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UNDERSTANDING MOUSE RESISTANCE IN A MEDITERRANEAN CITY: IMPLICATIONS FOR RODENT CONTROL

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Abstract Anticoagulant rodenticides are the most commonly used pesticides worldwide that inhibit blood coagulation by suppressing the activity of the vitamin K epoxide reductase (VKOR). Resistance to these compounds has been reported in multiple rodent populations in different countries, and numerous studies have linked several resistance phenotypes to mutations in the gene Vkorc1, which codifies for the VKOR complex subunit 1. The identification of resistance in rodent populations is key for the efficacy of their control. Here we sequenced exons 1, 2 and 3 of the Vkorc1 gene of 111 mice (Mus musculus domesticus) captured across the city of Barcelona. All the sampled individuals harbored resistant genotypes. Most of the SNPs identified were associated with resistance to first-generation anticoagulants (coumetralyl and chlorophacinone) and second-generation anticoagulants (bromadiolone and difenacoum). We also found one SNP in exon 3 associated with resistance to brodifacoum. Our results show a widespread occurrence of resistant mice in Barcelona, posing a unique challenge for rodent control given the widespread use of bromadiolone based products. Public health managers, pest control companies and citizens should be aware that most currently commercialized products are ineffective for mouse control.

Key words Rodenticides, anticoagulants, Vkorcl 1 gene, resistance

INTRODUCTION

Mus musculus is one of the 100 most invasive species in the world (Global Invasive database 2022) and a constitute major pest because its impacts on the environment, public health and economy. Nowadays, the control of mice populations is mainly carried out using anticoagulant rodenticides that inhibit blood coagulation by suppressing the activity of the vitamin K epoxide reductase (VKOR). When the anticoagulants block VKOR, there is a decrease in bioavailable vitamin K which leads to the absence of gamma-carboxilated clotting factors and compromises the coagulation process, causing internal hemorrhages and eventually death (Rost et al. 2004). The use of anticoagulants started in the early 1950s, with the development of warfarin, diphacione and coumatralyl (Buckle and Eason, 1994), commonly referred as first-generation anticoagulants. However, there is wide evidence of resistance in mice and other rodents to some of these compounds (McGee et al., 2020). To overcome this resistance, second-generation anticoagulants were developed, including bromadiolone, difenacoum or brodifacoum. Nevertheless, over time mice populations have also developed resistance against several second-generation compounds. The most studied resistance mechanism to anticoagulant rodenticides involves the enzyme VKOR. In particular, it has been shown that mutations in the gene vkorc1 that codes for the complex subunit 1 of the VKOR enzyme (VKORC1) affect the efficiency of both first- and second-generation anticoagulant rodenticides (Pelz et al., 2005; Rost et al., 2004). Therefore, to carry out an adequate control of rodent populations and minimize the health, ecological and economic impacts, it is critical to evaluate both the prevalence and the potential for resistance to anticoagulant rodenticides.

In the city of Barcelona, the Agència de Salut Pública de Barcelona carries the control of rodent populations since 2003. From 2003 to 2015 the control was based on the use of bromadiolone while afterwards, the preferential use of brodifacoum was implemented. For short periods during 2018 and 2019, bromadiolone was occasionally used, but the reports indicated that the treatments were not successful and there was suspicion of the presence of resistance. Here we identified vkorc1 gene mutations in *M. musculus domesticus* in Barcelona to monitor resistance to anticoagulant rodenticides.

MATERIALS AND METHODS

We sampled 111 M. musculus between 29/10/2018 and 31/01/2020 as part of the municipal pest surveillance and control programme carried out by Agència de Salut Pública de Barcelona in the city of Barcelona. We identified the species (Gosàlbez, J, 1987) and took a tail fragment that was stored at -20ºC for subsequent molecular analysis. Genomic DNA for all samples was extracted using the Maxwell®16 LEV system Research (Promega, Madison, WI) following manufacturer's protocol. We analyzed the mutations in the Vkorc1 gene in exon 1, exon 2 and exon 3 that are 174 bp, 110 bp and 202 bp long, respectively, and encode in total 161 amino acids. We used specific PCRs for each exon and the amplified products were sequenced on both strands (Madrid, Spain). The sequences of each exon were analyzed using Geneious v. 2020.0.3 (Kearse et al., 2012) and all the sequences were mapped to the vkorc1 gene of both *M. m. domesticus* (GenBank accession number: GQ905715.1) to screen for mutations.

RESULTS AND DISCUSSION

Genetic characterization of the vkorc1 gene showed that 100% of mice analyzed carried at least one missense mutation in one of the three exons of the Vkorc1 gene. Thus, the mice population in Barcelona has a high frequency of SNPs that alters the amino acid sequence of the enzyme VKOR and has the potential to confer resistance to anticoagulant rodenticides. We found seven different mutations in the Vkorc1 gene in the mice sampled, four mutations in the exon 1, one in the exon 2 and two in the exon 3. Overall 94.60% of individuals carried at least one mutation in exon 1; 85.59% of individuals carried at least one mutation in exon 2; and 13.51% of individuals carried at least one mutation in exon 3 (Table 1). All of the mutations have been previously described in different mouse populations and have been shown to provide resistance to both first and second generation anticoagulant rodenticides (Blažić et al., 2018, 2017, Mooney et al., 2018, Pelz et al., 2005, Rost et al., 2009, Šćepović et al., 2016, Song et al., 2011). Mutations found in exon 1 have been previously associated with resistance to both first-generation anticoagulants (coumetralyl and chlorophacinone) and second-generation anticoagulants (bromadiolone and difenacoum) (Goulois et al., 2017, Song et al., 2011). In addition, out of the two mutations in exon 3, one of them confers resistance to first generation anticoagulants and bromadiolone, and the other one can also confer resistance to brodifacoum (Blažić et al., 2018; Goulois et al., 2017; Mooney et al., 2018; Pelz et al., 2005; Šćepović et al., 2016).

Results from this study have important management implications. They provide strong evidence of the widespread resistance of mice to bromadiolone in Barcelona. This is particularly problematic because bromadiolone is widely used in private pest control. In addition, when using alternative rodenticides, the use of second-generation compounds such as brodifacoum needs to be carefully monitored because given that some individuals already carry resistance to this compound it might lead to the spread of resistance in the future. These results further highlight the importance of using molecular tools to better understand the prevalence of resistance in mice populations. Furthermore, it is critical to bring these results to the attention of pest control companies, manufacturers and distributors to make rational use of active substances such as bromadiolone and achieve effective control of mice in Barcelona.

Table 1. Mutations found in exons 1, 2 and 3 of the Vkorc1 gene in mice from Barcelona and information regarding the resistance to different anticoagulants.

Exon	Mutation	Codon Wild Type	Codon mutated	Resistance second generation anticoagulant
Exon 1	Arg12Trp	CGG	TGG	All combined confer resistance to Bromadiolone
	Ala26Ser	GCA	TCA	
	Glu37Glu	GAA	GAG	
	Ala48Thr	GCC	ACC	
Exon 2	Arg61Leu	CGG	CTG	Bromadiolone (with Exon 1 mutations)
Exon 3	Leu128Ser	TTA	TCA	Bromadiolone, Brodifacoum
	Tyr139Cys	TAT	TGT	Bromadiolone

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