

**House Mouse Field Trials to Assess Resistance to Warfarin and Difenacoum 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 much in their susceptibility to anticoagulants, and several sequence variants of the vitamin K epoxide reductase subcomponent 1 gene (*vkorc1*) were found in Germany. It was the aim of our study to characterise the degree of resistance in relation to genotypes in local mouse groups, and to prove if certain genotypes were selected by sequential treatments with two anticoagulant rodenticides.

Two successive treatments were conducted with baits containing warfarin and difenacoum, respectively, and their effect was determined on local sub-groups of mouse infestations in the respective sub-units on two livestock farms in Germany. The frequency of *vkorc1* genotypes was put into relation 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* wildtype mice. Only the R58G *vkorc1* variant was found, which appears not to be a resistance marker in house mice.

The study was funded by the Rodenticide Resistance Action Committee (RRAC) of CropLife International.

**Introduction**

House mice (*Mus musculus domesticus*) vary much in their susceptibility to anticoagulants. In contrast to rats, several sequence variants of the vitamin K epoxide reductase subcomponent 1 gene (*vkorc1*) were found in Germany (Rost et al. 2009). Only little information is available on the susceptibility to certain anticoagulants in mouse strains marked by these variants, and on the effect of practical treatments with rodenticides.

With the introduced study, the effectiveness of two anticoagulant rodenticides should be investigated in two populations of the house mouse suspected to harbour resistant animals.

Genetic analysis of the *vkorc1* gene should provide information about type and frequency of sequence variants occurring in sub-units of the populations before and after the treatments. It was the aim to characterise the degree of resistance in relation to genotypes in local mouse groups, and to prove if certain genotypes were selected by the treatments.

The study was funded by the Rodenticide Resistance Action Committee (RRAC) of CropLife International.

## Materials and Methods

Two successive treatments were conducted and monitored for their effect on local sub-groups of mouse infestations in the respective sub-units on two livestock farms in Westphalia, Germany. Grain baits containing common strength warfarin for the first treatment, and difenacoum for the second treatment, were systematically distributed at certain structural elements according to the standard rat control program BayTool for 28 days. Bait consumption was recorded during regular controls, and the effect of the treatments was determined by feeding census. Mice were trapped prior to, in-between, and after the treatments for tissue sampling. The *vkorc1* gene was amplified and sequenced for the detection of 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

3.0kg of warfarin bait and 1.2kg of difenacoum bait were consumed by mice during the first trial, and 3.7kg and 1.6kg 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.

Almost all mice were *vkorc1* wildtype on farm 1, only 2% were R58G. On farm 2, all mice were homozygous for R58G in tolerance type A (n = 14, warfarin-susceptible, sub-unit 1). 10 mice trapped in sub-unit 2 were wildtype, 6 homozygous and 16 heterozygous for R58G. A high degree of resistance was observed in *vkorc1* wildtype mice. Only the R58G *vkorc1* variant was found, which appears not to be a resistance marker in house mice.

**Table 1:** Results of anticoagulant treatments according to sub-units in 2 trial sites.

Tolerance type (sub-unit No.)	Site 1		Tolerance type (sub-unit No.)	Site 2	
	Survival rate (%)			Survival rate (%)	
	Warfarin	Difenacoum		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

## Discussion

Resistance to warfarin was present in both investigated mouse populations. Resistance to difenacoum occurred only in one sub-unit on one farm. Even there, no *vkorc1* variant was found which could explain resistance. We therefore conclude that different levels of resistance to anticoagulants are determined by other genes than the *vkorc1* gene in house mice.

## References

Rost S, Pelz H-J, Menzel S, MacNicol AD, León V, Song K-J, Jäkel T, Oldenburg J, Müller CR (2009): Novel mutations in the VKORC1 gene of wild rats and mice – a response to 50 years of selection pressure by warfarin? BMC Genetics 10 (4), doi:10.1186/1471-2156-10-4