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Anticoagulant Resistance in Rats and Mice in the UK – Current Status in 2017

Report from the Campaign for Responsible Rodenticide Use (CRRU) UK for the Government Oversight Group

Test Facility
The Vertebrate Pests Unit
School of Biological Sciences
The University of Reading
Whiteknights
Reading RG6 6AJ, UK

Sponsor
Campaign for Responsible Rodenticide UK
Crown House
85-89 St George's Road,
Leeds LS1 3BR, UK

Report prepared by:

Colin Prescott, Mhairi Baxter, Emily Coan, Clare Jones, David Rymer and Alan Buckle

Dr C.V. Prescott  Date: 7th November 2017

Associate Professor and Director of the Vertebrate Pests Unit

DISTRIBUTION:

University of Reading

Professor Robert Jackson – Head of School

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SUMMARY

1. The status of anticoagulant resistance in the UK is unique in several ways. Most importantly, more than fifty years of continuous research into this phenomenon, both in Norway rats and house mice, has provided an extensive platform of knowledge upon which to base practical advice on anticoagulant use and recommendations on resistance management. Regrettably, an axiom of resistance management, not to use active substances in areas where they are resisted, has been difficult to apply in practice because of a long-standing regulatory policy, virtually unknown elsewhere, wherein the most potent anticoagulant rodenticides were precluded from use in the management of resistant Norway rats because of perceived risks to the environment. Finally, and again uniquely, the UK is home to more anticoagulant resistance mutations in Norway rats than any other country world-wide, with five having practical impacts.

2. Developments in the last decade have revolutionised the study of anticoagulant resistance, in terms of our understanding of its genetic basis, physiological mechanisms and geographical distribution. New resistance tests based on DNA extraction and sequencing, permit rapid, cheap, accurate and humane resistance monitoring. These tests, however, still rely on older techniques, involving laboratory studies using either live rodents or blood samples taken from them and field efficacy testing, to understand practical impacts of resistance mutations on the outcome of anticoagulant applications. Fortunately, these two information threads come together in the UK.

3. Among UK Norway rats we have identified a total of nine genetical mutations in areas of the genome that are known to be important for the action of anticoagulants. Among these, three (L120Q, Y139C, Y139F) confer resistance to the first-generations anticoagulants (FGARs) and to one or more of the second-generation anticoagulants (SGARs). Among the remainder, two (Y139S, L128Q) confer significant levels of resistance to FGARs, one (N33P) has been found to confer resistance to warfarin in the laboratory, two (F63C, Y39N) impair protein function and one (A26T) is thought to have no practical consequences. Both mutations found in UK house mice (Y128S, Y139C) confer resistance to FGARs and to one or more SGARs.

4. This report presents the results of all anticoagulant resistance monitoring conducted to date at the Vertebrate Pests Unit, the University of Reading, for both Norway rats and house mice. It shows the massive extent of L120Q resistance in Norway rats, the most severe form of resistance in this species, across the whole of central southern England. The ubiquity of Y139F resistance among rats in Kent and East Sussex is also apparent. Of further concern are isolated records of these mutations, far from their core areas, suggesting either transportation of resistant rodents or the *de novo* development of new foci. Y139C, another relatively severe form of resistance, is also widely dispersed. Much of the UK remains untested because our laboratory has been unable to obtain biological material from many areas. It is unsafe to assume, however, that the absence of a sample showing resistance from any particular area indicates that resistance is absent. Furthermore, the scarcity of wild-type (i.e. fully susceptible) Norway rats, particularly in central-southern and south-east England, suggests that it is reasonable to assume that almost any rodent infestation in those areas will contain rats carrying one or other of the severe L120Q or Y139F mutations. A sample of house mice from south-east England has been tested and results are given in this report for the first time. It is perhaps not surprising that, although the sample is small, both known house mouse resistance mutations (L128S, Y139C) were found at high frequency, with some individuals worryingly possessing both mutations.

5. Recommendations in this report about the use of anticoagulant rodenticides against resistant rodent infestations are reproduced from resistance management guidelines published by the UK Rodenticide Resistance Action Group (RRAG).

1. Introduction

Information about anticoagulant resistance rodents inherited from earlier researchers in the UK is more extensive than for any other country worldwide (Buckle, 2013; Pelz and Prescott, 2015). The first case of anticoagulant resistance in Norway rats (*Rattus norvegicus*) was discovered in 1958 (Boyle, 1960) and research in government laboratories, universities and industry has continued ever since. The first national survey of anticoagulant resistance in the UK was conducted and reported by Greaves and Rennison (1973), showing the distribution of resistance prior to the introduction of the second-generation anticoagulant rodenticides (SGARs). Work has continued since then on the geographical distribution, the severity and practical consequences of anticoagulant resistance.

For almost 30 years, rodenticide regulatory policy in the UK was such that the most potent SGARs, and those widely used in resistance management elsewhere, namely brodifacoum, flocoumafen and latterly difethialone, were unavailable for use against resistant Norway rats (*Rattus norvegicus*). This was caused by a restriction of the use of these active substances to 'indoors only', with an associated stringent definition on what could be considered compliant with this term. This restriction was itself driven by concern, held by the UK regulatory authority and other agencies, about the exposure of, and possible subsequent impacts on, a wide variety of UK wildlife caused by anticoagulants (Newton *et al*., 1999: Burn et *al*., 2002; Buckle, 2013; Smith and Shore, 2015). The practical effects of this policy on the distribution of resistance in the UK can only be speculated upon. However, the obvious consequence was the continued, indeed almost exclusive, use of resisted anticoagulants in foci where they were either partially or wholly ineffective. Greaves (1994) predicted that nothing was more likely to promote the spread of anticoagulant resistance, and increase its severity, than such a situation.

The Health and Safety Executive (HSE), the UK Competent Authority for biocides, in consultation with a wide range of stakeholder organisations, contemplated a programme of risk mitigation that, if comprehensively applied, might permit the indoor only restriction to be lifted, so that the SGAR active substances that are the most effective for resistance management might be used against Norway rats in an outdoor situation for the first time in the UK (HSE 2013). The outline of this programme, termed a 'stewardship regime', was provided by HSE and included among its five key 'principles' the objective to retain effectiveness of SGAR treatments and manage resistance (HSE 2013).

The programme proposed then is now implemented as the 'UK Rodenticide Stewardship Regime' (Buckle *et al*, 2017a) and is managed by CRRU UK under the review of HSE and a Government Oversight Group (GOG) comprising representatives from all interested departments of government and the devolved administrations (see Buckle *et al*. 2017b). In its overview of the initial phases of the regime, the GOG has requested an annual report of currently available information on anticoagulant resistance in the UK (GOG 2107). The present document is provided by CRRU UK, and commissioned from the University of Reading, in fulfilment of this request.

A previous report from the UK Rodenticide Resistance Action Group (Buckle and Prescott 2012a) addressed a wide range of topics relevant to anticoagulant resistance in the UK, including definitions of relevant terms, methods of resistance testing, resistance mechanisms and alternatives to anticoagulant rodenticides. These subjects will not be dealt with further in this report.

2. Anticoagulant Resistance and the VKORC1 gene

Our understanding of anticoagulant resistance has been considerably enhanced by research on underlying changes in DNA sequences of the VKORC1 gene that have the potential to confer anticoagulant resistance on individuals that possess them (see Pelz *et al*., 2005; Rost *et al*., 2009; Hodroge *et* al., 2011). In this Report, both Norway rats and house mice will be described that possess mutations of the VKORC1 resistance gene. Table 1 presents a list of the VKORC1 mutations (i.e. single nucleotide polymorphism or SNPs) that are known to occur in UK populations of the two species, and goes on to summarise their geographical locations known to date (see section 2.4 for more on this). The first data entry is for Norway rats that possess the VKORC1 mutation, L128Q; indicting that at location 128 of the VKORC1 gene, the wildtype amino acid, Leucine (abbreviated as L) has been replaced by the amino acid Glutamine (abbreviated as Q). Such animals will subsequently be referred to as 'L128Q Rats'; and a similar convention will be applied for both species with each of the VKORC1 SNPs (see Table 1).

