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Rats, residues and rodenticide resistance: Hepatic concentration of anticoagulant rodenticides in genetically susceptible wild brown and black rats

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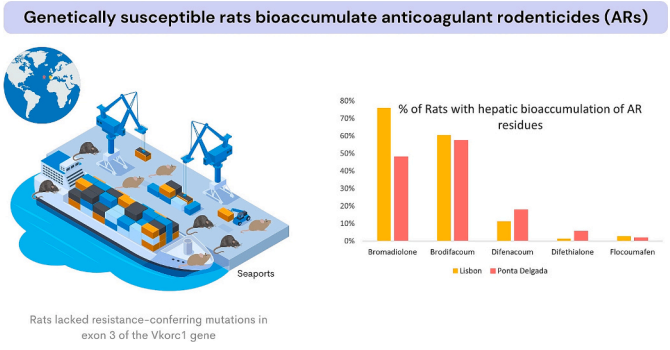
HIGHLIGHTS

- No known *Vkorc1* resistance mutations in Exon 3 were found in Lisbon and Ponta Delgada rats.
- Exon 3 *Vkorc1* wild-type rats bioaccumulate high concentrations of anticoagulant rodenticides.
- Residues of at least one SGAR were detected in 80.4% of the analysed rats.
- Older rats have a higher prevalence of bromadiolone residues.
- Black rats with higher body weight showed greater concentrations of total rodenticide residues.

GRAPHICAL ABSTRACT



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ABSTRACT

Invasive rats are major pests worldwide, posing economic and public health risks. Since the 1950s, efforts have been made to control or eradicate these animals by using anticoagulant rodenticides (ARs). Initially effective, ARs quickly lost efficacy due to the emergence of resistance-associated mutations in the *Vkorc1* gene. When consumed in (sub-)lethal doses, these biocides bioaccumulate in the liver, becoming a risk for non-target predators.

First, this study aims to assess genetic resistance to ARs in two synanthropic rat species, *Rattus rattus* and *Rattus norvegicus*, from port urban areas in Lisbon (mainland Portugal) and Ponta Delgada (São Miguel Island, Azores, Portugal). Secondly, we aim to detect and quantify the hepatic concentration of first- (FGARs) and second-generation anticoagulants (SGARs). We analysed 203 sequences of exon 3 of the *Vkorc1* gene and found no known resistance-conferring mutations in either species or locations. A subsample of 177 liver tissues from genotyped rats was examined for AR residues via Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS). No FGARs were detected, but five SGARs were identified with 80.4% of individuals accumulating at least one of them. The absence of genetically mediated resistance via exon 3 of *Vkorc1* mutations does not necessarily mean that the risk of AR exposure to rat predators is low, as 16.0% of the live-trapped rats bioaccumulated high levels of ARs, above the 100 ng/g toxicosis threshold. This finding underscores significant ecological risks, particularly for non-target wildlife apex-predators, that are more susceptible to AR exposure and may bioaccumulate lethal concentrations through repeated consumption of contaminated prey.

1. Introduction

Brown rats (*Rattus norvegicus*) and black rats (*Rattus rattus*) have a global distribution, mostly due to their close association with humans. Black rats, in particular, were once typically restricted to seaports and coastal regions when beyond their native Asian range, owing to their ability to inhabit seafaring ships. With increasing levels of global movement of people and goods, the transport of rats via ship navigation is still a contemporary issue (Morand et al., 2015) but its consequences understudied. In addition to public health costs, there are further burdens, including crop damage, food supply losses, and management efforts, which collectively sum up to millions of dollars spent annually worldwide. Specifically, costs associated with the genus *Rattus* spp. are estimated at 500.4 million USD between 1930 and 2022, accounting for 14% of the total costs attributed to invasive rodents (Diagne et al., 2023).

Given all these challenges associated with rats, pest management is a critical tool and a global task for mitigating these impacts. Since the early 1950s, rodent pest control has relied almost exclusively on anticoagulant rodenticides (ARs). However, their initial remarkable success was quickly hampered by the identification of resistant rats within a few years of introduction of these biocides (Boyle, 1960). The evidence of resistant animals prompted the development of second-generation anticoagulant rodenticides (SGARs, e.g., brodifacoum, bromadiolone, difenacoum, difethialone and flocoumafen), but resistance to these also soon emerged (McGee et al., 2020). SGARs exhibit higher toxicity when compared to first-generation anticoagulant rodenticides (FGARs), with greater potential for bioaccumulation and persistence due to their enhanced affinity for the vitamin K1-epoxide reductase (VKOR) enzyme in the liver (Watt et al., 2005). A prolonged elimination terminal half-life leads to tissue persistence for durations between 6 and 12 months (Laakso et al., 2010 and references therein).

In recent years, a significant number of reports of resistance to anticoagulants in rat populations around the world has been associated with several mutations in the vitamin K epoxide reductase complex subunit 1 (*Vkorc1*) coding gene (Pelz et al., 2005; Rost et al., 2009), predominantly in Europe [e.g. Germany (Jacob et al., 2012), France (Grandemange et al., 2010), Belgium (Baert et al., 2012), the Netherlands (Krijger et al., 2023), or the UK (Prescott et al., 2018)], but also documented in other regions such as, e.g., New Zealand (Cowan et al., 2017), Japan (Tanaka et al., 2012), and USA (Díaz and Kohn, 2021). Among the mutations known to have significant impacts on anticoagulant efficacy, the most widespread occur on the third exon of the *Vkorc1* gene, in codons 120, 128 and 139 (Buckle, 2013; Rost et al., 2009). Rats carrying these mutations not only can survive longer in the presence of first-generation anticoagulant rodenticides than susceptible ones (Baxter et al., 2022), but they can also potentially bioaccumulate these biocides for long periods (Vein et al., 2013).

Moreover, rodents undergo behavioural changes after consuming anticoagulant rodenticides, including a slower response time, increased movement in open areas and daylight activity, which increase vulnerability to predation by wildlife (Brakes and Smith, 2005). Considering the possible AR resistance of rats and the behavioural changes that can occur in consequence of AR consumption, poisoned rats pose an increased risk of exposure to predatory birds and other carnivores (López-Perea and Mateo, 2018; Walther et al., 2021a). In recent years, numerous studies have addressed the topic of secondary poisoning, revealing a high prevalence of anticoagulant rodenticides in the liver of predatory species (Nakayama et al., 2019), namely, reptiles (Lettoof et al., 2020; Martín Cruz et al., 2024a), raptors (Broughton et al., 2022; Carromeu-Santos et al., 2025; Christensen et al., 2012; Martín Cruz et al., 2024b), and carnivorous mammals (Keating et al., 2024 and references therein). Aiming to minimise the impact of rodent control on wildlife species, some countries have implemented restrictive or prohibitive measures regarding the use of the most toxic of these rodenticides under specific conditions. In the UK, until very recently, the use of bromadiolone and difenacoum was permitted “outdoors”, around buildings, however brodifacoum and flocoumafen could only be used “indoors” (Buckle, 2013). As of January 2025, the outdoor use (in open areas) of all SGARs is now illegal in the UK (CRRU 2024). The USA restricted the acquisition of biocides that contain second generation anticoagulants to general consumers and stipulated the mandatory use of bait stations for outdoor applications (Environmental Protection Agency (EPA), 2008). In the European Commission Regulation (EU 2016/1179) (European Parliament and the Council of the European Union, 2016) regulatory frameworks do not dictate the specific allocation of certain active ingredients based on indoor/outdoor use. Nevertheless, it is specified that active ingredients exceeding 30 ppm (parts per million) are not allowed for general public use, being restricted to professional usage (European Parliament and the Council of the European Union, 2016).

As described, extensive reports have assessed AR resistance mediated by mutations in the *Vkorc1* gene, as well as the impacts of ARs on wildlife. However, there is still a gap regarding the assessment of bioaccumulation levels of ARs in free-living rats inhabiting areas undergoing management actions. To our knowledge, studies specifically addressing hepatic AR bioaccumulation in wild pest rodent populations are very scarce (but see Buckley et al., 2024; Desvars-Larrive et al., 2017

and Pitt et al., 2015). Also, experimental studies have focused on quantifying AR residues under controlled conditions as part of laboratorial no-choice feeding tests (Frankova et al., 2004), free-fed or animals subject to oral gavage (of twofold LD50 for rats) (Walther et al., 2021a). In these studies, where the ingestion period and/or the amount of rodenticide consumed is known, results indicate significant AR bioaccumulation, with hepatic concentration reaching mean values of 14,910 ng/g [wet weight] (Frankova et al., 2004), 6011 ng/g and 2115 ng/g [wet weight] (free-fed and oral gavage, respectively) (Walther et al., 2021a). However, applying these findings to wild rodent populations is impossible. Even when samples are collected shortly after control actions, the exact amount of rodenticide each animal has consumed remains unknown. This limitation makes it difficult to directly extrapolate these laboratory-obtained results to wild rodent populations. Therefore, assessing AR bioaccumulation in wild rats where pest control measures are routinely implemented is essential. Pitt et al. (2015) reported average concentrations of 18.860 ng/g in dead rats after an eradication action, and also found a live rat, two weeks after the bait drop, with a hepatic concentration of 6.800 ng/g of brodifacoum. This is particularly significant considering that poisoned target rodents serve as the primary route of exposure to ARs for secondary-poisoning of predators and scavengers (Walther et al., 2021b). Understanding the AR bioaccumulation status of free-living rodents provides critical insights into the risks that management actions pose to wildlife.

