyl and oxamyl were detected in only one sample each.

The anticoagulant rodenticides were the next in frequency of use, with a total of 21 samples containing one rodenticide and 4 samples with a combination. Brodifacoum was the most detected, with 14 samples in total (4 of them combined with other anticoagulants), bromadiolone was detected in 9 baits (alone in 6 and mixed with brodifacoum in 3 samples), and difenacoum was detected in 2 baits (mixed with brodifacoum in 1 of them).

Some other compounds occasionally found were phenobarbital and strychnine, in 2 baits (1%) each; ethoxyquin, and the pyrethroids cypermethrin and deltamethrin, in 1 bait each (0.5%). Fig. 6 shows the trend regarding the use of the main substances between 2002 and 2018.

It should be pointed out that, in addition to the 5 baits that presented
a mixture of aldicarb and carbofuran (above-mentioned), 11 baits (5.6%) revealed a combination of substances. So, a mixture of chlorpyrifos, methomyl and endosulfan (Fig. 7) was present in 3 baits, as was a mixture of bromadiolone and brodifacoum; other mixtures were found in 1 bait each: aldicarb and lindane; fenamiphos and aldicarb; methiocarb, imidacloprid, metalaxyl, thiram and atrazine; brodifacoum and difenacoum; and another consisting of a mixture of one pyrethroid and three organophosphates (cypermethrin, chlorfenvinphos, chlorpyrifos and fenitrothion).

4. Discussion

Extremadura is a region of exceptional predator richness and has also an important livestock and hunting activity (Fig. 1), factors which are related to the deliberate use of poisons (Reynolds and Tapper, 1996; Villafuerte et al., 1998; Graham et al., 2005; Marquez et al., 2012; De Roma et al., 2018).

Monthly distribution of positive baits over the 17 years (Fig. 2) demonstrate that highest incidence peaks appear in spring and autumn, coinciding with the periods of more intense livestock activity, more natural food resources for cattle and wildlife, and/or with hunting activity; regarding to wildlife these periods also coincide with breeding season of most of birds and with main migration seasons.

The high percentages of different meat baits (pieces of fresh meat, sausages and cured dried meats, animal corpses, viscera) and their

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Types of poisoned baits (positives) based on material preparation, and presentation.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of bait</td>
<td>Baits: n (%)</td>
</tr>
<tr>
<td>Pieces of fresh meat</td>
<td>61 (30.9%)</td>
</tr>
<tr>
<td>Commercial chemical substances</td>
<td>32 (16.2%)</td>
</tr>
<tr>
<td>Sausages and dried cured meat</td>
<td>28 (14.2%)</td>
</tr>
<tr>
<td>Commercial baits (rodenticides)</td>
<td>21 (10.1%)</td>
</tr>
<tr>
<td>Impregnated objects (perches)</td>
<td>15 (7.6%)</td>
</tr>
<tr>
<td>Small animal carcasses</td>
<td>13 (6.6%)</td>
</tr>
<tr>
<td>Cooked human food</td>
<td>11 (5.6%)</td>
</tr>
<tr>
<td>Viscera (organs and placenta)</td>
<td>9 (4.6%)</td>
</tr>
<tr>
<td>Covered seeds</td>
<td>3 (1.5%)</td>
</tr>
<tr>
<td>Fish and seafood</td>
<td>3 (1.5%)</td>
</tr>
<tr>
<td>Vegetables</td>
<td>2 (1.0%)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>197 (100%)</td>
</tr>
</tbody>
</table>

Fig. 3. Monthly distribution of poisoned baits detected in this study.

Fig. 4. Poisoned bait consisting of cured dried sausage (typical Spanish product) filled with microgranules of the carbamate insecticide aldicarb.

