CONFIDENTIAL

REPORT SERIES:
VPU/19/012

VERTEBRATE PESTS UNIT
UNIVERSITY OF READING

Anticoagulant Resistance in Rats and Mice in the UK – Summary Report with new data for 2019

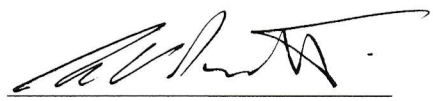
Report from the Campaign for Responsible Rodenticide Use (CRRU) UK for the Government Oversight Group

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:

Clare Jones, Montse Talavera, Alan Buckle and Colin Prescott

Dr C.V. Prescott  Date: 8th October 2019

Associate Professor and Director of the Vertebrate Pests Unit

DISTRIBUTION:

University of Reading

Professor Robert Jackson – Head of School

Contents

	page
Contents	2
Summary	3
1. Introduction	4
2. Materials and Methods	5
2.1 Origins of samples	5
2.2 Methods of DNA analysis	5
2.3 The Rodenticide Resistance Action Committee (RRAC) interactive global resistance map	6
3. Results	7
3.1 Norway rats	7
3.2 House mice	10
4. Discussion	15
5. References	17

SUMMARY

1. New resistance data are presented for tissue samples from Norway rats (*Rattus norvegicus*) and house mice (*Mus musculus*) collected in the period September 2018 to September 2019. Particular efforts were made to obtain samples in geographical areas in the UK from which none had been collected in the past.
2. A total of 140 Norway rat tissue samples were analysed, among which 55 were anticoagulant-susceptible and 85 carried one of five different resistance mutations (Y139S, Y139C, Y139F, L120Q, L128Q) in either the homozygous or heterozygous form. Therefore the prevalence of anticoagulant resistance in this Norway rat sample was 60.7%.
3. These new Norway rat resistance records extended the known area of the extensive L120Q resistance across the south of England, provided for the first time information about the prevalence of resistance in rats in Greater Manchester and identified a third new resistance mutation (Y139F) among rats in Greater London. The records also appear better to define the extent of a Y139C focus in the western counties along the entire course of the river Severn and the extent of a focus of the same mutation among the sub-counties of Yorkshire. Also, for the first time, we record the occurrence of the Y139S mutation from sites far removed from its origin on the Anglo-Welsh border.
4. A total of 35 house mouse tissue samples were collected, all showing one or other of the highly prevalent Y139C and L128S mutations. Although the total number of records for house mouse is small, these new data show the wide extent of house mouse resistance to anticoagulants across the UK and bring to 93.2% the prevalence of resistance in that species.
5. Attention is drawn to the situation in which permanent anticoagulant baiting is the predominant method for the control of the house mouse among professional pest control practitioners, house mice are widely resistant to difenacoum and bromadiolone, these two active substances are not recommended for use against house mice but are the only ones permitted for use in permanent baiting.

1. Introduction

Previous reports produced for the Campaign for Responsible Rodenticide Use (CRRU) (UK) on the status of anticoagulant resistance among Norway rats (*Rattus norvegicus*) and house mice (*Mus musculus*) in the UK have presented background information on resistance mutations, explained resistance testing methodologies and provided information on the occurrence and geographical distribution of resistance (see Prescott *et al*., 2017 and 2018). This previously-presented information will not be reproduced in this report; rather a summary is provided of new information that has been obtained since the last report was prepared as the result of genomic resistance testing conducted at the University of Reading and funded by the Rodenticide Resistance Action Committee of CropLife International (http://www.rrac.info/).

This report has been prepared for CRRU in response to a requirement of the Health and Safety Executive (HSE) and the Government Oversight Group (GOG) to provide resistance monitoring information on an annual basis to support their evaluation of the progress of the UK Rodenticide Stewardship Regime (HSE, 2019) under the heading "Competent Workforce".

2. Materials and Methods

2.1 Origins of samples

The tissue samples analysed for genetical mutations were either submitted by pest control technicians or were collected after trapping by staff of the Vertebrate Pests Unit (VPU) at the University of Reading. Thus, samples were generally received from areas in which technicians had experienced difficulties in obtaining effective control with anticoagulants, possibly because of resistance or, in the case of VPU sampling, were taken from the borders of known resistance areas in an attempt to identify their boundaries.

During 2019 additional effort was expended in obtaining samples from areas of the UK from which samples had not previously been received. The maps presented in previous reports had shown that samples have not been obtained, for example, from a large area in the centre of the country, including many counties of the Midlands. This area is of particular interest because, from the very few samples that have been received, there appears to be a low incidence of anticoagulant resistance among Norway rats. Consequently, calls were put out in the magazines serving the UK professional pest control community asking for samples from these areas (see for example Jones and Talavera, 2019; https://www.thinkwildlife.org/free-tests-and-new-guide-tackle-spread-of-resistant-rats/).

