

Large-scale identification of rodenticide resistance in *Rattus norvegicus* and *Mus musculus* in the Netherlands based on *Vkorc1* codon 139 mutations

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Abstract

BACKGROUND: Resistance to rodenticides has been reported globally and poses a considerable problem for efficacy in pest control. The most-documented resistance to rodenticides in commensal rodents is associated with mutations in the *Vkorc1* gene, in particular in codon 139. Resistance to anticoagulant rodenticides has been reported in the Netherlands since 1989. A study from 2013 showed that 25% of 169 Norway rats (*Rattus norvegicus*) had a mutation at codon 139 of the *Vkorc1* gene. To gain insight in the current status of rodenticide resistance amongst *R. norvegicus* and house mice *Mus musculus* in the Netherlands, we tested these rodents for mutations in codon 139 of the *Vkorc1* gene. In addition, we collected data from pest controllers on their use of rodenticides and experience with rodenticide resistance.

RESULTS: A total of 1801 rodent samples were collected throughout the country consisting of 1404 *R. norvegicus* and 397 *M. musculus*. In total, 15% of *R. norvegicus* [95% confidence interval (CI): 13–17%] and 38% of *M. musculus* (95% CI: 33–43%) carried a genetic mutation at codon 139 of the *Vkorc1* gene.

CONCLUSION: This study demonstrates genetic mutations at codon 139 of the *Vkorc1* gene in *M. musculus* in the Netherlands. Resistance to anticoagulant rodenticides is present in *R. norvegicus* and *M. musculus* in multiple regions in the Netherlands. The results of this comprehensive study provide a baseline and facilitate trend analyses of *Vkorc1* codon 139 mutations and evaluation of integrated pest management (IPM) strategies as these are enrolled in the Netherlands.

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1 INTRODUCTION

Commensal rodents live in close proximity to humans, leading to gnawing damage and risks for food storage and human and animal health.^{1–3} Therefore, rodent pests need to be managed although rodent management is complex and the need for innovative control strategies subsists.^{4–6} Since the accidental discovery of anticoagulant rodenticides in 1944, the use of anticoagulants has been widespread. This has gradually led to the development of rodenticide resistance.^{7,8} In order to manage rodent pests, concepts such as integrated pest management (IPM) and ecologically based rodent management (EBRM) were designed. Both concepts strive to minimize the use of rodenticides, but IPM is a more broadly used concept in agriculture for pest management in general (including insects), whereas EBRM is focused on rodent pests and uses specific knowledge, for

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example about behaviour, to reach its goals.⁹ IPM and EBRM do not exclude anticoagulants, but require targeted use after assessing environmental risks and resistance. Although IPM and EBRM are currently advocated and implemented, anticoagulant rodenticides are still frequently used by both professionals and nonprofessionals.

Rodenticide resistance can be caused by mutations in the *Vkorc1* gene, which encodes for a protein involved in the uptake and re-use of vitamin K in the body.^{10–13} Vitamin K is essential for the clotting of blood.¹¹ Rodenticides inhibit the coagulation of blood by inhibiting Vitamin K epoxide reductase (VKOR) activity, leading to internal haemorrhages. Another important enzyme in the vitamin K antagonist metabolism is CYP2C9, for which mutations linked to rodenticide resistance also have been identified.^{14–16} Multiple genetic variances of both enzymes are known. However, the *Vkorc1* gene is considered the most important gene for mutations with respect to rodenticide resistance.^{7,17}

It is known that mutations of one or more nucleotides on the *Vkorc1* gene can lead to various degrees of rodenticide resistance. These mutations are widespread amongst many mice and rat populations by altered amino acids on *Vkorc1* position 139 were found to be of global importance.¹⁴ The development of rodenticide resistance in the most common commensal rodent species such as *R. norvegicus* and *M. musculus* was studied over the past decades in many countries from all continents.¹⁷ In the Netherlands, resistance to second-generation anticoagulant rodenticides was reported in 1989 for a small number of samples collected close to the German border.¹⁸ Results from a larger study in the Netherlands reported in 2013 were that 25% of the *R. norvegicus* droppings (n = 169) showed a mutation in *Vkorc1* codon 139 associated with resistance.¹⁹ For some mutations the link between efficacy of active substances is known (see Supplement 1), for example the effective substance bromadiolone has lost its efficacy for the Y139F and Y139C mutations.^{20,21} Based on results of studies on rats from neighbouring countries, in this study we limited the tests to testing for occurrence of *VKORC1*-polymorphisms in amino-acid position 139.^{22,23}

