Proceedings of the Seventh International Conference on Urban Pests
William H Robinson and Ana Eugênia de Carvalho Campos (editors) 2011
Printed by Instituto Biológico, São Paulo, SP. Brazil.

DEVELOPMENT OF RODENTICIDES AND THE IMPACT OF RESISTANCE ON ANTICOAGULANT RODENTICIDES

COLIN V PRESCOTT

Director of the Vertebrate Pests Unit, School of Biological Sciences ,
University of Reading, Whiteknights, Reading, Berkshire RG6 6AS, UK

Abstract Cost effective methods are now available to identify physiological resistance in wild populations of Norway rat and House mice that are proving difficult to control. The new molecular methodology is a significant development for resistance management.
Key Words Norway rats, BCR technology, mutations

INTRODUCTION

The anticoagulants provide the principal method for controlling rodents. Prior to the 1950s a range of non-anticoagulants were used for rodenticides, and although their activity was based on a number of different modes of action, they all shared a number of properties. They were fast acting, there was no antidote, and they posed a significant risk to man, his companion animals, and wildlife. Because of their fast action, they are often referred to in the literature as acute rodenticides.

FEEDING HABITS

Rodents are intelligent pest species. Many rodent species are known to be shy of new object placed in their environment (a phenomenon known as Neophobia), and this often leads to a delay before a population of rodents readily consume rodenticide bait. Neophobia has the effect of reducing the rate of consumption of rodenticide, thus increasing the proportion of sub-lethally dosed animals. Such animals will often develop symptoms, and are then able to associate cause and effect, linking the symptoms with the rodenticide bait. Not only do they stop feeding, but they actively avoid the bait in the future; a behaviour referred to as 'conditioned bait aversion' or 'bait shyness'. This is a well-documented behaviour in many rodent species, and has been reported to last for months; some authors suggest that it lasts for the rest of the rodents life.

The practice of prolonged pre-baiting with a non-toxic bait has been developed in an attempt to overcome the 'conditioned bait aversion' (Buckle 1994). In effect, experienced rodent control operators are attempting to trick a large proportion of the rodent infestation into consuming a lethal dose. However, in practice, despite prolonged pre-baiting, it is unusual to achieve more than 70-80% control of an infestation, and because of their high productivity, such populations quickly return to pre-treatment levels. In addition, pre-baiting is labour intensive, expensive, and effectiveness relies heavily on the expertise of the rodent control operator.

ANTICOAGULANTS

In contrast, the anticoagulants have an indirect mode of action. They block a biochemical pathway (the Vitamin K cycle) which is involved in the activation of four blood clotting factors (factor II, VII, IX and X), and there is a time delay for endogenous levels of these blood clotting factors to become depleted before coagulation is compromised. This delay is critical, as it prevents the rodents associating cause and effect, and thus prevents the development of 'conditioned bait aversion' or 'bait shyness'. The ability to achieve 100% control without the labour intensive process of pre-baiting revolutionised rodent control. The first of the anticoagulants to appear on the market (ie. warfarin, coumachlor, diphacinone, coumatetralyl, and chlorophacinone) are now collectively known as the "first-generation anticoagulants".

Resistance

Since 1958, resistance to the first-generation anticoagulants has been found in Britain, Denmark, Holland, France, Germany, Japan, the USA and Brazil (Greaves, 1994). The development of resistance was a major setback for rodent control, and encouraged research into alternative modes of action and into the development of more potent anticoagulants. From 1975, a further five anticoagulants were developed and commercialised as resistance breaking compounds; and these compounds (difenacoum, bromadiolone, brodifacoum, flocoumafen and difethialone) collectively became known as the "second-generation anticoagulants".

Subsequent research revealed some degree of resistance to the second generation anticoagulants bromadiolone and difenacoum in England and Denmark (Greaves et al., 1982; Lund, 1984), although there was considerable doubt as to the practical significance of these reports, with control failures using these compounds increasingly being attributed to baiting problems (Greaves and Cullen-Ayres, 1988; Quy et al. 1992a; Quy et al. 1992b).

