Characteristics of the local distribution of the Y139C resistance gene in Norway rats (*Rattus norvegicus*) in a focus of resistance in Westphalia, Germany.

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SUMMARY

The Westphalian resistant Norway rat strain is characterised by the possession of the Y139C variant of the *vkorc1* gene, and practical resistance occurs in rat infestations at different frequencies to the anticoagulants warfarin, coumatetralyl, bromadiolone and difenacoum. Within the present study we investigated whether there was an obvious pattern in the distribution of resistance in relation to the distance to an identified hot spot of resistance from the site of sampling and whether the frequency of the resistance gene was connected with local conditions, such as rodent control history.

Rats were trapped at a single infested site in each of 12 squares (1km x 1km) in a line including a resistance hot spot. Tissue samples were taken from all trapped rats, and genotyped for the Y139C variant of the *vkorc1* gene. The frequency of the resistance gene was determined for each site sampled. Data were also collected about rodent control measures applied in the past and other relevant local conditions.

The frequency of the resistance gene varied considerably between < 20% and > 80%. There was no obvious correlation of the frequency of the resistance gene and the distance to the hot spot, and there was no increase or decrease of the gene frequency in west-east direction.

Permanent baiting and poor rodent control practice seemed to increase the incidence of resistance in the respective site. The implementation of good rodent control practice is recommended to prevent an increase in the frequency of resistance.

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

INTRODUCTION

The Muensterland/Westphalia focus of anticoagulant resistance has been well investigated in terms of the extent of the area, where resistant Norway rats may appear, the genetics of the resistance gene, and the impact of resistance on the practical outcome of treatments using anticoagulants (Pelz *et al*. 1995; Rost *et al*. 2004; Endepols *et al*. 2011). The Westphalian resistant rat strain is marked by the Y139C variant of the *vkorc1* gene, and practical resistance occurs at different frequencies to the anticoagulants warfarin, coumatetralyl, bromadiolone and difenacoum. Studies investigating the nature of resistance were performed only on farms, which were peculiar for their rat control problems, obviously being hot spots of resistance. Such hot spots might be centres from where resistant rats disperse. In the present study, it was investigated whether there was an obvious pattern of the distribution of resistance correlating with the distance to such a hot spot. Further, it was assessed whether the frequency of the resistance gene was connected with local conditions such as geography and rodent control practice applied in the respective site.

A livestock farm known to be a hot spot of resistance was selected and situated at the centre of 12 squares, each measuring 1km x 1km, distributed in a west-east line near a small town in the eastern part of the Muensterland resistance area, Westphalia, Germany. In every square, a site was located with a rat infestation which permitted the trapping of at least 10 rats. We tried to avoid the selection of sites with extensive rat problems, but to find sites with less conspicuous rat infestations.

Tissue samples were taken from all trapped rats, and genotyped for the Y139C variant of the *vkorc1* gene by the amplification refractory mutation system (ARMS)-PCR test. The frequency of the resistance gene was determined for each site. Data were collected by questionnaire about the rodent control practice applied in the past, local geographical conditions, and characteristics of livestock keeping (if applicable). These information was used to allocate factors, which are suspected of being connected with the occurrence of resistance. Statistical analysis was not possible due to the sample size and data quality.

RESULTS: Parameters, influencing the frequency of Y139C

Of all parameters noted for each site, those of rat control practice appeared to be most connected with the frequency of resistance. The three trial sites with the lowest frequency of resistance employed a good rodent control parctice (see table 1). Those sites with insufficient rodent control practice showed the highest frequency of resistance. No relation to resistance was observed for factors like the kind of livestock keeping and the distance to the resistance hot spot. The following table provides a rough qualitative summary of information collected regarding the rodent control practices applied in sites with the lowest and the highest frequency of resistance:

Table 1: Control practice on trial sites with the lowest and the highest resistance level.

Resistance level	Control practice
Low frequency (18-36%) *Samples from 1, 3, 8*	• Control operations ca. 2 times per year or when necessary • One anticoagulant per control • Good control practice (sufficient bait points, regular refilling bait points, good coverage of the infestation area)
High frequency (82-88%) *Samples from 6, 9,10*	• Permant baiting or controls not well-timed • High frequency of changes in active ingredients and products • Insufficient control practice (insufficient number of bait points, no regular refill of bait points)

CONCLUSIONS

The frequency of Y139C anticoagulant resistance varies much within short distances between infestated sites in the Muensterland area, Westphalia focus of anticoagulant resistance. It is therefore difficult to make any conclusion about the incidence of resistance at one site based on resistance tests conducted on a site nearby. Most of the sites in this study were livestock farms. Permanent baiting and poor rodent control practice appeared in connection with increased incidence of resistance at the respective site. The implementation of good rodent control practice is recommended to prevent an increase in the frequency of resistance. If an anticoagulant was applied, to which resistance may exist, it is recommended to eradicate surviving rats using one of the most potent anticoagulants or a non-anticoagulant rodenticide.

REFERENCES

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