Another side effect of rodenticides is the negative effect on the environment, including environmental hazards, uptake by nontarget animals and secondary poisoning in animals of prey.^{24–34} A recent study in the Netherlands showed rodenticide residues in 43% of 143 tested nontarget species, including 61 rodent predators such as the common buzzard (*Buteo buteo*), common kestrel (*Falco tinnunculus*), little owl (*Athene noctua*), barn owl (*Tyto alba*), European polecat (*Mustela putorius*), stoat (*Mustela erminea*), pine marten (*Martes foina*), red fox (*Vulpes vulpes*) and weasel (*Mustela nivalis*).³⁵ To complicate the assessment of these adverse effects, every country considers different rodent species as pest animals, which leads to the need of specific rodent management. In England, the wood mouse (*Apodemus sylvaticus*) is considered a pest species.³⁶ In other countries, such as the Netherlands, the wood mouse is not considered a pest (although problems occasionally occur) and is classified as nontarget by law. To reduce the development of rodenticide resistance and the negative effects on biodiversity, the use of rodenticides is restricted in the Netherlands as part of the IPM strategy. From 2023 onwards, the use of anticoagulant rodenticides will be restricted to IPM-certified companies, and IPM training will be mandatory for all professionals who use rodenticides. For the general public this will have quite some impact as a study indicated that in 2019 around 330 000 packs of rodenticides were purchased at retail outlets in the Netherlands.²⁴ The restrictions in the use of

rodenticides and the implementation of IPM might influence the occurrence, spread and distribution of rodenticide resistance.

In order to gain insight in the current status of rodenticide resistance induced by altered amino acids on *Vkorc1* position 139 among commensal rodent species in the Netherlands, this study assessed rodenticide resistance of *R. norvegicus* and *M. musculus*. Moreover, it facilitates quantification and trend watching as IPM strategies are enrolled in the Netherlands. In addition, we report the results of a questionnaire sent to pest controllers for current practices for their experiences and perceptions on use and complication in the use of rodenticides in the current IPM strategies.

2 MATERIALS AND METHODS

2.1 Sample collection

Ear or tail tissue of *R. norvegicus* and *M. musculus* was collected between September and December 2021 by professional pest controllers from all over the Netherlands. Also, ear tissue and DNA extractions from ear tissue from both *R. norvegicus* and *M. musculus* collected from May to October in both 2020 and 2021 (permission animal ethics committee AVD 32600 20 172 104) by the National institute for Public Health and the Environment (RIVM) were used. In addition, *M. musculus* tail tissue collected by pest controllers and given to Utrecht University between February and May 2019 were used. From each individual rodent the trapping/finding location was recorded based on the first three digits of the postal code, as well as the cause of death (i.e. trap, roadkill, rodenticide, ferrets).

Each rodent was visually identified to species level during sampling by the pest controllers or the researchers. All samples (carcass, ear or tail) from the period September–December 2021 were stored at -18°C . If rodents could not be frozen immediately, they were transported to the Dutch Pest and Wildlife Expertise Centre in a coolbox and frozen at -18°C until 4-mm diameter ear punches could be taken using a punch tool (Vanem Equipment Manufacturing, Hoek van Holland, the Netherlands). Samples collected by the RIVM in 2020 and 2021 were stored at -80°C until further analysis. The tail tissue of *M. musculus* collected by Utrecht University was stored at room temperature in 70% ethanol.

2.2 DNA extraction

DNA extraction was performed on ear and tail 4-mm diameter punches, with a commercial extraction kit according to the manufacturer's protocol (nexttec™ 1-Step Tissue & Cells; nexttec Biotechnologie GmbH, Leverkusen, Germany). DNA samples from the RIVM were extracted using the DNeasy blood and tissue kit (Qiagen GmbH, Hilden, Germany) following the manufacturer's protocol.