2.2 Methods of DNA analysis

As in the previous studies described above, 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.

A list of the VKORC1 mutations found in Norway rats and house mice in the UK is shown in Table 1.

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, Essex, Norfolk and elsewhere**
NR	**Tyrosine139Cysteine**	**Y139C**†	**Gloucestershire, Norfolk, Lincolnshire, Yorkshire, SW Scotland and elsewhere**
NR	**Tyrosine139Phenylalanine**	**Y139F**†	**Kent, Sussex, Norfolk, Suffolk**
NR	Argenine33Proline	R33P‡	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

2.3 The Rodenticide Resistance Action Committee (RRAC) interactive global resistance map

The results from this study were provided to the funding body, the Brussels-based RRAC of CropLife International (http://www.rrac.info/). The results are collated with those obtained from other global studies and presented in an interactive form on the RRAC web-site. The maps available (see example for the UK at: http://guide.rrac.info/resistance-maps/united-kingdom/) use Google 'heatmap' technology to ascribe different weightings to records depending on the numbers of positive samples and the frequencies of their closest neighbours. Users of the maps are able to scroll in to find their own location, that of the nearest confirmed incidence of anticoagulant resistance, the mutation of that record and to obtain advice about the correct use of anticoagulants in the area. It is anticipated that this scheme will help pest control practitioners to make informed choices about which anticoagulant active substance to use and will support a 'competent workforce'.

3. Results

3.1 Norway rats

During the period September 2018 and September 2019 a total of 140 Norway rat tissue samples was received that were capable of analysis using the gene sequencing technique. Among these, 85 were found to possess one of the five known Norway rat resistance mutations and 55 were found to be susceptible animals (Table 2). Hence, 60.7% of the samples received possessed one of the resistance mutations, in either their homozygous or heterozygous form.

Table 2. The numbers of Norway rats tissue samples received and analysed and their status of resistance or susceptibility. (See Table 1 for further explanations of the different resistance mutations.)

Resistance Mutation	Homozygous	Heterozygous	Total
L120Q	16	9	25
L128Q	12	15	27
Y139S	3	8	11
Y139F	3	5	8
Y139C	5	9	14
Susceptible	55	0	55
Total	**94**	**46**	**140**

The geographical origins of these new samples are shown in Figure 1. The discovery of several new resistance foci are revealed when a comparison is made of these finding and those published in the previous report (Prescott *et al*., 2018). Of course, it is impossible to determine whether these are newly-developed resistance foci or have been present for some time but had long been undetected.

One of the most interesting findings was the discovery of the Y139F mutation in rats taken in Central London. This mutation had previously been found only in Kent, East Sussex and East Anglia, with an outlier in north-west Shropshire. Rats from Greater London were previously found to be susceptible or to possess the L120Q or Y139C mutations.

In the north of England, rats possessing the L128Q mutation were found for the first time on the north-east coast in the counties of Durham, Northumberland and Tyneside. The first survey of rats from Greater Manchester revealed a complicated resistance picture. The dominant mutation was once again, the L128Q genotype. This was found mostly in its homozygous form, which suggests that the focus had been established for some time. However, some rats from the locality carried the L120Q and Y139C mutations, both in their heterozygous form. No susceptible rats were found in the samples received from Greater Manchester, which totalled 20 rat tissue samples. This presents a complex picture of Norway rat resistance in this very large conurbation but one in which anticoagulant resistance must be considered with more attention.

Elsewhere, the Y139S mutation that previously had been found almost exclusively on the Anglo-Welsh border was found in three new locations. A rat from North Yorkshire was found to be homozygous resistant for Y139S, and heterozygous Y139S rats were also found in Merseyside and in Essex.

Figure 1. Map showing the geographical locations of Norway rat tissue samples submitted to the Vertebrate Pests Unit in the period September 2018 to September 2019 and their resistance status.

