Institution: University of Reading

Unit of Assessment: UoA 5

Title of case study:
Anticoagulant rodenticide resistance: raising awareness, improving practical rodent control and prolonging product shelf life

Period when the underpinning research was undertaken: Between 2003 and 2020

Details of staff conducting the underpinning research from the submitting unit:

Name(s):	Role(s) (e.g. job title):	Period(s) employed by submitting HEI:
Dr Colin Prescott	Associate Professor	1989 to present
Dr Alan Buckle	CRRU Chairman & Visiting Research Fellow, University of Reading (Category C)	2004 to Present

Period when the claimed impact occurred: Between August 2013 and 2020

Is this case study continued from a case study submitted in 2014? Yes

1. Summary of the impact

The development of anticoagulant rodenticides (ARs) revolutionised rodent control in the 1950s, and in combination with recent regulatory restrictions, are now the most common control method for these pests; however, rodent physiological resistance to ARs is now widespread. Rodents are major global pests: they consume our foods, damage structures through gnawing, cause contamination, transmit many diseases, and impact on wildlife globally, creating concerns for conservation. Since 2005, the University of Reading's Vertebrate Pests Unit (VPU) has developed and applied new methodologies to identify the genetic basis for AR resistance in Norway rats and house mice, to map resistance across the UK, Europe and elsewhere, and to quantify resistance at the population level for each of the resistance mutations. Reading researchers submit data directly to the Health and Safety Executive (HSE), in support of the UK Rodenticide Stewardship Regime, resulting in new regulated guidance for the control of resistance by pest controllers, farmers and gamekeepers. This has enabled tailored and efficient control of rodent populations across the UK, thus prolonging the commercial lives of these important products.

2. Underpinning research

The VPU at the University of Reading conducts research in all aspects of vertebrate pest management. Dr Prescott is the Director of the VPU, and Dr Buckle is a Visiting Research Fellow at the University. Together, Prescott and Buckle's research has focused on the ecology and control of rodents with emphasis on ARs. The development of ARs in the early 1950s revolutionised rodent control. By the late 1950s and early 1960s, resistance to these first-generation ARs (FGARs) had developed in some Norway rat and house mouse populations, resulting in ineffective pest control across extensive areas of Europe and elsewhere. In the 1970s, the more potent, second-generation ARs (SGARs) were found to be more effective. However, within a few years, resistance had developed in both species, and it has been spreading ever since. In recent years, our excessive use of ineffective ARs has been key in the development and spread of resistance.

In 2003, Prescott was commissioned by the Rodenticide Resistance Action Committee (RRAC) of CropLife International (an association which promotes agricultural technologies such as pesticides) to develop a new standard Blood Clotting Response (BCR) methodology, as current methods had inconsistencies that made comparison of results between ARs impossible [1]. In 2007, Prescott published a reappraisal of the BCR tests, proposing a standardised methodology for identifying physiological resistance in rodent populations [2]. The methodology uses susceptibility baselines both to identify and assess the magnitude of physiological resistance. Prescott has established susceptibility baseline data for the FGARs warfarin, diphacinone, chlorophacinone, coumatetralyl, and for the SGARs bromadiolone, difenacoum, difethialone, flocoumafen and brodifacoum against Norway rats and house mice [2].

Also in 2007, Prescott and Buckle investigated the impact of resistance on treatment outcome in a Welsh population of Norway rats. Bromadiolone resistance factors were estimated, and the incidence of resistance was found to be high. A proprietary rodenticide containing 50ppm

bromadiolone was used. The research team estimated treatment success to be between 87% and 93% [3], suggesting that bromadiolone was still effective against Welsh resistance (subsequently identified as VKORC1 mutation Y139S).

