Science of the Total Environment 954 (2024) 176321

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Short Communication

No evidence of mutations associated with anticoagulant resistance in gene *Vkorc1* in brown and black rats from Barcelona

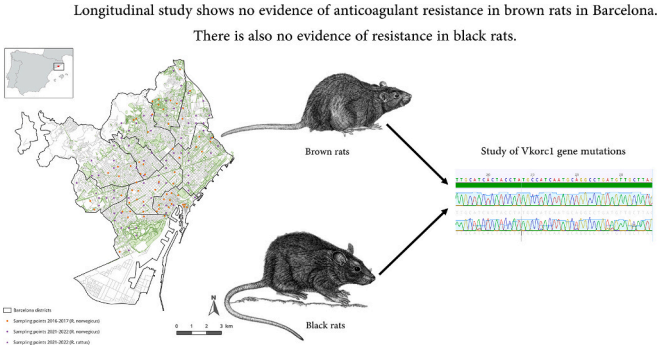
María José Ruiz-López [a,c,*], Sandra Franco [b], Josué Martínez-de la Puente [a,c], Martina Ferraguti [a,c], Emanuele Miccolis [d,e], Robert Petit [b], Laura Barahona [b,f], Jordi Figuerola [a,c], Tomas Montalvo [b,c,f]

[a] Estación Biológica de Doñana (EBD), CSIC, Avda. Américo Vespucio 26, 41092 Seville, Spain
[b] Agència de Salut Pública de Barcelona (ASPB), Pl. Lesseps, 1, 08023 Barcelona, Spain
[c] CIBER Epidemiología y Salud Pública (CIBERESP), Madrid, Spain
[d] Brighton Third Sector Training LTD, 161 Surrenden Road, BN16NN Brighton, United Kingdom
[e] Third Sector International (3Si), C. Leonardo da Vinci, 18, Planta 5ª - Módulos 1 y 2, 41092 Sevilla, Spain
[f] Institut de Recerca Sant Pau (IR SANT PAU), Sant Quintí 77-79, 08041 Barcelona, Spain

HIGHLIGHTS

- This is the first longitudinal study of anticoagulant resistance in brown rats in Spain.
- We found no evidence of anticoagulant resistance in Barcelona's brown rats.
- Rotating anticoagulant rodenticides likely helps prevent resistance in brown rats.
- *Vkorc1* gene in Barcelona black rats lacks anticoagulant resistance mutations.
- These results are a baseline for future analysis of *Vkorc1* mutations in Barcelona rats.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Rafael Mateo

Keywords:
Commensal rodents
Pest control
Rattus norvegicus
Rattus rattus
Vkorc1
Urban

ABSTRACT

Synanthropic rodents such as the brown rat (*Rattus norvegicus*) and black rat (*Rattus rattus*) are a source of disturbance in urban areas and the focus of control programs. Control measures often rely on anticoagulant rodenticides, but their broad use is compromised by the emergence of resistance. Here we studied the prevalence of anticoagulant resistance genotypes in the *Vkorc1* gene in rats in the metropolitan area of Barcelona. In this area, part of the management practices to control brown rats include anticoagulant rodenticide use, but rodenticides with different active ingredients are used in rotation. Brown rats were sampled from the sewage system during two periods: from December 2016 to November 2017 when difenacoum and brodifacoum were used, and from August 2021 to July 2022 when bromadiolone was used. Because black rats have just recently been detected in Barcelona, we only studied them during the latter sampling period, with samples obtained from a control action carried out in a green urban area. Exon 3 of the *Vkorc1* gene was characterized in both species, while exon 1 was additionally analyzed in black rats. Synonymous mutations, not resulting in amino-acid

* Corresponding author at: Estación Biológica de Doñana (EBD), CSIC, Avda. Américo Vespucio 26, 41092 Seville, Spain.
E-mail address: mjruiz@ebd.csic.es (M.J. Ruiz-López).

https://doi.org/10.1016/j.scitotenv.2024.176321
Received 30 April 2024; Received in revised form 13 September 2024; Accepted 14 September 2024
Available online 26 September 2024

changes, were found in both exons, indicating no evidence of anticoagulant resistance in the rats of Barcelona. This finding indicates that the current rodent management plan in Barcelona, which involves anticoagulant rotation for brown rats and the use of life capture traps in specific situations for black rats, has helped to prevent the emergence of resistance to anticoagulant rodenticides in rats in Barcelona. Future actions should aim to diversify the control measures included in the rodent management plan.

1. Introduction

Brown rats (*Rattus norvegicus*) and black rats (*Rattus rattus*) are two of the most important invasive species in the world (Global Invasive Species Database, IUCN, 2024a, 2024b). Both are widely distributed due to their high fecundity, varied eating habits, and adaptation to natural and anthropogenic environments. Rats are a source of disturbance in urban areas, citizen complaints and the focus of population control efforts (Diagne et al., 2023). The control of these synanthropic species presents a major challenge. Nowadays, anticoagulant rodenticides are widely used worldwide to control rodent populations (McGee et al., 2020), and are an important control tool in urban settings. However, despite their efficacy, their use is sometimes compromised by the emergence of resistance. One of the most studied resistance mechanisms involves the vitamin K epoxide reductase (VKOR) enzyme. This enzyme is the target of anticoagulant rodenticides (Rost et al., 2004), and when anticoagulants block it, the lack of bioavailable vitamin K results in the absence of gamma-carboxylated coagulation factors and compromises the coagulation process. As a result, anticoagulant rodenticides act to cause internal bleeding and ultimately death. Mutations in the *Vkorc1* gene encoding the complex 1 subunit of the VKOR enzyme (*Vkorc1*) affect the efficacy of both first-generation anticoagulant rodenticides (FGARs) such as warfarin, diphacione, and coumatryl, and some of the second-generation anticoagulant rodenticide (SGARs) like bromadiolone or difenacoum (Boyle, 1960; Buckle, 2013; Endepols et al., 2007; McGee et al., 2020; Pelz et al., 2005; Rost et al., 2009).