2.3 Molecular verification of species

For molecular verification of species identification, amplicon sequencing of cytochrome oxidase I (COI) was used. To amplify 750 bp of COI, the primers BatL5310 and R6036R were used.³⁷ The amplification reaction (30 μL) contained: GoTaq Reaction buffer 1x containing 1.5 mM MgCl_2 ; 0.75 U of GoTaq® G2 (Promega, Leiden, the Netherlands); forward and reverse primer at 333 nM each; dNTPs at 200 μM each; DNA polymerase; 1 μL DNA template. The PCR cycling conditions consisted of 5 min at 94°C , followed by 40 cycles of 94°C for 30 s, 48°C for 45 s and 72°C for 45 s and one final extension step of 72°C for 10 min.

After checking the PCR products on gel 1% agarose in TAE, all unpurified PCR products were sent to Macrogen Europe B.V. (Amsterdam, the Netherlands) for Sanger sequencing. Identification of the species was investigated using a BLAST search of the GenBank nucleic acid database.

2.4 Detection of mutations at the *Vkorc1* gene

A single tube tetra allelic TaqMan assay was performed for both rats and mice to amplify the nuclear region of the *Vkorc1* gene and specific detection for the homozygous and heterozygous variant of both Y139C and Y139F and the nonmutant variant. The multiplex reaction (25 μ L) contained: PerfeCTa Multiplex qPCR ToughMix (Quantabio, Beverly, MA, USA); forward and reverse primer at 300 nM each; four probes in different concentrations Rat_Mm-139_A-P (50 nM), Rat_Mm-139_C-P (6.25 nM), Rat_Mm-139_G-P (25 nM), Rat_Mm-139_T-P (100 nM); 1 μ L DNA template (Table 1). All primers and probes were obtained from Integrated DNA Technologies (Leuven, Belgium).

Thermal cycling conditions was for 1 min at 95 °C, followed by 40 cycles with temperature steps of 95 °C for 10 s and 60 °C for 30 s using the Quantstudio 12 K flex real-time PCR machine (Life Technologies, CA, USA). Afterwards, profiles were scored based on the profiles compared to a set of references haplotypes using synthetically DNA molecules gBlocks Gene Fragment (Integrated DNA Technologies) (Table 2) in mixtures of 10⁶ copies μ L⁻¹.

2.5 Statistical analyses

Confidence intervals (CI) of population proportions were obtained using the asymptotic Wald method based on a normal approximation, using the EPITOOLS online programme.³⁸

3 RESULTS

3.1 Samples collected and verification of species

Between September and December 2021 a total of 1801 rodent samples were collected, consisting of 1404 *R. norvegicus* and 397 *M. musculus*. This included 207 tails from *M. musculus* collected by Utrecht University, and 230 *R. norvegicus* and 25 *M. musculus* samples collected by the RIVM. For both *R. norvegicus* and *M. musculus* samples a reasonably even spread over the whole country was realized, with samples originating from (respectively) 60 and 43 two-digit postal code areas out of 90 in total. The amplicon sequence results of COI confirmed samples to be *R. norvegicus* and *M. musculus*.

3.2 Detection of mutations at the *Vkorc1* gene

Of the 1404 *R. norvegicus* samples, 215 (15%; 95% CI: 13–17%) carried a genetic mutation linked to rodenticide resistance, classified homo- and heterozygously for all four possible different amino acids in position 139 (Fig. 1; Table 3; detailed maps per mutation type are in Supplement S2). Of the 397 *M. musculus* samples, 151 (38%, 95% CI: 33–43%) carried a genetic mutation in amino acid position 139 linked to rodenticide resistance. Each mutation type showed a different geographical pattern (see detailed maps per class in Supplement 2). The scoring of genotypes in a tetra allelic probe assay for *Vkorc1*-139 on *R. norvegicus* samples is visualized and can be found in Supplement 4.