However, in the early 1990's, an infestation located in North Berkshire, between Oxford and Reading was identified that possessed a "practical resistance" to the second generation anticoagulant bromadiolone. The site was a small isolated livestock farm (with pigs, cattle, sheep and chickens). Two monitored field trials were conducted on the site, during which a total of 832 kg of 50 ppm bromadiolone bait was consumed. During the course of the second trial, the rodent population was monitored using tracking patches, and no significant reduction in tracking patch scores was recorded during the course of the treatment. This infestation has subsequently been referred to as the North Berkshire Resistance Focus.

Field Populations

The identification of resistance to anticoagulants in field populations of rodents is not straight-forward, and usually relies on the laboratory testing of wild caught rodents (EPPO 1999), either by feeding the animals on field strength formulation for a number of days and monitoring survival or mortality (the Lethal Feeding Period [LFP]Test), or by dosing the animals with active ingredient, and after a time delay of between 24 h and 96 h, measuring the coagulation time (the Blood Clotting Response [BCR] Test). In the latter case, resistant animals would be expected to have shorter coagulation times than susceptible animals.

More recently, a standardised BCR Test methodology has been developed at the University of Reading, and susceptibility data has been generated for nine active ingredients against Norway rat and five active ingredients against House mice (Prescott et al., 2007). An important advantage of this methodology is that it can be used to measure the magnitude of the resistance by providing an estimate of the Resistance Ratio.

RESISTANCE MUTATIONS

For some time it has been known that resistance is inherited from parent to offspring in a Mendelian fashion. Genetic studies indicate monogenic, dominant, autosomal inheritance of resistance in the Norway rat, with the resistance gene located on chromosome 1 (Greaves and Ayres 1967). Similarly, in House mice, the resistance gene has been located on chromosome 7, which is analogous to chromosome 1 in the rat (Greaves, 1994).

In 2004, the genetic mutation that causes warfarin resistance was identified and sequenced (Rost et al., 2004). Using this new and sophisticated DNA sequencing technology, it as possible to identify the genetic code in Norway rats and House mice, that is the DNA sequence (or gene) that becomes altered, in rodents resistant to anticoagulants (Pelz et al., 2005). As a result, a number of resistance mutations have been identified across the UK and Europe using DNA sequencing. The gene in question was given the name VKORC1, and selected mutations of this gene, are presented in the table below for Norway rats trapped in the UK and elsewhere (Pelz et al., 2005; Rost et al., 2009).

Transcription of the DNA produces a protein consisting of a series of amino acids in an order determined by the order of nucleotide based in the DNA. In the Table below, the name of the resistance mutation provides a numerical indication of the mutation's location on the VKORC1 gene, preceded by the name or code of the amino acid found in the 'wild type' susceptible animal, and followed by the name or code of the amino acid found in the resistant animal.

Mutations of the VKORC1 Gene in Norway rats from a range of geographical locations, as presented by Pelz et al. (2005) and Rost et al. (2009).

Resistance area	Resistance mutation	Abbreviated mutation name
Scotland	Leucine128Glutamine	L128Q
Wales	Tyrosine139Serine	Y139S
Hampshire	Leucine120Glutamine	L120Q
Berkshire	Leucine120Glutamine	L120Q
Gloucestershire	Tyrosine139Cysteine	Y139C
Norfolk	Tyrosine139Cysteine	Y139C
Lincolnshire	Tyrosine139Cysteine	Y139C
Lancashire	Leucine128Glutamine	L128Q
Yorkshire	Leucine128Glutamine	L128Q
Yorkshire	Tyrosine139Cysteine	Y139C
Cambridge/Essex	Phenylalanine63Cysteine	F63C
Nottinghamshire	Argenine33Proline	N33P
Denmark	Tyrosine139Cysteine	Y139C
Belgium	Tyrosine139 Phenylalanine	Y139F
France	Tyrosine139 Phenylalanine	Y139F
France	Argenine35Proline	N35P
Germany	Tyrosine139Cysteine	Y139C
Germany	Serine56Proline	S56P
Hungary	Tyrosine139Cysteine	Y139C
Korea	Tyrosine139 Phenylalanine	Y139F
USA	Isoleucine90Leucine	I90L
USA	Argenine35Proline	N35P
Argentina	Isoleucine90Leucine	I90L
Argentina	Tryptophan59Argenine	W59N
Japan	Glutamic acid67Lysine	E67K