In brown rats, the *Vkorc1* gene encodes a 161 amino acid protein and is located on chromosome 1 (Grandemange et al., 2009). Mutations in this gene have been shown to confer resistance in brown rats (*Rattus norvegicus*, Pelz et al., 2005; Meerburg et al., 2014; Cowan et al., 2017; Iacucci et al., 2018; Boitet et al., 2018; Díaz and Kohn, 2021). There is clear evidence of anticoagulant resistance associated with mutations occurring in codons 120, 128, and 139 of exon 3 of the *Vkorc1* gene (Pelz et al., 2005; Grandemange et al., 2010). These mutations have been associated with resistance to FGARs and SGARs in different countries including Belgium, Denmark, France, Germany, the United Kingdom, Italy, the Netherlands, Canada, and the United States (Pelz et al., 2005; McGee et al., 2020). Although other non-synonymous mutations have been identified in the *Vkorc1* gene (Rost et al., 2009; Yiğit et al., 2023; Díaz and Kohn, 2021; Bermejo-Nogales et al., 2022), there is no clear evidence of their association to anticoagulant resistance (https://guide.rrac.info/rodenticide-molecules/norway-rat.html). Despite the importance of understanding anticoagulant resistance in different rodent populations, much less information is available for other rat species. Mutations associated with resistance have been identified in black rats (*Rattus rattus*, Tanaka et al., 2012; Goulois et al., 2016; Takeda et al., 2016; Cowan et al., 2017; Díaz and Kohn, 2021; Yiğit et al., 2023; Mahamat et al., 2023; Marquez et al., 2019), buff-breasted rats (*Rattus flavipectus*, Huang et al., 2011), kiore rats (*Rattus exulans*, Cowan et al., 2017) and house rats (*Rattus tanezumi*, Huang et al., 2022). In the case of black rats, both synonymous and non-synonymous mutations have been detected in exon 3, but there is no data on whether they confer resistance (McGee et al., 2020; Chua et al., 2022). However, for exon 1 cases of anticoagulant resistance have been detected in Japan, New Zealand, and Spain (Cowan et al., 2017; Tanaka et al., 2012; Goulois et al., 2016), for exon 2, in New Zealand and Japan (Cowan et al., 2017; Takeda et al., 2016; Tanaka et al., 2012). Out of these mutations, most have been associated with resistance to the FGAR warfarin, and only the mutation in exon 1 Tyr25Phe has been associated with resistance to SGARs, particularly bromadiolone, difenacoum, and difethialone in *in-vitro* studies, and failure of bromadiolone control *in vivo* (Goulois et al., 2016).

The frequency and distribution of the different mutations, both for black and brown rats, vary greatly among countries. While studies in France or The Netherlands, show widespread resistance, with the frequency of mutations above 50 % (Meerburg et al., 2014; Desvars-Larrive et al., 2017), a low frequency of mutations associated with resistance has been found in other countries such as Ireland and Finland (Mooney et al., 2018; Aivelo et al., 2023). In Spain, to our knowledge, only four studies have analyzed the polymorphisms in the *Vkorc1* gene associated with anticoagulant resistance in rats. Goulois et al. (2016) found evidence of resistance to anticoagulants in black rats on a farm in Zaragoza associated with the mutation Tyr25Phe in exon 1. Subsequently, Iacucci et al. (2018) analyzed the frequency of *Vkorc1* mutations in different European populations of brown rats and found no evidence of mutations associated with anticoagulant resistance in the Spanish samples. A more recent study analyzed *Vkorc1* SNPs in exon 3 in rodents across Spain and found several polymorphisms in both brown and black rats (Bermejo-Nogales et al., 2022). None of these polymorphisms were in codons 120, 128, or 139, the ones commonly associated with major resistance to anticoagulants. However, some of the polymorphisms found in both species that cause amino acid changes (Ser149Ile-Ser149Thr, Glu155Lys and Glu155Gln) were predicted to have a reduced binding affinity to different SGARs (Bermejo-Nogales et al., 2022). Finally, Damin-Pernik et al. (2022), found several mutations associated with moderate resistance to anticoagulants *in vitro* in black rats in three regions in the center and North of Spain.

However, none of these studies have detailed information on management practices and rodenticides used. Rodenticide resistance is the result of rapid evolution under a novel selective pressure that acts either on standing variation or *de novo* mutations (Hawkins et al., 2019). Thus, rodenticide resistance emerges due to the complex combination of exposure to rodenticides, genetic variation in the rodent population and behavioral and environmental factors that can alter the exposure to rodenticides or the genetic variation in the population either through migration or genetic drift. Rodenticide rotation has been suggested to be an effective measure to help prevent the emergence of resistance (RRAC guidelines on anticoagulant rodenticide resistance management, 2015). In the city of Barcelona, even though anticoagulant rodenticide rotation was used to manage the mice population in municipal facilities, anticoagulant resistance is widespread among mice because there is a high prevalence of mutations associated with an adaptive introgression event (Ruiz-López et al., 2022). Here, we carry out a new study on the prevalence of anticoagulant resistance genotypes in the city of Barcelona but focus on brown rats inhabiting the sewage system and black rats inhabiting green spaces. Between the years 2014 and 2022 three anticoagulant rodenticides have been used in rotation, bromadiolone, brodifacoum and difenacoum. To evaluate the efficacy of the management strategy and assess the potential development of resistance over time we carried out a longitudinal study for brown rats (*Rattus norvegicus*). We examined brown rats over two time periods, from December 2016 to November 2017, and from August 2021 to July 2022. In addition, in recent years, there has been an increase in sightings of black rats, especially in forest environments of the city (large historical parks), probably due to colonization of these urban areas from nearby forest areas where they naturally inhabit. Therefore, we also monitored