In this study, we aim to assess the relationship between anticoagulant rodenticide concentration and potential genetically-mediated resistance in synanthropic rat populations from two port cities in Portugal: Lisbon (mainland), and Ponta Delgada (São Miguel Island, Azores archipelago). Given the regular maritime transportation to and from (and between) these two capital city ports, one continental and one insular, we seek to determine whether resistance-conferring mutations are arriving and spreading through these gateways. Specifically, we intend to identify the level of rodenticide resistance through the analysis of the third exon of the *Vkorc1* gene while quantifying the levels of hepatic concentration of first- and second-generation anticoagulant rodenticides in the same wild rats. This study attempts to improve the limited body of research addressing both genetic resistance and AR concentrations in target rodent populations. Additionally, we aim to address the influence of the *Vkorc1* genotype, sampling location, rat species, age or sex in AR detection. We hypothesise that, if present, genetic resistance will reveal higher AR concentrations in animals. Moreover, we anticipate that insular rats will show higher levels of AR exposure and that older animals will exhibit higher AR hepatic concentrations.

2. Material and methods

2.1. Ethics statement

This study was conducted within the scope of the research project PTDC/SAU-PUB/29254/2017, approved by the Animal Welfare Body at the Faculty of Sciences University of Lisbon — ORBEA (approval number 04/2018, 12 December 2018).

2.2. Sampling

Brown rats (*Rattus norvegicus*) and black rats (*Rattus rattus*) were live-trapped in Ponta Delgada (São Miguel Island, Azores archipelago) and Lisbon (mainland Portugal) in 2019–2020. Sampling sites were located within a 10 km radius from each city's seaport. Rats were captured in 30 sites in Ponta Delgada and 14 sites in Lisbon (details in Table S1).

Animals were captured in live Tomahawk traps, transported to the lab and euthanized following national/European regulations using isoflurane intoxication/overdose. Animals were then weighed, measured and sexed. Body weight and body length measurements (body, tail, posterior paw, ear) were used to confirm the identification of species and age. Specifically, age was determined by using the degree of eruption and wear of the upper molar cusps, and five relative age classes were established, according to Ringani et al. (2022): I — young, II — subadult, III — adult, IV — adult, and V — old. Liver samples and ear clips were collected from all animals: the liver was stored frozen at −80 °C and the ear tissue sample was preserved in absolute ethanol and kept at −20 °C until further analyses. When rats were found dead but without obvious signs of decomposition, both tissue samples were obtained in the same way. The details on individuals with liver samples, such as sex, age and status at trapping (alive/dead) are provided in Table S1 and summarised in Table 1.

2.3. DNA extraction and PCR conditions

A total of 203 rat tissue samples – ear clips – (122 brown rats, 90 from Ponta Delgada and 32 from Lisbon, and 81 black rats, 41 from Ponta Delgada and 40 from Lisbon) were used for screening exon 3 of the *Vkorc1* gene. Genomic DNA was extracted with the E.Z.N.A Tissue DNA Kit, omega BIO-TEK and diluted to an average concentration of 50 ng/μL.

Exon 3 of the *Vkorc1* gene was amplified by PCR (Polymerase Chain Reaction) following the protocol described by Grandemange et al. (2010), with minor modifications using the forward primer 5′-TTTCACCAGAAGCACCTGCTGCC-3′ and the reverse primer 5′-ACACTTGGGCAAGGCTCATGTG-3′. The PCR master mix and cycling conditions were the same for both rat species, with 12.5 μL of Taq 2× Master Mix, 0.2 mM MgCl (VWR, Leuven, Belgium), 10 μL of molecular grade water, 0.5 μL of each forward and reverse primer (at 10 μM), and 1 μL of template DNA. Amplification followed an initial denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 63 °C for 30 s and extension at 72 °C for 60 s, and a final extension for 10 min at 72 °C. PCR products were purified with ExoSAP-IT (ThermoFisher Scientific) and commercially sequenced at STABVIDA on an ABI Prism™ 3730xl DNA sequencer.

2.4. Genetic screening analysis

Sequence chromatograms of 203 animals were manually checked for mutation presence, in homozygosity and heterozygosity with Sequencher 4.1 software (Gene Codes Corporation). Reference sequences were downloaded from GenBank (BC166413.1 for *Rattus norvegicus* and HM181981.1 for *Rattus rattus*) and used as wild type for comparison. Final sequences were translated into codons by MEGA 10.1.8 (Kumar et al., 2018) for identification of the mutated codons. Rats were classified as susceptible if they lacked any resistance-conferring mutations previously identified in the literature (L120Q, L128Q, Y139C, Y139F, Y139S) (Buckle et al., 2022).

2.5. Quantification of hepatic anticoagulant rodenticides

2.5.1. Sample preparation

Rodenticide concentrations were measured in a subset of 177 rodents having at least 1 g of liver mass for this chemical analysis. The extraction of ARs was performed following the protocol described by Rial-Berriel et al. (2021), a validated method for the simultaneous quantification of four FGARs (warfarin, chlorophacinone, diphacinone, coumatetralyl) and five SGARs (bromadiolone, brodifacoum, difenacoum, difethialone and flocoumafen). Briefly, 1 g of rat liver was homogenised in 7 mL tubes at 6500 rpm for 1 min in a Precellys Evolution homogenizer (Bertin Technologies, Rockville, Washington D.C., USA). The homogenised sample was diluted with MiliQ pure water in a proportion of 1 g of liver per 4 mL of water. One gram of homogenate was then transferred to a 5 mL tube with 20 μL of internal standard P-IS mix (certified AR standards — warfarin, diphacinone, chlorophacinone, coumatetralyl, brodifacoum, bromadiolone, difethialone, difenacoum, and flocoumafen, 98.0–99.8% purity) and Warfarin-d5 (obtained from Dr.

Table 1
Summary table of liver samples used for quantification of anticoagulant rodenticides.
N — number of individuals; Alive/Dead — status at the time of trapping/collection.

	Rattus norvegicus					*Rattus rattus*				
	N	*Sex*		*Status*		N	*Sex*		*Status*	
		Male	Female	Alive	Dead		Male	Female	Alive	Dead
Lisbon	34	19	15	32	2	37	23	14	34	3
Ponta Delgada	92	53	37	84	8	14	7	7	13	1

Ehrenstorfer, Germany) and vortexed. After 15 min, 2 mL of acetonitrile (acidified with 0.5% formic acid) was added and the tubes vortexed again. The tubes were then subjected to 30 min of an ultrasound bath at a frequency of 40 Hz. A mixture of 480 mg of anhydrous magnesium sulphate and 120 mg of sodium acetate was then added to the tube, vortexed and manually shaken for 1 min. Finally, samples were centrifuged at 3300 *g* for 15 min at 0 °C and the supernatant removed and filtered using 0.2 μm Chromafil PET-20/15 (Macherey-Nagel, Düren, Germany) syringe filters.

The same procedure, using chicken liver (with no exposure to AR), was followed to make the QC (quality control samples at 5 ng/g ww) and the calibration curve, fortified with 10 different known concentrations [100, 50, 25, 12.5, 6.25, 3.125, 1.563, 0.781, 0.391, 0.195] ng/g. All rodenticide concentrations in the liver are given in ng/g wet weight.

2.5.2. Instrumental analysis

The identification and quantification of all potential nine anticoagulant rodenticides was performed by liquid chromatography coupled to triple quadrupole mass spectrometry (LC-MS/MS), as extensively described in Rial-Berriel et al. (2021). Briefly, analysis was conducted with a 1290 Infinity II LC System coupled to a Triple Quad 6460 mass spectrometer (Agilent Technologies, Palo Alto, USA). The column for the chromatographic separations was a Poroshell 120 EC-C18 column (2.1 mm, 100 mm, 2.7 m), the mobile phase A consisted of ultrapure water with 0.1% formic acid (FA) and 2 mM of ammonium acetate, and the mobile phase B consisted of 2 mM of ammonium acetate in methanol (MeOH), programmed in a binary gradient. A volume of 8 μL of each sample was injected with a flow rate of 0.4 mL/min. MS/MS analyses were performed using the Agilent Jet Stream Electrospray Ionization Source (AJS-ESI), in negative ionization mode. The total run time was 18 min. The limits of detection, quantification, and instrumental conditions of the anticoagulant rodenticides analysed can be found in Table S2 of the supplementary material.

