Field trials to assess resistance to warfarin and difenacoum of house mice in relation to the occurrence of variants in the *vkorc1*-gene before and after the treatments

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Abstract

House mice (*Mus musculus domesticus*) vary considerably in their susceptibility to anticoagulants, and several non-synonymous sequence variants in the coding region of the vitamin K epoxide reductase subcomponent 1 gene (*vkorc1*) were found in Germany (Rost et al., 2009). It was the aim of our study to characterize the degree of resistance in relation to *vkorc1*genotypes in local mouse groups, and to test whether certain genotypes were selected by sequential treatments with the two anticoagulant rodenticides warfarin and difenacoum.

Two successive treatments were conducted, the first with bait containing warfarin followed by a second using difenacoum. Their effects were determined on local sub-groups of mouse infestations in different sub-units on two livestock farms in Westphalia, Germany. The frequency of different *vkorc1* genotypes, as determined by Sanger sequencing, was considered relative to the rodenticide treatment results for each sub-group and sampling period.

Three tolerance types were identified on farm one: A=warfarin-susceptible, B=resistant to warfarin, but susceptible to difenacoum, C=approx. one half of animals resistant to both anticoagulants. On farm 2, only type A and B were identified. A high degree of resistance was observed in *vkorc1* wild-type mice. In all cases, only the R58G *vkorc1* variant was found, which appears not to be a resistance marker in house mice. Hence, in these mouse infestations, practical resistance to anticoagulants was not accompanied by any identifiable *vkorc1* resistance marker.

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Keywords: difenacoum, house mouse, *Mus musculus domesticus*, resistance to anticoagulants, rodent control, rodenticide treatment, *vkorc1*, warfarin

Introduction

House mice (*Mus musculus domesticus*) vary considerably in their susceptibility to anticoagulants. In contrast to rats (*Rattus norvegicus*) in Northern Germany, where virtually all anticoagulant resistant rats carry a Y139C mutation in vitamin K epoxide reductase subcomponent 1 gene (*vkorc1*), several other non-synonymous coding sequence variants of the *vkorc1* appear to segregate in mouse populations from Germany including the Y139C variant (Rost et al., 2009). However, with the exception of the Y139C mutation, little information from field studies is available on the susceptibility to anticoagulants in mouse strains marked by these variants, and on the effect of practical treatments with rodenticides. In the present study, the effectiveness of two anticoagulant rodenticides was investigated in two populations of the house mouse suspected to harbor resistant animals. Genetic analysis of *vkorc1* was used to provide information about type and frequency of *vkorc1* sequence variants occurring in sub-units of the populations before and after the treatments. The aim was to characterize the degree of resistance in relation to genotypes in local mouse groups, and to test whether certain *vkorc1* genotypes were selected by the treatments, i.e. increased or decreased in allele frequency as a result of rodenticide use.

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Materials and Methods

Two successive treatments with anticoagulant rodenticides were conducted and monitored for their effect on local sub-groups of mouse infestations in the different sub-units on two livestock farms in Westphalia, Germany. Grain baits containing 0.05% warfarin for the first treatment, and 0.005% difenacoum for the second treatment, were systematically distributed at certain structural elements according to the standard rodent control program BayTool for 28 days (www.baytool.info). Bait consumption was recorded during regular visits to the treated sites, and the effect of the treatments was determined by census baiting. Mice were trapped prior to, between the two treatments, and after the treatments for tissue sampling (tail clips). Since most non-synonymous mutations of *vkorc1* in mice were found in the first and third exons (Rost et al., 2009), the two segments were amplified using the primers and conditions described in Song et al. (2008). All PCR products were cleaned by ExoSAP-IT (USB, Cleveland, OH) and sequenced with the Sanger-method for the detection of coding sequence variants in the gene. The frequency of *vkorc1* genotypes was put into relation to the rodenticide treatment results for each sub-group and sampling period.

Results

Mice consumed 3.0 kg of warfarin bait and 1.2 kg of difenacoum bait during the first trial, and 3.7 kg and 1.6 kg respectively of the two baits during the second trial. The level of resistance differed markedly between sub-units on both farms (Table 1). Three tolerance types were identified on farm one: A=warfarin-susceptible, B=resistant to warfarin, but susceptible to difenacoum, C=approx. one half of animals resistant to both anticoagulants. On farm 2, only type A and B were identified.

Tab. 1 Results of anticoagulant treatments according to sub-units in 2 trial sites.

Site 1			Site 2		
Tolerance type	**Survival rate (%)**		**Tolerance type**	**Survival rate (%)**	
(sub-unit No.)	**Warfarin**	**Difenacoum**	**(sub-unit No.)**	**Warfarin**	**Difenacoum**
A (1)	7.2	0	A (1)	7.1	0
B (2)	92.9	0	B (2)	104.0	7.2
C (3)	59.3	94.5	B (3)	92.0	5.7

We obtained *vkorc1* sequences for 41 mice on farm 1 and 54 mice on farm 2. Only one non-synonymous mutation R58G was found in exon 1 in all the sequenced mice from the two farms. On farm 1 almost all mice (n=39) were *vkorc1* wild-type, and only 2 mice carried the heterozygous mutation R58G (i.e. allele frequency 2.4%). On farm 2, all mice were homozygous for R58G in tolerance type A (n =18, warfarin-susceptible, sub-unit 1). 12 mice trapped in sub-unit 2+3 were wild-type, 6 were homozygous and 18 were heterozygous for R58G. Thus, we observed a high degree of resistance in *vkorc1* wild-type mice. Only the R58G *vkorc1* variant was found, which appears not to be a resistance marker in house mice.

Discussion

Resistance to warfarin was present in both mouse populations studied. Resistance to difenacoum occurred only in one sub-unit on one farm. Even there, no *vkorc1* variant was found that could explain *vkorc1*-mediated resistance, at least at the level of protein coding mutations. We therefore conclude that resistance to anticoagulants can be manifested in field populations of mice without the presence of *vkorc1* coding mutations. In addition to the comparatively well-understood *vkorc1*-mediated resistance (e.g., Y139C variant), our results suggest the possible involvement of other genes and/or other genetic mechanisms (e.g., gene regulatory or epigenetic changes) in anticoagulant resistance in mice. It is also conceivable that food rich in vitamin K mediated a fully or partially non-genetic form of resistance.

References

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