What is resistance (and why does it matter?)

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In the 1920's, cattle farmers in Alberta, Wisconsin started to lose cattle to minor routine procedures like castrations and dehorning. These normally simple procedures were frequently ending in the death of the animal through major haemorrhaging. It turned out that the sweet-clover feed, that the animals had been eating, had been naturally producing a chemical called dicoumarol, as it began to rot. It wasn't until a decade later however, that anyone attributed the animal feed to the unexplained deaths in the animals and it was not until 1939 when it was fully understood which molecule was responsible for the reduction in clotting factors. By the 1940's, dicoumarol had been fully isolated and synthesised and the more potent synthetic version had been named warfarin (after the institute which undertook the work, the Wisconsin Alumni Research Foundation).

By the late 1940's, warfarin was being widely used as an oral rat poison. It was used to great success for a decade, until the first reported instances of resistances in brown rats in 1958 (Boyle, 1960) and then subsequently in mice (Dodsworth, 1961). With the onset of resistance to warfarin growing rapidly (Müller, C.R. & Rost, S., 2011), a flurry of research was undertaken to find a replacement. Initially research was led by screening a catalogue of chemicals which had been rejected by the pharmaceutical industry for their toxic properties. This process proved to be costly and overly time-consuming, with only a few active compounds making it to field trials – notably norbormide and parachlorphenyl silatrane. Neither of these proved to be promising replacements.

Instead, a revision of the original, anticoagulant compounds was undertaken by Hadler and Shadbolt. They looked at the resistance mechanisms and what was understood about the coagulation theory at the time and set about to create a molecule specific to the task. What they succeeded in creating was the hydroxycoumarin-based anticoagulants, the first of which was called difenacoum. As with the first generation anticoagulants, widespread use lead to history repeating itself with resistance loci starting to appear in populations of brown rats.

What is resistance?

Resistance as we know it, is a largely misunderstood concept. So often it is used interchangeably in conversation with words such as tolerance and immunity. In reality all three words mean very specific and very different things. Resistance, as far as we are concerned, was best defined by Greaves who said;

> "*Anticoagulant resistance is a major loss of efficacy in practical conditions where the anticoagulant has been applied correctly, the loss in efficacy being due to the presence of a strain of rodent with a heritable and commensurately reduced sensitivity to the anticoagulant.*" (Greaves 1994)

Resistance therefore is the measure of difficulty that a chemical or strategy has at controlling a specific pest population, with the key term, in Greave's definition, being 'in practical conditions'. Often what is recorded in the laboratory is only an indicator of what occurs under the more dynamic conditions of fieldwork. This can be for many reasons, but primarily because resistance can come in many guises; behavioural, biochemical and genetic, all of which will confer varying degrees of protection to the target population from treatment.

Behavioural resistance is where an animal alters its behaviour, and in doing so increases the likelihood of its survival when faced with a certain treatment or situation. Aversion to bait formulations and other equipment is the most common form of behavioural resistance. A classic example of this is found in the behaviour of mice in Birmingham. Populations of mice have become averse to carbohydrate rich foods, leading to near total bait aversion of conventional formulations.

Biochemical resistance is where an animal's metabolic pathways detoxify and remove poisons at a rate fast enough, to allow them to survive what would normally be considered a lethal dose. Biochemical variations, in the presence and abundance of these detoxifying elements, are what is largely responsible for the variation in the amount of bait required to achieve a lethal response between different sexes within the same species (Sébastien Lefebvre, et al., 2016). Although the instances where this form of resistance becomes of practical impact in the anticoagulant roden-

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ticides is rare, it has been observed with other active compounds used for rodent control, both in the past and present.

Genetic resistance is by far the most prevalent of the three forms of resistance seen today. In rats, it allows for the greatest degree of protection from the bait formulations commonly used. Both first and second generation anticoagulants work by binding to and subsequently inhibiting, the function of the Vitamin K epoxide reductase enzyme (Müller, C.R. & Rost, S., 2011). This inhibition effectively breaks the Vitamin K cycle, denying the animal the ability to produce chemicals which are (amongst other processes) essential to the clotting of blood. The effectiveness of a molecule to inhibit this enzyme, is directly linked to the strength with which it binds to it. With genetic resistance, the coding responsible for the enzyme alters slightly, not so much that the change causes the enzyme to cease functioning entirely, but enough so that the binding site for the anticoagulant becomes altered or obscured. This alteration to the structure of the enzyme reduces the binding affinity of the anticoagulant to the enzyme and with this loss in sensitivity comes a loss in efficacy.

How does effecting one gene cause so much trouble?

In the simplest terms, genes code for proteins. More specifically genes code for strings of amino acids in a very specific order. Under normal circumstances a gene will provide the template for a string of amino acids which, when exposed to certain conditions, will fold itself from a string into a complex three dimensional shape, a protein. Some of these proteins are structural and many others like enzymes are functional in the biochemical processes of the cell. Sometimes the genetic code, which corresponds to a specific protein, becomes damaged, with certain amino acids missing, replaced or their order altered. Each of these changes is known as a mutation and each mutation has the capacity to drastically alter the shape of the protein it codes for. If a mutation has a severe effect on the structure of the protein it is coding for, the shape may be altered so dramatically that it may not function at all, causing the mutation to become lethal. When a mutation occurs that alters the shape of the protein enough to reduce the binding of poisons to it – but not so much that it ceases to function altogether – then resistance occurs.

So far, there have been multiple individual gene mutations which are shown to confer resistance to the second generation anticoagulants in rats, many of which are known to cause practical resistance in the field. Despite the vast geographical areas where these genes are found, it is interesting to note that the genes which confer resistance tend to move in 'pockets' which is indicative of the rodents' population dynamics. This means that resistances, although found over a wide area, might be dramatically different between neighbouring farms. With one farm showing high levels of resistance whilst a neighbouring farm shows no specific resistance at all.

Resistances usually come at a price. Deviating from a gene type which has been selected and refined over thousands of generations usually comes with a loss in efficacy. In some populations of bromadiolone resistant rats for example, there is a higher requirement for dietary Vitamin K in order to reproduce effectively (J. Jacob, et al., n.d.). Other issues, such as retardation to growth and even a reduction in survival, means that resistance to rodenticides is often only any use to a population whilst they are under selective pressure from that rodenticide.

How does this affect us?

As rodenticide resistance increases, rodents need to consume more of a certain bait type before they succumb to the treatment. In some extreme cases, the amount of bait required to kill that population will be practically unfeasible inside a workable timeframe. The lack of mixing between populations at larger geographical scales, revealed in a study by Mohd and Haniza, is likely to intensify the effect of local selection pressure imposed by sustained anticoagulant use. Reversing these processes is therefore likely to be slow and difficult to achieve (Mohd Z.H. Haniza, et al., 2015). At this point it will be necessary to modify the strategy in order to circumvent the resistance.

In conclusion, there is evidence of resistance of varying degrees around the globe. The resistance to a specific rodenticide is proportional to the efficiency with which is binds to the VKOR enzyme. As the sensitivity of the enzyme to the specific anticoagulant molecule reduces, the amount of bait required to achieve a lethal effect increases. When the level of bait needed to elicit a lethal effect increases beyond a certain level it will become economically and technically more effective to swap over to an alternative active ingredient. In the rare instance that the resistance is applicable to both difenacoum and bromadiolone, then an escalation to brodifacoum, flocoumafen or difethialone should be considered - there is as yet no documented resistance to these molecules.

References

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