4 DISCUSSION

Our results demonstrate that multiple genetic mutations at codon 139 of the *Vkorc1* gene occur in both *R. norvegicus* (15.3%, 95% CI: 13–17%) and *M. musculus* (38.1%, 95% CI: 33–43%). As these mutations are associated with rodenticide resistance this means that rodenticide resistance is widespread in commensal rodent populations in the Netherlands. Of all *R. norvegicus* samples analyzed, 215 (15.3%) carried a genetic mutation that is associated with rodenticide resistance. This number is lower than reported in our previous study in 2012–2013 on rodenticide resistance in rodent droppings where 42 of 169 samples showed genetic mutations (25%, 95% CI: 18–31%).¹⁹ The current 15.3% is a countrywide average, based on many more samples and thereby providing better statistical power to observe trends and quantitative differences. We observed noticeable differences between the sampled regions. In some areas only rats without mutations were found, in some only rats with rodenticide resistance-associated mutations, and in most areas both *R. norvegicus* with and without mutations were identified. Such spatial differences also have been observed in other studies. In 250 mice and rats from nine countries (the UK, Hungary, Portugal (Azores), Korea, Indonesia, Thailand, Japan, Argentina and the USA) a prevalence of 72% mutations on the *Vkorc1* gene, consisting of 23 different mutation types, was detected.

When looking for mutations at codon 139 per species individually, for *R. norvegicus* a British study from 2020 found a prevalence of 86.9% homozygous resistant ($n = 107$) and only one individual (0.01%) without mutations.³⁹ Another study from the UK in 2015 found 124 (67.4%) samples from *R. norvegicus* with genetic mutations associated with rodenticide resistance ($n = 184$).⁴⁰ In France, 86 *R. norvegicus* rats were tested for the presence of mutations on the *Vkorc1* gene, and a prevalence of 55.8% of the Y139F mutation

Table 1. List of primers and probes and their sequence for tetra allelic TaqMan assay

Target	Primer/probe*	Sequence (5' → 3')	Dye	Quencher
<i>R. norvegicus</i> / <i>M. musculus</i>	Rat-VKORC1-F	ACGTTGGGCCTCTATCCTA		
<i>R. norvegicus</i>	Rat-VKORC1-R	GGCAAAGCAAGTCATGTCAG		
<i>M. musculus</i>	Mm-VKORC1-R	GCCAAGGCAAGCAAGTTAG		
<i>R. norvegicus</i> / <i>M. musculus</i>	Rat_Mm-139_A-P	CA + C + CT + A + T + GCCA	ATTO550	IABkFQ
<i>R. norvegicus</i> / <i>M. musculus</i>	Rat_Mm-139_C-P	CA + CC + T + C + T + GCC	ATTO647	IABRQSp
<i>R. norvegicus</i> / <i>M. musculus</i>	Rat_Mm-139_G-P	CAC + C + T + G + TGCCA	FAM	IBFQ
<i>R. norvegicus</i> / <i>M. musculus</i>	Rat_Mm-139_T-P	CA + C + CT + TT + G + CCA	HEX	IBFQ

*Suffix F = forward primer, R = reverse primer, and P = hydrolysis probes. Integrated DNA Technologies, Leuven, Belgium.