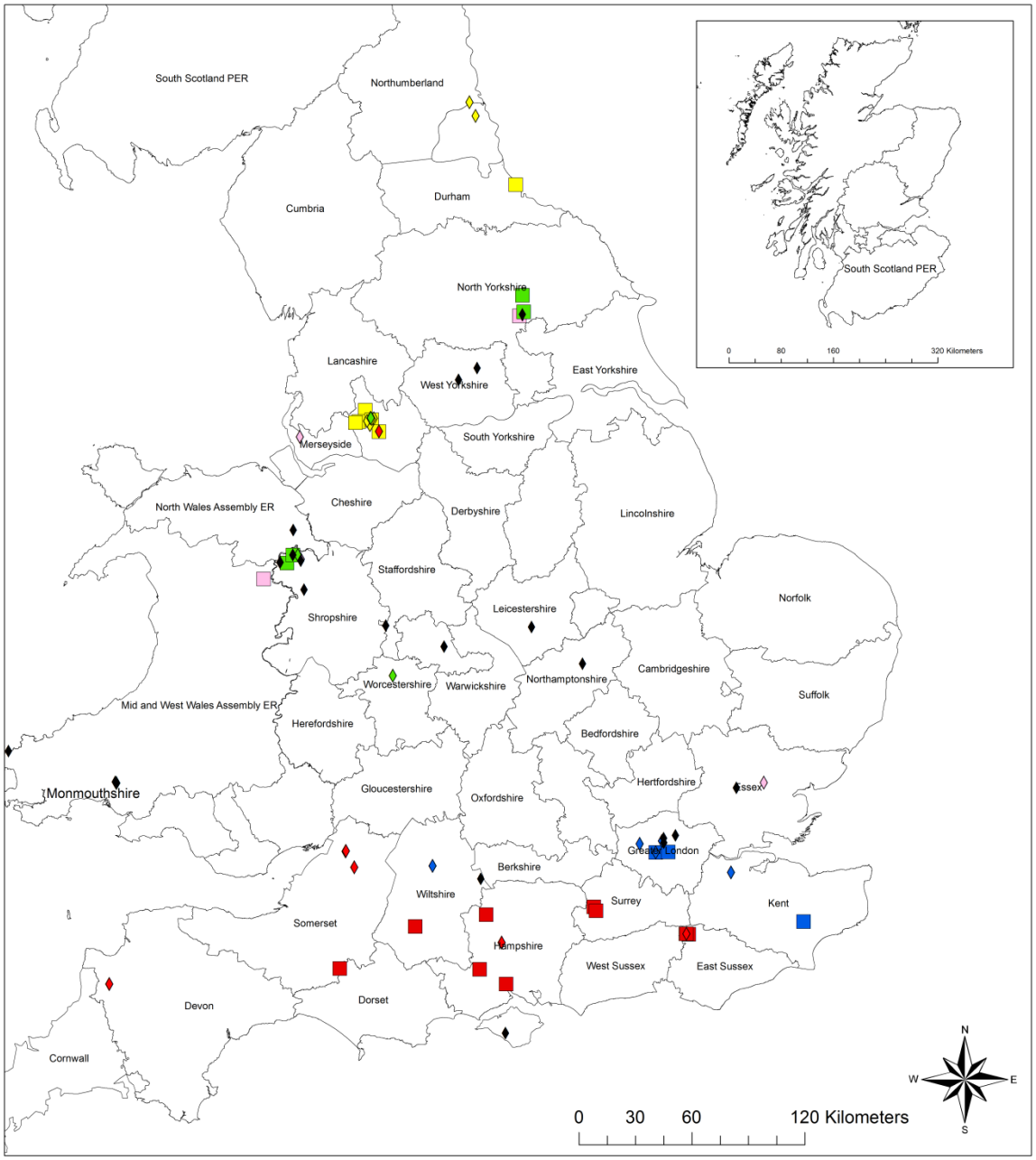
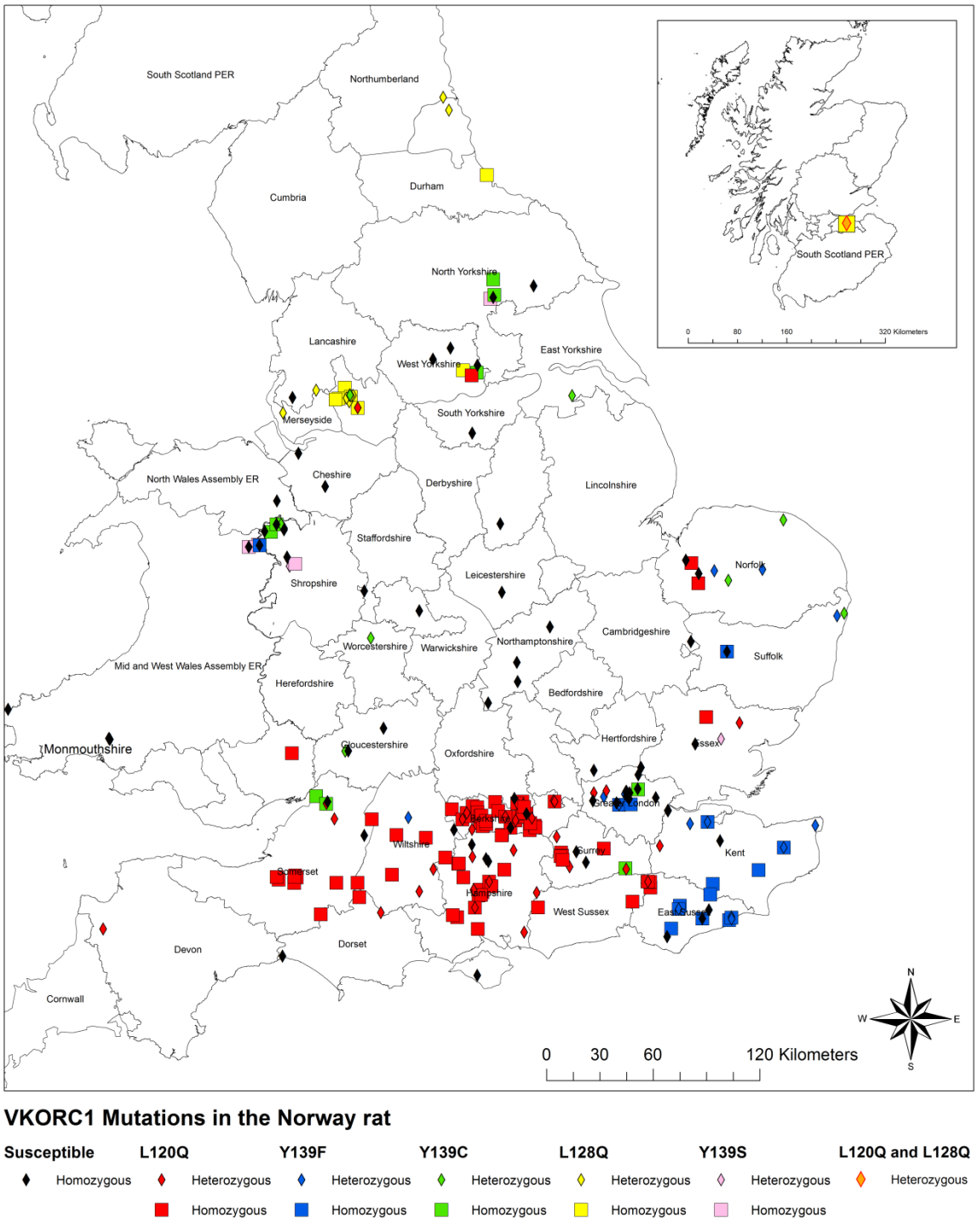


