RRAC Seminar:

ADVANCES IN ANTICOAGULANT RODENTICIDE RESISTANCE RESEARCH

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Genetic Research in Anticoagulant Rodenticide Resistance: A summary of the 2008 RRAC seminar

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Knowledge on anticoagulant resistance has made considerable progress during recent years, due in particular to the investigation of the genetic basis of the enzyme-complex involved, and the introduction of new test methods. The Rodenticide Resistance Action Committee (RRAC) of CropLife International therefore invited leading scientists from six countries to a seminar with the objective to review current results of genetic research with special regard to the expression of resistance in wild rodents.

Significant Advances in Resistance Research

Recently the gene which codes for the enzyme complex I of the vitamin K epoxide reductase (VKORC1), a crucial enzyme in the vitamin K cycle and the one blocked by all anticoagulant rodenticides was described. Knowing the gene's DNA sequence (Rost et al. 2004), it became possible to look for variants that may result in anticoagulant resistance in rodents, without bringing live animals to the laboratory for *in-vivo* testing.
Anticoagulant resistance in Norway rats has evolved over the years, with different mutations occurring on the VKOR gene (Table 1). Occasionally the same VKORC1 variant was found in rats from different countries indicating that either the same mutation emerged several times or the rat populations developed from the same original stock. One such situation is the same resistance found in Denmark and Germany is the same as that found in north-west England. But is the proof for this variant and others, respectively, sufficient to predict the levels of susceptibility to certain anticoagulants in the respective rat populations? The seminar should help to understand the options and constraints of genetic testing in relation to resistance management based on today's knowledge.

The RRAC seminar

The meeting was opened by Stefan Endepols of Bayer CropScience, who described the objectives and work of RRAC (http://www.rrac.info). He began with an example of Bayer's research, showing differing proportions of advanced resistant rats (resistant to coumatetralyl and bromadiolone) amongst rats heterozygous and homozygous for the Y139C variant in the VKORC1 gene, which introduced the topics of the presentations and discussions, in particular the relation of VKORC1 genotype and resistance-phenotype.

Alan MacNicoll (UK, Central Science Laboratory) summarised the status of genetic resistance research as presented at the 6th European Vertebrate Pest Management Conference in Reading, UK in September 2007, which highlighted areas of uncertainty and defined those requiring resolution so that results could be interpreted for practical use. He posed a series of questions such as: why are there so many different resistance mutations, do all the mutations now found actually confer resistance, do they affect the biological fitness of rodents so that resistant individuals are less likely to survive than their susceptible counterparts, and what do mutations of VKORC1 really tell us about practical resistance to compounds other than warfarin, such as bromadiolone and difenacoum? These questions, and many others, were answered during the seminar.

Etienne Benoit (National College of Veterinary Medicine, Lyon) introduced the genetic and biochemical work on *in-vitro* VKOR suppression and genetic analysis conducted by his research team. They identified a single major resistance mutation (tyrosine139phenyalanine), found throughout France and Belgium. The team conducted a survey of resistance using samples sent by professional pest control technicians from all over France and identified a total of 9 different variants of the VKORC1 in Norway rats.

Kristoff Baert (Belgium, Institute for Nature and Forestry) and Anne-Charlotte Heiberg (Danish Pest Infestation Laboratory) gave talks on the distribution of resistance in their respective countries. Resistance is widespread in western and eastern Belgium but, surprisingly, not in the central part of the country. Resistance to bromadiolone, and to a lesser extent difenacoum, is found in Belgium. In Denmark, the distribution and intensity of resistance is well known. Resistance to warfarin is widespread, and advanced resistance appears in some districts. But the main point of Heiberg's presentation was that resistance is very complex and not fully explained by single mutations of the VKORC1 gene. The Danish group provided evidence of differential expression of the cytochrome P450 genes between susceptible and resistant rats which supports the idea that increased detoxification is another principle in rodenticide resistance (Markussen et al. 2008).

H.-Joachim Pelz (Germany, JKI Federal Research Centre for Cultivated Plants) reviewed all of the currently-known VKORC1 variants in rats, mice and humans (Pelz et al. 2005). A total of about 30 variations have now been identified, but not all result in effective resistance.

Michael Kohn (USA, Rice University, Houston) reported on a cardiovascular phenotype in wild-derived warfarin resistant rats of the Y139C genotype, particularly in homozygous males, which causes mineralization of the aorta. This is important as it may mean that these rats could be at an evolutionary disadvantage due to their fitness costs for resistance. This and other costs such as an increased demand in nutritional vitamin K were discussed in order to understand why anticoagulant resistance appears only locally, although anticoagulants are widely used, and warfarin-resistance appeared in the 1950s only a few years after the introduction of warfarin.

The discussions during the seminar were summarised by addressing the questions posed by MacNicoll earlier (see above):

- Is the VKORC1 gene the one coding for resistance or is it a linked marker? No clear answer can be given yet.
- Why are there so many variants of the VKORC1 gene? High selection pressure may be one explanation. An evolutionary advantage such as gene variability in the rat due to exposure to toxic plants.
- Do (all) the variants of the VKORC1 gene influence the phenotype? Not all do, e.g. in the Scottish resistant rat strain, *in-vitro* inhibition of VKOR is the same as in susceptible rats.
- What does the detection of a VKORC1 variant tell about advanced levels of resistance? In well documented resistant strains and areas it can be a good marker. The frequency of resistance to certain compounds in relation to the respective genotype should be known, and heterozygousity/homozygousity must be considered.
- Does the VKORC1 genotype influence the vitamin K requirements of rats from different strains? In particular in homozygous rats which is considered one of the physiological fitness costs for resistance.

- Are we in danger of over-interpreting genetic findings when predicting resistance? Yes, genetic findings must be considered in accordance with further resistance-tests and on the empirical data of the expression of resistance in the respective area and rodent strain.

These and other topics discussed gave much inspiration for future research. Current knowledge and the availability of new research tools provide some chance for a better understanding of the emergence, nature, and, finally, practical management of rodenticide resistance. We are convinced that another seminar on anticoagulant rodenticide resistance will be held in the not so distant future.

Table 1. Some of the VKORC1 mutations in Norway rats (*Rattus norvegicus*) and house mice (*Mus musculus domesticus*) from different areas of resistance. Data provided by H.-J. Pelz.

Rodent Species	Resistance Area	Amino-acid Position	Original Amino-acid	Mutated Amino-acid
Norway rat				
	Hampshire, UK	120	Leucine	Glutamine
	Berkshire, UK	120	Leucine	Glutamine
	Scotland, UK	128	Leucine	Glutamine
	Wales, UK	139	Tyrosine	Serine
	Yorkshire, UK	139	Tyrosine	Cysteine
	Yorkshire, UK	128	Leucine	Glutamine
	Nottinghamshire, UK	33	Argenine	Proleine
	Denmark	139	Tyrosine	Cysteine
	Germany	139	Tyrosine	Cysteine
	Hungary	139	Tyrosine	Cysteine
	Belgium	139	Tyrosine	Phenylallanine
	France	139	Tyrosine	Phenylallanine
	USA	35	Argenine	Proleine
House mouse				
	UK	139	Tyrosine	Cysteine
	UK	128	Leucine	Serine

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References

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Photo. The speakers at the Seminar. From left to right: back row, Alan MacNicoll, Anne-Charlotte Heiberg, Jo Pelz, Etienne Benoit; front row, Michael Kohn, Kristoff Baert, Stefan Endepols.