For European Norway rats, it is perhaps only in France where a similar (though somewhat lesser) number of mutations is present (Grandemange *et al*., 2010). While in other territories, some with much larger land areas (e.g. Germany), only one or two resistance mutations have been identified to date (Pelz and Prescott, 2015). In others, for example in Ireland, Italy and Spain, there is no known occurrence of anticoagulant resistance in Norway rats, although no studies have been conducted there to look for them. Therefore, the profile of resistance in UK Norway rats is unique in that virtually all known resistance mutations are present and there is one, Welsh resistance (Y139S), that is present nowhere else.

Table 1. VKORC1 mutations in Norway rats (NR) and House mouse (HM) in UK. From: Pelz *et al*. 2005; Rost *et al*. 2009; Prescott *et al*. 2010; Pelz and Prescott, 2015;Clarke and Prescott, 2015 unpublished report. Major resistance mutations with known practical consequences shown in bold.

Species	Mutation	Abbreviations	Where present
NR	**Leucine128Glutamine**	**L128Q†**	**Central Southern Scotland, Yorkshire, Lancashire**
NR	**Tyrosine139Serine**	**Y139S†**	**Anglo-Welsh border**
NR	**Leucine120Glutamine**	**L120Q†**	**Hampshire, Berkshire**
NR	**Tyrosine139Cysteine**	**Y139C†**	**Gloucestershire, Norfolk, Lincolnshire, Yorkshire, SW Scotland**
NR	**Tyrosine139Phenylalanine**	**Y139F†**	**Kent**
NR	Argenine33Proline	N33P‡	Nottinghamshire
NR	Phenylalanin63Cysteine	F63C*	Cambridge/Essex
NR	Tyrosine39Asparagine	Y39N*	Cambridge/Essex
NR	Alanine26Threonine	A26T#	Cambridge/Essex
HM	**Tyrosine139Cysteine**	**Y139C†**	**Reading**
HM	**Leucine128Serine**	**L128S†**	**Cambridge**

† Known either from field experiments and/or field experience to have a significant practical effect on anticoagulant efficacy
‡ Known from laboratory experiments to confer warfarin resistance
* Shown in laboratory experiments to have a significant impact on protein function
# Unlikely to confer any significant degree of resistance

In European house mice, both Y139C and L128S mutations have been recorded, particularly in Germany, and there is a third VKORC1 sequence variant that involves four SNPs (A12T; A26S;

A48T and A61L) that is also associated with a substantial loss of efficacy against anticoagulants (Pelz and Prescott, 2015), although to date, this VKORC1 sequence variant has not been identified in the UK.

3. Resistance in Norway rats

3.1. Background

Publications have been produced periodically over the last forty years which document the development and spread of anticoagulant resistance in Norway rats in the UK (see Greaves and Rennison, 1973: MacNicoll *et al*., 1996; Buckle and Prescott, 2012a: Buckle, 2013; Rymer *et al*., 2015; Clarke and Prescott, 2015 unpublished report). Reference may be made to these for an historical account of anticoagulant resistance in UK Norway rats.

It is assumed that each VKORC1 mutation was originally produced by a random mutation event within the DNA of wildtype susceptible animals. It is possible that these mutations have occurred in the rodents quite recently, following exposure to anticoagulant, although it is probably more likely that they were present in wild populations at a very low incidence prior to the introduction of anticoagulants. The occurrence of these mutations in wild populations of rodents could either result from intense selection by the prolonged use of ineffective anticoagulants, or by transportation of established resistant animals (for example in bedding between farms, or on ships between continents). The occurrence in Kent and East Sussex of Y139F resistance, in an area that is proximate to the occurrence of the same mutation on the continent of Europe (i.e. Belgium and France), suggests that transportation may have been relevant in that case. The geographical distribution of Y139C in UK Norway rats is also suggestive of transportation as a cause because it is present on the east coast, which faces towards Denmark and Germany where it is the only mutation present (Pelz and Prescott, 2015), and elsewhere close to large port cities such as Bristol and Hull. The prolonged and intensive use of anticoagulants as the principal method of rodent control by a wide range of users ensures that resistant genotypes are at a competitive advantage over wild-type individuals and therefore become established and spread.

Knowledge of the severity and distribution of anticoagulant resistance has important uses in the practical application of rodent pest management in the UK. It is a fundamental tenet of effective resistance management that resisted active substances should not be used against infestations that are resistant to them (Greaves, 1994; Buckle, 2013) and this cannot be implemented unless practitioners know where resistance occurs. Also, there are correlations between the potency of SGARs, their effectiveness against the different resistant strains of rodent and their ecotoxicological risks (Smith and Shore, 2015). Therefore, consideration of 'risk hierarchy' in the application of the available different chemical interventions would dictate that the less potent anticoagulants should be used preferentially where no resistance to them is known to exist (Berny *et al*., 2014). Hence, the effective communication to practitioners of information on resistance is valuable both in terms of managing anticoagulant resistance and minimising ecotoxicological risks.

3.2. UK Resistance Mutations in Norway rats

Nine different SNPs have been found in Norway rat infestations the UK (Table 1). So far as we currently know, some of these SNPs are restricted in the UK to single foci. For example, Y139S is restricted to a large area of the West Midlands, the Anglo-Welsh border and central Wales and Y139F is largely restricted to Sussex, Kent, Suffolk and Norfolk. Other SNPs, such as Y139C, and especially the most severe, L120Q, are more widely dispersed.

Among these nine mutations, five are known to have significant detrimental practical impacts on anticoagulant efficacy (see the following sections). With the exception of Y139S which is only known from the UK, all the others (i.e. L128Q, L120Q, Y139C and Y139F) are found elsewhere in the EU where they are known to have similar practical impacts on rat control (Buckle, 2013). In other words they are reliable markers for practical resistance to one or more anticoagulant rodenticides. In the remainder of this section we describe each of these resistance mutations present in UK Norway rats that are known to affect efficacy and what is known from previous research about the effectiveness of anticoagulants against them. Information on current knowledge of their geographical distribution is given in subsequent sections of the report.

3.2.1 'L128Q Rats' ("Scottish resistance")

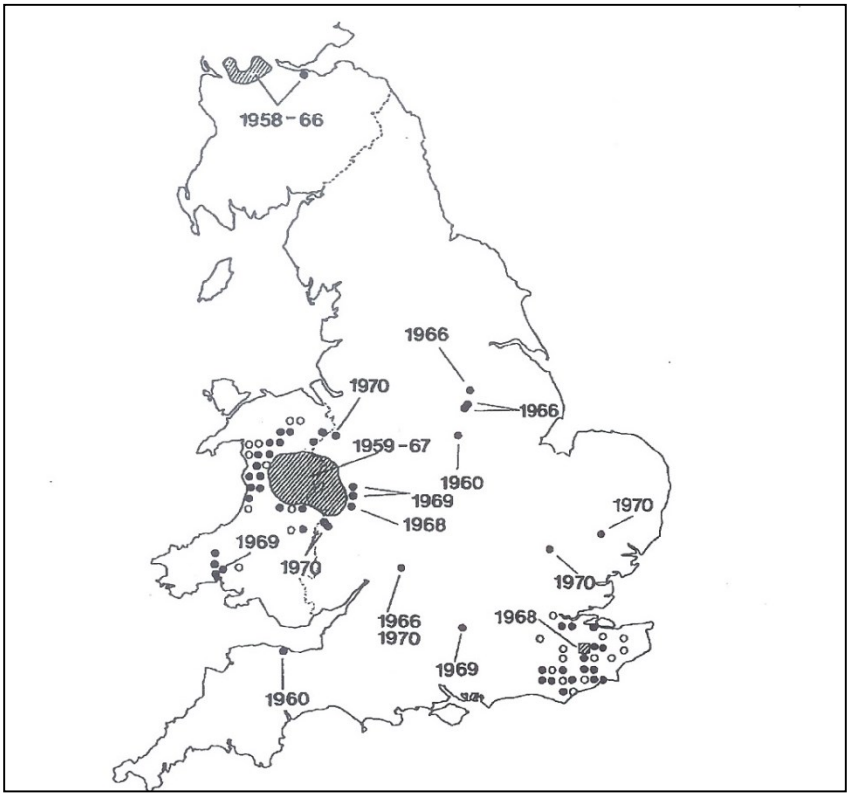
This SNP was the one found at the site of the first occurrence of Norway rat anticoagulant resistance in Scotland (Figure 1) (Greaves and Ayres, 1976and has been subsequently found in rats in parts of the north-west of England and in Yorkshire. 'L128Q Rats' appears to confer practical resistance to warfarin and diphacinone, with warfarin resistance factors for males and females of 51.5 and 115.9 respectively (Greaves and Cullen-Ayres, 1988). At first, coumatetralyl was found to retain some effectiveness against such rats but resistance factors were high, 34.0 in males and 56.2 in females (Greaves and Cullen-Ayres, 1988) and efficacy is certainly impaired. Second-generation anticoagulants are considered to be effective against this resistance strain. Evidence for this is provided by laboratory tests on difenacoum conducted at UK government laboratories (Hadler *et al*., 1975) and by Greaves and Cullen-Ayres (1988), who reported resistance factors for difenacoum, bromadiolone and brodifacoum that are all below 3.4. However, no scientific evidence from field testing has been published to corroborate these laboratory studies.

