Institution: University of Reading

Unit of Assessment: 5 Biological Sciences

Title of case study: Identification and quantification of anticoagulant resistance in Norway rats and house mice: informing guidance and risk mitigation strategies.

1. Summary of the impact

Local authorities, the UK government and the European Commission have benefitted from the widespread application of new molecular methodologies, developed in 2005 and applied by the University of Reading's Vertebrate Pests Unit (VPU) to identify and quantify anticoagulant rodenticide resistance in rodent populations. Rodents are a major global pest that consumes our food, causes contamination with urine and faeces, damages structures through gnawing, transmits diseases, and impacts on species of conservation concern. Due to historical success and recent regulatory restrictions, anticoagulant rodenticides are the most common control method for these pests. However, physiological resistance to anticoagulants is now widespread and the VPU has been involved in mapping this resistance and identifying the genetic basis for the resistance. Their research has led to new methodologies to identify anticoagulant resistance that have been adopted by the global plant science industry and to new guidance in treating resistant populations that has been adopted by the European biocides industry.

2. Underpinning research

The Vertebrate Pests Unit (VPU) at the University of Reading provides expertise in all aspects of vertebrate pest management and control and is an accredited facility for research and development. The Unit is also a well renowned service provider for screening services and policy appraisal. Dr Prescott, a small mammal expert, joined the University of Reading in 1989 as Research Manager of the VPU and is currently a Principal Research Fellow and Director of the VPU. Dr Buckle has been a Visiting Research Fellow at the University since 2004. Together, their research has focused on the ecology and control of rodents. In particular, Prescott and Buckle have worked on anticoagulant rodenticides that act by blocking the enzymatic reactions required to activate vitamin K, which is necessary in blood coagulation. They have looked at how these rodenticides act within the body, how they are eliminated and distributed within the tissues, how animals develop a physiological resistance to these chemicals and their impact on primary and secondary non-target species.

First generation anticoagulant rodenticides (FGAR) were introduced in the 1950s and 1960s and revolutionised rodent control, achieving 100% control. However, the development of physiological resistance in Brown rats and house mice in the early 1960's rendered the anticoagulants ineffective across extensive areas of Europe and elsewhere. In the 1970s, second generation anticoagulant rodenticides (SGAR) were developed specifically to control resistant populations of rodents.

Proving resistance to second generation anticoagulant rodenticides in UK rats:

In the early 1990's, Prescott identified a population of Norway rats that possessed a level of physiological resistance to the SGAR, bromadiolone, which had never been observed before. After several unsuccessful treatment regimes, Prescott approached the Central Science Laboratory (an Agency of the then Ministry of Agriculture, Forestry and Fisheries) and the agrochemical company Zeneca to collaborate on extensive field study experiments on the unusual population. Prescott and his colleagues unequivocally established for the first time that an unusually high prevalence and degree of resistance to bromadiolone had resulted in the treatment failure [1]. This resistance has now been shown to be widespread across southern England, and extensive studies are on-going to establish the geographical extent of this and other types of resistance [2, 3].

Using new molecular methodologies to identify genetic basis for resistance:

Though it had already been established that resistance was a dominant trait that could be inherited from one parent, the specific genetic mutations remained unknown. Prescott sent tissue samples of his rodenticide resistant strains of rats to Hans-Joachim Pelz, from Germany's Federal Biological Research Center for Agriculture and Forestry, who had experience with mutations in the VKORC1 gene - the gene responsible for encoding the enzyme that reduces vitamin K into its active form. In

2005, Pelz, Prescott and others reported eight different mutations in the VKORC1 gene in both Prescott's anticoagulant resistant strains as well as other laboaratory resistant strains and wild-caught rats [4]. This work not only identified the genetic mutations responsible for anticoagulant resistance in wild populations of Norway rats and house mice, it also described a new molecular methodology for identifying anticoagulant resistance.

Prescott applied this new molecular methodology in Reading with Buckle [4] to study a population of Norway rats from Kent where the application of bromadiolone had been unsuccessful. In 2011, they reported that this resistance was the result of a common resistance mutation – Y139F – that had been found in rat populations in France and Belgium, but had not been previously reported in the UK [3]. The VPU is currently using this molecular methodology to monitor physiological resistance in Norway rats in the UK [2], Europe, the US and elsewhere.

Development of new standardised methodologies:
The drawback of the molecular methodology is that it gives no indication of the likely impact of a particular VKORC1 mutation on treatment outcome. For this, other methodologies that help establish the likely outcome of resistance in the field, known as resistance assessment methodologies, are necessary. In 2001, Prescott conducted a comprehensive assessment of existing resistance assessment methodologies, which were based on blood clotting response (BCR), and concluded that they were invalid for both practical and statistical reasons. He subsequently developed a new standardized methodology that could be used to both identify and quantify physiological resistance in Norway rats and house mice [5].

