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Interspecific and geographical differences in anticoagulant rodenticide residues of predatory wildlife from the Mediterranean region of Spain

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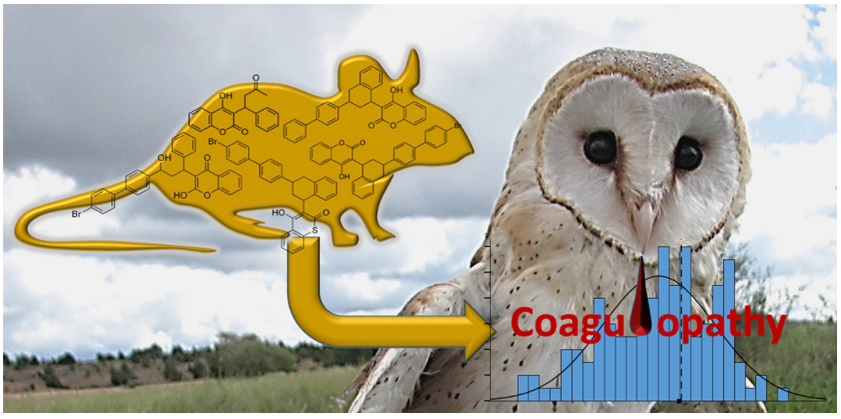
HIGHLIGHTS

- Anticoagulant rodenticides were found in 62.8% of the studied animals.
- Rodenticide occurrence was positively correlated with human population density.
- Scops owls were more exposed to rodenticides in Majorca Island than in Catalonia.
- Birds showed lower levels of bromadiolone than mammals.
- Rodenticide levels were compatible with lethal poisoning in 23.3% of the animals.

GRAPHICAL ABSTRACT



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ABSTRACT

We studied the prevalence of anticoagulant rodenticides (ARs) in the liver of 344 individuals representing 11 species of predatory wildlife that were found dead in the Mediterranean region of Spain (Catalonia and Majorca Island). Six different ARs (brodifacoum, bromadiolone, difenacoum, flocoumafen, difethialone, warfarin) were found in the liver of 216 (62.8%) animals and >1 AR co-occurred in 119 individuals (34.6%). The occurrence of ARs was positively correlated with the human population density. Catalonia and Majorca showed similar prevalence of AR detection (64.4 and 60.4%, respectively), but a higher prevalence was found in the resident population of Eurasian scops owl (*Otus scops*) from Majorca (57.7%) compared to the migratory population from Catalonia (14.3%). Birds of prey had lower levels of bromadiolone than hedgehogs, whereas no difference was found for other ARs. The risk of SGAR poisoning in wild predators in NE Spain is believed to be elevated, because 23.3% of the individuals exhibited hepatic concentration of ARs exceeding 200 ng/g.

1. Introduction

The diet of many wild predators is based on rodents that are commonly the target of campaigns for control or eradication with anticoagulant

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rodenticides (ARs), which makes these predators susceptible to secondary poisoning by AR-contaminated prey (Brakes and Smith, 2005; Sage et al., 2008; Rattner et al., 2014b). In the last decades, several studies have been performed that reveal the prevalence of exposure in non-target wildlife species at risk. The prevalence of AR residues in the liver of diurnal and nocturnal species of birds of prey ranged between 29–100% in the United States (Stone et al., 2003; Murray, 2011), 23–92% in Canada (Albert et al., 2010; Thomas et al., 2011), 10–38% in the United Kingdom (Newton et al., 1990; Shore et al., 2006), 14–100% in France (Berny et al., 1997; Lemarchand et al., 2010) and 33–57% in Spain (Sánchez-Barbudo et al., 2012). In some circumstances, predatory mammals have been also found to be at high risk of AR poisoning, especially Mustelidae (Shore et al., 2003; Gabriel et al., 2012) and Erinaceidae (Dowding et al., 2010). These monitoring studies have been usually conducted in animals found dead, so it is likely that these data are biased towards overestimation of AR exposure. This chronic exposure implies a risk of mortality (Ruder et al., 2011; Gabriel et al., 2012; Coeurdassier et al., 2014), that may resemble the case of cyclodiene insecticides some decades ago (Walker and Newton, 1998).

Monitoring studies of ARs in wildlife show differences in the use patterns in various geographic areas, and may reveal differences in sensitivity and bioaccumulation among species. The development of the resistance by rodents to the first generation ARs (FGARs) led to the development of second generation ARs (SGARs) of very low LD_{50} and high persistence in the hepatic tissue of rodents and their predators (Watt et al., 2005; Ishizuka et al., 2008). Some of these SGARs, such as brodifacoum, have similar acute LD_{50} in mammals (0.16–25 mg/kg) and birds (0.2–4.6 mg/kg); but in other cases, such as for bromadiolone, mammals seem to be more sensitive (0.49–25 mg/kg) than birds (81–261 mg/kg) (EPA, 2004). In terms of toxicokinetics, hepatic half-life of ARs ranges in birds from 11.7 days (FGARs) to 155 days (SGARs) (Newton et al., 1994; Rattner et al., 2014a) and in mammals from 8 days (FGARs) to 307 days (SGARs) (Veenstra et al., 1991; Nelson and Hickling, 1994; Vandenbroucke et al., 2008).