'L128Q Rats' were also found in a sample of Norway rats taken for DNA resistance testing in France (Grandemange *et al*., 2010).

3.2.2 'Y139S Rats' ("Welsh resistance")

Resistance was found in Norway rats on farms on the Anglo-Welsh border centred on the town of Welshpool soon after the original discovery of resistance in Scotland (Figure 1). Welsh resistant rats are now known to carry the Y139S mutation and have very high resistance factors to the first-generation anticoagulants warfarin and coumatetralyl. Extensive fieldwork was conducted in an attempt to curtail the spread of this focus, but the work was ineffectual and was eventually abandoned (Greaves, 1995).

Figure 1. Sites of anticoagulant resistance in the UK from surveys conducted during the years 1959 to 1970. Filled symbols show where resistant Norway rats were found, open symbols where resistance was not found. From Greaves and Rennison (1973).



Evidence for the effectiveness of the second-generation anticoagulants against 'Y139S Rats' is extensive, from both laboratory and field studies, because this resistant strain of Norway rats was the one mainly used to evaluate difenacoum, bromadiolone, brodifacoum and flocoumafen for their effectiveness at controlling resistant Norway rats (Table 2). The second-generation compounds are considered to be effective against 'Y139S Rats' although bromadiolone may be the least effective (Buckle *et al*., 2007), with a resistance factor for female animals of 6.9 (Greaves and Cullen-Ayres, 1988). Trials were conducted using restricted placements of baits containing bromadiolone, difenacoum and brodifacoum against field infestations of 'Y139S Rats' (Greaves *et al*., 1988). The results showed that bait points containing 50 g of 50ppm brodifacoum bait, replenished weekly or twice weekly, gave complete control of 'Y139C Rats' in 14-25 days. Difenacoum and bromadiolone were less effective.

To date this mutation has only ever been found in the original focus, although the current geographical extent of the focus is unknown. Very few samples have been obtained from this focus for DNA sequencing. However, resistance foci are not known to recede spontaneously, so this resistance is unlikely to cover an area smaller than that shown in Figure 1. Indeed, it is likely to extend over a large part the counties of Powys and Shropshire, and to extend to portions of the counties of Gwynedd, Herefordshire and Staffordshire.

Table 2. Laboratory and field studies of the efficacy of second-generation anticoagulants against Y139C-resistant Norway rats conducted by industry and government scientists during the commercial development of these active substances.

Active substance	Laboratory study	Field study
difenacoum	Hadler *et al*., 1975	Rennison and Hadler, 1975
brodifacoum	Redfern *et al*., 1976	Rennison and Dubock, 1978
bromadiolone	Redfern and Gill, 1980	Richards, 1981
flocoumafen	Bowler *et al*., 1984	Buckle, 1986

3.2.3 'Y139C Rats' ("Gloucestershire resistance")

Anticoagulant resistant Norway rats have been present in Gloucestershire since 1969 (Figure 1) and are now known to carry the Y139C mutation. This mutation is also found in the UK in such widely separated areas as Yorkshire, Norfolk and south-west Scotland (Table 1 and Clarke and Prescott, 2015, unpublished report). We know little about the development of these resistance foci and no research has been published on 'Y139C Rats' from studies conducted in the UK. However, this SNP has also been present for decades over large parts of Jutland, and elsewhere, in Denmark (Lodal, 2001) and in North-west Germany around the city of Műnster (Pelz *et al*, 1995; Pelz and Prescott, 2015). It is now also found in The Netherlands (van der Lee *et al*., 2011), Hungary (Pelz and Prescott, 2015) and France (Grandemange *et al*., 2010).

Most of what we know about the efficacy of anticoagulants against 'Y139C Rats' comes from work carried out in Germany and Denmark, where this SNP confers strong practical resistance against the first-generation anticoagulants; for example, resistance factors to coumatetralyl in Germany are 34 for males and 54 for females (Endepols *et al*., 2012). The strain shows resistance to the second-generation anticoagulants, particularly where there is a high incidence of resistance, and a high frequency of homozygous animals. In particular, the efficacy of bromadiolone is poor against animals carrying this mutation (Endepols *et al*., 2012) and, although difenacoum is generally more effective, acceptable control may be difficult to achieve (Buckle *et al*., 2012). These studies, and practical experience in the UK, have resulted in the UK Rodenticide Resistance Action Group (RRAG) advice that bromadiolone and difenacoum should not be used against Norway rat populations possessing this mutation (RRAG, 2010).

It has been confirmed in field experiments conducted at a German focus of 'Y139C resistance that applications of 50 g bait placements containing 0.005% brodifacoum are fully effective against 'Y139C Rats' (Buckle and Prescott, 2012b). Using the 'pulsed baiting' application technique, very small quantities of brodifacoum bait were required for complete elimination of 'Y139C Rat' infestations. It is to be anticipated that the same would be the case in the UK. A summary of the German field trials against 'Y139C Rats', using either bromadiolone, difenacoum or brodifacoum is given in Table 3.

The extent of the various foci of this resistance mutation in the UK is unknown. However, rats carrying this SNP have been identified in the counties of Gloucestershire, Yorkshire, Lincolnshire, Norfolk and Surrey. Additionally, Clarke and Prescott (2015, unpublished report) record 'Y139C Rats' from Gwynedd, from sites in the West Midlands and from Dumfries and Galloway.

Table 3. The quantities of anticoagulant baits used and estimated efficacy when bromadiolone, difenacoum and brodifacoum were used to control Y139C resistant rats on farms in the Münsterland

Active substance	Site number	Maximum daily pre-treatment census bait take (kg)[#]	Quantity of bait consumed (kg)	Estimated % efficacy[*]
Bromadiolone[†]	1	2.66	9.95	71.50
	2	2.14	43.40	0.00
	3	1.51	25.50	20.00
	4	4.52	38.38	69.00
Difenacoum[‡]	1	6.89	28.20	86.80
	2	1.62	8.10	59.90
Brodifacoum[§]	1	2.98	4.00	99.20
	2	1.63	1.45	100.00

[*] maximum daily census bait used to estimate efficacy

[#] provides a relative estimate of initial rat population size

[†] from Endepols *et al.*, 2012
[‡] from Buckle *et al.,* 2012
[§] from Buckle and Prescott, 2012b

3.2.4 'Y139F Rats' ("Kent resistance")

Resistance in Kent once covered a large part of that county and neighbouring East Sussex (Figure 1). A recent practical failure of a bromadiolone treatment resulted in the DNA sequencing of tissue samples from rats at the site. The Y139F mutation, never before found in the UK, was identified (Prescott *et al*., 2010). This SNP is found in several European countries including France, Belgium and The Netherlands (Buckle, 2013; Pelz and Prescott, 2015). There is little published evidence, however, about the practical effectiveness of anticoagulants against Y139F. Grandemange *et al*. (2009) conducted laboratory experiments using BCR testing to determine the effectiveness of chlorophacinone, bromadiolone, difenacoum, and difethialone against rats possessing the VKORC1 mutation Y139F. From this, the authors recommended that the first-generation compounds and bromadiolone should not be used against these 'Y139F Rats', and that difenacoum might be effective, but its use would be expected to increase the frequency of the resistance mutation. They proposed that the highly potent compounds, such as difethialone (and by extrapolation presumably brodifacoum and flocoumafen), may be effective against the strain. The work of Grandemange *et al*. (2009) was used to develop the RRAG recommendations that neither first-generation anticoagulants nor bromadiolone and difenacoum should be used against 'Y139F Rats' (RRAG, 2010).

Once again, the precise current extent of this resistance in the UK is unknown. However, rats carrying this SNP have been identified in the counties of Kent, East Sussex, Suffolk and Norfolk.