Table 2. Sequences of the gBlocks used in this study

Target	gBlock haplotype	Sequence (5' → 3')
<i>R. norvegicus</i> wild-type 280 bp	gBlock-Rat-VKORC1-A:	TTCTACACCATACAGCTGTTGTTAGGTTGCTTGAGGGGACGTTGGGCCTCTATCCTACTGA TCCTGAGTTCCTGGTGTCTGTCGCTGTTCTCTGTACCTGGCCTGGATCCTGTT CTTTGCTGTATGATTTCTGCATTGTTTGCATCACCACCT AT GCCATCAATGCGGGC CTGATGTTGCTTAGCTCCAGAAGGTGCCAGAACACAAGGTCAAAAAGCCCTGAGGT CCCACCTCATGCCAGGCTGACATGACTTGCTTTGCCTTAGCACATGAGC
	gBlock-Rat-VKORC1-C:	TTCTACACCATACAGCTGTTGTTAGGTTGCTTGAGGGGACGTTGGGCCTCTATCCTACTG ATCCTGAGTTCCTGGTGTCTGTCGCTGTTCTCTGTACCTGGCCTGGATCCTGTTCTT TGCCTGTATGATTTCTGCATTGTTTGCATCACCACCT CT GCCATCAATGCGGGCCTGA TGTTGCTTAGCTCCAGAAGGTGCCAGAACACAAGGTCAAAAAGCCCTGAGGTCCCA CCTCATGCCAGGCTGACATGACTTGCTTTGCCTTAGCACATGAGC
	gBlock-Rat-VKORC1-G:	TTCTACACCATACAGCTGTTGTTAGGTTGCTTGAGGGGACGTTGGGCCTCTATCCTACTGA TCCTGAGTTCCTGGTGTCTGTCGCTGTTCTCTGTACCTGGCCTGGATCCTGTTCTTTG TCCTGTATGATTTCTGCATTGTTTGCATCACCACCT GT GCCATCAATGCGGGCCTGATG TTGCTTAGCTCCAGAAGGTGCCAGAACACAAGGTCAAAAAGCCCTGAGGTCCACC TCATGCCAGGCTGACATGACTTGCTTTGCCTTAGCACATGAGC
	gBlock-Rat-VKORC1-T:	TTCTACACCATACAGCTGTTGTTAGGTTGCTTGAGGGGACGTTGGGCCTCTATCCTACTGA TCCTGAGTTCCTGGTGTCTGTCGCTGTTCTCTGTACCTGGCCTGGATCCTGTTCTTTG TCCTGTATGATTTCTGCATTGTTTGCATCACCACCT TT GCCATCAATGCGGGCCTGATGT TGCTTAGCTCCAGAAGGTGCCAGAACACAAGGTCAAAAAGCCCTGAGGTCCACCT CATGCCAGGCTGACATGACTTGCTTTGCCTTAGCACATGAGC
<i>M. musculus</i> wild-type 289 bp	gBlock-Mm-VKORC1-A:	GATATACCATTACTGACCGTCTCTTTTACAGGTTGCTTGAGGGGACGTTGGGCCTCTA TCCTACTGGTGTGAGTTCCTGGTGTCCGTCGCTGGTCCGTGTACCTGGCCTGGATC CTGTTCTTTGTGCATATGATTTCTGCATTGTGTGCATTACCACCT AT GCCATCAATGTG GGTCTGATGTTGCTTAGCTCCAGAAGGTACCAGAACACAAGACCAAAAAGCACTG AGTCCACCTCATGCCAGACTAACCTA ACT TGCTTTGCCTGGCACATGACC
	gBlock-Mm-VKORC1-C:	GATATACCATTACTGACCGTCTCTTTTACAGGTTGCTTGAGGGGACGTTGGGCCTCTAT CCTACTGGTGTGAGTTCCTGGTGTCCGTCGCTGGTCCGTGTACCTGGCCTGGATCCT GTTCTTTGTGCATATGATTTCTGCATTGTGTGCATTACCACCT CT GCCATCAATGTGGGT CTGATGTTGCTTAGCTCCAGAAGGTACCAGAACACAAGACCAAAAAGCACTGAGTTC CACCTCATGCCAGACTAACCTA ACT TGCTTTGCCTGGCACATGACC
	gBlock-Mm-VKORC1-G:	GATATACCATTACTGACCGTCTCTTTTACAGGTTGCTTGAGGGGACGTTGGGCCTCTAT CCTACTGGTGTGAGTTCCTGGTGTCCGTCGCTGGTCCGTGTACCTGGCCTGGATCCT GTTCTTTGTGCATATGATTTCTGCATTGTGTGCATTACCACCT GT GCCATCAATGTGGGT CTGATGTTGCTTAGCTCCAGAAGGTACCAGAACACAAGACCAAAAAGCACTGAGTTC CACCTCATGCCAGACTAACCTA ACT TGCTTTGCCTGGCACATGACC
	gBlock-Mm-VKORC1-T:	GATATACCATTACTGACCGTCTCTTTTACAGGTTGCTTGAGGGGACGTTGGGCCTCTATC CTACTGGTGTGAGTTCCTGGTGTCCGTCGCTGGTCCGTGTACCTGGCCTGGATCCTG TTCTTTGTGCATATGATTTCTGCATTGTGTGCATTACCACCT TT GCCATCAATGTGGGTCT GATGTTGCTTAGCTCCAGAAGGTACCAGAACACAAGACCAAAAAGCACTGAGTTCCTCA CCTCATGCCAGACTAACCTA ACT TGCTTTGCCTGGCACATGACC

bold nucleotides are those that differentiate.