The diagnosis of AR poisoning in wildlife has been usually based on the observation of hemorrhages and the detection of ARs in the liver (Berny et al., 1997; Murray, 2011). Following these criteria, fatal incidences of AR poisoning of predators and other non-target species have been documented in many countries around the world (Walker et al., 2008; Albert et al., 2010; Gabriel et al., 2012; Christensen et al., 2012; Sánchez-Barbudo et al., 2012). However, it is also known that in some cases the animals poisoned by ARs do not develop macroscopic hemorrhages (Sarabia et al., 2008; Rattner et al., 2010, 2011). Therefore, the establishment of a threshold level of AR hepatic residues associated with toxicity and lethality would be essential for the correct diagnosis of some cases, but again sensitivity may vary among species and individuals. Thomas et al. (2011) have suggested a probabilistic model to calculate the probability of becoming symptomatic as a function of AR residue concentrations. They found than some species like great horned owl (*Bubo virginianus*) would have a 5% probability of exhibiting signs of toxicosis with AR liver residues of 20 ng/g, which is below the toxicity threshold suggested by Newton et al. (1999) at 100–200 ng/g (w.w.). Recently, Rattner et al. (2014a) carried out an experiment to study the toxicokinetics and the development of hemorrhages produced by diphacinone exposure in eastern screech owls (*Megascops asio*) and they found signs of coagulopathy associated with liver diphacinone levels exceeding 100 ng/g (w.w.).

In Spain, AR poisoning in wildlife has been observed after large-scale treatments during population peaks of common voles (*Microtus arvalis*) in agricultural areas (Sarabia et al., 2008; Olea et al, 2009; Vidal et al., 2009), and also in other areas of Spain where ARs are regularly used against commensal rodents (Sánchez-Barbudo et al., 2012). The present work focused on determining the prevalence of ARs in predators from two highly populated areas in Spain, Catalonia and Majorca Island. The study of these two areas permitted determination of differences in AR exposure between island and mainland populations. Moreover, the study of different species of diurnal and nocturnal birds of prey and mammals will be used to detect differences in the distribution of AR levels between these groups that could reflect differences in bioaccumulation and/or sensitivity.

2. Materials and methods

2.1. Sample collection

We analyzed liver samples of wild animals (n = 344) received by the Laboratory of Toxicology of IREC between 2011 and 2013 corresponding to animals attended in Wildlife Rehabilitation Centres (WRC) in the Majorca Island and Catalonia. This sampling included seven species of birds of prey and two species of mammals. Birds included barn owl (*Tyto alba*; n = 41), Eurasian eagle owl (*Bubo bubo*; n = 14), tawny owl (*Strix aluco*; n = 27), Northern long-eared owl (*Asio otus*; n = 12), common buzzard (*Buteo buteo*; n = 56), little owl (*Athenea noctua*; n = 7), and Eurasian scops owl (*Otus scops*; n = 33). These species are widely distributed in Spain and frequently inhabit open areas associated with towns and rural areas. Most are residents in Spain, and only the mainland population of scops owl is mostly a sub-Saharan migrant (Martí and Del Moral, 2003). Barn owl, common kestrel, eagle owl, tawny owl and long-eared owl feed more frequently on small mammals. Common buzzard also feed on medium-sized birds. Little and scops owls feed on invertebrates, mainly insects, but can also feed on rodents. In summary, all the studied bird species include rodents in their diet (Martí and Del Moral, 2003). The mammals studied include European hedgehog (*Erinaceus europaeus*; n = 48) and Algerian hedgehog (*Atelerix algirus*; n = 106). The European hedgehog is widely distributed across continental Spain, and eats earthworms, gastropods, insects, reptiles, rodents and bird carcasses (Palomo et al., 2007). The Algerian hedgehog is restricted to the warmest areas of the South and East of continental Spain and it is introduced in Balearic Islands; preys on invertebrates and occasionally on small vertebrates (mostly lizards) (Palomo et al., 2007).

All the animals were found dead or moribund. Necropsies were performed by the veterinary staff of the wildlife rehabilitation centers from Catalonia and Majorca and the presence of trauma, hemorrhages and non-clotted blood was recorded. Liver samples were taken and immediately frozen at −20 °C until AR analysis at the Spanish Institute of Game and Wildlife Research (IREC).

2.2. Rodenticide analysis

The analysis of ARs was carried out following the method described by Sánchez-Barbudo et al. (2012) with some modifications. Briefly, 1 g of liver was ground in a mortar with 9 g of anhydrous sodium sulfate (Prolabo, Leuven, Belgium), then the homogenate was transferred to a Teflon-capped 30 mL-glass tube and 20 mL of a mixture of dichloromethane:acetone (70:30) (HiperSolv Cromanorm Gradient grade, Prolabo, Leuven, Belgium) was added, horizontally shaken for 10 min and sonicated for 5 min. The extract was filtered through a Whatman paper filter and collected in a conical tube for solvent evaporation in a rotary evaporator. The extraction was repeated with 5 mL of the solvent mixture, and the supernatant obtained was pooled with the previous one. After solvent evaporation, the dry extract was dissolved in 2 mL of dichloromethane:acetone (70:30). Then, this extract was cleaned-up in a solid phase extraction (SPE) column filled with neutral alumina (SPE ALN 500 mg/3 mL, Upti-clean Interchrom, Montluçon, France). The SPE column was conditioned with 5 mL of dichloromethane and 10 mL of dichloromethane:acetone (70:30). The sample was added to the column and washed with 3 mL of dichloromethane:acetone (25:75). Finally, the anticoagulant rodenticides were eluted with 3 mL of methanol:acetic acid (95:5) (Prolabo, Leuven, Belgium). The solvent was evaporated