Fig. 5. Fenthion-impregnated perches prepared to poison European bee-eaters by skin contact in a beekeeping area.

in 1 bait each: aldicarb and lindane; fenamiphos and aldicarb; methiocarb, imidacloprid, metalaxyl, thiram and atrazine; brodifacoum and difenacoum; and another consisting of a mixture of one pyrethroid and three organophosphates (cypermethrin, chlorfenvinphos, chlorpyrifos and fenitrothion).
elaborate preparation, makes clear the intentionality against target species (predators such as birds of prey and carnivores) that are usually affected by the deliberate use of poison (Marquez et al., 2013; Berny et al., 2015; Marquez et al., 2016; Di Blasio et al., 2020). It is well known that these practices can cause their decrease (Marquez et al., 2012) and can also affect their distribution (Mateo-Tomas et al., 2012). The extensive use of meat baits also indicates its effectiveness in attracting the ‘target species’, low economic cost, easy management, and resistance to degradation when facing with weather conditions (especially when it comes to dry-cured meat). Unlike similar studies (Giorgi and Mengozzi, 2011), a direct relationship between the material used (high fat content, elaborated or raw food, etc.) and the type of substance involved was not found. Objects impregnated with the organophosphate fenthion were found in 15 samples. This substance was on the surface of different objects such as sticks, branches, ropes and wires used as perches by birds in beekeeping areas (Fig. 5). Birds such as the Bee-eater Merops apiaster are considered harmful for this economic activity and the intention is to poison them by skin contact, as this pesticide is easily absorbed by the skin; similar cases have been reported in other regions of Spain (Cano et al., 2016).

Commercial chemicals were sent to our laboratory as they were left in the field after the preparation of the corresponding food-supported or -impregnated bait or when found in facilities (warehouses, farms) in which the preparation of baits was suspected.

Cooked human food is a common and varied material used in the preparation of baits, as found in other studies (Giorgi and Mengozzi, 2011); this material appears covered, impregnated or filled with the poison, this being an inexpensive and easy way to prepare a tasty matrix for the baits. It is usually spoiled or leftover food that is used for this purpose, such as the fish and seafood baits.

In one case, seeds covered with permitted pesticides were placed intentionally on the ground with criminal intent, while in 2 cases seeds were not properly buried during sowing, which led to the accidental exposure of fauna to the pesticides. At this point it is worth remembering that circumstantial poisonings have traditionally been classified as accidental events with permitted substances, poor application or use, or criminal actions (Mineau, 2002; Berny, 2007; Martinez-Haro et al., 2008).

A high number of the analysed baits (80%) were positive for toxic compounds, indicating a high level of selectivity by well-trained forest rangers collecting the baits in the field. Recently, a study on bait poisoning data from Northwest Italy collected between 2012 and 2017

<table>
<thead>
<tr>
<th>Substance</th>
<th>Baits: n (%)</th>
<th>Cases: n</th>
</tr>
</thead>
<tbody>
<tr>
<td>No toxic substance detected (negatives)</td>
<td>49 (19.9%)</td>
<td>42</td>
</tr>
<tr>
<td>Aldicarb</td>
<td>43 (17.5%)</td>
<td>31</td>
</tr>
<tr>
<td>Aldicarb (microgranules)</td>
<td>38 (15.4%)</td>
<td>26</td>
</tr>
<tr>
<td>Carbofuran</td>
<td>25 (10.2%)</td>
<td>22</td>
</tr>
<tr>
<td>Carbofuran (microgranules)</td>
<td>14 (5.7%)</td>
<td>9</td>
</tr>
<tr>
<td>Fenthion</td>
<td>15 (6.1%)</td>
<td>12</td>
</tr>
<tr>
<td>Brodifacoum</td>
<td>10 (4.1%)</td>
<td>1</td>
</tr>
<tr>
<td>Fenamiphos</td>
<td>7 (2.8%)</td>
<td>7</td>
</tr>
<tr>
<td>Bromadiolone</td>
<td>6 (2.4%)</td>
<td>4</td>
</tr>
<tr>
<td>Aldicarb + carbofuran</td>
<td>5 (2.0%)</td>
<td>3</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>4 (1.6%)</td>
<td>1</td>
</tr>
<tr>
<td>Malathion</td>
<td>4 (1.6%)</td>
<td>3</td>
</tr>
<tr>
<td>Bromadiolone + brodifacoum</td>
<td>3 (1.2%)</td>
<td>2</td>
</tr>
<tr>
<td>Chlorpyrifos + methyldiolone</td>
<td>3 (1.2%)</td>
<td>2</td>
</tr>
<tr>
<td>Triflumuron</td>
<td>2 (0.8%)</td>
<td>2</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>2 (0.8%)</td>
<td>1</td>
</tr>
<tr>
<td>Styroxine</td>
<td>2 (0.8%)</td>
<td>1</td>
</tr>
<tr>
<td>Chlorpyrifos (+ sulfoate)</td>
<td>2 (0.8%)</td>
<td>2</td>
</tr>
<tr>
<td>Methomyl</td>
<td>1 (0.4%)</td>
<td>1</td>
</tr>
<tr>
<td>Difenacoum</td>
<td>1 (0.4%)</td>
<td>1</td>
</tr>
<tr>
<td>Ethoxyquin</td>
<td>1 (0.4%)</td>
<td>1</td>
</tr>
<tr>
<td>Metaldaldehyde</td>
<td>1 (0.4%)</td>
<td>1</td>
</tr>
<tr>
<td>Oxamyl</td>
<td>1 (0.4%)</td>
<td>1</td>
</tr>
<tr>
<td>Monocrotophos</td>
<td>1 (0.4%)</td>
<td>1</td>
</tr>
<tr>
<td>Deltamethrin</td>
<td>1 (0.4%)</td>
<td>1</td>
</tr>
<tr>
<td>Aldicarb + lindane</td>
<td>1 (0.4%)</td>
<td>1</td>
</tr>
<tr>
<td>Fenamiphos + aldicarb</td>
<td>1 (0.4%)</td>
<td>1</td>
</tr>
<tr>
<td>Methiocarb + imidacloprid + metalaxyl + thiram + atrazine</td>
<td>1 (0.4%)</td>
<td>1</td>
</tr>
<tr>
<td>Brodifacoum + difenacoum</td>
<td>1 (0.4%)</td>
<td>1</td>
</tr>
<tr>
<td>Cypermethrin + chlorfenenphos + chlorpyrifos + fenitrothion</td>
<td>1 (0.4%)</td>
<td>1</td>
</tr>
<tr>
<td>TOTAL</td>
<td>246 (100%)</td>
<td>183</td>
</tr>
</tbody>
</table>