was found.⁴¹ A recent study from Finland reports two of 48 (4.2%) *R. norvegicus* carrying a rodenticide resistance mutation, both a rare type (Arg33Pro).²² So far, UK rats have demonstrated the greatest diversity of alterations in the *Vkorc1* gene. The high prevalence can be explained by the fact that rodents were collected from anticoagulant-exposed areas.³⁹ By contrast, during a study in 2018 in Ireland, 65 *R. norvegicus* individuals from the eastern part of the island were tested: no mutations linked to rodenticide resistance were found.⁴²

In the current study multiple mutation variants were found in both *R. norvegicus* and *M. musculus*. Animals carrying the homozygous Y139C genotype ('German' mutation) were not only found in areas already known to accommodate resistant animals (Twente, Achterhoek), but also around Rotterdam (a major sea port in the

west of the country) and in the province of Noord-Holland. These findings are in line with the Dutch study from 2012–2013. Animals with the homozygous Y139F genotype ('French' mutation) also were found in several areas in the south of the Netherlands (Zuid-Brabant, Noord-Limburg). The heterozygous German- and French-type resistant animals may have spread further over the country compared to the study from 2012–2013. However, the larger sample size could have increased the detection of animals with mutations. The Y139F genotype is found more frequently in the southern part of the Netherlands compared to the Y139C genotype. The risk of presence of animals with heterozygous genotype mutations is that during reproduction selection towards homozygosity could occur, leading to more resistant populations.⁴³

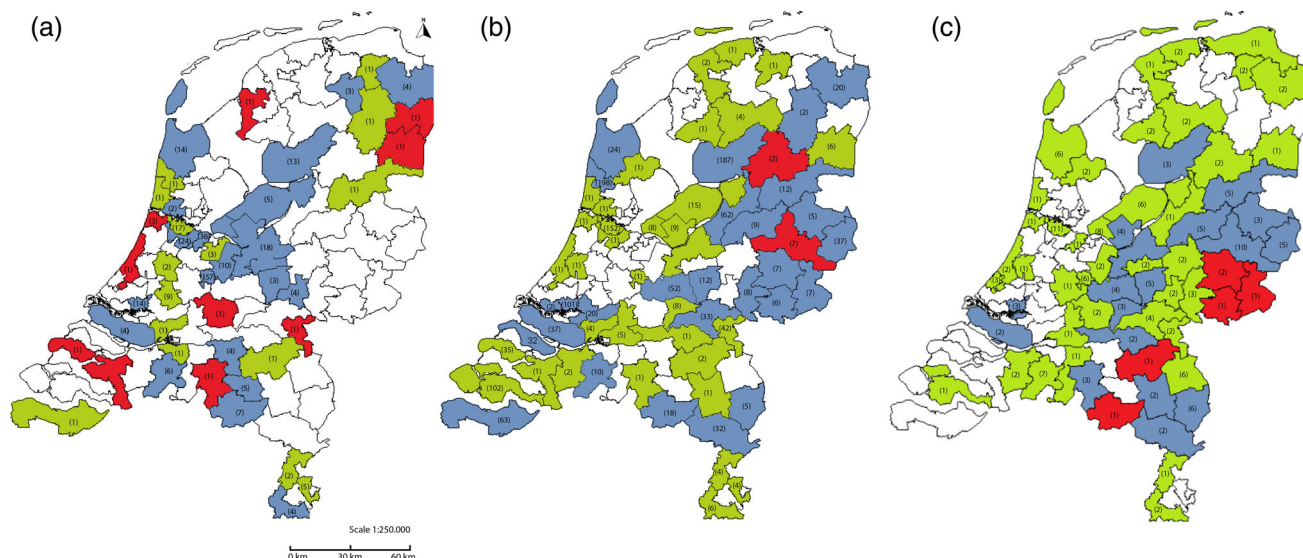


Figure 1. Map of the Netherlands divided into two digit postal code areas showing regions where rodents were collected for analysis for mutations in the *Vkorc1* gene codon 139 in 2021 (left and middle) and 2012 (right). Numbers in parentheses show the number of animals tested in that region. Area coloration: green, no genetic mutation found; blue, animals with and without genetic mutations; red, all tested animals had mutations. (A) *M. musculus* (B) *R. norvegicus*, (C) *R. norvegicus* 2012, adjusted from Meerburg *et al.* 2014.¹⁹