2.5.3. Anticoagulant rodenticide data analyses

Generalized Linear Models (GLM) with Wald test (z-statistics) were used to assess the influence of various predictor variables (sampling city, rat species, sex, age and body weight) on the likelihood of detection of anticoagulant rodenticide and the level of rodenticides quantified. Concentrations were log-transformed to meet the assumptions of normality and re-tested. The model used the sampling city, rat species, sex, age and body weight as predictors, including the interaction term of species with sex and body weight. To avoid multicollinearity, we calculated the Variance Inflation Factor (VIF) for all variables and confirmed all values were below 4 (Dormann et al., 2013). We used a two-step approach. For each rodenticide, we first analysed its presence/absence by using a GLM with binomial distribution and logit function. We then performed a second analysis, to evaluate rodenticide concentration, excluding individuals where the rodenticide was absent and used a GLM with Gaussian distribution. All analyses were performed in the R software, version 4.3.3, with stats package (R Core Team, 2024).

Of the 177 rat livers used to quantify all anticoagulant rodenticides, 14 samples were excluded from the analysis because they were obtained from animals found dead. Although all animals had died recently, and their livers remained fresh, this exclusion was applied to prevent any overestimation of rodenticide bioaccumulation of the analysis and therefore potentially introducing a bias in the study's conclusions. Nevertheless, the AR concentrations for these specimens were determined and can be consulted in Table S1.

3. Results

3.1. Vkorc1 genotyping

Populations of *Rattus norvegicus* and *R. rattus* were analysed for genetic resistance to anticoagulant rodenticides in Lisbon and Ponta Delgada. A total of 203 sequences of the *Vkorc1* exon 3 were obtained. No resistance-conferring mutations, as described in the literature, were detected in any of the rat species or locations analysed in this study. Only synonymous mutations were detected in sequences of brown rats, *R. norvegicus*, and black rats, *R. rattus* from Lisbon. For brown rats, a set of 3 variants, Ile107Ile, Thr137Thr and Ala143Ala, were recorded. In black rats, only the synonymous polymorphism Ala143Ala was detected, in six sequences. Considering all variations detected in both species, none have been, so far, associated with any effects regarding anticoagulant rodenticide resistance. All variations detected are summarised in Table 2.

3.2. Anticoagulant rodenticide quantification

The same rat populations were analysed for the presence of hepatic anticoagulant rodenticides. Among the 163 analysed liver samples from live-trapped animals (66 from Lisbon and 97 from Ponta Delgada), no FGARs were detected. On the other hand, SGARs were identified in most samples. Specifically, at least one SGAR was detected in the liver of 80.4% of the analysed rats, two SGARs in 33.7% and three SGARs in 9.8% of the rats (Fig. 1A). Residues of four different rodenticides (brodifacoum, bromadiolone, difethialone and flocoumafen) were identified in the liver of a single rat. Moreover, 26 rats (16.0% of all analysed animals) exhibited concentrations of ∑ARs above the 100 ng/g toxicosis threshold. In both cities, the most detected anticoagulant rodenticides were bromadiolone and brodifacoum, followed by difenacoum, with minimal detection of difethialone and flocoumafen (Fig. 1B). Among all SGARs, bromadiolone was the most recorded in Lisbon (detected in 77.3% of the livers), whereas brodifacoum was the most prevalent in Ponta Delgada (detected in 55.7% of the samples) (Fig. 2C, Table 3).

Considering the total anticoagulant rodenticides detected in each individual, a higher detection rate was observed in *Rattus norvegicus* from Lisbon (93.8%), with a median ∑AR of 10.3 ng/g (and maximum ∑ of all detected rodenticides, 2607.7 ng/g). For *Rattus norvegicus* in

Table 2
Genetic variants found in exon three of the Vkorc1 gene in both rat populations analysed.

Species	Location	N	Ile107Ile	Thr137Thr	Ala143Ala
Rattus norvegicus	Lisbon	32	5	5	5
	Ponta Delgada	90	–	–	–
Rattus rattus	Lisbon	40	–	–	6
	Ponta Delgada	41	–	–	–

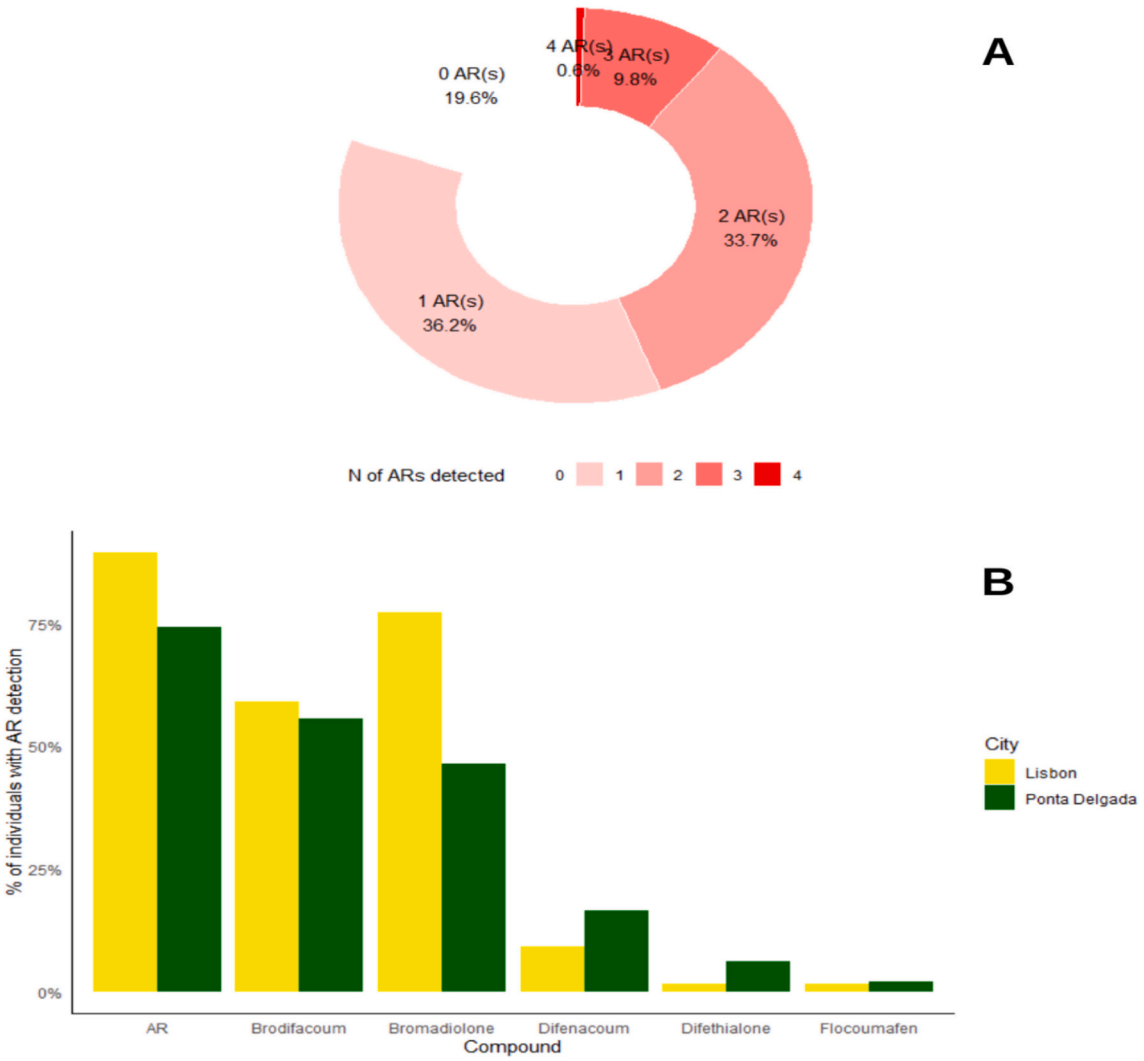

Fig. 1. (A) Distribution of individuals ($N = 163$) based on the number of different ARs detected in each; (B) proportion of individuals from each city (Lisbon and Ponta Delgada) with detected ARs.

Ponta Delgada the detection rate was 76.4% and the maximum concentration detected was 1363.9 ng/g. In contrast, *Rattus rattus* from Lisbon and Ponta Delgada had lower detection rates (85.3% and 53.8%, respectively). These results were summarised in Table 3.

Results of model analyses of ARs presence/absence and model analyses of ARs concentration are presented in Tables S3–S4. No significant differences in ARs presence were found between the two rat species for ∑ARs ($p = 0.2093$ — Mann-Whitney test, and Fig. 2A). No differences in AR presence and bioaccumulation levels were observed between sexes for any compound or their sum. A lower probability of finding ∑AR residues ($p = 0.0028$) and bromadiolone ($p = 0.0001$) was significantly lower in rats from Ponta Delgada when compared with Lisbon. Similarly, when bromadiolone was present in rats from Ponta Delgada, its concentrations were significantly lower ($p = 0.0389$) than those found in Lisbon.