Fig. 6. Number of baits containing the main substances found in the study.

Fig. 7. Bait consisting of meat and skin sprayed with a mixture of pesticides (the organophosphate chlorpyrifos, the carbamate methomyl and the organochlorine endosulfan were detected).
showed that 40.4% of the baits (294 out of 728) were classified as positive for toxic substances with a poisoning prevalence fluctuating between 32.1% and 45.6% (Di Blasio et al., 2020).

As in similar studies in Spain (Sanchez-Barbudo et al., 2012) and in other European countries, insecticides were found to be the pesticides most commonly involved in poisoned baits (Table 2). Among insecticides, anticholinesterase compounds were the most frequently detected, as reported in the European literature (Guitart et al., 1999; Motas-Guzman et al., 2003; Guitart et al., 2010; Giorgi and Mengozzi, 2011; Ruiz-Suarez et al., 2015; Chiari et al., 2017; Bertero et al., 2020), particularly those with granular shape (facilitating handling) and very high toxicity (which increases effectiveness) (Berny, 2007; Martínez-Haro et al., 2008). Similar data have been published in the U.S. by Fleischli et al. (2004) who reviewed the U.S. Geological Survey National Wildlife Health Center (NWHC) mortality database from 1980 to 2000 to identify cases of poisoning by pesticides and attributed 0.96% of all avian mortality events to anticholinesterase poisoning. However, a more recent retrospective study performed in Southern Italy over a five-year period (De Roma et al., 2018), revealed that the toxic compounds on baits sent for toxicological analysis showed a trend different from that of other countries, as well as from that reported for Northern Italy. Methaldehyde, a molluscicide, was the most common substance detected (63.9%) followed by organochlorine insecticides (29.2%, mainly endosulfan), organophosphate insecticides (11.1%) and anticoagulant rodenticides (9.7%). Other rodenticides, such as strychnine and zinc phosphide were detected only once in baits.

