Field trials of calciferol against warfarin resistant infestations of the Norway rat (*Rattus norvegicus* Berk.)*

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SUMMARY

The effectiveness of calciferol (vitamin D₂) against *Rattus norvegicus* was investigated in field trials on twenty-three farms with rat infestations partly resistant to warfarin. At 0.01 % combined with warfarin at 0.025 % no rodenticidal effect of the calciferol was discernible. At 0.025 % with warfarin at the same concentration, results were better, but not appreciably better than is often obtained with warfarin alone against resistant rat populations. When the concentration of calciferol was stepped up to 0.1 %, four out of five treatments in which the poison was applied directly gave complete control. The fifth may have partly failed because of poison shyness caused by under-baiting. Five out of six more treatments done after 'pre-baiting' were also successful. The sixth failed for reasons unconnected with the choice of poison.

Six further infestations that were not responding adequately to warfarin treatments were quickly controlled when, in three instances, calciferol at 0.1 % was used instead and, in three more, it was used together with warfarin. It is concluded that calciferol at 0.1 % is an effective poison against *R. norvegicus* either combined with warfarin or not, but that because at 0.1 % its effect is sub-acute rather than chronic, there may be a case in some environments for using it only after pre-baiting.

INTRODUCTION

The rodenticidal potentialities of calciferol (vitamin D₂) alone and in combination with the anticoagulant warfarin have been discussed from the laboratory aspect by Greaves, Redfern & King (1974). The present paper describes the result of a number of field treatments in Montgomeryshire against *Rattus norvegicus* with calciferol, in most cases combined with warfarin, the form in which the manufacturer developing calciferol was proposing to market it when the tests began. And since, as Greaves *et al.* (1974) point out, the current problem in rodent control is to overcome the setback caused by the appearance of resistance to the otherwise highly successful anticoagulants, the treatments were done on farms with a history of warfarin resistance.

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Fig. 1. The results of poison baiting with 0.01 % calciferol plus 0.025 % warfarin on Farms 1-3.

Fig. 2. The results of poison baiting with 0.025 % calciferol plus 0.025 % warfarin on Farms 4-6.

METHODS AND RESULTS

The presence of resistant rats on 17 of the 23 farms that were used was demonstrated by trapping animals on each of the 17 sites at least 4 weeks before the treatments began and subjecting them to the same laboratory test for warfarin resistance that is mentioned by Greaves *et al.* (1974).

The course of each poison treatment was monitored by the method described by Drummond & Rennison (1973) for testing anticoagulant resistance in the field. This essentially involves comparing, on a standard graph, the rate of fall in the number of daily takes of bait with that shown by experience to occur in treatments with 0.025 % warfarin against susceptible populations.

All the farms were surveyed and baiting points selected, and protected wooden bait trays were put in place 2-4 days before baiting commenced. If at the end of a treatment no baits had been touched for 4 days the farm was then resurveyed to make sure that no rats had survived.

Early trials

When the first six farms (1–6) were treated there were few laboratory data available on calciferol plus warfarin to indicate the most likely optimal concentrations at which to use these poisons together in the field. In the event, Farms 1–3 were treated by one pair of operators with 0.01 % calciferol plus 0.025 % warfarin (the normal concentration against *R. norvegicus*) and Farms 4–6 by a second pair of operators with the calciferol at 0.025 %. The six farms were treated simultaneously.

The poison baits were made up by mixing 1 part by weight of a fine oatmeal-based master-mix containing 0.5 % warfarin and either 0.5 or 0.2 % calciferol with 19 parts by weight of 'stabilized' medium oatmeal. Because of the instability of calciferol under damp conditions, all uneaten bait was replaced by freshly prepared material every time the farms were visited (on Mondays, Wednesdays and Fridays) throughout the period of the trials.

The results of the six treatments (1–6) are shown in Figs. 1 and 2. In the case of Farms 1–3 (calciferol at 0.01 %) each of the treatments progressed satisfactorily at first. The poison bait was being accepted well on days 2 and 4 and dead rats were found on all three farms on days 4 and 7 (and day 9 on Farm 1). However on day 7 the bait takes were very much smaller than they had been previously and on the ninth and subsequent days about 70 % of the takes recorded were so small that it was impossible to determine by eye whether anything had been eaten, although the bait had clearly been disturbed by rats. The disturbed baits were nevertheless counted as 'takes' for the purposes of recording the progress of each treatment, for clearly they denoted continuing rat activity.