Black rats accumulate significantly lower concentrations of ∑ARs ($p = 0.0063$) and bromadiolone ($p = 0.0003$) compared to brown rats. However, heavier black rats bioaccumulate higher concentrations of ∑AR ($p = 0.0207$) and bromadiolone ($p = 0.0033$) than the lighter ones. Older rats show higher probability of bioaccumulating bromadiolone ($p = 0.023$) and a tendency for difethialone ($p = 0.0919$). However, there were no significant differences for total AR accumulation between age groups (Fig. 2B).

4. Discussion

All animals analysed in the present study were genetically characterised as susceptible to anticoagulant rodenticides based on the analysis of the third exon of the *Vkorc1* gene. Nevertheless, these individuals were captured alive, and some exhibited notably high hepatic concentrations of second-generation anticoagulant rodenticides, posing important risks to non-target wildlife targeting them as prey.

4.1. *Vkorc1*-mediated rodenticide resistance of analysed rat populations in the context of Europe

Our results suggest that both maritime ports of Lisbon and Ponta Delgada do not appear to be significant exchange points for the arrival and spread of rodenticide resistance. Nonetheless, this is important information in terms of pest control targeting rat populations, as it allows flexibility in the choice of active substances, since, in the absence of resistance, any of them could be used (Damin-Pernik et al., 2022).

All brown and black rats sampled in both port cities were wild-type or had synonymous mutations in this exon, with unknown associations with anticoagulant rodenticide resistance. Prior to this study, no data on *Vkorc1* variation in rats from mainland Portugal had been published. Only a few individuals of *R. norvegicus* from the Azorean archipelago, São Miguel Island (Iacucci et al., 2018) and Terceira island (Rost et al.,

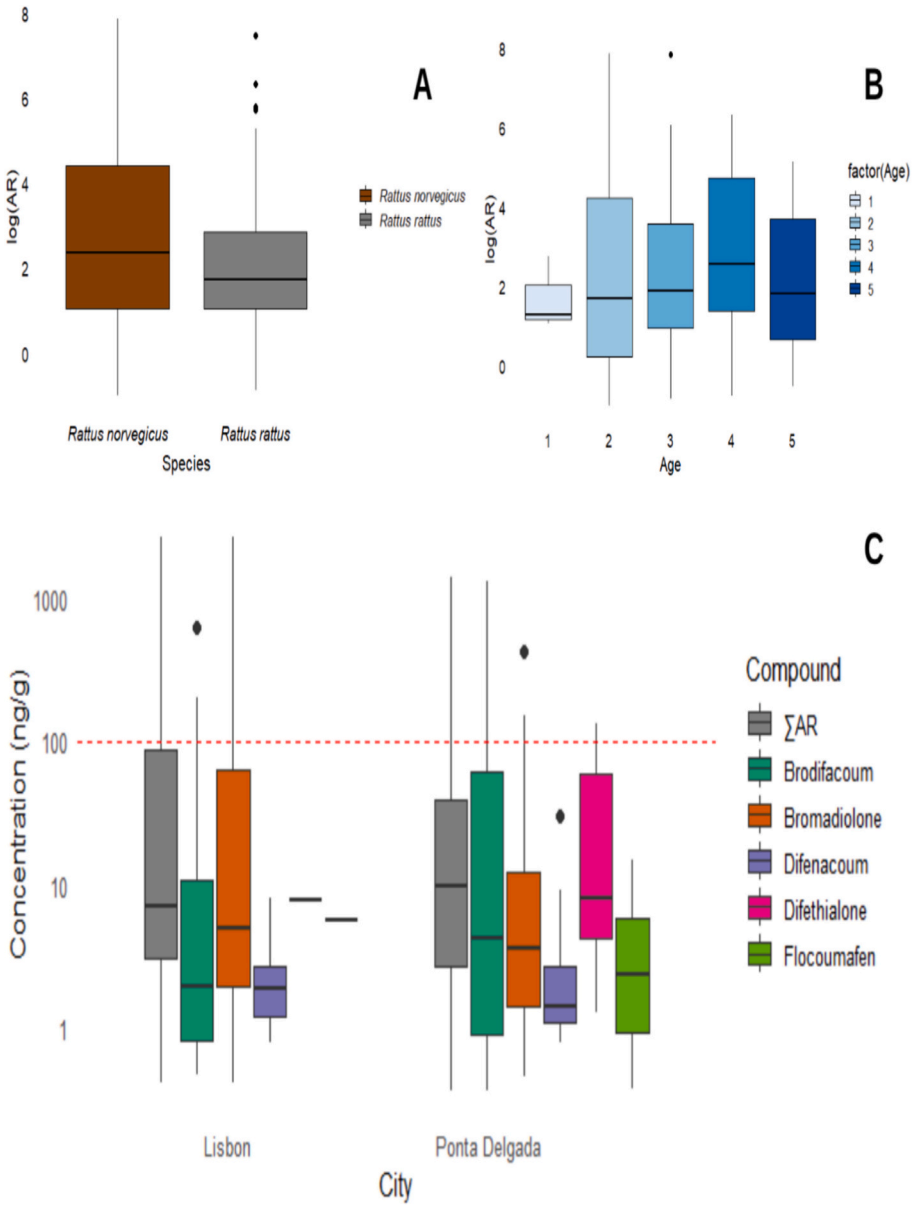

Fig. 2. A — Boxplot depicting total anticoagulant rodenticide (AR) accumulation in the two study species, *Rattus norvegicus* and *R. rattus*. B — Boxplot depicting total AR accumulation across age categories (1 = youngest, 5 = oldest). C — Quartiles of hepatic concentration of second-generation anticoagulant rodenticides in rats from Lisbon (difethialone and flocoumafen were only detected in a single individual) and Ponta Delgada.
Note: A and B* — The lower and upper hinges correspond to the first and third quartiles (the 25th and 75th percentiles). This differs slightly from the method used by the boxplot() function, and may be apparent with small samples. The upper whisker extends from the hinge to the largest value no further than 1.5 * IQR from the hinge (where IQR is the inter-quartile range, or distance between the first and third quartiles). The lower whisker extends from the hinge to the smallest value at most 1.5 * IQR of the hinge. Data beyond the end of the whiskers are called "outlying" points and are plotted individually. C* — The central thick line in the boxplots represents the median, the box represents the interquartile range, and the whiskers extend to the minimum and maximum values. The red dashed line represents the threshold above which the animals are expected to exhibit toxicosis symptoms (100 ng/g).

2009) had been analysed, with no detection of known resistance-conferring mutations. In these studies, some synonymous mutations (Leu94Leu, Ile107Ile, Thr137Thr, Ala143Ala) and non-synonymous mutations (Ile90Leu, Val112Leu), with no known role on resistance, were described (Rost et al., 2009). Three of these synonymous mutations (Ile107Ile, Thr137Thr, Ala143Ala) were observed in this study in *R. norvegicus* from Lisbon. When observed together in the same animal, these three mutations, described in the Azores (Terceira Island) and also in the USA, Argentina (Rost et al., 2009), New Zealand (Cowan et al., 2017) and France, have the denomination of Haplotype C (Grandemange et al., 2010). Despite the large sampling, the observed genetic susceptibility in both species in Lisbon and Ponta Delgada was somewhat unexpected, especially considering the observed pattern throughout Europe, in the Netherlands (Krijger et al., 2023), Germany (Jacob et al., 2012), Belgium (Baert et al., 2012), UK (Buckle et al., 2022), France (Grandemange et al., 2010; Pelz et al., 2005; Rost et al., 2009) and for the rodent pest *Mus musculus* in Portugal (Carromeu-Santos et al., 2023), for which resistance is completely widespread. In France, mutations have been detected in codons 120, 128 and 139 (L120Q, L128, Y139C, Y139F); in Belgium, resistance-conferring mutations were restricted to codons 120 and 139 (L120Q, Y139F); whereas in Germany, Denmark, Hungary, Italy and the Netherlands, only variations in codon 139 (Y139C, Y139F, Y139S) have been recorded. The UK remains the only country where all known resistance-conferring

Table 3
Frequency of detection and descriptive statistics of the presence of all five SGARs (second generation anticoagulant rodenticides) (ng/g) individually quantified as well as their sum (∑).