Figure 2. Map showing all available data on the occurrence of resistance mutations among Norway rats in the UK.



A new focus of Y139C resistance was demonstrated in North Yorkshire, within the same sampling area that contained the homozygous Y139S rat that was mentioned previously. Although this is the first record of Y139C for this county, it had previously been recorded in the neighbouring counties of West, South and East Yorkshire and probably confirms a large contiguous focus. The Y139C mutation was also found for the first time in Worcestershire and this suggests a possible large focus associated with the valley of the River Severn, with Y139C rats having been found previously in north Somerset, Gloucestershire and Shropshire. Y139C is an advanced form of resistance and one against which the UK Rodenticide Resistance Action Group (RRAG) recommends that only brodifacoum, difethialone or flocoumafen should be used (RRAG, 2018).

Finally, the dimensions of the very large focus of the severe L120Q resistance, which covers much of the south of England, was extended into a new county, namely Devon. A heterozygous resistant rat carrying this resistance gene was found in the extreme east of the county near the town of Holsworthy. Previously, the furthest west this mutation had been found was in central Somerset near Taunton.

A special effort was made this year to obtain samples from some of the Midland counties that had previously been unrecorded. This was not especially successful because only four samples were received from those locations but these efforts will be continued. However, those limited rat tissue samples received from south-west Staffordshire, the West Midlands, Leicestershire and Northamptonshire were all found to have come from susceptible Norway rats.

The map shown in Figure 2 gives all accumulated data on the distribution of anticoagulant resistance for Norway rats in the UK and includes the 2019 data.

3.2 House mice

The results from the analysis of a total of 35 house mouse tissue samples submitted in the period September 2018 to September 2019 are shown in Table 3. Among 35 samples examined, none carried the fully susceptible genotype. Table 1 shows that one or other of the two resistance mutations commonly found among house mice in the UK were present in all animals. Either Y139C or L128S was found in homozygous form in 21 animals and in heterozygous form in a further 11 animals, while three individuals carried both mutations each heterozygous.