Using new molecular methodologies to identify the genetic basis for resistance

In 2004, the gene conferring physiological resistance to ARs in humans and rodents (VKORC1) was identified. VKORC1 is associated with the vitamin K epoxide reductase, an enzyme involved in the activation of certain blood clotting factors (II, VII, IX and X), and which is the target enzyme for anticoagulants such as warfarin. Pelz, who was a co-author of the original 2004 paper, began collaborating with Prescott, and subsequently investigated tissue samples obtained from resistant strains of rats and mice maintained at the University of Reading. In 2005, Pelz, Prescott and others reported eight different VKORC1 mutations in both Prescott's resistant strains, and in wild-caught rodents from across Europe [4]. The findings provide the basis for DNA-based monitoring of anticoagulant resistance in rodents. Notably, this work led to the shift away from live animal testing, thus contributing to the principles of the 3Rs (Replacement, Reduction and Refinement) for animal welfare in research. In 2011, Prescott investigated tissue samples from a population of Norway rats from Kent, where the SGAR bromadiolone had been unsuccessful. He tested the animals and discovered they possessed the VKORC1 mutation Y139F, which was known to be widespread in France and Belgium, but unknown in the UK [5]. Historically, this resistance was referred to as 'Kent resistance'. To validate the resistance methodologies described above, Prescott and colleagues carried out six fully monitored field trials against Norway rats in Hampshire/Berkshire, UK, where 87% of individuals tested were found to be homozygous for the L120Q mutation [6].

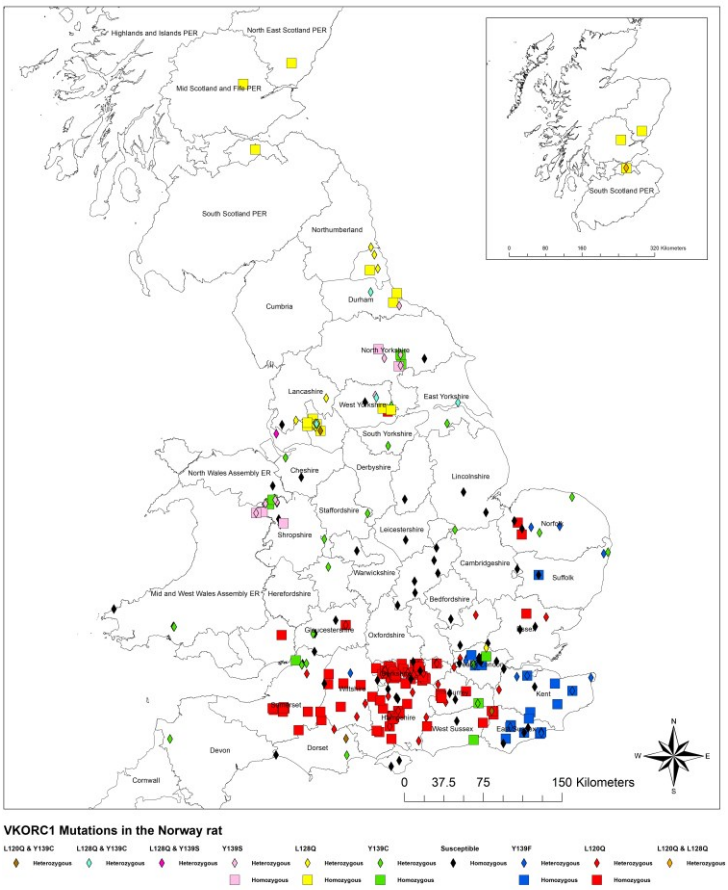

Figure 1. UK Resistance Mutations in Norway Rats [8]

They carried out two field trials using AR containing each of the active ingredients: bromadiolone, difenacoum and brodifacoum. They found that ARs containing 50ppm bromadiolone and 50ppm difenacoum were ineffective in each trial, while the AR containing 23ppm brodifacoum was fully effective in both trials, and with much lower anticoagulant environmental emissions than for bromadiolone or difenacoum. High resistance factors for bromadiolone and difenacoum, and a low resistance factor for brodifacoum, as determined by Prescott for the L120Q mutation, provides strong supporting evidence for the resistance management strategies now promoted by the RRAC and the Campaign for Responsible Rodenticide Use (CRRU) [7]. To validate the new molecular methodology, Prescott tested established BCR susceptibility baselines for nine ARs [2] against Norway rats and house mice homozygous for the VKORC1 mutations L120Q and Y139C, respectively. In addition, the RRAC commissioned a German laboratory to validate other Norway rat VKORC1 mutations using the Reading methodology.