Establishing susceptibility baselines:
Prescott carried out a series of studies to establish susceptibility baselines for Norway rats and house mice, which could then be used to identify and quantify physiological resistance in wild populations of these two species globally. Prescott established susceptibility baselines for nine rodenticides used against Norway rats and for five rodenticides used against house mice [5]. Previous to this, there were baselines for only five rodenticides for rats, which had been established using flawed methods, and there were no baselines for mice whatsoever. Prescott has used these susceptibility baselines to validate the new Reading methodologies in the field [e.g. 6].

3. References to the research

[1] Quy, R.J., Cowan, D.P., Prescott, C.V., Gill, J.E., Kerins, G.M., Dunsfold, G., Jones, A., MacNicoll, A.D. (1995) Control of a population of Norway Rats resistant to anticoagulant rodenticides. Pest Sci, 45: 247-256. DOI: 10.1002/ps.2780450308 (WoS Citations=13)

[2] Buckle, A., Prescott, C.V. (2012) The Current Status of Anticoagulant Resistance in Rats and Mice in the UK, Report from the Rodenticide Resistance Action Group of the United Kingdom to the Health and Safety Executive. <http://www.bpca.org.uk/assets/RRAG-ReportAnticoagulantResistanceintheUK.pdf>

[3] Prescott, C. V., Buckle, A.P., Gibbings, J. G., Allan, N.W., Stuart, A.M. (2011) Anticoagulant resistance in Norway rats (*Rattus norvegicus* Berk.) in Kent - a VKORC1 single nucleotide polymorphism, tyrosine139phenylalanine, new to the UK. *Int J Pest Mang*, 57: 61-65. DOI: 10.1080/09670874.2010.523124

[4] Pelz, H-J, Rost, S., Hünerberg, M., Fregin, A., Heiberg, A-C, Baert, K., MacNicoll, A., Prescott, C.V., Walker, A-S, Oldenburg, J., Müller, C.R. (2005) The genetic basis of resistance to anticoagulant rodenticides. Genetics 170: 1839-1847. DOI: 10.1534/genetics.104.040360 (WoS Citations=93)

[5] Prescott, C.V., Buckle, A.P., Endepols, S., Hussain, I. (2007) A standardised BCR-resistance test for all anticoagulant rodenticides. International Journal of Pest Management, 53 (4): 265-272. DOI: 10.1080/09670870701245249 (WoS Citations=14)

[6] Buckle, A.P., Endepols, S., Prescott, C.V. (2007) Relationship between resistance factors and treatment efficacy when bromadiolone was used against anticoagulant-resistant Norway rats (*Rattus norvegicus* Berk.) in Wales. International Journal of Pest Management, 53: 291-297. DOI: 10.1080/09670870701469872

The research has featured in the respected journals above, and has been peer reviewed as being of at least 2* quality.

4. Details of the impact

Resistance mapping informs local authority pest management strategies

Resistance survey data generated by the VPU using the new molecular methodology was commissioned by West Berkshire Council and used in their application to the Health and Safety Executive (the Competent Authority in the UK responsible for the registration of rodenticides) for the limited emergency use of anticoagulants containing either brodifacoum, flocoumafen or difethialone, in areas where there was resistance. The key contact was Sue Gore, Team Leader and Principal Environmental Health Officer, who has provided the following statement: "Verification of resistance in the West Berkshire area has enabled the Council to advise it's residents more accurately on the best methods to try and reduce rat populations".

As the new molecular methodology for identifying anticoagulant resistance does not require testing on live animals, it is extremely useful as a tool for mapping the distribution of different mutations across the globe. In the UK, the VPU is using the molecular methodology [4] to map the distribution of resistance in Norway rats and is providing this information to the Industry and local authorities. VPU is working with the University of Huddersfield on this project, with the VPU focusing on central southern England and Huddersfield focusing on areas further north. The local authorities benefit from this information as it helps them to manage their rodenticide usage and tailor their pest management strategies accordingly.

The VPU, with input from Dr Buckle, is involved in Norway rat surveys in the UK, Libya, US, Russia, Mexico and Brazil, and in house mouse surveys in the UK, the US and the Azores.

The School currently has a long-standing agreement with a well-recognized international agro-chemical company which stems from earlier contracts (beginning in 1989) with predecessors of that Company. In addition, there have been numerous commercial contracts with UK and European companies, as well as work for local authorities in the UK. Recently, expert opinion has been provided to a US law firm representing the interests of an international company regarding the use of its products in the US. Further information about these activities may be requested and, insofar as is possible, will be made available if the confidentiality restrictions in the contracts allow.