Table 3. The numbers of house mouse tissue samples received and analysed and their status of resistance or susceptibility. (See Table 1 for further explanations of the different resistance mutations.)

Mutation	**Homozygous**	**Heterozygous**	**Total**
L128S	14	6	20
Y138C	7	5	12
L128S and Y139C	0	3*	3
Susceptible	0	0	0
Total samples	**21**	**14**	**35**

*These three animals were heterozygous for each of two the resistance mutations.

The geographical distribution of the 35 samples analysed during September 2018 to September 2019 and reported here is shown in Figure 3. The combined data for all years is shown in Figure 4. Resistance distribution data for house mice recorded in the previous reports (Prescott *et al*., 2017 and 2018) were mainly from Greater London and the south-east of England. The samples now reported were, for the first time, much more widely dispersed and demonstrate conclusively for the first time the extent of anticoagulant resistance in UK house mice.

Figure 3. Map showing the geographical locations of house mouse tissue samples submitted to the Vertebrate Pests Unit in the period September 2018 to September 2019 and their resistance status.

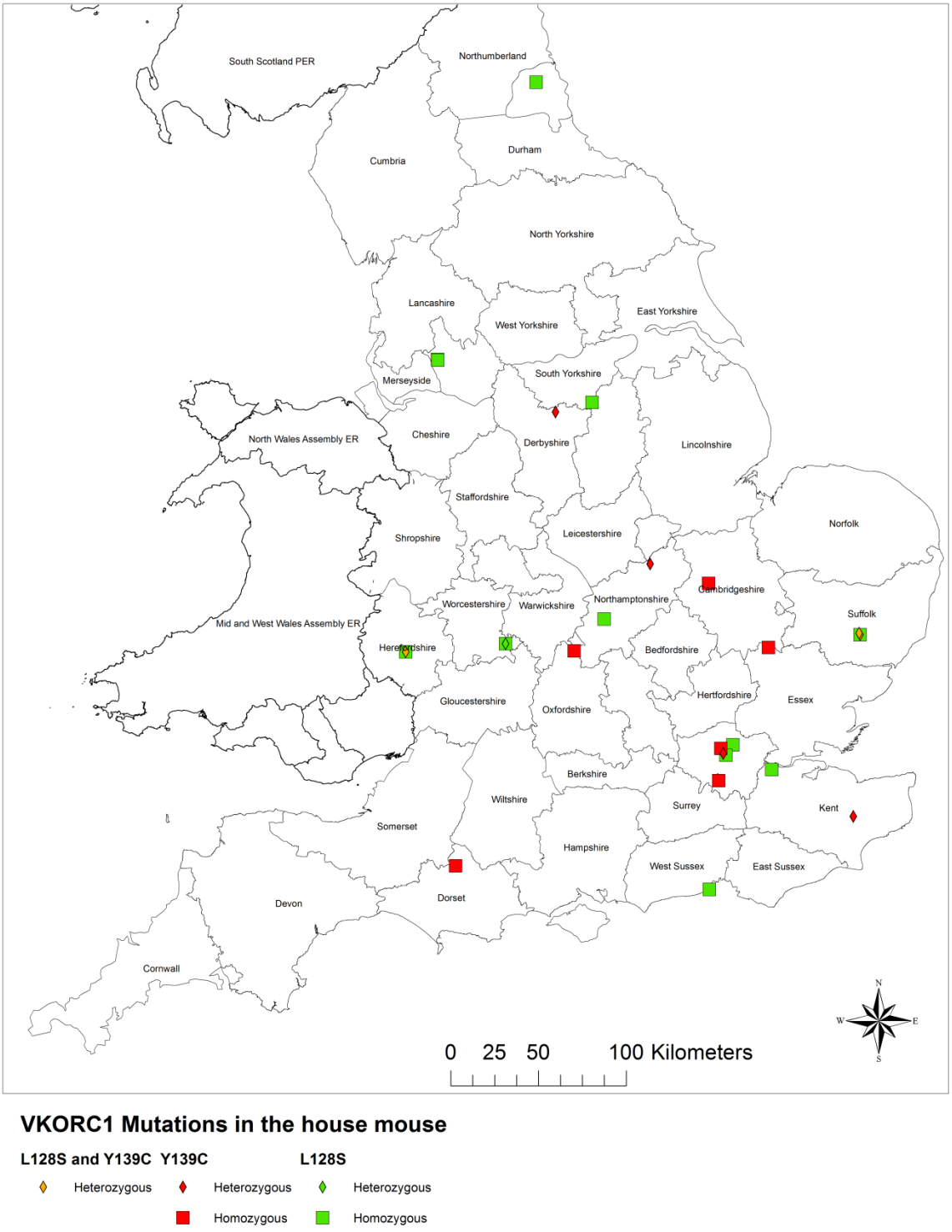
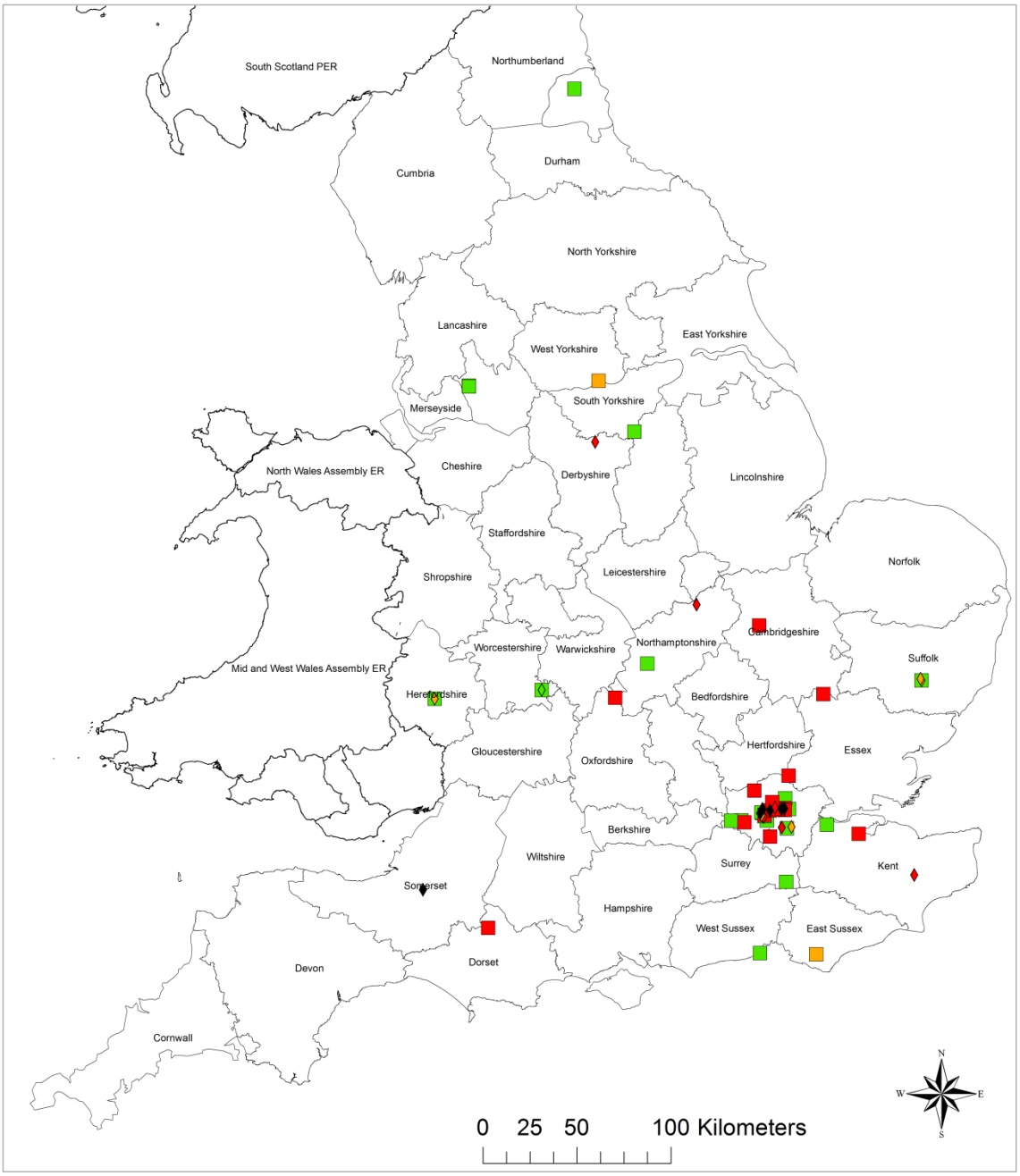


Figure 4. Map showing all available data on the occurrence of resistance mutations among house mice in the UK.



The L128S mutation appears to be very widely distributed across much of England, from Tyneside in the north-east to the Channel coast of East and West Sussex, whereas the Y139C mutation seems to be somewhat more restricted in distribution to the southern and eastern counties. However, we still lack data for the house mouse and many of the records are for either single animals or very small samples.