anticoagulant resistance in black rats during the second period. This study represents the first longitudinal study looking at resistance in brown rats in Spain.

2. Material and methods

2.1. Sampling method and study area

Brown rats were captured in the public sewers of the city of Barcelona using dead capture devices, in the framework of the BCNRats Project led by the Agència de Salut Pública de Barcelona (ASPB), the competent institution in the city for pest surveillance and control. As part of the program, treatment is always initiated when there is clear evidence of the presence of rodents during inspection or when there is consistent suspicion of rat activity (*e.g.* rodent faeces, gnawed material, worker observations or reliable citizen observations). In the public sewage system rodenticide is applied in manholes or storm drains (secured with a rope and a nail to the inside). Every treated manhole or storm drain is checked every two weeks, and the rodenticide is replaced if it has been consumed or is damaged. When the rodenticide does not show signs of rodent activity, it is checked one last time after a month and if there is no activity detected, it is removed. Between the years 2014 and 2022, three different active substances were used for rat control in rotation: bromadiolone, difenacoum and brodifacoum. Specifically, from January 2014 to September 2014 bromadiolone was the active ingredient used in the sewage system (885 kg), changing to brodifacoum from July 2014 to February 2016 (2715 kg); difenacoum was the main rodenticide from March 2016 to July 2017 (6405 kg), when it changed to brodifacoum, being the active ingredient used until March 2019 (5600 kg); finally, from April 2019 to December 2022 bromadiolone was the active ingredient chosen (23,100 kg). The percentage of active substance in the baits has always been 0,005 % (Fig. 1).

Captures of brown rats were conducted between December 2016 and November 2017 and between August 2021 and July 2022. During the first sampling period, the anticoagulant rodenticides used were difenacoum and brodifacoum, and during the second sampling period, it was bromadiolone (Fig. 1). In both randomized studies, 135 sites were selected: 63 in the 2016–2017 period and 72 in the 2021–2022 period (Fig. 2). During the first period, the stratification factor was the number of citizen complaints (Pascual et al., 2020) (Supplementary Fig. 1), and in the second period, the sewerage density (m of sewerage per m^2) was used (Supplementary Fig. 2). In each stratum, points were randomly selected using QGIS software. For each point, a 90 m long section was selected and, in each section, 30 traps (death traps) were placed during the period 2016–2017 and 20 during the period 2021–2022. Black rats were captured within the rodent control program in a large historic forested city park using *in vivo* trapping devices incorporating a GSM tracking camera that sent a real-time photograph when there was a capture (Supplementary Fig. 2). On average, ten devices were deployed during the period 2021–2022, as these sites were home to protected fauna (Red squirrel), thus minimizing collateral effects. All the rats were transferred to the facilities of the ASPB. Black rats were euthanized in a CO_2 chamber following the current legislation. Their tails were separated and stored at −20 °C until molecular analyses. In total, 569 brown rats were captured between December 2016 and November 2017, and 183 brown rats and 22 black rats between 2021 and 2022.

2.2. Genomic DNA extraction and Vkorc1 sequencing

Genomic DNA was individually extracted from tail samples using a maximum of 50 mg of tissue with tail clippings shorter than 0.5 cm. Samples collected in 2016–2017 were extracted using a Chloroform/Isoamyl Alcohol protocol (Gemmell and Akiyama, 1996). Samples collected in 2021–2022 were extracted using the Maxwell®16 LEV system Research (Promega, Madison, Wisconsin, USA) with the Maxwell®16 LEV Blood and Tissue DNA kit following the manufacturer's protocol. Polymerase chain reactions (PCR) were carried out to amplify *Vkorc1*-exon 3 in *Rattus norvegicus* and *Rattus rattus* and *Vkorc1*-exon 1 in *Rattus rattus*. Amplifications for exon 3 were performed with the primers exon3-Forward (5′-TTTCACCAGAAGCACCTGCTGCTGCC-3′) and exon3-Reverse (5′-ACACTTGGGCAAGGCTCATGTG-3′) that yielded a fragment of 354 base pairs (bp) (Grandemange et al., 2010). Amplification reactions were performed in a 25 μl volume containing approximately 50 ng of genomic DNA, 1× Buffer, 3 mM MgCl2, 2 mM dNTPs (Bioline, London, United Kingdom), 0.16 mg/ml BSA (Roche Diagnostics, Basel, Switzerland), 0.5 μM of each primer, and 1 Unit of Taq polymerase (BIOTAQTM DNA polymerase, Bioline). The amplification reaction was performed using polymerase activation at 94 °C for 3 min followed by 35 cycles at 94 °C for 30 s, 63 °C for 30 s, and 72 °C for 1 min, followed by a final extension for 10 min at 72 °C. Amplifications