City	Species	Statistics	BRODI	BROMA	DFC	DFT	FLO	∑AR
Lisbon	*Rattus norvegicus*	Average ± SD	24.98 ± 108.09	216.32 ± 630.69	0.47 ± 1.56	0.24 ± 1.38	0.18 ± 1	242.2 ± 636.3
		Median	0.73	6.28	0	0	0	10.3
		Minimum	0	0	0	0	0	0
		Maximum	608.43	2590.33	8.1	7.81	5.64	2607.7
		% Detection	65.6%	81.2%	12.5%	3.10%	3.10%	93.8%
	Rattus rattus	Average ± SD	16.6 ± 45.98	87.62 ± 310.72	0.05 ± 0.22	0 ± 0	0 ± 0	104.3 ± 319.6
		Median	0.49	1.62	0	0	0	4.6
		Minimum	0	0	0	0	0	0
		Maximum	200.18	1712.24	1.03	0	0	1773.6
		% Detection	52.9%	73.5%	5.9%	0%	0%	85.3%
Ponta Delgada	*Rattus norvegicus*	Average ± SD	39.35 ± 150.08	12.35 ± 51.27	0.71 ± 3.47	2.99 ± 17.76	0.18 ± 1.61	55.57 ± 169.1
		Median	0.67	0	0	0	0	3.92
		Minimum	0	0	0	0	0	0
		Maximum	1285.33	421.51	30.29	130.77	14.76	1363.9
		% Detection	57.1%	47.6%	17.9%	6.00%	2.40%	77.4%
	Rattus rattus	Average ± SD	1.36 ± 3.13	3.92 ± 9.22	0.23 ± 0.83	0.1 ± 0.35	0 ± 0	5.61 ± 9.7
		Median	0	0	0	0	0	1.04
		Minimum	0	0	0	0	0	0
		Maximum	11.28	32.8	2.98	1.28	0	34.4
		% Detection	46.2%	38.5%	7.70%	7.70%	0%	53.8%

BRODI — brodifacoum; BROMA — bromadiolone; DFC — difenacoum; DFT — difethialone; FLO — flocoumafen; ∑AR — sum all anticoagulant rodenticides.

mutations (L120Q, L128Q, Y139C, Y139F, Y139S) have been reported (Buckle et al., 2022). Similarly to our study, in Spain, no resistance-conferring mutations on codons 120, 128 or 139 have been identified for brown or black rats in the scientific literature (Bermejo-Nogales et al., 2022; Damin-Pernik et al., 2022; Goulois et al., 2015). Only two faecal samples of *R. norvegicus* originating from central Spain have been registered in the Rodenticide Resistance Action Committee (RRAC) (2024), carrying the Y139F mutation. Although mutations on exons 1 and 2 (Y25F, A26P, W59R) have been described in black rats (Damin-Pernik et al., 2022; Goulois et al., 2015), these mutations are associated with a much lower resistance factor compared to those in codons 120, 128 or 139. The minimal effect of mutations on exons 1 and 2 towards AR resistance suggests that even if present in both study areas, we would not expect them to play a major role on rodenticide resistance levels.

4.2. Anticoagulant rodenticide bioaccumulation in Vkorc1-susceptible rat populations

Hepatic quantification of rodenticides revealed no FGARs in rats from either Lisbon or Ponta Delgada, which is consistent with the current trend of minimal usage of these active ingredients across Europe (Jacob and Buckle, 2018). Nowadays, FGARs represent less than 3.5% of anticoagulant rodenticides in the European market (Commission Implementing Decision (EU) 2024/816, European Parliament and the Council of the European Union (2024). This is also corroborated by the limited availability of commercial FGARs' containing biocides in the Portuguese market. On the other hand, all currently authorised SGARs were detected in Lisbon and Ponta Delgada, revealing a common intense use, except for flocoumafen and difethialone which were only detected in a few samples from both cities, yet more expressively in Ponta Delgada. Differences in rodenticide detection between locations reflect rodenticide availability in the environment, which is also dependent on regional and governmental pest control strategies. The most frequently recorded ARs were, by far, bromadiolone and brodifacoum, which is in line with data collected from numerous raptor species from Portugal (Carromeu-Santos et al., 2025). It is also concordant with information gathered from pest controllers nationwide which highlight the same compounds as the most common for professional use (data not published). The detection rate of difethialone was surprisingly low, particularly for Ponta Delgada, despite being the rodenticide acquired by the Azorean Regional Government (145 tons of difethialone 0.0025%, between 2018 and 2020) as part of the archipelago's pest management strategy over the past few years. Difethialone was either distributed free of charge to farmers or used in government-led pest management initiatives (Direção Regional de Agricultura, S.R. da A. e do D.R, 2021).The obtained data reveals a higher probability of finding AR residues in rats caught in Lisbon than in Ponta Delgada. The denser urban matrix of Lisbon compared with the more rural Ponta Delgada certainly impacts the type of rodent control applied in each city. In the agricultural and more open areas of Ponta Delgada, the intake of ARs by rodents may be reduced due to high availability of alternative food sources (Patergnani et al., 2010), such as grains (corn and corn silage). On the other hand, in Lisbon, food availability is highly dependent on anthropic sources, like waste disposal sites, increasing the likelihood of rats consuming the rodenticide bait when present (Patergnani et al., 2010), therefore, reaching higher concentrations of hepatic ARs.

Body weight was also a significant factor associated with levels of AR concentration, at least in black rats, as heavier rats showed significantly higher ∑AR and bromadiolone concentrations. This is actually the opposite tendency to that observed by Wang et al. (2019), suggesting that animals exposed to other pesticides (insecticides, herbicides and fungicides) showed a decrease in body weight for increased exposure time.

The GLM analysis also shows that older rats are more likely to accumulate bromadiolone residues than younger rats. This suggests a continued consumption of sub-lethal doses throughout their lifespan and highlights the high persistence of these compounds in the liver. However, a similar pattern was not observed for brodifacoum, despite it being the most persistent AR in the liver, with an elimination half-life of 307 days (Vandenbroucke et al., 2008). One possible explanation is that bromadiolone is less toxic than brodifacoum (Špakauskas et al., 2005), potentially resulting in greater bioaccumulation if consumed in small non-lethal doses.

One of the few studies focusing on wild rodent pests (Desvars-Larrive et al., 2017) monitored AR bioaccumulation in a park in France, where the use of ARs had been banned. While most rats showed low levels of AR bioaccumulation, three of the analysed animals had AR concentrations exceeding 500 ng/g. In fact, the specimen carrying the Y139F resistance-conferring mutation in the *Vkorc1* gene (in homozygosity) exhibited the highest AR concentration (1500 ng/g ww), highlighting the impact of this well-known resistance mechanism.

While the use of summed AR concentrations (∑ARs) provides a practical metric to describe overall exposure, this approach has limitations. Anticoagulant rodenticides differ in both toxicity and persistence

— brodifacoum, for instance, is more potent and persistent than bromadiolone. Therefore, applying a general ∑AR threshold may underestimate or overestimate potential toxicosis (Rattner and Harvey, 2021). In addition, hepatic residues reflect past exposure rather than active toxicosis, which depends on dose, frequency of intake and the condition of the individual (Rattner and Harvey, 2021). Thus, although ∑ARs are useful for comparisons across sites or age classes, their interpretation must be made with caution and in light of these constraints.

4.3. Potential alternative mechanisms of rodenticide resistance

Variation in the *Vkorc*1 gene has been closely associated with the resistance to ARs' phenotype, although there is evidence suggesting that resistance to second-generation rodenticides may be polygenic (Gill et al., 1993). Tolerance to ARs has also been documented, not necessarily linked to *Vkorc1* variation. Numerous studies have considered other putative mechanisms contributing to resistance to first-generation anticoagulant rodenticides in rats. The overexpression of the Cyp3A gene subfamily (Ishizuka et al., 2007) or the increased activity of NADPH cytochrome *c* reductase (Ishizuka et al., 2008) are examples of changes in warfarin metabolism responsible for reduced susceptibility to ARs in rats. Similarly, Sugano et al. (2001) demonstrated enhanced warfarin metabolism in *Rattus rattus* during the early stages of exposure. Enhanced metabolic activity towards FGARs has also been reported on voles (Horak et al., 2015). Moreover, Garg et al. (2017) found no mutations in the *Vkorc1* gene in animals suspected to be resistant to bromadiolone. These animals exhibited survival periods ranging from 26 to 73 days in a feeding test trial with LD50 for bromadiolone, using 0.005% and 0.05% baits, suggesting the presence of alternative tolerance mechanisms. Markussen et al. (2007, 2008) suggested that resistance to bromadiolone in *Rattus norvegicus* may not only be attributed to mutations in the *Vkorc1* gene, but also be explained by an increase in metabolism of this compound due to overexpression of cytochrome P450 enzymes. Among our dataset, a total of fourteen rats exhibited hepatic concentration of bromadiolone above 100 ng/g, while carrying no known resistance-conferring mutations to bromadiolone (Y139C, Y139F and L120Q). If alternative AR resistance mechanisms are present, they could lead to unsuccessful rodent control in rats that appeared susceptible, based on the *Vkorc1* exon 3 analysis. Even though alternative mechanisms of resistance to FGARs or bromadiolone have been associated with increased metabolic rates, rats would still bioaccumulate ARs and retain them within their tissues. As predators typically consume multiple rats, sublethal concentrations could progressively accumulate throughout the food web. Furthermore, there is currently no evidence regarding how such mechanisms might operate against the more toxic and persistent SGARs.