Earlier reports provided information on a total of 53 house mouse samples and these are now augmented by a further 35 samples. Among the previous 53 a total of 47 (88.7%) carried one or more resistance mutations. With the addition of the 35 samples reported here, all resistant, the prevalence of resistance in UK house mice is now 82 resistant samples out of a total of 88, or 93.2%.

4. Discussion

This report is the third in a series compiled for CRRU UK by the University of Reading to document the distribution of resistance to anticoagulants among Norway rats (*Rattus norvegicus*) and house mice (*Mus musculus*) in the UK. When new resistance foci are discovered it is impossible to know if these have only just developed or if they have in fact existed in the recorded localities for some time. This is because no consistent and wide-scale inventory of resistance foci has ever been attempted using the modern DNA sequencing technique (se Pelz and Prescott, 2015). Instead, samples are received on an *ad hoc* basis, mainly from technicians in the professional pest control industry who have experienced difficulty in achieving control using their customary methods and products. This scheme for the acquisition of samples, of course, causes bias because, if the cause of the difficulty is indeed resistance, it increases the likelihood that resistance mutations will be found.

With this important proviso, it is possible to draw some conclusions with a fair degree of certainty from the data that has been collected over the past three years and presented here. Firstly, anticoagulant resistance in Norway rats is predominant and widespread across the whole of southern England, either in the form of the highly resistant L120Q genotype or, further to the east, as the only slightly less resistant Y139F genotype. With these resistances dominant over such a large area, and an area in which so much of the country's commercial and agricultural activity occurs, it is hardly surprising that these resistance mutations are spreading. This appears to be happening, firstly, on the boundaries of the main focus, hence the finding of L120Q in the far west in Devon and, in the far east, on the borders of Surrey and West Sussex with Kent and East Sussex. A similar 'spread' phenomenon may be found in the discovery of rats carrying the Y139F mutation in central London, which is on the western boundary of the known focus of that mutation.

However, new L120Q Norway rat resistance foci now appear in places often far removed from the original heartland' of Hampshire and Berkshire, such as in East Anglia and West Yorkshire and it is hard to conceive that the true extent of the focus is actually contiguous with these outliers. Rather it seems likely that new foci have either developed *de novo* or resistant rats have been transported from the main focus and have flourished in new localities. Rats carrying Y139F are also found far from the original Kent focus in the north of East Anglia and the far west of Shropshire.

Elsewhere, the data reported here support the likely existence of other large, but previously unknown, resistance foci. For example, we now have Y139C resistant rat samples from all of the counties along the Severn Valley, from Somerset in the south to the north western-most edge of Shropshire and this probably indicates a single focus, rather than several small foci. Similarly, there appears to be a substantial Yorkshire Y139C focus, since this resistance has been found in all the Yorkshire sub-counties. Figure 2 appears to show an association between the rivers Severn, Thames, Humber, Mersey and Dee with this resistance mutation, which is the only one to be found in both Germany and Denmark (Pelz and Prescott, 2015). But the observations might as easily be explained by the courses of the motorways M2, M4, M5 and M62.

There remains a central core of the country, including most of the Midland counties, from which we have very few Norway rat samples but in which we have only detected susceptible animals. Why the centre of the country should retain anticoagulant susceptibility, while almost completely surrounded by resistance foci, is a question that may only be answered by more detailed genetical studies.

The purpose of these resistance studies is to provide information that will permit professional pest control technicians to make informed decisions about their choice of rodenticide active substances. However its effect in documenting the increasingly extensive occurrence in the UK of highly anticoagulant-resistant Norway rats is likely to be that there is a shift towards the use of the three most potent 'resistance-breaking' compounds, brodifacoum, difethialone and flocoumafen. The fact that the latter two active substances remain proprietary to single commercial entities, while brodifacoum is much more widely available, makes it likely that this latter active substance that will be the one in predominant use in the UK's growing Norway rat resistance foci.

So far this discussion has been restricted to the situation regarding Norway rats. The extent of resistance in house mouse seems to be even more pervasive. None of the 35 samples collected during the period of the current study (September 2018 to September 2019) carried any susceptible genetic material. If these are added to the samples previously reported (Prescott *et al*., 2017 and 2018) we arrive at a prevalence of resistance among UK house mice of 93.2%.