In the UK, a pilot study was launched collaboratively at the University of Huddersfield and the University of Reading in 2009, to provide preliminary data on the geographical distribution of resistance mutations in Norway rats across the UK. Similar studies are being conducted across Europe and elsewhere.

With a number of the mutations identified to date we have a very good idea of their likely impact on field control, but with others we do not; and some mutations seem to have a variable impact on field efficacy (for example Leucine120 Glutamine in Hampshire and in Berkshire). In these situations there will still be a requirement to assess the magnitude of the resistance in the laboratory, most probably by assessing Resistance Ratios using the new BCR methodology (Prescott et al., 2007).

REFERENCES CITED

Buckle, A.P. 1994. Rodent Control Methods: Chemical. In: Rodent Pests and their Control, (Buckle AP, Smith RH, eds). Wallingford:CAB International, 127-160.

EPPO. 1999. Testing rodents or resistance to anticoagulant rodenticides. Paris, France:European and Mediterranean Plant Protection Organization.

Greaves, J.H. 1994. Resistance to anticoagulant rodenticides. In: Rodent Pests and their Control, (P BA, H SR, eds). Wallingford:CAB International, 197-217.

Greaves, J.H and Ayres, P. 1967. Heritable Resistance to Warfarin in Rats. Nature 215: 877.

Greaves, J.H and Cullen-Ayres, P.B. 1988. Genetics of difenacoum resistance in the rat. *In*: Current Advances in Vitamin K Research, (W SJ, ed). Amsterdam:Elsevier, 389-397.

Greaves, J.H, Shepherd, D.S, and Quy, R. 1982. Field trials of second-generation anticoagulants against difenacoum-resistant Norway rat populations. The Journal of hygiene 89(2): 295-301.

Lund, M. 1984. Resistance to the second-generation anticoagulant rodenticides. In: Proceedings of the 11th Vertebrate Pest Conference, 1984. Sacramento, California, (Clark DO ed). University of California, Davis, 89-94.

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

Prescott, C.V, Buckle, A.P, Hussain, I, and Endepols, S. 2007. A standardised BCR resistance test for all anticoagulant rodenticides. International Journal of Pest Management 53(4): 265 - 272.

Quy, R.J, Shepherd, D.S, and Inglis, I.R. 1992a. Bait Avoidance and Effectiveness of Anticoagulant Rodenticides against Warfarin-Resistant and Difenacoum-Resistant Populations of Norway Rats (*Rattus norvegicus*). Crop Protection 11(1): 14-20.

Quy, R.J, Cowan, D.P, Haynes, P, Inglis, I.R, and Swinney, T. 1992b. The influence of stored food on the effectiveness of farm rat control. The British Crop Protection Council Brighton Crop Protection Conference: Pests and Diseases, Vols 1, 2 and 3: 3) 291-300.

Rost, S, Pelz, H.J, Menzel, S, MacNicoll, A.D, Leon, V, Song, K.J, Jakel, T, Oldenburg, J, and Muller C.R. 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.

Rost, S, Fregin, A, Ivaskevicius, V, Conzelmann, E, Hortnagel, K, Pelz, H.J, Lappegard, K, Seifried, E, Scharrer, I, Tuddenham, E.G.D, Muller, C.R, Strom, T.M, and Oldenburg, J. 2004. Mutations in VKORC1 cause warfarin resistance and multiple coagulation factor deficiency type 2. Nature 427(6974): 537-541.