Second-generation anticoagulant rodenticides (SGARs) were the second largest group we detected (Table 2), coinciding with similar studies (Giorgi and Mengozzi, 2011; Chiari et al., 2017). However, anticoagulant rodenticides did not play a primary role of importance among the toxic agents we found, unlike in similar studies such as the ones reported by Guitart et al. (1999), Motas-Guzmán et al. (2003), Berny (2007), Billé et al. (2016) and Chiari et al. (2017). These authors detected a higher prevalence of the use of SGARs, including bromadiolone, brodifacoum and difenacoum due to its widespread use. SGARs are toxic at low doses and after a single ingestion (Petterino and Biancardi, 2001; Berny, 2007), increasing the potential for primary and secondary poisoning (Chiari et al., 2017) although their persistence in the field can be affected by different factors (Sage et al., 2007). It is worth mentioning that in the last few years in Spain the debate about the use of these poisons in pest control has reappeared as a result of the mitigation measures applied to control the plagues of the common vole Microtus arvalis in Castilla y León Region. These measures involved the use of products containing chlorophacinone (used in cereal grains) in 2007 and bromadiolone (in cereal grains and baits prepared in a solid waxy matrix) in 2013; these practices caused severe damage to birds, hares and canines, among others (Vinuela et al., 2010; Vinuela et al., 2014).

In our study, 8.1/8.5% of the cases/baits included more than one toxic compound, which agrees with Chiari et al. (2017), who reported 9.3% of baits to contain more than one toxic compound in northern regions in Italy.

The number of baits in which strychnine was implicated was very low (only 2 baits), which agrees with the data reported by De Roma et al. (2018) in Italy. Strychnine poisonings can be highly variable from place to place depending on different factors (Blakley, 2009), and in the last report of wildlife poisoning in Spain (De la Bodega et al., 2020) it is stated that strychnine is used mainly in the North of Spain, its use being restricted in the other parts of the country, which agrees with our results. Pharmaceuticals such as the phenobarbital (detected in 2 baits) are considered emerging contaminants but are associated to deliberated poisonings in terms of impacts on wildlife mainly in scavengers due to the consumption of carcasses from euthanized livestock. Moreover, they are also detected in isolated poisoned baits in similar studies (Wells et al., 2020; Herrero-Villar et al., 2021).

Over the years, the trend regarding the use of the main substances to elaborate baits (Fig. 6) shows an upturn of the aldicarb and carbofuran during the years immediately after their ban (2003 and 2008 respectively), demonstrating their criminal intentionality. The use of fenthion is related to an isolated concentration of cases with specific characteristics and the use of brodifacoum start to appear in the last years of the study (coinciding with other studies above mentioned).

For all the above-mentioned, the intentionality of these criminal practices against target species such as carnivores and scavengers using edible baits (but also against other species such as bee-eaters using baits/materials to facilitate skin absorption of the poison) is evident (Fig. 5).

It is also known that poison is the most generalised method to kill predators worldwide (Marquez et al., 2012). In Spain, it was already reported by Blanco and Villafuerte (1993) that many hunters believed that predators were the main cause of the rabbit population decrease, and some admitted the use of illegal methods to kill them. Later, Villafuerte et al. (1996) found that there was no correlation between rabbit density and carnivore abundance and that hunters have the general impression that there were ‘too many kites’ in Spain. It is obvious that intentional application of poisoned baits is a threat to fauna and may cause far-reaching consequences to populations locally and around the world (Guitart et al., 2010; Fleischli et al., 2004; Mateo-Tomás et al., 2012). Poisoned baits may cause regional or national impacts, with declines or extinction of different wild carnivore mammals, such as wolves (Ripple et al., 2014), lynx (Rodríguez and Delibes, 1990) or wild cats (Lozano and Malo, 2012). Toxicosis related to this illegal activity has been identified as the main threat to the conservation of different species of raptors in Europe and Asia, such as, for example the Egyptian vulture (Hernández and Margalida, 2009). Moreover, poisoned baits have been identified as the primary limiting factor in the expansion of the reintroduced population of some raptors such as red kites and of the golden eagle (Aquila chrysaetos) in Scotland (Smart et al., 2010; Whitfield et al., 2008). Recently, Mateo-Tomás et al. (2020) demonstrated the existence of a direct relationship over the last 20 years between the decline in breeding pairs of red kite (Milvus milvus) in Spain and the poisoning of fauna, showing that the increase in the poisoning of red kites in each locality decreases the reproductive population of the species and increases the risk of local extinction.