Table 3. The presence of genetic mutations at codon 139 of the *Vkorc1* gene in sampled *M. musculus* and *R. norvegicus*

Species	Total no. rodents	Resistance mutations found				
		No mutation found	Y139C		Y139F	
			Heterozygote	Homozygote	Heterozygote	Homozygote
House mouse (<i>M. musculus</i>)	397	246 (61.9%)	105 (26.4%)	46 (11.6%)	0	0
Norway rat (<i>R. norvegicus</i>)	1404	1189 (84.7%)	135 (9.6%)	28 (1.9%)	32 (2.3%)	20 (1.5%)

In the current study, genetic resistance to anticoagulants was more prevalent in *M. musculus* (38%) than in *R. norvegicus* (15.3%). For *M. musculus* there are three major strains of proven resistant mice known (139C, 128 S, Spretus-introgression).^{14,15,17,44–48} In the *M. musculus* samples of this study only two types of mutations were detected: the heterozygous Y139C (26%) and homozygous Y139C variant (12%). For *M. musculus* we observed variation in prevalence between regions, which also has been found in other studies.²² Interestingly, resistance in mice was detected in different areas to resistance in rats. The difference applies to both mutation types found; the heterozygous and homozygous German-type mutation (Y139C). For example *M. musculus* from the centre of the country carried the Y139C mutation type, whereas *R. norvegicus* trapped in the same areas did not carry this specific mutation. Unfortunately, there is little research published where the prevalence per species is compared between locations. However, we expect these regional differences to occur everywhere. In Ireland 84% of 50 *M. musculus* individuals tested positive for genetic mutation on the *Vkorc1* gene, with mutation types Y139C and L128S.⁴² In Finland, 65% of the mice tested (n = 48) showed a mutation on the *Vkorc1* gene with three mutation types found (Y139C, both heterozygous and homozygous, and L128S).²² On the island of Martinique, 40% of 59 *M. musculus* individuals showed Y139C mutations of the *Vkorc1* gene, which is in line with our findings.⁴⁹ Reports from Germany and Switzerland also record rodenticide resistance in *M. musculus* and in the same

two mutation types (Y139C and L128S).⁸ The Y139C mutation indicates resistance against first-generation anticoagulants and two second-generation anticoagulants (active substances bromadiolone and difenacoum; Tables 1 and 2).^{22,50}

However, genetic resistance comes at a certain cost, as the health of the animals may be affected in a negative way. Rats with a genetic mutation on the *Vkorc1* gene, have a greater need for vitamin K in their diet than wild-type animals.⁵¹ The heterozygote Y139C mutation also was suggested to have a negative effect on reproduction, possibly with vitamin K as underlying factor.^{52–54}

In future studies in the Netherlands on rodenticide resistance, more mutation types should be included, for example L120 and L128. Particularly for *M. musculus*, mutation L128S could increase the percentage of resistant individuals detected significantly. However, the current study is the first report of rodenticide resistance in *M. musculus* in the Netherlands and the high percentage of resistant animals detected already allows a review of the strategies of control using rodenticides.

Based on the current findings, it appears that rodenticide resistance in *M. musculus* is a bigger issue than in *R. norvegicus*. This also is in line with the findings of the questionnaire responses from pest controllers who indicated that they experience more resistance in mice than in rats. This could be the result of more frequent use of rodenticides in mouse control. Further research on

the prevalence of rodenticide resistance in *M. musculus* is needed to monitor the resistance status on a national scale. It also is recommended to research other possible mutation types in *M. musculus*, and to assess rodenticide presence or resistance in other common mice species in the Netherlands.

The widespread rodenticide resistance detected in two common rodent species underlines the need for applying IPM and EBRM. Pest controllers need to be educated more on behaviour of the specific pest species to be able to manage conform IPM and EBRM to manage rodent populations. More insight in the use of rodenticides by pest controllers will facilitate the interpretation of the occurrence, distribution, dynamics and consequently mitigation of rodenticide resistance.

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CONFLICT OF INTEREST DECLARATION

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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