This observation draws attention to a regulatory anomaly. The UK Rodenticide Resistance Action Group published guidance on the use of anticoagulant rodenticides to permit effective rodent pest management and the prevention of the spread of resistance (RRAG, 2012 and 2018). In its guidance on the control of house mice with anticoagulants (RRAG, 2012), RRAG recommends that bromadiolone and difenacoum should not be used against house mice because of the occurrence of resistance to them. The predominant method for the management of house mice in all commercial and (especially) in food storage/preparation/sale premises is the deployment of permanent tamper-resistant mouse bait boxes containing anticoagulant baits. This use is fundamental to the protection of human health and hygiene. However, we draw attention to the new rules on permanent baiting, embodied in current product labels, which only permit the resisted bromadiolone and difenacoum to be used in permanent baiting programmes (CRRU, 2019). This situation requires immediate attention. It seems particularly unfortunate that we have just emerged from the virtual prolonged ban on the use of effective resistance-breaking anticoagulants against Norway rats, which has undoubtedly contributed to the massive spread of resistant Norway rats in the UK, and we now find ourselves in a similarly contrary regulatory position with House mice.

5. References

Clarke, D. and C. Prescott. (2015). *Investigation of the current status of anticoagulant resistance in UK Norway rats by VKORC1 genotyping. Summary of results – February 2015*. University of Huddersfield, University of Reading. Confidential report. 22 pp.

CRRU UK. (2019). CRRU UK Guidance Permanent Baiting. Revised August 2019. The Campaign for Responsible Rodenticide Use (CRRU) UK. 11 pp.

HSE (2019). Report on the Rodenticides Stewardship Regime - Assessment of Implementation. Rodenticides Stewardship Government Oversight Group. Health and Safety Directorate, Redgrave Court, Merton Road, Bootle, Merseyside. January 2019. 9 pp. Available from: http://www.hse.gov.uk/biocides/eu-bpr/rodenticides.htm. Date accessed: 30.09.19.

Jones, C. and Talavera, M. (2019). Rodenticide resistance: Will you help find the gaps? *PEST Magazine*, Issue August/September 2019, pp 10-11, Available at: http://www.pestmagazine.co.uk/. Date accessed: 01.10.19.

Pelz, H.-J., S. Rost, M. Hűnerburg, A. Fregin, A-C. Heiberg, K. Baert, A. MacNicoll, C. Prescott, A-S. Walker, J. Oldenberg and C. Muller. (2005). The genetic basis of resistance to anticoagulants in rodents. *Genetics* 170 (4): 1839-1847.

Pelz, H.-J., and C. V. Prescott. (2015). Chapter 9. Resistance to Anticoagulants. Pp 187-208. In: *Rodent Pests and their Control*. A. P. Buckle and R. H. Smith (eds.). CAB International, Wallingford, Oxon, UK. 422 pp.

Prescott, C. V., A. P. Buckle, J. G. Gibbings, E. N. W. Allan and A. M. Stewart. (2010). Anticoagulant resistance in Norway rats (*Rattus norvegicus* Berk.) in Kent – a VKORC1 single nucleotide polymorphism, tyrosine139phenylalanine, new to the UK. *International Journal of Pest Management* 57(1): 61-65.

Prescott, C., Baxter, M., Coan, E., Jones, C., Rymer, D. and Buckle, A. (2017). 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. Vertebrate Pests Unit, University of Reading, UK. 30 pp. Available at: http://www.thinkwildlife.org/downloads_resources/. Date accessed: 30.09.19.

Prescott, C., Coan, E., Jones, C., Rymer, D. and Buckle, A. (2018). Anticoagulant Resistance in Rats and Mice in the UK – Current Status in 2018. Report from the Campaign for Responsible Rodenticide Use (CRRU) UK for the Government Oversight Group. Vertebrate Pests Unit, University of Reading, UK. 35 pp. Available at: http://www.thinkwildlife.org/downloads_resources/. Date accessed: 30.09.19.

RRAG. (2012). *RRAG House Mouse Resistance Guideline*. Rodenticide Resistance Action Group of the UK. 15 August 2012. 15 pp.

RRAG. (2018). *Anticoagulant resistance in the Norway rat and Guidelines for the management of resistant rat infestations in the UK*. Rodenticide Resistance Action Group of the UK. Revised September 2018. 8 pp.

Rost, S., H.-J. Pelz, S. Menzel, A. MacNicoll, V. León, K-J. Song. T. Jäkel, J. Oldenburg and C. Műller. (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): 9.

RRAG (2012). *RRAG House Mouse Resistance Guideline*. Rodenticide Resistance Action Group, UK. Derby. 11pp.

RRAG (2018). Anticoagulant resistance in the Norway rat and Guidelines for the management of resistant rat infestations in the UK. Rodenticide Resistance Action Group, UK. Derby. 9pp.