Even though there have been different attempts to fight the use of poisoned baits, such as the Convention on the Conservation of Migratory Species of Wild Animals for the protection of migratory animals and their habitats, no legal framework exists to ban these practices at an international level. At the European level, the Council of Europe’s Convention on the Conservation of European Wildlife and Natural Habitats (1979), or Bern Convention, was the first international agreement to protect both species and habitats and to bring countries together to decide how to act on nature conservation. Directive 2009/147/EC on the conservation of wild birds and Directive 92/43/EC on the conservation of natural habitats and of wild fauna and flora are legal frameworks in the EU to apply the provisions contained in the Bern Convention. In Spain, the first legal regulation of the use of poisons to control livestock predation (by the wolf) is almost 200 years old. In the 19th century the Real Decreto de 3 de mayo de 1834 promoted and financed the use of poisons to eliminate ‘harmful’ species, and subsequent legal regulations continued to encourage the use of poisons to control predators with the authorisation of the state administration. These policies caused the persecution of many species that today are catalogued as threatened or endangered. In the 1970s and 1980s legal proposals were made, and the use of poisons was prohibited until the last 27 de marzo (España, 1989) and the Penal Code, penalised the use of poisons for hunting and fishing as a ‘non-selective’ medium or practice, and in 2007 the Ley 42/2007 de 13 de diciembre established the prohibition of all large-scale or non-selective procedures to capture or kill animals. Subsequently, a monitoring
scheme, the ANTIDOTO programme, was established in Spain (De la Bodega et al., 2020) to provide straightforward evidence of the impact of toxic substances on wild species over large spatiotemporal scales, and different regional administrations developed legal rules aimed at these malpractices. Particularly, in Extremadura, it is regulated by the Ley 8/1998 de 26 de junio (España, 1998) (of Conservation of Nature and Natural Spaces of Extremadura) and the Ley 14/2010 de 9 de diciembre (Extremadura, 2010) (of hunting in Extremadura). Furthermore, this legal framework includes strategies and plans that at both regional and national level have developed measures to prevent and prosecute this offense. An example of that is the previously mentioned ‘Extremadura’s Strategy Against the Illegal use of Poisoned Baits in Natural Environment’ which covers five crucial items: increase in and ready flow of information, improvement of knowledge, development and execution of specific operations intended to prevent and deter, research and prosecution of offenses, and criminal and administrative proceedings, as well as other complementary actions (Extremadura. Orden de 27 de marzo de 2015) (Extremadura, 2015). These legal restrictions and efforts to fight against these malpractices are allowing species to recover gradually, but old economic and environmental conflicts are also being resurrected. These conflicts may become more serious in areas where important game animal activity, extensive livestock and a rich fauna of birds of prey and carnivorous mammals merge into a common area (Virgós and Travaini, 2005; Márquez et al., 2012).

5. Conclusions

This survey provides, for the first time, updated and useful epidemiological data on the use of poison baits in Extremadura (Spain), a region with a very high ecological value for biodiversity in Europe and where agriculture, livestock and hunting coexist. Anticholinesterase pesticides are the compounds most frequently used to elaborate poisonous baits, being the most implicated the carbamates aldicarb and carbofuran, showing a clear intentionality of poisoning of target and nontarget species (raptors, carnivores and others). Their use can cause a decrease, or affect distribution, of several endangered species inhabiting Extremadura, including mammals as the Iberian lynx (the world’s most endangered feline species) and Iberian wolf Canis lupus signatus, as well as birds such as the Iberian imperial eagle (one of the rarest and most threatened birds in the world), Red kite, Black vulture Ciconia nigra or Bonelli’s Eagle Aquila fasciata). This is a consequence of a lasting conflict between humans and predators, which has been legally and culturally supported in Spain for decades. Even if these criminal practices are banned, no legal framework exists to address it at an international level. In areas where important game activity, extensive livestock and a rich fauna of birds of prey and carnivorous mammals merge, these conflicts have resurrected at the same time as legal restrictions and efforts against these malpractices try to be effective. The incidence of poisoned baits is extremely worrying, and actions (worldwide legal and educational measures) to reduce this illegal practice and its environmental impact are still urgent. Our findings may be useful to improve prevention and control measures.

Credit author statement

Yolanda Ibáñez-Pernía: Methodology, Investigation, Writing – original draft, Writing – review & editing; David Hernández-Moreno: Methodology, Investigation; Marcos Pérez-López: Investigation, Conceptualization; Francisco Soler-Rodríguez: Conceptualization, Methodology, Validation, Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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References