As Fig. 1 shows, this quickly had the effect of carrying the lines for each of the Treatments 1–3 over the upper bound of the standard graph – a result typical of ordinary warfarin treatments that have failed owing to resistance. It may be concluded that the calciferol made a negligible contribution to the final levels of control obtained (estimated at 20, 32 and 46 %, respectively) and may even have affected them adversely by depressing the appetite of the rats for the poison bait.

In the treatments with 0.025 % calciferol and warfarin (4–6) the number of takes fell steadily over the first 11 days and then the picture (Fig. 2) followed that of Treatments 1–3. The probable interpretation of Fig. 2 is that after day 11 warfarin-resistant rats were recovering from sub-lethal doses of calciferol. Consistent with this is the fact that Treatment 4, which showed the greatest reduction in takes of bait and went on for 31 days, took place on the farm from which was obtained the rat sample with the lowest proportion of resistant animals. The estimated kills on Farms 4, 5 and 6 were 87, 65 and 52 %, respectively.

Trials with calciferol at 0.1 %

Treatments 1 and 6 indicated that if calciferol plus warfarin was going to be effective against the common rat in the field, the concentration of calciferol would need to be increased: and this was confirmed by toxicity data obtained at this stage by Greaves *et al.* (1974). Accordingly in all subsequent treatments with

Fig. 3. The results of poison baiting with 0.1 % calciferol plus 0.025 % warfarin on Farms 7-11.

calciferol the concentration used was 0.1 %. At this concentration in the laboratory some rats succumb after 1 day of exposure to the poison and it was feared that in the field the phenomenon of 'poison shyness' as a result of sub-lethal feeding might impair the results of treatments. In the next series of treatments therefore, six were done after 'pre-baiting' with plain bait for 4 days and five, as before, by putting the mixture of 0.1 % calciferol and 0.025 % warfarin down immediately.

The infestation on Farms 7-9 and 12-14 were treated simultaneously and before those on Farms 10 and 11 and 15-17. The two pairs of operators responsible for the work were allocated the method (pre-baiting or direct poisoning) at random so that the team that directly poisoned three of the first six infestations, pre-baited and poisoned three of the second six, while the second team pre-baited on three of the first farms and directly poisoned on two of the second. A sixth direct poisoning treatment that had been planned was not carried out because the pre-treatment trapping was found to have virtually eradicated the rats.

To obtain an even mix of the poison in the bait the best procedure was found to be to mix together 1 part of 0.5 % warfarin in fine oatmeal and 1 part of a 2 % solution of calciferol in an edible oil that by now had become available and then slowly add to this 18 parts of stabilized medium oatmeal. As in the earlier trials the poison bait was replaced by fresh bait at each visit but it has since been ascertained that calciferol in an oil formulation does not deteriorate during the course of a treatment of normal length (M. R. Hadler, personal communication). For the pre-baiting, stabilized medium oatmeal mixed with 5 % maize oil was chosen.

The results of the five treatments in which direct poisoning was used are shown in Fig. 3 and those of the six that followed pre-baiting in Fig. 4. At all eleven sites the considerable 'takes' of poison bait recorded at the first inspection indicated that it had been readily accepted, but thereafter the quantities taken were small.

Dead rats were found only intermittently from the 3rd to the 8th day where poisoning followed pre-baiting, but at every visit from the 4th to the 9th day on

Fig. 4. The results of poison baiting with 0.1 % calciferol plus 0.025 % warfarin after pre-baiting on Farms 12-17. The poison baits taken were counted for the first time after 1 day instead of the usual 2 days in these treatments.

each of the other farms. This suggests that pre-baiting induced more rapid feeding with the result that the majority of rats became moribund within the first 24 hr. of the treatment and died under cover.