New methodologies adopted by global plant science industry

Crop Life International is a global federation representing leaders in the plant science industry such as Bayer, Syngenta, Rentokil, Sorex, BASF, LiphaTech and PelGar. In 2001, the Rodenticide Resistance Action Committee (RRAC) of Crop Life International funded Prescott's reappraisal of resistance assessment methodologies, which led to his development of a new standardized methodology for identifying and quantifying physiological resistance in Norway rats and house mice [5]. In 2003, the RRAC adopted this new methodology and released it as a technical monograph [a, cites Prescott et al., (2007) *International Journal of Pest Management,* 53 (4): 265-272]. This standardised methodology is currently being used at the University of Reading, University of Lyon, France, and the Julius Kuhn Institut, Germany, to determine the Resistance Factors for each "species/VKORC1 mutation / anticoagulant active ingredient" combination, in order to provide the key link between molecular mutation of the VKORC1 gene and impact on field efficacy. This work is being funded by the RRAC.

Informing guidance on treating resistant rat populations in the UK

In 2010, Buckle and Prescott were co-authors on a report for the Rodenticide Resistance Action Group (RRAG), which provided guidance on treating resistant rat populations in the UK [b]. The RRAG is a UK-based group consisting of representatives from Universities, government agencies and all sectors of the pest control industry, with expertise in rodenticide resistance. The guidance describes the need to monitor rodent infestations and how the new molecular methodologies can help achieve this. It also describes how anticoagulant substances that are resisted by certain populations should cease to be used and alternative control methods explored. These two guiding principles were then incorporated into guidance from the European Biocidal Products Forum (EBPF), which represents 60 companies in the European biocides industry [c].

Influencing UK and European Union risk mitigation strategies

A RRAG report, containing detailed resistance survey data generated by the VPU [2], was

submitted to the Health and Safety Executive (HSE), which is the UK Competent Authority responsible for the European Biocidal Products Directive and its replacement as of September 1st 2013, the EU Biocides Regulation. The report was then passed on to the European Commission in response to a request prompted by The Netherlands that all European Union Member States provide an update on rodenticide resistance. The VPUs data formed part of the UKs response to the Commission's request [d].

Prescott subsequently collaborated with Professor Berny (University of Lyon, France) and Dr Jacob (Julius Kuhn Institut, Germany) on a bid for a European Commission Tender to review the Risk Mitigation Measures for anticoagulant rodenticide use across Europe. They were awarded the contract in December 2012. A member of the European Commission stated that "*the teams recruited for this project include the three most prominent European academic research teams in the field of AR expertise, both with respect to resistance detection, monitoring and management and non-target poisoning"* [e].

Buckle holds the following positions through which VPU expertise impacts and informs rodent pest management across the EU:

- Chair and Director, Campaign for Responsible Rodenticide Use UK. (http://www.thinkwildlife.org)
- Vice-Chair and Director, Campaign for Responsible Rodenticide Use Ireland
- Vice-Chair, Rodenticides Working Group, European Biocidal Products Forum (EBPF) Cefic
- Chair, Rodenticide Resistance Action Group UK (http://www.bpca.org.uk/pages/index.cfm?page_id=53)
- Vice-Chair, Rodenticide Resistance Action Committee, CropLife International (http://www.rrac.info)
- Recent achievements in these roles:
    - The Health and Safety Executive has requested Dr Buckle, in his role of Chair of CRRU UK, to co-ordinate a nationwide programme of rodenticide stewardship in all user sectors including farming, local authorities, professional pest control and amateur.
    - Influencing documents authored by Buckle in the last 12 months:
        - Sustainable use of rodenticides as biocides in the EU. http://www.cefic.org/Documents/Other/EBPF%20Sustainable%20use%20of%20rodenticides%20as%20biocides.pdf
        - Guideline on best practice in the use of rodenticide baits as biocides in the EU (http://www.cefic.org)
        - Rat Control and Game Management (http://www.thinkwildlife.org/crru-guideline-on-responsible-rat-control-by-gamekeepers)

5. Sources to corroborate the impact

[a] RRAC Technical Monograph (2003) A Reappraisal of Blood Clotting Response Tests for Anticoagulant Resistance and a proposal for a standardised BCR Test Methodology. http://www.croplife.org/view_document.aspx?docId=444

[b] RRAG (2010) Anticoagulant resistance in the Norway rat and Guidelines for the management of resistant rat infestations in the UK. http://www.bpca.org.uk/assets/RRAG_Resistance_Guideline.pdf

[c] EBPF (Cefic) Sustainable use of rodenticides as biocides in the EU. http://www.bpca.org.uk/assets/ceficdoc.pdf

[d] British Pest Control Association (2013) 'New Initiative in Anticoagulant Resistance by the European Commission', RRAG News [website accessed 28 Aug 2013] http://www.bpca.org.uk/pages/index.cfm?page_id=55

[e] DG Environment, Unit D.2 (Chemicals, Biocides and Nanomaterials, European Commission.