Monitoring and mapping

Since 2009, the VPU genetically screens tail samples from the UK (Figure 1), Italy, Portugal, Poland, USA, Libya, Singapore and Russia. The UK rat and mouse data for all identified mutations to date are freely available on the RRAC website (https://www.rrac.info/) along with a mapping tool (https://guide.rrac.info/resistance-maps.html) that can be freely interrogated by researchers and field operators working across Europe.

3. References to the research

The Reading researchers are confident that the research meets at least the 2* quality level. The research has been published within the main peer reviewed, international journals of the field. Each of the outputs were assessed in the University of Reading's internal peer review process and were further validated by external peer review. Many of the papers have been highly accessed and are well cited. The research provides significant advances to the knowledge in the field. It has also provided logical and coherent arguments that have contributed to new theories, which have influenced policy impacting on public health and safety, food security and animal health and welfare, leading to changes in management and practice.

[1] **Prescott, C.V.** (2003). 'A Reappraisal of Blood Clotting Response Tests for Anticoagulant Resistance a proposal for a standardised BCR Test Methodology. RRAC of CropLife International'. Technical Monograph: https://about.rrac.info/fileadmin/downloads/technical_monograph_2003_BCR .pdf

[2] **Prescott, C.V.**, **Buckle, A.P.**, Hussain, I., Endepols, S. (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)

[3] **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 norgegicus Berk.) in Wales'. *International Journal of Pest Management*. **53**, 291-297. DOI: 10.1080/09670870701469872

[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.**, 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, tryosine139phenylalanine, new to the UK'. *International Journal of Pest Management*. **57**, 61-65. DOI: 10.1080/09670874.2010.523124

[6] **Buckle, A.P.**, Jones, C.R., Rymer, D.J., Coan, E.E., **Prescott, C.V.** (2020). 'The Hampshire-Berkshire focus of L120Q anticoagulant resistance in the Norway rat (Rattus norvegicus) and field trials of bromadiolone, difenacoum and brodifacoum'. *Crop Protection*. **137**. DOI: https://doi.org/10.1016/j.cropro.2020.105301

[7] **Buckle, A.P.** (2013). 'Anticoagulant resistance in the UK and a new guideline for the management of resistant infestations of Norway rats (Rattus norvegicus Berk.)'. *Pest Management Science*. **69**, 334-341.

[8] Jones, C., Talavera, M., **Buckle, A.** and **Prescott, C.** (2019). Anticoagulant Resistance in Rats and Mice in the UK – Summary Report with new data for 2019. University Report. https://www.thinkwildlife.org/downloads/

4. Details of the impact

Anticoagulant Rodenticides Stewardship Regime: All FGARs and SGARs fail environmental risk assessment for use outdoors; however, the UK Government recognises that their use is sometimes necessary to control pests because of the threats they can pose, for example to public health [A], plus the limited availability of safer alternatives (to humans, animals and the environment). The HSE is the lead agency and Statutory Competent Authority for government policy on biocides/rodenticides in the UK. For HSE to authorise the use of AR products, it asked the industry to explore ways to improve and spread good practice and self-policing of professional use of these products. In late 2012, the HSE asked the Campaign for Responsible Rodenticide Use (CRRU) to coordinate this task on behalf of its members (48 people from 32 stakeholder organisations). The CRRU has been chaired by Buckle since 2004. The main output of this task is the UK's AR Stewardship Regime, or SR, with Prescott as lead of the Monitoring

Work Group between 2012 and 2018, and subsequently as member of the Monitoring Work Group and Technical Advisor for the CRRU. The CRRU SR is currently the only SR in practice and has three main target user groups: professional pest controllers, farmers and gamekeepers [A]. The key benefits from the SR are a competent workforce, monitoring compliance and governance of the supply chain.