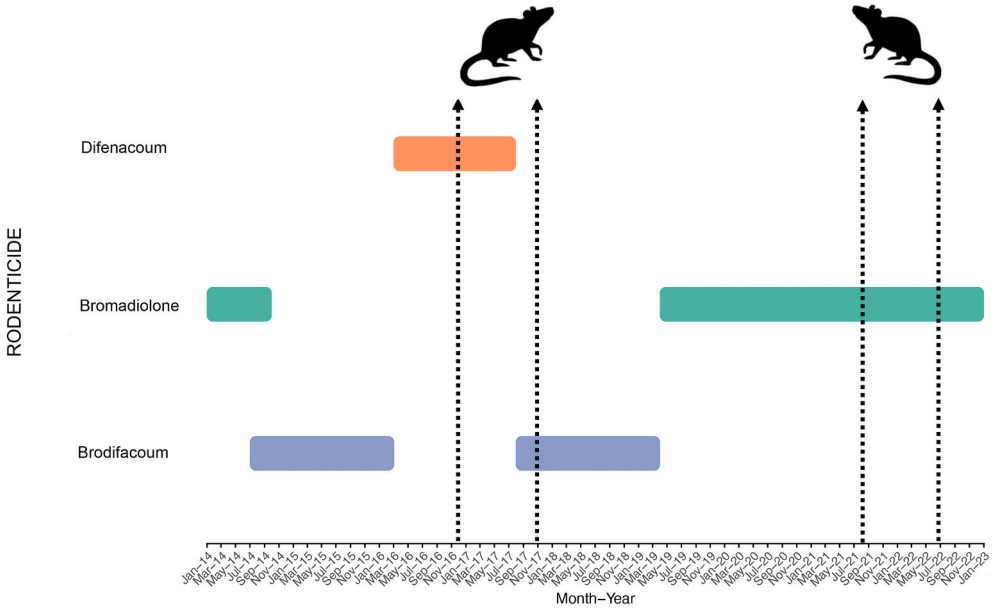


Fig. 1. Active ingredients used between January 2014 and December 2022. The arrows represent the start and end of the two sampling periods in 2016–2017 and 2021–2022. Percentage of the active ingredient in the bait was always 0.005 %.

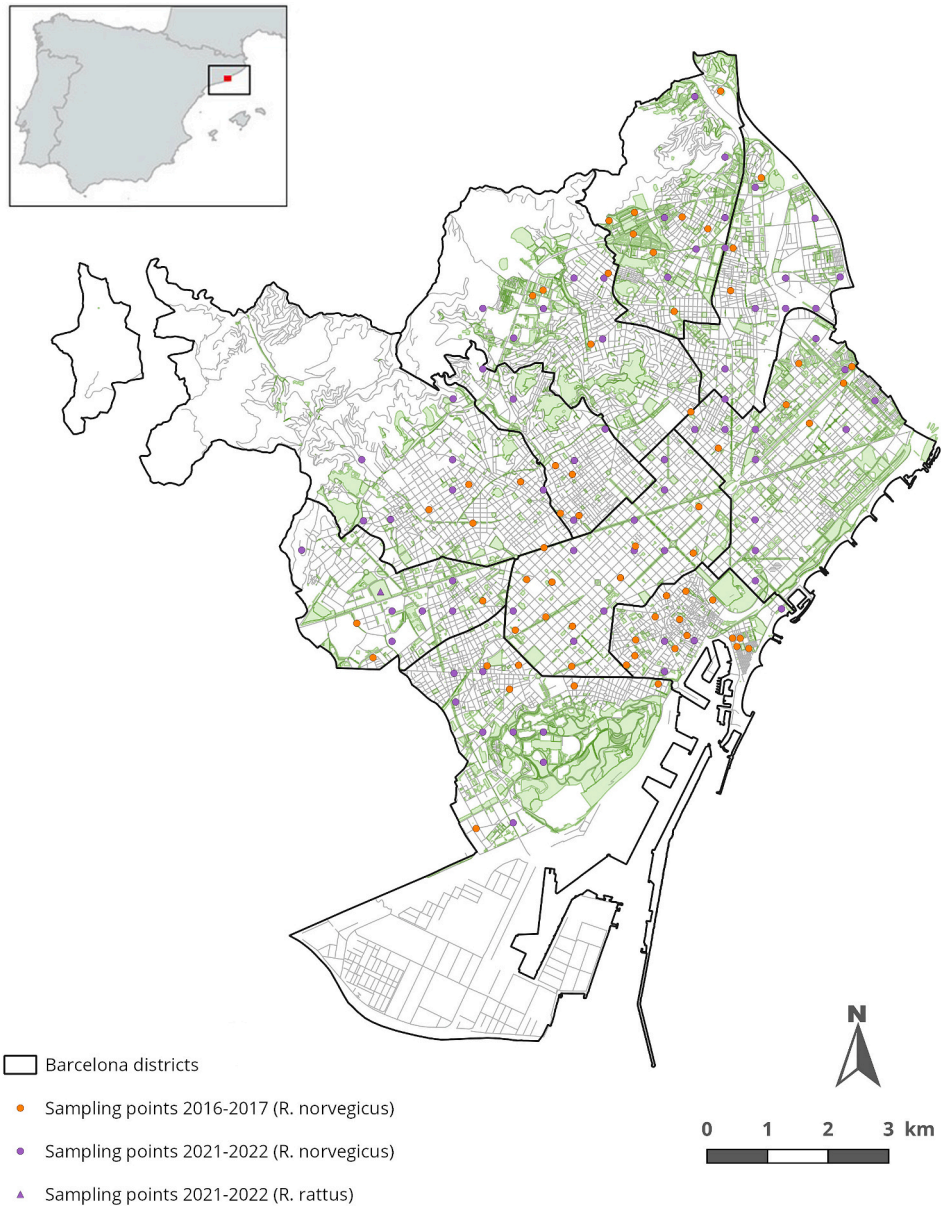


Fig. 2. Sampling locations for brown and black rats in the period 2016–2017 and 2021–2022.