Apart from specific mechanisms of resistance, there are natural levels of tolerance that could allow high concentrations of anticoagulant rodenticides in rats. For instance, it is documented that female rats exhibited a higher tolerance than males to many ARs (Lefebvre et al., 2016). This increased tolerance in females is attributed to higher VKOR activity in the liver (2.4 fold). Additionally, the basal activity combined with the longer half-life of vitamin K-dependent clotting factors allows for greater accumulation of ARs in the liver of female rats (Lefebvre et al., 2016). However, this association was not found within our dataset, as hepatic concentration of ARs was not significantly higher in females than in males for any of the quantified rodenticides or their sum.

4.4. Consequences for non-target wildlife

Hepatic concentrations of ARs above 100 ng/g may lead to alterations in blood coagulation in susceptible rats (Desvars-Larrive et al., 2017). In our study, 26 rats exhibited ∑ARs concentrations above 100 ng/g. For brodifacoum alone, the maximum concentration recorded in a single individual was 1285 ng/g and for bromadiolone, 2590 ng/g. All these *Vkorc1* wild-type animals were highly contaminated, posing serious risks for non-target wildlife in both study places. This is highly concerning, especially for birds of prey (Nakayama et al., 2019) that feed predominantly on rodents and are particularly susceptible to ARs (Khidkhan et al., 2024). This consumption of contaminated rats leads to the phenomenon of biomagnification through the flow of anticoagulant rodenticides from rats (prey) to their common predators. SGAR values above 200 ng/g could be potentially lethal for raptors (Christensen et al., 2012), while levels around 100 ng/g for most raptor families (40 ng/g for Tytonidae family) are strongly associated with a high probability of developing coagulopathy (Elliott et al., 2024). Some reports revealed adverse effects on local bird populations after rodent management actions (Ebbert and Burek-Huntington, 2010; Walther et al., 2021b) and many recent studies have documented hepatic AR residues in many species that include rats in their diet (Martín Cruz et al., 2024a, 2024b; Moriceau et al., 2022). In our study, this highlights the risk of exposure of *Buteo buteo* (present in Lisbon and São Miguel) and *Falco tinnunculus* (present just in Lisbon).

5. Conclusions

This study addressed a knowledge gap regarding wild rat populations living under natural conditions in urban environments exposed to rodent control practices. Our findings indicate that despite the absence of the most common resistance-conferring mutations in the third exon of the *Vkorc1* gene observed in Europe, rats still bioaccumulate significant levels of SGARs. Moreover, older rats have a higher probability of accumulating bromadiolone residues, reflecting an overtime exposure. This underscores the need for effective mitigation measures for rodent pest control. The implications of these findings raise concerns and align with documented cases of secondary-poisoning in non-target wildlife worldwide, particularly in predator species that consume rodents as prey on a daily basis. While this study provides valuable insights into SGARs concentration in deemed susceptible rat populations, further research is needed to evaluate whether mutations on other exons of the *Vkorc1* gene could play a role in resistance to ARs or explore alternative resistance mechanisms beyond *Vkorc1* mutations.

CRediT authorship contribution statement

Ana Carromeu-Santos: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Beatriz Martín-Cruz:** Writing – review & editing, Methodology, Investigation. **Tomé Neves:** Writing – review & editing, Methodology, Formal analysis. **Elizandra Matos Cardoso:** Writing – review & editing, Methodology. **Naiara Guimarães Sales:** Writing – review & editing, Investigation. **Andrea Acosta Dacal:** Writing – review & editing, Methodology. **Ana Macías-Montes:** Writing – review & editing, Methodology. **Rita Pacheco:** Writing – review & editing, Resources. **Bastiaan G. Meerburg:** Writing – review & editing, Conceptualization. **Allan D. McDevitt:** Writing – review & editing, Conceptualization. **Maria da Luz Mathias:** Writing – review & editing, Supervision, Resources. **Octavio Pérez Luzardo:** Writing – review & editing, Supervision, Resources. **Sofia Isabel Gabriel:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare no competing financial interests or personal relationships influencing the work reported in this study.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2026.181344.

Data availability

Data will be made available on request.

References

Baert, K., Stuyck, J., Breyne, P., Maes, D., Casaer, J., 2012. Distribution of anticoagulant resistance in the brown rat in Belgium. Belgian J. Zool. 142, 39–48.

Baxter, M.A., Buckle, A.P., Endepols, S., Prescott, C.V., 2022. Anticoagulant rodenticide blood-clotting dose-responses and resistance factors for Tyrosine139Cysteine (Y139C) heterozygous- and homozygous-resistant house mice (*Mus musculus*). Pest Manag. Sci. 78, 4480–4487. https://doi.org/10.1002/ps.7066.

Bermejo-Nogales, A., Rodríguez Martín, J.A., Coll, J., Navas, J.M., 2022. VKORC1 single nucleotide polymorphisms in rodents in Spain. Chemosphere 308. https://doi.org/10.1016/j.chemosphere.2022.136021.

Boyle, C.M., 1960. Case of apparent resistance of *Rattus*. Nature 4749, 517.

Brakes, C.R., Smith, R.H., 2005. Exposure of non-target small mammals to rodenticides: short-term effects, recovery and implications for secondary poisoning. J. Appl. Ecol. 42, 118–128. https://doi.org/10.1111/j.1365-2664.2005.00997.x.

Broughton, R.K., Searle, K.R., Walker, L.A., Potter, E.D., Pereira, M.G., Carter, H., Sleep, D., Noble, D.G., Butler, A., Johnson, A.C., 2022. Long-term trends of second generation anticoagulant rodenticides (SGARs) show widespread contamination of a bird-eating predator, the Eurasian sparrowhawk (*Accipiter nisus*) in Britain. Environ. Pollut. 314. https://doi.org/10.1016/j.envpol.2022.120269.

Buckle, A., 2013. Anticoagulant resistance in the United Kingdom and a new guideline for the management of resistant infestations of Norway rats (*Rattus norvegicus* Berk.). Pest Manag. Sci. 69, 334–341. https://doi.org/10.1002/ps.3309.

Buckle, A.P., Cawthraw, S., Neumann, J., Prescott, C., 2022. Anticoagulant Resistance in Rats and Mice in the UK—Summary Report with New Data for 2021 and 2022. https://doi.org/10.13140/RG.2.2.20232.90882.

Buckley, J.Y., Murray, M.H., de la Sancha, N.U., Fidino, M., Byers, K.A., Fyffe, R., Magle, S., 2024. Widespread exposure to anticoagulant rodenticides among common urban mesopredators in Chicago. Sci. Total Environ. 952. https://doi.org/10.1016/j.scitotenv.2024.175883.

Carromeu-Santos, A., Mathias, M.L., Gabriel, S.I., 2023. Widespread distribution of rodenticide resistance-conferring mutations in the Vkorc1 gene among house mouse populations in Portuguese Macaronesian islands and Iberian Atlantic areas. Sci. Total Environ. 900, 166290. https://doi.org/10.1016/j.scitotenv.2023.166290.

Carromeu-Santos, A., Martín-Cruz, B., Neves, T., Acosta-Dacal, A., Macías-Montes, A., Casero, M., Mathias, M.L., Luzardo, O.P., Gabriel, S.I., 2025. Toxic legacy: the hidden impact of anticoagulant rodenticides on Portuguese raptors. Sci. Total Environ. 1002, 180547. https://doi.org/10.1016/j.scitotenv.2025.18054.

Christensen, T.K., Lassen, P., Elmeros, M., 2012. High exposure rates of anticoagulant rodenticides in predatory bird species in intensively managed landscapes in Denmark. Arch. Environ. Contam. Toxicol. 63, 437–444. https://doi.org/10.1007/s00244-012-9771-6.

Cowan, P.E., Gleeson, D.M., Howitt, R.L.J., Ramón-Laca, A., Esther, A., Pelz, H.J., 2017. Vkorc1 sequencing suggests anticoagulant resistance in rats in New Zealand. Pest Manag. Sci. 73, 262–266. https://doi.org/10.1002/ps.4304.

Damin-Pernik, M., Hammed, A., Giraud, L., Goulois, J., Benoît, E., Lattard, V., 2022. Distribution of non-synonymous Vkorc1 mutations in roof rats (*Rattus rattus*) in France and in Spain — consequences for management. Pestic. Biochem. Physiol. 183. https://doi.org/10.1016/j.pestbp.2022.105052.

Desvars-Larrive, A., Pascal, M., Gasqui, P., Cosson, J.F., Benoît, E., Lattard, V., Crespin, L., Lorvelec, O., Pisanu, B., Teynié, A., Vayssier-Taussat, M., Bonnet, S., Marianneau, P., Lacôte, S., Bourhy, P., Berny, P., Pavio, N., Le Poder, S., Gilot-Fromont, E., Jourdain, E., Hammed, A., Fourel, I., Chikh, F., Vourc'h, G., 2017. Population genetics, community of parasites, and resistance to rodenticides in an urban brown rat (*Rattus norvegicus*) population. PloS One 12, 1–25. https://doi.org/10.1371/journal.pone.0184015.