3.2.5 'L120Q Rats' ("Hampshire and Berkshire resistances")

In 1969 (Figure 1), rats from a farm in north-east Hampshire were found in laboratory tests to possess a degree of resistant that was distinct from that of the Scottish and Welsh strains. Subsequently, rats were tested for resistance using feeding tests for warfarin (EPPO, 1995) and,

later difenacoum, and were found to be resistant to both compounds (Greaves and Cullen-Ayres, 1988). A laboratory breeding programme was carried out to product a pure line of Hampshire resistant rats called the Homozygous Hampshire (HH) strain. Later investigations (Figure 2) showed that rats putatively resistant to difenacoum were prevalent over an area of 1,200 square kilometres, including parts of the neighbouring counties of Berkshire and Wiltshire (Greaves *et al*., 1982a and b; MacNicoll and Gill, 1987).

This mutation is also known from France (Grandemange *et al*., 2009) and Belgium (Pelz and Prescott, 2015).

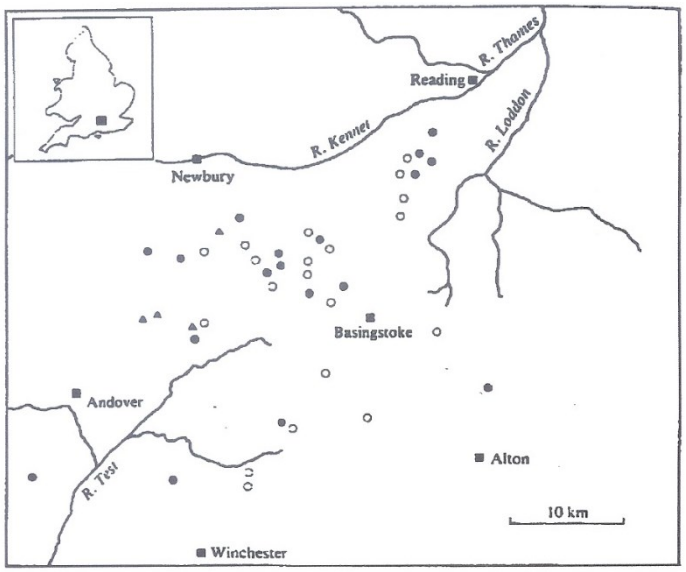
Greaves *et al*., (1982b) carried out a series of field trials on farmsteads near Basingstoke, at the centre of the resistance area shown in Figure 2. They conducted three sets of six experimental treatments using baits containing each the active ingredients difenacoum (50 ppm), bromadiolone (50 ppm) and brodifacoum (20 ppm). The difenacoum treatments were almost entirely ineffective and those of bromadiolone were only partially successful. All six treatments of brodifacoum were completely effective although the treatments were somewhat prolonged.

Subsequent investigations on another, nearby farm demonstrated unequivocally that Berkshire resistant rats show practical resistance to bromadiolone (Quy *et al*., 1995). In feeding trials conducted at the University of Reading, thirteen female rats from the farm survived doses of between 22.2 and 44.2 $mg.kg^{-1}$ of bromadiolone, and ten male rats survived doses of between 14.9 and 30.1 $mg.kg^{-1}$ of bromadiolone (Hussain, 1998). These values far exceed the quantities of bromadiolone bait that would be lethal to susceptible Norway rats (Greaves and Cullen-Ayres, 1988). A laboratory breeding programme was carried out to product a pure line of Berkshire resistant rats called the Homozygous Berkshire (HB) strain; and resistance factors for female Berkshire resistant Norway rats have been estimated to be: 18 for difenacoum and 35 for bromadiolone, but only 5 for both brodifacoum and flocoumafen (MacNicoll, personal communication, 2004). The HB strain is the most extreme form of anticoagulant resistance currently known in the UK, and elsewhere, and Norway rats with these resistance characteristics are now present across much of central-southern England (section 3.4).

The genetics of the Hampshire and Berkshire resistant strains is uncertain. Both carry the L120Q mutation but it is postulated that Berkshire resistance is conferred by the presence of this mutation as well as some other factor(s), possibly because of the combined effects of pharmacodynamically-based resistance (altered biochemistry of the target enzyme) and enhanced clearance (i.e. pharmacokinetically-based resistance) (Thijssen, 1995).

Recent studies on the VKORC1 mutation in Hampshire and Berkshire have shown that a very high proportion of animals are now Homozygous for L120Q. It may be that the increased levels of resistance recently detected in Hampshire and Berkshire are a reflection of 1) the increase in numbers of homozygous animals, and 2) the development of a selected line of resistance in the field as a result of the continued use of SGARs that are not totally effective. Both Greaves and Cullen Ayres (1988) and MacNicoll (unpublished report) produced selected lines of the Hampshire strain and Berkshire strain respectively. These had enhanced levels of resistance when compared with the unselected lines. Indeed, our recent studies on wild populations of Norway rats from Hampshire have demonstrated levels of resistance that are more characteristic of the Berkshire strain.

Figure 2. The distribution of farmsteads providing samples of rats that contained difenacoum-resistant (filled circles), warfarin-resistant (open circles) and non-resistant (closed triangles) Norway rats. From Greaves *et al*. (1982a).



Field trials of bromadiolone and difenacoum were conducted by workers from the University of Reading recently on farms near Newbury (Berkshire) and Winchester (Hampshire), where the rat infestations where almost entirely homozygous for the L120Q resistance mutation. Very large quantities of bromadiolone and difenacoum baits were used at these sites and poor levels of control were achieved (Rymer, 2017).

First-generation anticoagulants are likely to be ineffective against L120Q. Greaves *et al*. (1982b) showed that difenacoum is also ineffective and bromadiolone partially so. On at least one farm with 'L120Q Rats' near Newbury, bromadiolone was also entirely ineffective. However, a recent, carefully-monitored practical treatment has shown that brodifacoum is fully effective against 'L120Q Rats' (Meyer, 2009). Although the site was in central-southern Hampshire (Winchester), previous treatment records show the complete failure of difenacoum and bromadiolone baits and suggest that the resistance there was of the advanced Berkshire type. A total of 213 kg of (mainly) difenacoum and bromadiolone was used over a period of two years at this small site without any observable effect on the rat infestation. An application of 3.4 kg of brodifacoum bait, made under an emergency extension of approval, eradicated the infestation in 18 days.

Until recently the focus of 'L120Q Rats' in central southern England was thought to comprise a single contiguous focus, the extent of which comprised very large portions of Hampshire and Berkshire, with parts of the neighbouring counties of Wiltshire, Oxfordshire and Surrey also involved. Recent work conducted at the University of Reading has revealed the presence of 'L120Q Rats' in Sussex, Kent, Greater London, Essex, Norfolk, and in Dorset. It is not currently possible to say whether these foci are contiguous with the main one. An outlying focus has also been identified in south-west Scotland.

A noteworthy feature of the L120Q focus is the frequent occurrence of homozygous resistance, and the fact that there are few wild-type 'susceptible' animals (i.e. that do not possess a resistance

gene) within the resistant infestations. This implies that recent and historic rodenticide applications in the resistance area have operated an extreme selection pressure in favour of the L120Q resistant genotypes.

Consequently, bromadiolone and difenacoum are no longer advocated for use anywhere in this area against L120Q (RRAG, 2010) and only brodifacoum, flocoumafen and difethialone have been recommended for use against L120Q.

3.3 Resistance ratios for the some UK strains of resistant Norway rats

Resistance ratios given in the preceding sections of this report are extracted from the literature, principally (Greaves and Cullen-Ayres, 1988). However, the University of Reading has developed a methodology based on blood clotting activity that can be used to identify resistance and estimate the Resistance Factor for each active ingredient/species/sex combination (Prescott *et al*., 2007). With funding and technical support from the Rodenticide Resistance Action Committee (RRAC) of the industry trade association CropLife International, Resistance Factors have made estimates for both male and female 'L120Q rats' against all five second generation anticoagulants (RRAC, 2016). In further collaboration between RRAC and the Julius Kűhn Institute, Federal Research Centre for Cultivated Plants, Germany, the methodology of Prescott *et al*., (2007) has been used to estimate Resistance Factors for male and female animals of both 'Y139C Rats and 'Y139F Rats'. The summary results of all this work are shown in Table 4.