Fig. 3 shows that four of the five direct treatments were completely successful. On Farm 10 however an estimated minimum of 28 % of the population was surviving on day 16, when it was decided that no further success would be achieved and the baiting was temporarily discontinued. Beginning 2 weeks later, Farm 10 was pre-baited with dry, cracked wheat. At the end of 9 days three 200 g. takes and seven takes between 50 and 200 g. were recorded but when calciferol plus warfarin was then put down again for 14 days only three or four baiting points were found to have been disturbed at each visit. The only satisfactory explanation of these observations stems from the fact that the size of the rat population on Farm 10 was misjudged before the original treatment and on the first inspection after poison was laid several baits in the area that subsequently gave trouble were found to have been completely eaten. This inadvertent underbaiting might have resulted in a number of animals acquiring sub-lethal doses and developing shyness to one or both of the poisons present in the bait.

Similarly, in the six treatments following pre-baiting (Fig. 4), five were successfully concluded within 14 days. The sixth (Farm 17) fell short of completeness only because rats in a single burrow in a disused deep litter poultry shed regularly disturbed the nearby bait by throwing litter over it, but took neither the pre-bait nor the poison. Obviously this failure could not be attributed to the poison.

As a perhaps more satisfactory check of the effectiveness of calciferol against warfarin-resistant populations of *R. norvegicus* a further six farms (18-23) with a history of resistant rats were selected and treated first with warfarin at 0.025 % and the course of each treatment plotted on the standard graph. Figs. 5 and 6 show that within 7-18 days it could be concluded that five of the six treatments had been unsuccessful because of resistance to the poison, which was therefore removed. The sixth treatment on Farm 20 was more successful but even here it was apparent by day 14 that a few resistant rats were probably present, although

Fig. 5. The results of first poison baiting with 0.025 % warfarin (broken lines) and then adding 0.1 % calciferol to the poison bait (solid lines) on Farms 18–20.

Fig. 6. The results of first poison baiting with 0.025 % warfarin (broken lines) and then with 0.1 % calciferol (solid lines) on Farms 21–23.

it might have taken a rather longer period of baiting to prove it. The poison was therefore picked up on this farm too. On all six farms, immediately or within 2 days (determined by the need, for labour reasons, to synchronize the visits) treatments were then resumed, either with 0.1 % calciferol plus warfarin (Farms 18, 19 and 20: Fig. 5) or with 0.1 % calciferol alone (Farms 21, 22 and 23: Fig. 6). Figs. 5 and 6 show that within 2–9 days the change of poison led to the eradication of the rats on all six sites and this was confirmed by observations continued over several more days. The rapidity with which control was achieved on Farm 20 can be attributed to the small size of the residual infestation and the ease with which it could be baited.

It should be recorded that in some of the treatments done in frosty weather a number of dead house sparrows and hedge sparrows were found in the vicinity of baiting points, in spite of the fact that every precaution had been taken to protect the bait from interference by non-target animals. A similar occurrence has been recorded in trials of calciferol in Scotland (C. M. Boyle, personal communication).

CONCLUSIONS

The effectiveness of the anticoagulant poisons such as warfarin derives from their cumulative effect, and because they do not induce symptoms that inhibit feeding on bait until a lethal dose has been ingested. The above field trials were begun in the belief that calciferol could be used at such a concentration that the same advantages might accrue. In fact, at 0.1 % below which it proved to be ineffective (Treatments 1-6), calciferol seems to behave almost as a single-dose poison. There are indications that it fairly quickly reduces appetite and there is the possibility also that it may induce poison shyness. There is no proof of this, but it is not easy to find another explanation for the results obtained on Farm 10.

In either case, it is suggested that when calciferol is being used, with or without warfarin, the aim should be to encourage the rodents at risk to feed freely from the poison bait from the beginning. A surplus of bait must therefore be available and, in some situations, ensuring this may involve either test-baiting to discover more about the size and distribution of the rat population to be treated or – more effectively still – pre-baiting.

An alternative approach is to begin treatments with warfarin and then follow up with 0.1 % calciferol, thus reducing the risk to non-target animals and using less of the more expensive poison. This method was very successful on Farms 18-23.

In only 3 of the 23 treatments was calciferol used without warfarin, but there seems little doubt that it is effective alone. In fact it is likely that when it is combined with warfarin what happens is that the latter at the most only accounts, or helps to account, for the more warfarin-susceptible individuals while the more warfarin-resistant animals die of calciferol poisoning before a lethal amount of warfarin has been acquired.

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