Competent Workforce: Professional rodenticide products can only be purchased legally by those who are able to show they are sufficiently competent to use them for effective rodent control. Thus, all rodent control practitioners must go through the SR training and certification. Research at Reading directly feeds into the RRAC resistance mapping tool (see Figure 1) [B], which is utilised in the SR training and certification, and is partly funded by the RRAC [C].

Professional Pest Controllers: Since the beginning of the SR in 2015, approximately 26,000 people - of whom approximately 23,000 are practitioners - have taken CRRU-approved courses via the four Awarding Organisations (Lantra, City and Guilds, BASIS and RSPH), passed the exam and received a certificate for proof of competence [D, E]. Each course is mapped against the content requirements developed by CRRU [E]. In addition to the training, five CRRU modules have been developed for Continuing Professional Development (CPD). The CPD modules went online in July 2017, and since have been downloaded from the CRRU website 7,000 times - the module on environmental risk assessment alone has been downloaded 3,000 times [D].

The British Pest Control Association (BPCA) is the leading trade association for pest control. The BPCA CEO, Ian Andrew writes: "*The Research undertaken by the University of Reading helps provide a tangible tool for pest professionals across the UK tackling the issue of rodenticide resistance in their area. Through the use of the resistance map, professionals can tailor their service approach depending on the individual resistance issues in their area, thereby making their pest programmes more effective, whilst using less ineffective rodenticide, reducing the risk of non-target exposure.*" [F]. The BPCA survey was sent to 9,495 people with an open rate of 28% or 2,659 members, of which 255 completed the survey. With members responding from every county across the UK, they were asked what specific actions their resistance awareness has resulted in, and 143 people responded in freeform text. Over half of the comments were about "changing bait;" i.e., that resistance awareness has changed their practice directly: "*Withdrawal from use of actives where there is known resistance increased emphasis of environmental management measures and physical means of control, supported by use of more toxic actives (Brodifacoum) where appropriate*." Members highlighted their sources for awareness resistance, including: 37% citing the SR training certification course; 67% the CPD courses; 51% the CRRU website; 79% the BPCA website and articles; and of the magazines: 61% BPCA, 53% Killgerm, 45% Pest and 18% National Pest Technician's Association. The importance of Reading's research was commented upon positively by 115 people: "*Had it not been for the Reading research published in the trade magazines I would not have been aware the area of resistance had grown*.", "*The data proves that the pest management community's engagement with the University of Reading's research results in heightened awareness of resistance, subsequent change of practice; plus extensive use of CRRU's SR training and CPD courses*" [F].

Farmers & Gamekeepers: Across the UK, there are 17 Farm Assurance Schemes with standards for rodent pest management that comply with the CRRU Code of Best Practice and make direct reference to it in their published standards [D]. Over 94,000 premises in the farming sector are registered with these schemes. Prior to 2015, very few gamekeepers were qualified professionals. In 2015, the CRUU organised a meeting at the headquarters of the Game and Wildlife Conservation Trust (GWCT). All gamekeeper organisations attended: the National Gamekeepers Organisation (NGO), the Scottish Gamekeepers Association, the British Association for Shooting and Conservation (BASC) and the Countryside Alliance. Together, the group designed a special one-day rodent control course for gamekeepers based on a previous CRRU course called 'Wildlife Aware'. It was then mapped by the CRRU Training and Certification Working Group and approved. The Awarding Organisation, BASIS, includes this course on its roster and the GWCT, NGO and BASC became the course providers for gamekeepers [D, E].