for exon 1 were performed with the primers exon1-Forward (5′-GTGGCGGGTTCTTCCCTC-3′) and exon1-Reverse (5′-GACTCCAAAAT-CATCTGGCAACC-3′) that generate a fragment of 294 bp (Grandemange et al., 2010). Amplification reactions were performed using polymerase activation at 94 °C for 3 min, 10 cycles of 94 °C for 30 s, a touch down from 63 to 60 °C (with 0.2 °C steps) for 30 s and 72 °C for 1 min, and then 30 cycles of 94 °C for 30 s, 60 °C for 30 s and 72 °C for 1 min, followed by a final extension at 72 °C for 10 min. The amplified products were sequenced on both strands using Capillary Electrophoresis Sequencing by Macrogen (Madrid, Spain).

The sequences obtained from samples collected in 2016–2017 were analyzed using Sequencher software (v4.9; Genecodes). The sequences obtained from samples collected in 2021–2022 were analyzed using Geneious software v2020.0.3, (Kearse et al., 2012). Mutations were analyzed by mapping all sequences against the *Vkorc1* gene of either *Rattus norvegicus* (GenBank accession number: NC_051336.1, now NC_086019.1, https://www.ncbi.nlm.nih.gov/gene/309004) or *Rattus rattus* (GenBank accession number: NC_046155.1, https://www.ncbi.nlm.nih.gov/gene/116891450). The presence of heterozygotes was analyzed based on the presence of double peaks on both strands of the sequence. During the second period, potentially heterozygous individuals were sequenced twice in independent PCRs to confirm the results. The repeatability of the sequences was 100 %.

3. Results and discussion

Establishing effective management strategies for rodent control requires the adequate detection of resistance to anticoagulant rodenticides. In this regard, longitudinal studies play a key role in evaluating whether resistance is evolving over time. Here, we carried out the first longitudinal study of anticoagulant resistance in brown rats in an urban area in Spain and found no evidence of resistance. Similarly, we found no evidence of resistance in the black rats analyzed during the second sampling period. The absence of resistance mutations in rats contrasts with the situation observed in mice in Barcelona City, where there is widespread resistance to anticoagulant rodenticides (Ruiz-López et al., 2022).

The study of *Vkorc1*-exon 3 in brown rats showed differences over

time in the presence of mutations, but there was no association between the mutations and anticoagulant resistance. In the first sampling period (2016–2017), we successfully sequenced *Vkorc1*-exon 3 for 458 out of 569 samples and identified 26 individuals that carried a single nucleotide polymorphism (SNP) in codon 120. These individuals were heterozygous for the mutation C > T, but the resulting codons (CTG > TTG) are synonymous and code for the same amino acid (Leu). However, we did not detect this mutation or any other in the 183 individuals sequenced during the second sampling period in 2021–2022 (100 % of the captured individuals) (Table 1). These results agree with previous studies conducted on brown rat samples in Spain, suggesting that resistance to anticoagulants in rats is generally low (Iacucci et al., 2018; Bermejo-Nogales et al., 2022).

We only obtained black rat samples from captured individuals during the second sampling period in 2021–2022. We obtained sequences for the 22 individuals captured. Although we identified several mutations in the two exons studied during this period, none were associated with anticoagulant resistance. For exon 1, we found 2 synonymous mutations, specifically in codon 39 (TAC > TAT) and in codon 41 (GCG > GCA). In both cases, we observed heterozygote and homozygote individuals (Table 1). Previous studies have reported several mutations at codon 41 that are associated with warfarin resistance in Japan (Tanaka et al., 2012).

Regarding exon 3, we only found mutations in codon 143. In this case, 86,37 % of individuals were identical to the reference sequence, and 13,63 % were heterozygous for the mutation (GCA > GCG, Table 1). Despite being a synonymous mutation that does not lead to amino acid changes, polymorphisms at this codon have been found in other studies (Bermejo-Nogales et al., 2022; Chua et al., 2022). The absence of mutations associated with anticoagulant resistance in the *Vkorc1* exon 3 (codons 120, 128 and 139) of black rats is consistent with findings from previous studies. However, Bermejo-Nogales et al. (2022) identified black rats in the Barcelona province carrying the mutations Glu155Lys and Ser149Thr, which, according to computational analyses, may be associated with a reduction in SGAR activity. The difference in results is probably associated with differences in sample origin. While our samples were collected in an urban setting recently colonized by black rats, Bermejo-Nogales et al. (2022) samples were obtained from a farm where black rats have been present for a long time and subjected to control interventions using rodenticides. In animal farms, rodents may reach high densities due to the high availability of food and shelter, resulting in increased use of rodenticides (Leirs et al., 2004; Lambert et al., 2018). In contrast, the population of black rats in the city of Barcelona is currently low and has not been subject to any invasive interventions. When necessary, control of black rats is carried out by capturing them without the use of rodenticides. This allows for coexistence with protected fauna, which often makes it impossible to intervene with rodenticides. Consequently, intensive rodenticide treatments have not been applied to the urban black rat population in Barcelona, thus keeping the risk of developing resistance low, whereas in farms this risk is higher.