Table 4. Resistance Factors in male and female homozygous resistant Norway rats (*Rattus norvegicus*), generated against the three most important VKORC1 resistance mutations identified in the UK to date, using all five Second Generation Anticoagulants (RRAC, 2016). N.B. These Resistance Factors are for active substances *per se*. They do not take into consideration the concentrations of active substance used in baits.

VKORC1 mutation	Active Substance				
	Bromadiolone	Difenacoum	Brodifacoum	Flocoumafen	Difethialone
L120Q	10.0/14.0	4.8/12.0	2.8/6.7	2.5/3.2	2.2/2.3
Y139C	17.0/15.0	1.6/2.9	1.2/1.8	0.8/1.0	0.5/0.8
Y139F	7.0/9.0	1.4/1.9	1.3/1.3	1.0/1.0	0.9/0.8

Field experience would suggest that Resistance Factors that are less than five are unlikely to cause any significant discernible effect on the practical efficacy of an active substance against the resistant strain, although where bait uptake is a concurrent problem, perhaps caused by bait aversion, unpalatable baits or the abundance of alternative food, treatment difficulties may be encountered (e.g. Buckle *et al*., 2012). Resistance Factors that are greater than five may cause a loss of efficacy, particularly against infestations with a high proportion of homozygous resistant animals and where baiting problems co-occur. This loss of efficacy may be demonstrated by prolonged duration of treatments and the requirement to apply larger than normal quantities of rodenticide bait, although complete control may be achieved (e.g. Greaves *et al*., 1982b). Resistance Factors that are greater than 10 are likely to presage a significant detrimental effect on anticoagulant efficacy

3.4 Norway rat resistance surveys in the UK

3.4.1 Background

Surveys of anticoagulant resistance in Norway rats in the UK have been conducted since the 1960s. Results from surveys conducted during the period 1959 to 1970 are shown in Figure 1. Many different resistance foci were identified at a very early stage and the majority of these exist to this day. Another early review of the distribution and significance of resistance among Norway rats in England and Wales was provided by MacNicoll *et al*., (1996).

For many years our knowledge of the distribution of anticoagulant resistance in the UK was hampered by cumbersome, expensive and inhumane resistance detection measures, which involved the capture and maintenance of wild rodents in the laboratory for long periods (EPPO, 1995). This situation changed with the pioneering work of H-J Pelz and his co-workers (Pelz *et al*., 2005), who sequenced the VKORC1 'resistance gene' and then identified a number of genetic mutations of that gene that were found in many of the historic UK resistance foci previously identified. This new DNA sequencing technology, which relied heavily on developmental work in the field of human medicine (Rost *et al*., 2004; Li *et al*., 2004; Tie *et al*., 2005) has revolutionised the detection of anticoagulant resistant in wild populations of rodent.

However, the detection of a particular mutation of the VKORC1 resistance gene does not permit any conclusions to be drawn about the potential impact of that mutation on our ability to use anticoagulants to control the rodents that carry them. To some extent, this information has been provided by historic UK research conducted in the laboratory and field over the past fifty years, although this may be of limited value, as the magnitude of the resistance in certain locations (such as Hampshire; see Section 3.2.5 above) is known to have increased markedly in recent years.

With funding from RRAC of CropLife International, methodologies developed at the University of Reading are now being used across Europe to quantify the magnitude of the resistance by estimating both male and female Resistance Factors conferred by a number of the VKORC1 mutations against all five second generation anticoagulants (see Section 3.3; RRAC, 2016).

3.4.2 DNA Sequencing of resistant Norway rats in the UK

The technique of DNA sequencing for the detection of rodents carrying anticoagulant resistance SNPs is relatively new. In collaboration with scientists in the UK, Pelz *et al*., (2005) sequenced DNA material from UK resistant Norway rats and, thereby, provided researchers in the UK with an understanding of the SNPs underlying the majority of the established UK anticoagulant resistance foci.

DNA sequencing has been used for surveys of resistance in several EU countries including Germany (Pelz, 2007; Pelz *et al*., 2011), The Netherlands (van der Lee *et al*., 2011) and Belgium (Baert *et al*., 2011). In the UK, the technique was used for the first time to identify the resistance SNP present in the Kent resistance focus (Prescott *et al*., 2010).

3.4.3 Data Sources and GIS analysis

The information presented here comes primarily from the Vertebrate Pests Unit of the University of Reading, and in particular, the PhD studies of David Rymer and Mhairi Baxter.

These data have been entered onto a Global Information System (ArcGIS) database in which positional data for each rat DNA sample is entered either as a UK National Grid Reference or as a UK postcode. Additional data entered are: 1) resistance mutation found, 2) whether the sample was homozygous or heterozygous for the SNP found. The resistance maps prepared in this way, therefore, contain accumulated data from all data sources.

3.4.4 Materials and Methods

Genetical material was obtained from the field in the form of either tail tip samples or fresh droppings. Where possible, samples were placed in tubes containing 80% alcohol and then stored at -20°C as quickly as possible. Some unfrozen samples were shipped to the laboratory using a courier service, surface mail or by hand delivery, and were frozen on receipt.

Genomic DNA was extracted using the Qiagen DNeasy tissue extraction kit following the manufacturer's recommendations (Qiagen Ltd., Crawley, West Sussex, UK). Briefly, a small quantity of tissue (approximately 3mm x 2mm x 2mm) was shaved from each tail using a sterile sharp razor blade, and then placed in a 1.5ml microtube. Pre-warmed extraction buffer ATL (180 µl) was added, followed by 20 µl of proteinase K. The mixture was vortexed and incubated at 55 ˚C on a rocking platform overnight (approx. 17 h). Genomic DNA was then purified and eluted from spin-purification columns in 80 µl of elution buffer and the quality and yield were assessed spectrophotometrically using a nano-drop instrument.

The three exons of the VKORC1 gene, designated 1, 2 and 3, were amplified by PCR following the methodology of Rost *et al*. (2004). PCR products were purified using the QIAquick PCR purification kit (Qiagen Ltd., Crawley, West Sussex, UK). Product samples (3.5µl) were then sequenced with BigDye version 3.1 terminator chemistry (ABI) on a 9700 ABI thermal cycler, and the terminated products were resolved on an ABI 3130XL capillary sequencer. The sequence trace files were visually analysed and any ambiguous bases were edited using the DNASTAR Lasergene software. The sequence alignments were compiled using ClustalW2.

3.4.5 Results.

The Norway rat DNA sequencing results are presented in Figure 3. The majority of records are from central southern England, and to a lesser extent, south east England. Records elsewhere across the UK are much less frequent and as a result, provide little information on the incidence of the mutation in the free-living populations.

Available DNA sequencing data is most extensive for the VKORC1 mutation L120Q, primarily from the areas of West Berkshire and Hampshire. Historically, this resistance was first found on farms in north-east Hampshire and West Berkshire (Figure 2) and for some time it was considered that the focus remained relatively confined. However, recent DNA screening has demonstrated the presence of the mutation over a very large part of central southern England. As well as having a widespread distribution across Berkshire and Hampshire, it has been found to be distributed north into Oxfordshire, west to the Dorset/Somerset boarders, and east to Surrey and the western boarders of Kent and East Sussex. Additional occurrences were found in Greater London, Essex and Norfolk, and there was one occurrence near Edinburgh, where a single animal was heterozygous for two VKORC1 mutations, L120Q and L128Q. The occurrence of this mutation in the homozygous state across much of its range clearly indicates considerable selection for L120Q resistance in these populations over a prolonged time period. The homozygous animals have clearly had a selective advantage over heterozygous (and susceptible) animals when exposed to anticoagulant rodenticides. Based on research conducted in the UK, RRAG now recommends that bromadiolone and difenacoum should not

be used against ‘L120Q Rats’. Although, of course, until recently UK regulatory policy made their use almost unavoidable.

It is highly likely that the focus of resistance is contiguous over much of central southern England, and there is no evidence that the incidence of resistance in West Berkshire exceeds that in other neighbouring counties. Indeed, it is important to note that resistant rats are present, and may even predominate, in the conurbations of Reading, Newbury, Winchester, Basingstoke, Andover and Salisbury; and that effective rodent control is severely compromised in this entire area.