Monitoring Compliance: Progress with the SR is monitored by a Government Oversight Group (GOG) chaired by the HSE, and represents other government stakeholders, including: HSE Northern Ireland, DEFRA, Public Health England, Natural England, the Welsh and Scottish Governments and an independent scientific adviser. Monitoring compliance of the Stewardship Regime comes in many forms, including annual reports to GOG [G], and Knowledge, Attitude and Practice (KAP) Surveys that look at behaviour change for the three target rodenticide user groups: pest controllers, farmers and gamekeepers. The 2017 KAP Survey data has been compared to that obtained in 2015 (note that 2019 is not yet available). The comparison [H] shows that the percentage of users holding rodenticide use qualifications increased across all user sectors, but especially among gamekeepers (from 37% to 60%). Awareness of the SR increased across all sectors: in farming (from 20% to 35%); gamekeeping (from 30% to 56%); and pest controllers (from 56% to 89%). There is evidence of more training being taken up in each of the three sectors, especially in gamekeeping, which appears to have made significant progress in the last two years in terms of increased professionalism. Uptake of training and attendance at seminars on responsible rodenticide usage increased between 2015 and 2017 among farmers (from 11% to 19%), gamekeepers (from 14% to 49%) and pest controllers (from 71% to 83%) [H].

Governance of the Supply Chain: Reading research has informed the CRRU guidance, which carries regulatory weight. There are >700 rodenticide products available on the market, from 15 manufacturers and distributors. Each product must adhere to the CRRU guidance in order to make it to market, and this is evident on their authorisation labels. The HSE website stipulates that an SGAR product cannot be sold in the UK unless evidence is provided that the seller is part of a stewardship programme. It also sets out the 'High Level Principles' that must be met by an HSE-approved stewardship regime, and it states that the CRRU regime meets these principles [I]. The CRRU can also be credited with influencing government policy – starting with the introduction of new guidelines for the management of resistant infestations of Norway Rats [7]. The working groups and SR have also contributed to further understanding of the science behind resistance, so much so that since 31 January 2015, the HSE have said that all five SGARs (bromadiolone, difenacoum, difethialone, flocoumafen and brodifacoum) can be used in and around farm buildings, which can be corroborated through the HSE database [J].

Research at Reading is improving awareness of AR resistance through the SR, which is resulting in a definitive change in baiting practices amongst pest controllers and further standardised training for farmers and gamekeepers. As a result, more targeted and controlled use of existing AR products in the market can continue for the foreseeable future.

5. Sources to corroborate the impact

[A] Buckle, A., Prescott, C., Davies, M., Broome, R. (2017). The UK Rodenticide Stewardship Regime. A model for anticoagulant risk mitigation? *Proceedings of the Ninth International Conference on Urban Pests*. Davies, M., Pfeiffer, C., Robinson, W.H. (Eds.). Aston University, Birmingham, between 9 and 12 July 2017. 165-170.

[B] Resistance Maps for Norway Rat and House Mouse – Accessed: 31 July 2020

[C] 2015 RRAC Online Guide to Anticoagulant Rodenticide Resistance Management – Accessed: 31 July 2020.

[D] The UK Rodenticide Stewardship Regime 2019 Annual Report. Accessed: 22 June 2020

[E] CRRU Training and Certification in Rodent Pest Management and Associated Approved Certifications – Accessed: 31 July 2020

[F] Testimonial from British Pest Control Association (BPCA) CEO, June 2020

[G] 2017 & 2019-2020 CRRU Reports 'Anticoagulant resistance in rats and mice in the UK' - Date accessed: 31 July 2020

[H] Buckle, A., Broome, R., Bull, S., Christopher, P., Davies, M., Prescott, C. and Ward-Thompson, D (2017). *The UK Rodenticide Stewardship Regime 2017 Annual Report*: Section 3.6.2: Knowledge, Attitude and Practice (KAP) survey, 12-14.

[I] Health and Safety Executive Anticoagulant rodenticides and biocides legislation and most up-to-date 2019 Report on Rodenticides Stewardship Regime

[J] UK Authorised Rodenticide Product Database Search Database - Accessed: 31 July 2020