Diagne, C., Ballesteros-Mejia, L., Cuthbert, R.N., Bodey, T.W., Fantle-Lepczyk, J., Angulo, E., Bang, A., Dobigny, G., Courchamp, F., 2023. Economic costs of invasive rodents worldwide: the tip of the iceberg. PeerJ 11, 1–24. https://doi.org/10.7717/peerj.14935.

Díaz, J.C., Kohn, M.H., 2021. A VKORC1-based SNP survey of anticoagulant rodenticide resistance in the house mouse, Norway rat and roof rat in the USA. Pest Manag. Sci. 77, 234–242. https://doi.org/10.1002/ps.6012.

Direção Regional de Agricultura, S.R. da A. e do D.R, 2021. Relatório de atividades 2020.

Dormann, C.F., Elith, J., Bacher, S., Buchmann, C., Carl, G., Carré, G., Marquéz, J.R.G., Gruber, B., Lafourcade, B., Leitão, P.J., Münkemüller, T., Mcclean, C., Osborne, P.E., Reineking, B., Schröder, B., Skidmore, A.K., Zurell, D., Lautenbach, S., 2013. Collinearity: a review of methods to deal with it and a simulation study evaluating their performance. Ecography (Cop) 36, 27–46. https://doi.org/10.1111/j.1600-0587.2012.07348.x.

Ebbert, S., Burek-Huntington, K., 2010. Anticoagulant residual concentration and poisoning in birds following a large-scale aerial application of 25 ppm *Brodifacoum* bait for rat eradication on Rat Island, Alaska. Proc. Vertebr. Pest Conf. 24, 153–160. https://doi.org/10.5070/v424110700.

Elliott, J.E., Silverthorn, V., English, S.G., Mineau, P., Hindmarch, S., Thomas, P.J., Lee, S., Bowes, V., Redford, T., Maisonneuve, F., Okoniewski, J., 2024. Anticoagulant rodenticide toxicity in terrestrial raptors: tools to estimate the impact on populations in North America and globally. Environ. Toxicol. Chem. 43, 988–998. https://doi.org/10.1002/etc.5829.

Environmental Protection Agency (EPA), 2008. Risk Mitigation Decision for Ten Rodenticides, p. 60.

Parliament, European, the Council of the European Union, 2016. Commission regulation (EU) 2016/1179 of 19 July 2016. J. Eur. Union 195, 11–25.

Parliament, European, the Council of the European Union, 2024. Commission implementing decision (EU) 2024/816 of 5 March 2024. J. Eur. Union 1–10, 2024/3058.

Frankova, M., Radostna, T., Aulicky, R., Stejskal, V., 2004. Less brodifacoum in baits results in greater accumulation in the liver of captive *Rattus norvegicus* in a no-choice trail. J. Pest. Sci. 2024. https://doi.org/10.1007/s10340-023-01737-y.

Garg, N., Singla, N., Jindal, V., Babbar, B.K., 2017. Studies on bromadiolone resistance in *Rattus rattus* populations from Punjab, India. Pestic. Biochem. Physiol. 139, 24–31. https://doi.org/10.1016/j.pestbp.2017.04.005.

Gill, J.E., Kerins, G.M., Langton, S.D., Macnicoll, A.D., 1993. The development of a blood clotting response test for discriminating between difenacoum-resistant and susceptible Norway rats (*Rattus norvegicus*, Berk.). Comp. Biochem. Physiol. Pt. C Comp. 104, 29–36. https://doi.org/10.1016/0742-8413(93)90106-U.

Goulois, J., Chapuzet, A., Lambert, V., Chatron, N., Tchertanov, L., Legros, L., Benoît, E., Lattard, V., 2015. Evidence of a target resistance to antivitamin K rodenticides in the roof rat *Rattus rattus*: identification and characterisation of a novel Y25F mutation in the Vkorc1 gene. Pest Manag. Sci. 72, 544–550. https://doi.org/10.1002/ps.4020.

Grandemange, A., Lasseur, R., Longin-Sauvageon, C., Benoit, E., Berny, P., 2010. Distribution of VKORC1 single nucleotide polymorphism in wild *Rattus norvegicus* in France. Pest Manag. Sci. 66, 270–276. https://doi.org/10.1002/ps.1869.

Horak, K.E., Volker, S.F., Campton, C.M., 2015. Increased diphacinone and chlorophacinone metabolism in previously exposed wild caught voles, Microtus californicus. Crop Prot. 78, 35–39. https://doi.org/10.1016/j.cropro.2015.08.

Iacucci, A., Colangelo, P., Gamberi, V., Mori, E., Capizzi, D., Baert, K., Esther, A., Leirs, H., Petit, T., Ribas, A., Aloise, G., Annesi, F., Castiglia, R., 2018. VKORC1 mutation in European populations of *Rattus norvegicus* with first data for Italy and the report of a new amino acid substitution. Hystrix. Ital. J. Mammal. 29, 95–99. https://doi.org/10.4404/hystrix.

Ishizuka, M., Okajima, F., Tanikawa, T., Min, H., Tanaka, K.D., Sakamoto, K.Q., Fujita, S., 2007. Elevated warfarin metabolism in warfarin-resistant roof rats (*Rattus rattus*) in Tokyo. Drug Metab. Dispos. 35, 62–66. https://doi.org/10.1124/dmd.106.011775.

Ishizuka, M., Tanikawa, T., Tanaka, K.D., Heewon, M., Okajima, F., Sakamoto, K.Q., Fujita, S., 2008. Pesticide resistance in wild mammals — mechanisms of anticoagulant resistance in wild rodents. J. Toxicol. Sci. 33, 283–291. https://doi.org/10.2131/jts.33.283.

Jacob, J., Buckle, A., 2018. Use of Anticoagulant Rodenticides in Different Applications Around the World. doi:https://doi.org/10.1007/978-3-319-64377-9_2.

Jacob, J., Endepols, S., Pelz, H.J., Kampling, E., Cooper, T.G., Yeung, C.H., Redmann, K., Schlatt, S., 2012. Vitamin K requirement and reproduction in bromadiolone-resistant Norway rats. Pest Manag. Sci. 68, 378–385. https://doi.org/10.1002/ps.2273.

Keating, M.P., Saldo, E.A., Frair, J.L., Cunningham, S.A., Mateo, R., Jachowski, D.S., 2024. Global review of anticoagulant rodenticide exposure in wild mammalian carnivores. Anim. Conserv. 1–15. https://doi.org/10.1111/acv.12947.

Khidkhan, K., Yasuhira, F., Saengtienchai, A., Kasorndorkbua, C., Sitdhibutr, R., Ogasawara, K., Adachi, H., Watanabe, Y., Saito, K., Sakai, H., Horikoshi, K., Suzuki, H., Kawai, Y.K., Takeda, K., Yohannes, Y.B., Ikenaka, Y., Rattner, B.A., Ishizuka, M., Nakayama, S.M.M., 2024. Evaluation of anticoagulant rodenticide sensitivity by examining in vivo and in vitro responses in avian species, focusing on raptors. Environ. Pollut. 341, 122837. https://doi.org/10.1016/j.envpol.2023.122837.

Krijger, I.M., Strating, M., van Gent-Pelzer, M., van der Lee, T.A.J., Burt, S.A., Schroeten, F.H., de Vries, R., de Cock, M., Maas, M., Meerburg, B.G., 2023. Large-scale identification of rodenticide resistance in *Rattus norvegicus* and *Mus musculus* in the Netherlands based on Vkorc1 codon 139 mutations. Pest Manag. Sci. 79, 989–995. https://doi.org/10.1002/ps.7261.

Laakso, S., Suomalainen, K., Koivisto, S., 2010. Literature review on residues of anticoagulant rodenticides in non-target animals. J. Chem. Inf. Model. https://doi.org/10.6027/TN2010-541.

Lefebvre, S., Rannou, B., Besse, S., Benoit, E., Lattard, V., 2016. Origin of the gender differences of the natural resistance to antivitamin K anticoagulants in rats. Toxicology 344–346, 34–41. https://doi.org/10.1016/j.tox.2016.02.002.

Lettoof, D.C., Lohr, M.T., Busetti, F., Bateman, P.W., Davis, R.A., 2020. Toxic time bombs: frequent detection of anticoagulant rodenticides in urban reptiles at multiple trophic levels. Sci. Total Environ. 724, 138218. https://doi.org/10.1016/j.scitotenv.2020.138218.

López-Perea, J.J., Mateo, R., 2018. Secondary Exposure to Anticoagulant Rodenticides and Effects on Predators. https://doi.org/10.1007/978-3-319-64377-9_7.

Markussen, M.D., Heiberg, A.C., Alsbo, C., Nielsen, P.S., Kauppinen, S., Kristensen, M., 2007. Involvement of hepatic xenobiotic related genes in bromadiolone resistance in wild Norway rats, *Rattus norvegicus* (Berk.). Pestic. Biochem. Physiol. 88, 284–295. https://doi.org/10.1016/j.pestbp.2007.01.003.