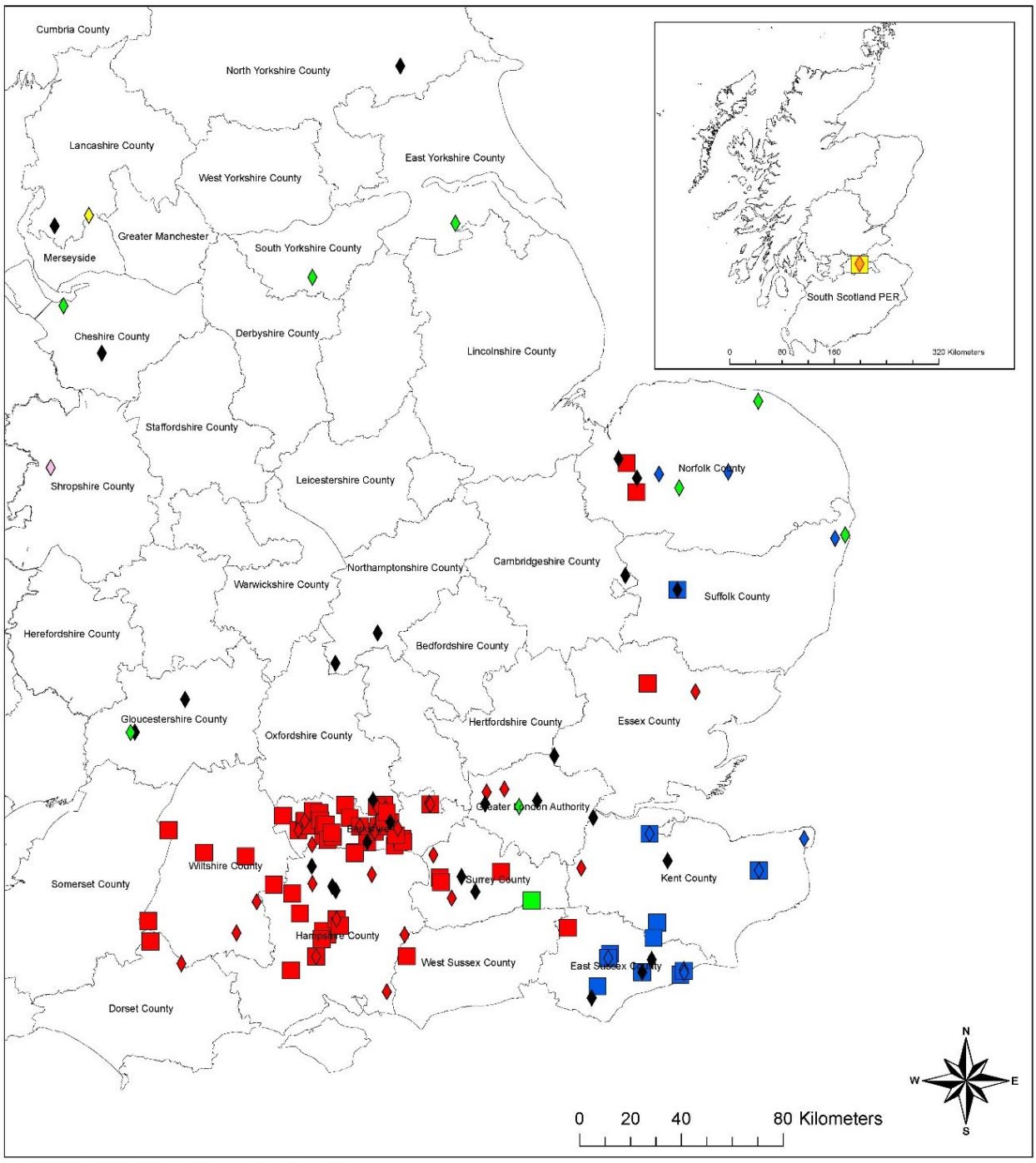
The VKORC1 mutation Y139F was found to have a widespread distribution across East Sussex and Kent, with other occurrences in Suffolk and Norfolk. The occurrence of this mutation in the homozygous state across much of its range in the south east clearly indicates considerable selection for Y139F resistance in these populations, and again, clearly indicates that homozygous resistant animals have had a selective advantage over heterozygous (and susceptible) animals when exposed to anticoagulants. Based on research primarily conducted across Europe, RRAG recommends that bromadiolone and difenacoum should not be used against ‘Y139F Rats’.

The VKORC1 mutation, Y139C was found to occur widely across the UK, but with only one occurrence in the homozygous state, at a Surrey location. In the heterozygous state it was found to occur in Gloucestershire, Cheshire, South Yorkshire, East Yorkshire, Norfolk, Suffolk and Greater London. With limited data available on VKORC1 mutations across much of the UK, it is not possible to draw conclusions on the likely impact of this mutation on rodenticide efficacy, but the low occurrence of homozygous resistant animals would suggest a much lower degree of selection when compared with the VKORC1 mutations L120Q and Y139F. Based on research conducted across Europe, RRAG recommends that bromadiolone and difenacoum should not be used against ‘Y139F Rats’.

If this situation persists, the intensive selection pressure towards the resistant genotype will result in the geographical spread of the mutation and a tendency towards the homozygous condition of the genotype coming to predominate in resistant infestations. Given the widespread current distribution of the mutation, it is possible that, in the foreseeable future, rats carrying Y139C may be present over the larger part of the UK.

The VKORC1 mutation, Y139S was found only to occur in Shropshire, within the extensive and well-known resistance focus on the Anglo-Welsh border. To date, this is the only location globally where the Y139S mutation has been located, although the current geographical extent of the focus is unknown. Currently RRAG recommends that any of the five second generation anticoagulants (including bromadiolone and difenacoum) can be used against ‘Y139S Rats’.

Figure 3. Available data on the geographical distribution of VKORC1 mutations in Norway rats across the UK.



3.4.6 Summary - recommended anticoagulant use against UK Norway rats

The very widespread occurrence of the VKORC1 mutations L120Q, Y139F and Y139C in Norway rats is most probably a reflection of restrictions on the permitted use of anticoagulants for rat control in the UK. Until recently, the use of the more effective anticoagulants (i.e. brodifacoum, difethialone and flocoumafen) was virtually precluded at all these foci, and as a result, the resisted active substances bromadiolone and difenacoum were predominantly used, and now appear to be largely ineffective against Norway rats that possess one of these three VKORC1 resistance mutations.

For these three VKORC1 mutations in Norway rats, it is the view of RRAG that continued use of bromadiolone and difenacoum will lead to the increasing spread of these severe forms of anticoagulant resistance, will not provide any satisfactory level of rat control and constitutes unnecessary risk to wildlife because of the large quantities of ineffective anticoagulants that are entering the environment (see Meyer, 2009). RRAG therefore recommends that bromadiolone and difenacoum should not be used against Norway rats that are shown to possess one of these three VKORC1 mutations.

3.5 Resistance surveys using DNA sequencing in other countries in the EU

Resistance surveys using DNA extraction and sequencing from Norway rat and house mouse tissue samples have been conducted in several countries of the European Union, including France, Germany, the Netherlands, Belgium and Hungary. A summary of these studies has been published by the RRAC (RRAC, 2015). In collaboration with their authors, including the University for Reading, RRAC has developed and interactive resistance management website (http://www.rrac.info/). This will allow those who experience difficulty in achieving acceptable levels of rodent control because of suspected resistance to interrogate the website to find how close to them the nearest known occurrence of anticoagulant resistance is (Endepols, 2017). The RRAC website will also provide information on the type of resistance present and will give resistance management advice. There is an additional facility inviting confirmatory testing by the submission of tissue samples for DNA sequencing at the University of Reading.

4. Resistance in House mice

4.1 Background

The house mouse (*Mus domesticus*) is known to possess a degree of natural tolerance to anticoagulant rodenticides (Buckle and Eason, 2015), and as a result, anticoagulants are generally less effective against house mice than they are against Norway rats. True resistance to anticoagulants, conferred by genetical mutation, has been known among house mice in the UK since the 1960s. Resistance is now so widespread it is often said anecdotally that it is harder to find fully susceptible house mice than resistant ones (RRAG, 2012).

The previous regulatory position adopted by HSE, in which there were no approvals for biocidal products containing first-generation anticoagulant active substances labelled for house mice in the UK, is no longer in force. No scientific information to explain this reversal of policy, involving several commercial products containing the first-generation anticoagulant coumatetralyl, is in the public domain.

The study of resistance to anticoagulants in the house mouse has long been a 'poor relation' in comparison to the quantity and quality of available information on anticoagulant resistance in Norway rats. Consequently, there are a number of important unanswered questions about resistance in UK house mice. In particular, no map of the distribution of anticoagulant resistance in house mice in the UK has ever been produced, due at least in part to its assumed widespread occurrence.