The absence of resistance to anticoagulant rodenticides in the two species of rats in the city of Barcelona contrasts with the widespread resistance found in house mice (Ruiz-López et al., 2022). A similar result was found in Irish populations of *Rattus norvegicus* and *Mus musculus* in Ireland (Mooney et al., 2018). Rodenticide resistance emerges due to the complex interplay of rodenticide exposure, genetic diversity within rodent populations and behavioral and environmental factors that can influence the exposure to rodenticides or the genetic variation in the population. Thus, the differences we find can probably be explained by a combination of factors that include the species' natural history and management practices. First, the main origin of pesticide resistance in the house mice in Barcelona has been linked to adaptive introgression with *Mus spretus*, a sympatric species (Song et al., 2011; Ruiz-López et al., 2022). Additionally, although rats' and mice's management and control strategies both rely on the rotation of anticoagulant rodenticides, they differ significantly due to their habitat preference and behavior. Rats primarily inhabit underground and sewage systems, occasionally coexisting with people, whereas mice are commensal with humans, often causing issues on private properties. This ecological and behavioral distinction has important implications for control efforts because rodenticide is applied differently following the Spanish legislation making it unlikely that rats and mice have access to the same baits.

For brown rats, the majority of treatments are administered in public spaces and the sewerage system by competent administration, allowing for controlled use of biocides and rotation as needed. Biocides are not used preventively, and rodenticides are deployed only upon detection of rodent activity. Control and surveillance points are periodically checked to remove rodenticides when activity ceases or to change the rodenticide if it is suspected to be non-functional. In contrast, because mice cause most problems in the private sphere, pest control companies and citizens are primarily responsible for applying treatments against mice. In this case, the availability of rodenticides is limited, with 70 % of publicly available rodenticides containing bromadiolone as the principal component (Registro Oficial de Biocidas, 2022). Moreover, in the private sphere there is no standardized strategy for use and product rotation. The continuous and broad exposure of mice to specific rodenticides has probably favoured the rapid development of resistance, while rats are rarely exposed to rodenticides used by people in domestic settings in urban environments.

When comparing our findings with other studies in Europe, it becomes evident that there is a significant variation in the prevalence of resistance across different countries and regions. It would be important to understand if these differences are due to differences in management practices or if other environmental and genetic factors contribute to them. Along these lines, it would also be important to understand the population genetic structure of rats in Barcelona. It was initially proposed that the movement of resistant rats expanding into new areas contributed to the spread of rodenticide resistance in European brown rat populations (Pelz et al., 2005). However, the study of rat population

Table 1
SNPs found for the *Vkorc1* exons 1 and 3 in brown rats (sampling period 2016–2017) and black rats (2021–2022). We report the percentage of homozygotes and heterozygotes for the mutations found and the number of individuals that carry the mutations (N individuals).

Brown rats (*Rattus norvegicus*)							
Exon	Codon position	Wild	Mutated	Aminoacid	% Homozygotes	% Heterozygotes	N Individuals
Exon 3	120	CTG	TTG	Leu107Leu	0	5.67	26

Black rats (*Rattus rattus*)							
Exon	Codon position	Wild	Mutated	Aminoacid	% Homozygotes	% Heterozygotes	N individuals
Exon 1	39	TAC	TAT	Tyr39Tyr	4.54	31.81	8
Exon 1	41	GCG	GCA	Ala41Ala	4.54	9.09	3
Exon 3	143	GCA	GCG	Ala143Ala	0	13.63	3

genetic structure in this context is often overlooked (Haniza et al., 2015). In addition, future studies should analyze the three exons of the *Vkorc1* gene to identify new potential mutations associated with rodenticide resistance.

In conclusion, our results confirm that rodenticide resistance in rats is not currently a concern in Barcelona City, and support the effectiveness of the product rotation strategy implemented by the local administration. Our findings also underscore the importance of conducting periodic evaluations of resistance in murid species to assess the consequences of management practices. In the future, rodent management plans in the city of Barcelona should continue to regularly rotate anticoagulant rodenticides. However, there should also be an effort to minimize the use of anticoagulant rodenticides, using whenever possible other rodenticide active ingredients that are less toxic to the environment. Futhermore, it will be important to strengthen alternative strategies for controlling rodents by involving other municipal departments and collaborating with citizens to reduce factors that favor rodent infestations, such as waste accumulation and improper disposal. This highlights the necessity of strengthening the collaboration among administration, producers, distributors, the pest control sector, and citizens. Such collaboration will help to regulate the use of effective active substances based on scientific evidence, thereby promoting good management practices and ensuring the rational use of biocides.

Ethical approval

We obtained an exceptional authorization from the regional Government of Catalonia (Ref.: SF/044) to use the snap traps in the Barcelona sewer system.

CRediT authorship contribution statement

María José Ruiz-López: Writing – review & editing, Writing – original draft, Methodology, Formal analysis. **Sandra Franco:** Writing – review & editing, Funding acquisition, Conceptualization. **Josué Martínez-de la Puente:** Writing – review & editing, Methodology, Formal analysis. **Martina Ferraguti:** Writing – review & editing, Formal analysis. **Emanuele Miccolis:** Writing – review & editing, Formal analysis. **Robert Petit:** Writing – review & editing, Funding acquisition. **Laura Barahona:** Writing – review & editing, Writing – original draft, Resources, Investigation, Funding acquisition, Conceptualization. **Jordi Figuerola:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Tomas Montalvo:** Writing – review & editing, Writing – original draft, Validation, Supervision, Funding acquisition, Conceptualization.