Markussen, M.D.K., Heiberg, A.C., Fredholm, M., Kristensen, M., 2008. Differential expression of cytochrome P450 genes between bromadiolone-resistant and anticoagulant-susceptible Norway rats: a possible role for pharmacokinetics in bromadiolone resistance. Pest Manag. Sci. 64, 239–248. https://doi.org/10.1002/ps.1506.

Martín Cruz, B., Cecchetti, M., Simbaña-Rivera, K., Rial-Berriel, C., Acosta-Dacal, A., Zumbado-Peña, M., Henríquez-Hernández, L.A., Gallo-Barneto, R., Cabrera-Pérez, M.Á., Melián-Melián, A., Suárez-Pérez, A., Luzardo, O.P., 2024a. Potential exposure of native wildlife to anticoagulant rodenticides in Gran Canaria (Canary Islands, Spain): evidence from residue analysis of the invasive California Kingsnake (*Lampropeltis californiae*). Sci. Total Environ. 911, 168761. https://doi.org/10.1016/j.scitotenv.2023.168761.

Martín Cruz, B., Rial Berriel, C., Acosta Dacal, A., Carromeu-Santos, A., Simbaña-Rivera, K., Gabriel, S.I., Pastor Tiburón, N., González González, F., Fernández Valeriano, R., Henríquez-Hernández, L.A., Zumbado-Peña, M., Luzardo, O.P., 2024b. Differential exposure to second-generation anticoagulant rodenticides in raptors from continental and insular regions of the Iberian Peninsula. Environ. Pollut. 362. https://doi.org/10.1016/j.envpol.2024.125034.

McGee, C.F., McGilloway, D.A., Buckle, A.P., 2020. Anticoagulant rodenticides and resistance development in rodent pest species — a comprehensive review. J. Stored Prod. Res. 88, 101688. https://doi.org/10.1016/j.jspr.2020.101688.

Morand, S., Bordes, F., Chen, H.W., Claude, J., Cosson, J.F., Galan, M., Czirják, G.A., Greenwood, A.D., Latinne, A., Michaux, J., Ribas, A., 2015. Global parasite and *Rattus* rodent invasions: the consequences for rodent-borne diseases. Integr. Zool. 10, 409–423. https://doi.org/10.1111/1749-4877.12143.

Moriceau, M.A., Lefebvre, S., Fourel, I., Benoit, E., Buronfosse-Roque, F., Orabi, P., Rattner, B.A., Lattard, V., 2022. Exposure of predatory and scavenging birds to anticoagulant rodenticides in France: exploration of data from French surveillance programs. Sci. Total Environ. 810. https://doi.org/10.1016/j.scitotenv.2021.151291.

Nakayama, S.M.M., Morita, A., Ikenaka, Y., Mizukawa, H., Ishizuka, M., 2019. A review: poisoning by anticoagulant rodenticides in non-target animals globally. J. Vet. Med. Sci. 81, 298–313. https://doi.org/10.1292/jvms.17-0717.

Patergnani, M., Mughini Gras, L., Poglayen, G., Gelli, A., Pasqualucci, F., Farina, M., Stancampiano, L., 2010. Environmental influence on urban rodent bait consumption. J. Pest Sci. 83 (2004), 347–359. https://doi.org/10.1007/s10340-010-0304-7.

Pelz, H.J., Rost, S., Hünerberg, M., Fregin, A., Heiberg, A.C., Baert, K., MacNicoll, A.D., Prescott, C.V., Walker, A.S., Oldenburg, J., Müller, C.R., 2005. The genetic basis of resistance to anticoagulants in rodents. Genetics 170, 1839–1847. https://doi.org/10.1534/genetics.104.040360.

Pitt, W.C., Berentsen, A.R., Shiels, A.B., Volker, S.F., Eisemann, J.D., Wegmann, A.S., Howald, G.R., 2015. Non-target species mortality and the measurement of brodifacoum rodenticide residues after a rat (*Rattus rattus*) eradication on Palmyra Atoll, tropical Pacific. Biol. Conserv. 185, 36–46. https://doi.org/10.1016/j.biocon.2015.01.008.

Prescott, C., Coan, E.E., Jones, C., Baxter, M., Rymer, D., Buckle, A.P., 2018. Anticoagulant Resistance in Rats and Mice in the UK—Current Status in 2018 Rodent Management View Project UK Rodenticide Stewardship Regime View Project.

Core Team, R., 2024. R: A Language and Environment for Statistical. In: R Foundation for Statistical Computing. https://www.R-project.org.

Rattner, B.A., Harvey, J.J., 2021. Challenges in the interpretation of anticoagulant rodenticide residues and toxicity in predatory and scavenging birds. Pest Manag. Sci. 77 (2), 604–610. https://doi.org/10.1002/ps.6137.

Rial-Berriel, C., Acosta-Dacal, A., Zumbado, M., Henríquez-Hernández, L.A., Rodríguez-Hernández, Á., Macías-Montes, A., Boada, L.D., Travieso-Aja, M.D.M., Cruz, B.M., Luzardo, O.P., 2021. A method scope extension for the simultaneous analysis of pops, current-use and banned pesticides, rodenticides, and pharmaceuticals in liver. Application to food safety and biomonitoring. Toxics. https://doi.org/10.3390/toxics9100238.

Ringani, G., Zengeya, T., Pirk, C., Chimimba, C., 2022. Assessment of craniometric sexual dimorphism and ontogenetic variation in invasive *Rattus norvegicus* and *R. rattus* from urban and peri-urban areas of Gauteng Province, South Africa. Mammalia 86, 601–614. https://doi.org/10.1515/mammalia-2021-0191.

Rodenticide Resistance Action Committee (RRAC), 2024. RRAC — Rodenticide Resistance Action Committee. Available at: https://rrac.info/index.html (Accessed: August 2025).

Rost, S., Pelz, H.J., Menzel, S., MacNicoll, A.D., León, V., Song, K.J., Jäkel, T., Oldenburg, J., Müller, C.R., 2009. Novel mutations in the VKORC1 gene of wild rats and mice — a response to 50 years of selection pressure by warfarin? BMC Genet. 10, 1–9. https://doi.org/10.1186/1471-2156-10-4.

Špakauskas, V., Klimienė, I., Ružauskas, M., Bandzaitė, V., 2005. Toxicity and effectiveness of indirect anticoagulants bramodiolone and brodifacoum. Biologija 77–81. https://doi.org/10.6001/biologija.vi4.610.

Sugano, S., Kobayashi, T., Tanikawa, T., Kawakami, Y., Kojima, H., Nakamura, K., Uchida, A., Morishima, N., Tamai, Y., 2001. Suppression of CYP3A2 mRNA expression in the warfarin-resistant roof rat, *Rattus rattus*: possible involvement of cytochrome P450 in the warfarin resistance mechanism. Xenobiotica 31, 399–407. https://doi.org/10.1080/00498250110060932.

Tanaka, K.D., Kawai, Y.K., Ikenaka, Y., Harunari, T., Tanikawa, T., Ando, S., Min, H.W., Okajima, F., Fujita, S., Ishizuka, M., 2012. The genetic mechanisms of warfarin resistance in *Rattus rattus* found in the wild in Japan. Pestic. Biochem. Physiol. 103, 144–151. https://doi.org/10.1016/j.pestbp.2012.04.011.

Vandenbroucke, V., Bousquet-Melou, A., De Backer, P., Croubels, S., 2008. Pharmacokinetics of eight anticoagulant rodenticides in mice after single oral administration. J. Vet. Pharmacol. Ther. 31, 437–445. https://doi.org/10.1111/j.1365-2885.2008.00979.x.

Vein, J., Vey, D., Fourel, I., Berny, P., 2013. Bioaccumulation of chlorophacinone in strains of rats resistant to anticoagulants. Pest Manag. Sci. 69, 397–402. https://doi.org/10.1002/ps.3367.

Walther, B., Ennen, H., Geduhn, A., Schlötelburg, A., Klemann, N., Endepols, S., Schenke, D., Jacob, J., 2021a. Effects of anticoagulant rodenticide poisoning on spatial behavior of farm dwelling Norway rats. Sci. Total Environ. 787, 147520. https://doi.org/10.1016/j.scitotenv.2021.147520.

Walther, B., Geduhn, A., Schenke, D., Jacob, J., 2021b. Exposure of passerine birds to brodifacoum during management of Norway rats on farms. Sci. Total Environ. 762, 144160. https://doi.org/10.1016/j.scitotenv.2020.144160.

Wang, M., Guckland, A., Murfitt, R., Ebeling, M., Sprenger, D., Foudoulakis, M., Koutsaftis, A., 2019. Relationship between magnitude of body weight effects and exposure duration in mammalian toxicology studies and implications for ecotoxicological risk assessment. Environ. Sci. Eur. 31, 1–7. https://doi.org/10.1186/s12302-019-0221-1.

Watt, B.E., Proudfoot, A.T., Bradberry, S.M., Vale, J.A., 2005. Anticoagulant rodenticides. Toxicol. Rev. 24 (4), 259–269. https://doi.org/10.2165/00139709-200524040-00005.