Recently, in Germany, a study of the distribution of resistance in house mice has been conducted using DNA sequencing for the detection of anticoagulant resistant mutations (Pelz *et al*., 2011). It revealed that resistant house mice are very widespread and frequent in Germany. More than 90% of the mice examined carried genetical resistance mutations and resistance was found at 29 of the 30 locations sampled. The two resistant house mouse strains that were found in the German study are also known to be present in the UK.

4.2 "Natural tolerance" and the early anticoagulants

The first anticoagulant extensively tested against house mice was warfarin. Groups of anticoagulant-naïve mice were offered in the laboratory 0.025% warfarin bait. It is apparent that complete mortality of house mice was not obtained unless the animals fed on warfarin bait, without choice, for very long periods (Rowe and Redfern, 1964). The data were used to calculate a series of values for the toxicity of warfarin expressed as lethal feeding periods (LFP). These are defined as a number of days of continuous, no-choice feeding required to kill a given percentage of the mice tested. For example the LFP_{50}, LFP_{90} and LFP_{99} are feeding periods required to achieve 50%, 90% and 99% mortality, and are analogous to the more well-known LD_{50}, LD_{90} and LD_{99} based on lethal doses. The analysis revealed that the LFP_{50} for 0.025% warfarin for house mice was 4.8 days and the LFP_{99} was 29.5 days. These results, in comparison with similar results obtained for Norway rats (*Rattus norvegicus*) whose LFP_{50} and LFP_{99} are 1.7 and 5.8 days respectively, showed that house mice possess a remarkable degree of tolerance to warfarin (Rowe and Redfern, 1965). This does not conform to the definition of resistance that is normally applied and is sometimes known as *natural tolerance*.

We also know that the feeding behaviour of house mice is such that they often do not feed consistently from any single food source and this characteristic would make it even less likely

that warfarin would be fully effective against house mice. Research on anticoagulants continued after the invention of warfarin, when other compounds, such as coumachlor, diphacinone, chlorophacinone and coumatetralyl came to the market. However, it is generally accepted that none of these perform significantly better than warfarin against house mice (RRAG, 2012). Therefore, the earlier regulatory policy not to permit the use of these active substances against house mice in the UK was apparently justified (see section 4.3 below; RRAG 2012).

4.3 Resistance to first-generation anticoagulants

In 1961, just ten years after the introduction of warfarin, reports were received of the failure of this compound to control mouse infestations from a number of widely separated locations in the UK. A resistance test was developed in which survival after 21 days of continuous feeding on 0.025% warfarin bait was considered to be indicative of resistance (EPPO, 1995). Using this test, the presence of warfarin resistance was confirmed in mouse infestations from many parts of the UK (RRAG, 2012). Tests of diphacinone and chlorophacinone against mice that had survived the 21-day warfarin resistance test showed that these compounds did not provide a solution to warfarin resistance in mice. Sometime later, a population of resistant house mice was discovered in Cambridge. These animals had a distinctive coat colour and it appears that the gene for this attribute was linked to that of resistance. A homozygous resistant laboratory strain of these 'Cambridge Cream' mice were developed and much subsequent assessment of the activity of anticoagulants against resistant house mice relied on tests on the progeny from this original breeding stock.

A report recently published by the European Commission (Berny *et al*., 2014) has concluded that the 'default' position should be not to use first-generation anticoagulants against house mice unless there is information available to the practitioner that anticoagulant resistance is absent at the site to be treated. To the knowledge of the authors of this report, such sites are not known to exist in the UK.

4.4 Resistance to second-generation anticoagulants

Difenacoum and bromadiolone were the first active substances to be tested against resistant house mice. Laboratory tests showed a useful level of activity of these compounds and both appeared to be substantially more effective than warfarin (Hadler *et al*., 1975: Redfern and Gill, 1980). Two days of no-choice feeding on 0.005% difenacoum resulted in 87% mortality and ten days of similar testing with bromadiolone gave 80% mortality.

Subsequently, a series of pen tests was carried out using families of warfarin-resistant house mice and field trials against natural infestations were also conducted (Rowe *et al*., 1981). A result observed in these trials was the frequent inability of difenacoum and bromadiolone to provide complete control, both in the case of resistant family groups in pen tests and of wild infestations in the field. Indeed, mice survived in five of the 12 field trials conducted. These survivors were removed to the laboratory and later offered either 0.005% bromadiolone or difenacoum for 21 days. Respectively 43% and 18% of the mice survived in these bromadiolone and difenacoum tests. These results appeared to show that some mice, substantially resistant to bromadiolone and difenacoum, were present in field infestations even before these two compounds came into widespread use. It is not clear whether this was just another manifestation of tolerance or whether resistance mutations were already present in some mouse populations. The tests also

showed that, for whatever reason, control was likely to be more problematic in the case of bromadiolone than difenacoum and this has subsequently proved to be the case.

Two more second-generation anticoagulants, brodifacoum and flocoumafen, were subsequently introduced and these were shown to be substantially more potent than bromadiolone and difenacoum against house mice (Rowe and Bradfield., 1976; Rowe *et al*., 1978; Rowe *et al*. 1985). In the laboratory, complete mortality of resistant house mice was achieved with both these compounds after one- and two-day periods of no-choice feeding. Six field trials with brodifacoum against wild house mouse infestations resulted in an average of 98.8% control and ten field trials with flocoumafen gave an average of 97.2% control. An advantage of these two compounds for resistant house mouse control is that only small quantities of bait are required to achieve a lethal dose, even for resistant mice, and this characteristic is important for house mice because of their sporadic feeding behaviour.

Rodenticide products containing a third potent second-generation anticoagulant active substance, difethialone, has also been introduced in the UK, and has been marketed as being effective against anticoagulant-resistant house mice (see RRAG, 2012).

Further research has been conducted at the University of Reading, using standardised blood clotting response test methodology (Prescott *et al*., 2007) to estimate Resistance Factors for male and female house mice that are homozygous for the VKORC1 mutation Y139C. The work was conducted in collaboration with the Rodenticide Resistance Action Committee (RRAC), who provided both technical support and funding. The results of this work are presented in Table 5.

Table 5. One of the most important polymorphisms of the VKOR proven to induce resistance to anticoagulants in the house mouse (*Mus musculus*), and resistance factors in male and female homozygous resistant mice. The data in this table are the result of work funded by RRAC and conducted by Dr C Prescott and Ms Mhairi Baxter (of the University of Reading, UK).

VKORC1 mutation	Active Substance				
	Bromadiolone	Difenacoum	Brodifacoum	Flocoumafen	Difethialone
Y139C	17/21	1.2/2.7	1.7/1.9	0.9/1.2	1.5/1.5

As resistance factors of less than five are unlikely to cause any significant discernible effect on the practical efficacy of an active substance against a resistant strain, the above results would suggest a high degree of resistance to bromadiolone with both male and female ‘Y139C Mice’. The results would further suggest that difenacoum, brodifacoum, flocoumafen and difethialone would be effective against this strain of mice.

4.5 Genetics of anticoagulant resistance in House mice

Resistance in house mice is now thought to be the same as that in Norway rats, with mutations of the VKORC1 gene complex governing anticoagulant resistance (Pelz and Prescott, 2015). Resistance mutations of the VKORC1 gene are known to occur at the same gene location in both species (Pelz *at al*., 2005); and homozygous resistant strains of both species are known to have a higher dietary requirements for vitamin K than susceptible animals (Pelz and Prescott, 2015).

Since the early genetical studies, a very limited amount of research work has been done on house mouse resistance in the UK. This early work was done on the so-called ‘Cambridge Cream’ resistance strain at the Central Science Laboratory (later the Food and Environment Research Agency and now the Animal and Plant Health Agency), and has been used in resistance research

in the UK since the 1980s. These animals are now known to carry the leucine128serine mutation, and are referred to as 'L128S Mice'.

In the 1980s, a population of resistant mice was discovered in the Reading area that had a high degree of resistance to bromadiolone, and studies were conducted on them which resulted in the development of a laboratory strain of homozygous resistant house mice (Prescott, 1996). The mutation for this strain was subsequently identified as tyrosine139cysteine (or Y139C). This'Y139C Mouse' strain is considered to be fully resistant to the first-generation anticoagulants and to the second-generation compound bromadiolone.