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.

Data availability

Data are available at DIGITAL.CSIC (http://hdl.handle.net/10261/368849).

Acknowledgements

We would like to thank Lokimica for their contribution and dedication in the fieldwork and the tail sample obtention and Barcelona Cicle de l'Aigua, who assisted in the sewage system task. We thank Cristina Perez for her help in the lab. This study has been funded by ASPB through a contract with EBD-CSIC. MF is currently funded by a Ramón y Cajal postdoctoral contract (RYC2021-031613-I) from the Spanish Ministry of Science and Innovation. EM was funded by the UK's Turing Scheme. We also would like to thank four anonymous reviewers for their insightful comments.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.176321.

References

Aivelo, T., Koivisto, E., Esther, A., Koivisto, S., Huitu, O., 2023. VKORC1-based resistance to anticoagulant rodenticides widespread in Finnish house mice but not in brown rats. Int. J. Pest Manag. 1–8. https://doi.org/10.1080/09670874.2023.2234324.

Bermejo-Nogales, A., Rodríguez-Martín, J.A., Coll, J., Navas, J.M., 2022. VKORC1 single nucleotide polymorphisms in rodents in Spain. Chemosphere 308, 136021. https://doi.org/10.1016/j.chemosphere.2022.136021.

Boitet, M., Hammed, A., Chatron, N., Debaux, J.V., Benoit, E., Lattard, V., 2018. Elevated difenacoum metabolism is involved in the difenacoum-resistant phenotype observed in Berkshire rats homozygous for the L120Q mutation in the vitamin K epoxide reductase complex subunit 1 (Vkorc1) gene. Pest Manag. Sci. 74, 1328–1334.

Boyle, C.M., 1960. Case of apparent resistance of *Rattus norvegicus* Berkenhout to anticoagulant poisons. Nature 188, 517. https://doi.org/10.1038/188517a0.

Buckle, A.P., 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.

Chua, C., Humaidi, M., Neves, E.S., Mailepessov, D., Ng, L.C., Aik, J., 2022. VKORC1 mutations in rodent populations of a tropical city-state as an indicator of anticoagulant rodenticide resistance. Sci. Rep. 12, 4553.

Cowan, P.E., Gleeson, D.M., Howitt, R.L.J., Ramon-Laca, A., Estherd, A., Pelz, H.J., 2017. Vkorc1 sequencing suggests anticoagulant resistance in rats in New Zealand. Pest Manag. Sci. 73, 262–266.

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, 105052. https://doi.org/10.1016/j.pestbp.2022.105052.

Desvars-Larrive, A., Pascal, M., Gasqui, P., Cosson, J.F., Benoit, E., Lattard, V., Crespin, L., Lorvelec, O., Pisanu, B., Teynie, A., Vayssier-Taussat, M., Bonnet, S., Marianneau, P., Lacote, 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, e0184015.

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, e14935. https://doi.org/10.7717/peerj.14935 (Mar 24).

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.

Endepols, S., Prescott, C.V., Klemann, N., Buckle, A.P., 2007. Susceptibility to the anticoagulants bromadiolone and coumatetralyl in wild Norway rats (*Rattus norvegicus*) from the UK and Germany. Int. J. Pest Manag. 53, 285–290. https://doi.org/10.1080/09670870701427045.

Gemmell, N.J., Akiyama, S., 1996. An efficient method for the extraction of DNA from vertebrate tissues. Trends Genet. 12, 338–339. https://doi.org/10.1016/s0168-9525(96)80005-9.

Global Invasive Species Database, 2024a. Species profile: *Rattus norvegicus*. Downloaded from. http://www.iucngisd.org/gisd/speciesname/Rattus+norvegicus. on 14-02-2024.

Global Invasive Species Database (2024b) Species profile: *Rattus rattus*. Downloaded from http://www.iucngisd.org/gisd/speciesname/Rattus+rattus on 14-02-2024.

Goulois, J., Chapuzet, A., Lambert, V., Chatron, N., Tchertanov, L., Legros, L., Benoita, E., Lattarda, V., 2016. 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.

Grandemange, A., Kohn, M.H., Lasseur, R., Longin-Sauvageon, C., Berny, P., Benoit, E., 2009. Consequences of the Y139F Vkorc1 mutation on resistance to AVKs: *in vivo* investigation in a 7th generation of congenic Y139F strain of rats. Pharmacogenetics Genom. 19, 742–750.

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.

Haniza, M.Z., Adams, S., Jones, E.P., MacNicoll, A., Mallon, E.B., Smith, R.H., Lambert, M.S., 2015. Large-scale structure of brown rat (*Rattus norvegicus*) populations in England: effects on rodenticide resistance. PeerJ 3, e1458. https://doi.org/10.7717/peerj.1458 (Dec 7).

Hawkins, N.J., Bass, C., Dixon, A., Neve, P., 2019. The evolutionary origins of pesticide resistance. Biol. Rev. 94, 135–155. https://doi.org/10.1111/brv.12440.

Huang, B.H., Feng, Z.Y., Yue, L.F., Yao, D.D., Gao, Z.X., Wang, D.W., Liu, X.H., 2011. Warfarin resistance test and polymorphism screening in the *VKORC1* gene in *Rattus flavipectus*. J. Pest. Sci. 84, 87–92. https://doi.org/10.1007/s10340-010-0330-5.