Thus, we can say with reasonable certainty that we have at least two different house mouse resistance mutations in the UK.

Preliminary studies are ongoing at the Vertebrate Pests Unit of the University of Reading, to identify and map the VKORC1 mutations in UK house mice. The main stumbling block for this survey is the supply of house mouse tissue samples. To date, 44 samples have been received and successfully genotyped, and their results are presented in Figure 4.

The tissue samples collected to date are primarily from the Greater London area. Both VKORC1 resistance mutations Y139C and L128S have been identified. Of the 44 animals samples:

- 5 samples were susceptible animals
- 19 samples had the VKORC1 mutation L128S
    - 15 samples were homozygous for L128S
    - 4 samples were heterozygous for L128S
- 17 samples had the VKORC1 mutation Y139C
    - 10 samples were homozygous for Y139C
    - 7 samples were heterozygous for Y139C
- 3 samples were heterozygous for both VKORC1 mutations L128S and Y139C

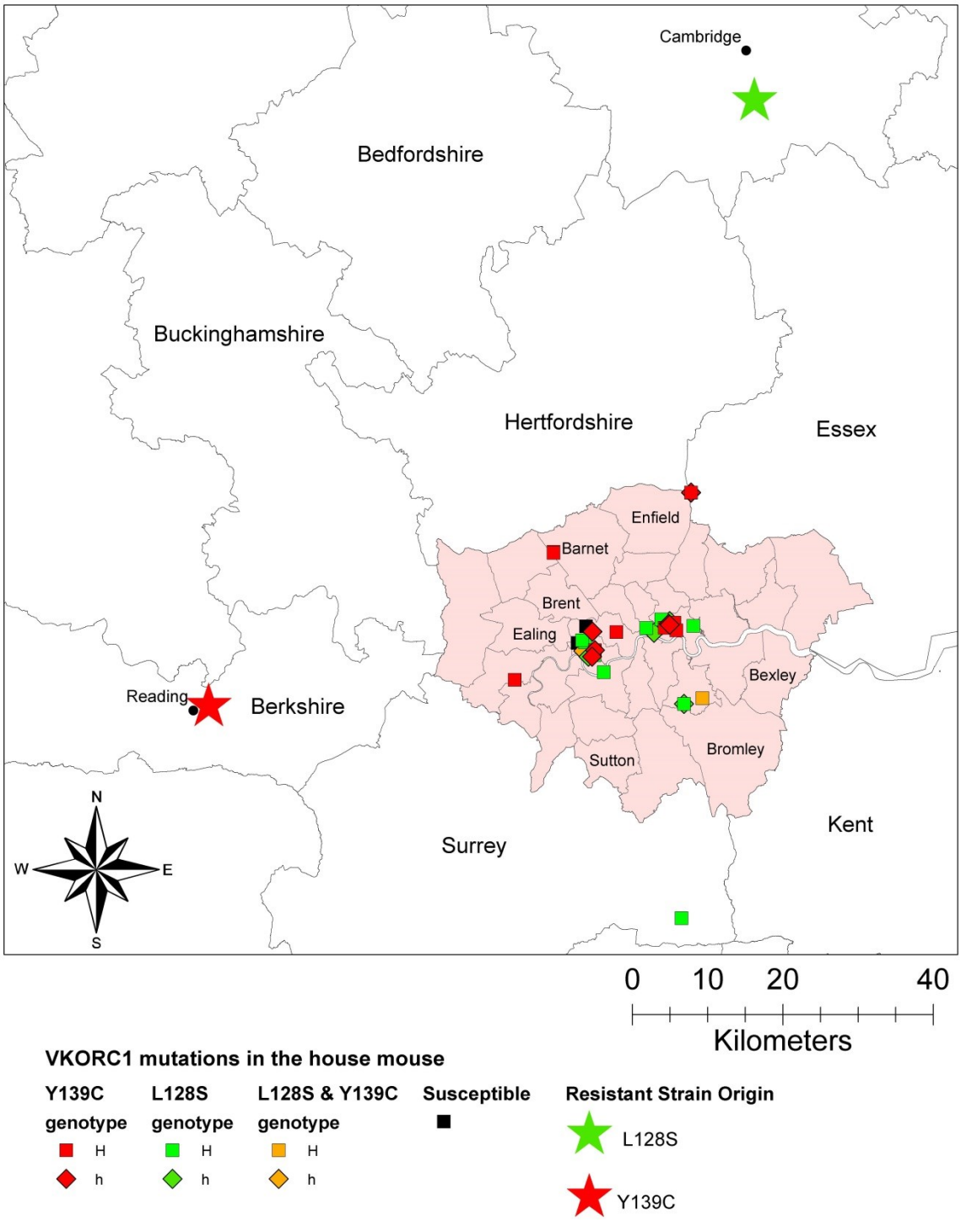
In total, over 88% of the animals sampled possessed a resistance gene, with 50% of animals possessing the VKORC1 mutation L128S and 45.5% possessing the VKORC1 mutation Y139C.

Of all animal sampled, 56.8% of animals were homozygous for a VKORC1 resistance mutation, and 11.3% were susceptible animals that did not possess a VKORC1 resistance mutation.

The above results indicate a very high degree of selection for anticoagulant resistance in the house mice that have been sampled, with a clear indication that homozygous resistance animals have a selective advantage over heterozygous resistant animals and susceptible animals. They also show marked similarities with those of (Pelz *et al*., 2011) for these two mutations among house mice in Germany.

The occurrence of resistant house mice that possess both VKORC1 resistance mutations is concerning, as it raises the possibility that the two mutations may have a cumulative effect of on the magnitude of the resistance; and with the future potential to develop resistance mice that are homozygous for both VKORC1 resistance mutations, if selection continues to occur.

Figure 4. Available data on the geographical distribution of VKORC1 mutations in house mice in the UK.



4.6 Summary - recommended anticoagulant use against UK House mice

It was a long-standing regulatory policy that first generation anticoagulants such as warfarin, chlorophacinone, diphacinone and coumatetralyl should not be used for the control of house mice in the UK. This was because the occurrence of resistance would be likely to render them widely ineffective and because the continued use of these substances is likely to increase the severity and spread of resistance among house mice (RRAG, 2012).

We know that one of the two strains of resistant mice present in the UK (Y139C) shows a significant degree of resistance to bromadiolone. There are also many anecdotal reports of the failure of bromadiolone to control house mice. While it is likely that some infestations may be controlled, at least in part, by applications of bromadiolone, the use of this active substance against house mice in UK is not recommended as it may not result in an inadequate level of control and will exacerbate future resistance problems (RRAG, 2012).

The situation for difenacoum is more equivocal. Mice carrying the Y139C mutation are known to possess a degree of resistance to difenacoum. However, this active substance is widely used in successful mouse control treatments, and the estimated Resistance Factor would suggest that it should be efficacious (see Table 5).

The situation with L128S is more uncertain. What is certain, however, is that 30 years ago some individuals within mouse infestations could not be controlled with difenacoum baits, and it is unlikely that this situation has improved in the intervening period. It would therefore be prudent, in areas where resistance in house mice is suspected, not to use products that contain difenacoum (RRAG, 2012).

Studies on the intrinsic activity of the second-generation anticoagulants demonstrate that brodifacoum and flocoumafen are the most potent active substances against susceptible house mice (Prescott *et al*, 2007). There is also good evidence from early field studies that brodifacoum and flocoumafen are effective against anticoagulant-resistant house mice.

Currently, there are no anecdotal reports of the failure of either of these compounds to control infestations of house mice in the UK. Therefore, products containing brodifacoum and flocoumafen should be the rodenticides of choice when carrying out control treatments against house mice in the UK (RRAG, 2012). This is because they offer the promise of the highest levels of control and are the least likely to result in further selection for anticoagulant-resistant mice.

Baits carrying the second-generation anticoagulant difethialone are now in the market in the UK. In studies conducted at the University of Reading on the potency of second-generation anticoagulants (including difethialone) against anticoagulant-susceptible house mice (Prescott *et al*. 2007) , the intrinsic activity of difethialone was greater than bromadiolone and similar to difenacoum against male mice, and was less than difenacoum and greater than bromadiolone against female mice. It should be held in mind, however, that difethialone baits contain 0.0025% of the active substance, while those carrying brodifacoum and flocoumafen can contain up to 0.005% of the active substance (RRAG, 2012).

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