Huang, E.Y.Y., Law, S.T.S., Nong, W., Yip, H.Y., Uea-Anuwong, T., Magouras, I., Hui, J.H.L., 2022. The screening for anticoagulant rodenticide gene VKORC1 polymorphism

in the rat *Rattus norvegicus*, *Rattus tanezumi* and *Rattus losea* in Hong Kong. Sci. Rep. 12, 12545. https://doi.org/10.1038/s41598-022-16550-3.

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 29, 95–99. https://doi.org/10.4404/hystrix-00055-2018.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P., Drummond, A., 2012. Geneious basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinforma. Oxf. Engl. 28, 1647–1649. https://doi.org/10.1093/bioinformatics/bts199.

Lambert, M., Bellamy, F., Budgey, R., Callaby, R., Coats, J., Talling, J., 2018. Validating activity indices from camera traps for commensal rodents and other wildlife in and around farm buildings. Pest Manag. Sci. 74, 70–77. https://doi.org/10.1002/ps.4668.

Leirs, H., Lodal, J., Knorr, M., 2004. Factors correlated with the presence of rodents on outdoor pig farms in Denmark and suggestions for management strategies. NJAS: Wagen J. Life Sci. 52, 145–161. https://doi.org/10.1016/S1573-5214(04)80010-1.

Mahamat, A.B., Groud, K., Djibrine, S.I., Soro, S.D., Fourel, I., Rached, A., Chatron, N., Benoit, E., Lattard, V., 2023. Exposure and resistance to anticoagulant rodenticides in invasive and endemic Chadian urban rodent species to develop a rational management strategy. Pest Manag. Sci. https://doi.org/10.1002/ps.7911.

Marquez, A., Khalil, R.A., Fourel, I., Ovarbury, T., Pinot, A., Rosine, A., Thalmensi, G., Jaffory, G., Kodjo, A., Benoit, E., Lattard, V., 2019. Resistance to anticoagulant rodenticides in Martinique could lead to inefficient rodent control in a context of endemic leptospirosis. Sci. Rep. 9, 13491. https://doi.org/10.1038/s41598-019-49661-5.

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.

Meerburg, B.G., van Gent-Pelzer, M.P.E., Schoelitsz, B., van der Lee, T.A.J., 2014. Distribution of anticoagulant rodenticide resistance in Rattus norvegicus in The Netherlands according to Vkorc1 mutations. Pest Manag. Sci. 70, 1761–1766.

Mooney, J., Lynch, M.R., Prescott, C.V., Clegg, T., Loughlin, M., Hannon, B., Moore, C., Faulkner, R., 2018. VKORC1 sequence variants associated with resistance to anticoagulant rodenticides in Irish populations of *Rattus norvegicus* and *Mus musculus domesticus*. Sci. Rep. 8, 4535. https://doi.org/10.1038/s41598-018-22815-7.

Pascual, J., Franco, S., Bueno-Marí, R., Peracho, V., Montalvo, T., 2020. Demography and ecology of Norway rats, *Rattus norvegicus*, in the sewer system of Barcelona (Catalonia, Spain). J. Pest. Sci. 93, 711–722.

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.

Registro oficial de Biocidas, 2022. Downloaded from. http://www.mscbs.es/ciudadanos/productos.do?tipo=biocidas. on 17-01-22.

Rodenticide Resistance Action Committee (RRAC), 2015. RRAC Guidelines on Anticoagulant Rodenticide Resistance Management. https://croplife.org/wp-content/uploads/2015/10/Rodenticide-Resistance-Strategy_Sept2015v3.pdf. Downloaded on July 29, 2021.

Rost, S., Fregin, A., Ivaskevicius, V., Conzelmann, E., Hörtnagel, K., Pelz, H.-J., Lappegard, K., Seifried, E., Scharrer, I., Tuddenham, E.G.D., Müller, C.R., Strom, T. M., Oldenburg, J., 2004. Mutations in VKORC1 cause warfarin resistance and multiple coagulation factor deficiency type 2. Nature 427, 537–541. https://doi.org/10.1038/nature02214.

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, 4. https://doi.org/10.1186/1471-2156-10-4.

Ruiz-López, M.J., Barahona, L., Martínez-de la Puente, J., Pepió, M., Valsecchi, A., Peracho, V., Figuerola, J., Montalvo, T., 2022. Widespread resistance to anticoagulant rodenticides in *Mus musculus domesticus* in the city of Barcelona. Sci. Total Environ. 845, 157192.

Song, Y., Endepols, S., Klemann, N., Richter, D., Matuschka, F.-R., Shih, C.-H., Nachman, M.W., Kohn, M.H., 2011. Adaptive introgression of anticoagulant rodent poison resistance by hybridization between old world mice. Curr. Biol. 21, 1296–1301. https://doi.org/10.1016/j.cub.2011.06.043.

Takeda, K., Ikenaka, Y., Tanikawa, T., Tanaka, K.D., Nakayama, S.M.M., Mizukawa, H., 2016. Novel revelation of warfarin resistant mechanism in roof rats (*Rattus rattus*) using pharmacokinetic/pharmacodynamic analysis. Pestic. Biochem. Physiol. 134, 1–7.

Tanaka, K.D., Kawai, Y.K., Ikenaka, Y., Harunari, T., Tanikawa, T., Ando, S., won Min, H., 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 (2), 144–151.

Yiğit, N., Duman, M.T., Çetintürk, D., Saygılı-Yiğit, F., Çolak, E., Çolak, R., 2023. Vkorc1 gene polymorphisms confer resistance to anticoagulant rodenticide in Turkish rats. PeerJ 11, e15055. https://doi.org/10.7717/peerj.15055.