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EFFICACY OF A CELLULOSE-BASED RODENTICIDE FOR CONTROL OF WARFARIN-RESISTANT BLACK RATS (*RATTUS RATTUS*)

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Summary

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The resistance of synanthropic rodents to anticoagulant rodenticides is an emerging problem whose spread is constantly increasing. This is at the background of the growing interest towards the implementation of various non-anticoagulant rodenticides in deratisation practice, including cellulose-based baits. The aim of this study was to evaluate the efficacy of the cellulose-based rodenticide Eradirat (Republic Mills, USA) for control of warfarin-resistant black rats *(Rattus rattus).*The palatability and efficacy of cellulose-based rodenticide Eradirat were evaluated in field tests and a series of lethal feeding tests with *(choice test)* and without choice *(no-choice test)* on wild warfarin-resistant black rats *(Rattus rattus)*. The palatability of Eradirat baits for studied black rats was extremely low, which resulted in zero efficacy of choice tests and field deratisation procedures. The 14-day no-choice feeding test with Eradirat, supplemented with additional attractants resulted in high efficacy (90%) in warfarin-resistant black rats. The results suggested a high deratisation potential of cellulose-based rodenticide Eradirat, but at the same time, outlined its low palatability for black rats as a very serious disadvantage thus making it inappropriate for use in practice.

Key words: anticoagulant resistance, cellulose, *Rattus rattus*

INTRODUCTION

The last decades have witnessed a marked change in the arsenal of means used for harmful rodent control. Almost all acute rodenticides as zinc phosphide, fluoro-organic, thiocarbamate compounds etc. were gradually banned and removed from the market, mainly because of the high risk they pose for human health, non-target animal species and the environment. They were sequentially replaced by anticoagulant rodenticides and at present, they are the most commonly used chemical means for rodent control. Unfortunately, their practical efficacy is strongly compromised by the appearance of genetic mutations in synanthropic rodents' genome resulting in onset of resistance. The latter is not a new phenomenon, as it was first reported in 1958 (Boyle, 1960). During the last years however, probably due to the common use of anticoagulants and the limited array of alternative means, the problem with anticoagulant resistance has become more serious – it has not only spread over a broader geographic area, but greater extents of resistance affecting different rodent species were also established (Brooks & Bowerman, 1973; Myllymäki, 1995; Wang *et al.*, 2008; Rost *et al.*, 2009; Huang *et al.*, 2011; Baert *et al.*, 2012). This negative trend has pointed out to the necessity of implementation of different non-anticoagulant rodenticides in the deratisation practice. It is assumed that their efficacy is not affected by the resistance due to the different mechanism of action and that is why they are recommended for control of anticoagulant-resistant rodent populations.

Cellulose-based baits are interesting representatives of non-anticoagulant means for control of rodents. These rodenticides contain cellulose from pelletised powdered corn cobs and a flavour enhancer. A significant advantage is that they are entirely harmless for domestic animals and birds, pets and men, are ecologically safe as they do not contain hazardous chemicals and are 100% biodegradable (Anonymous, 2008).

The mechanism of action of cellulose-based rodenticide baits is based on the ability of cellulose to absorb large amounts of water on one part, and to the specific unique features of the alimentary tract of rodents. After ingestion of baits, cellulose binds large amounts of water provoking a severe bowel impaction and systemic dehydration in rodents. These events are accompanied by hypovolaemia, hypotension, tissue dehydration, multiorgan failure, circulatory shock, and death (Anonymous, 2007; 2008 Vukša *et al.*, 2012).

This experiment was performed to evaluate the efficacy of the cellulose-based rodenticide Eradirat (Republic Mills, USA) for control of warfarin-resistant black rats *(Rattus rattus)*.

MATERIAL AND METHODS

Wild black rats *(Rattus rattus)* caught in animal production facilities were used as experimental subjects. The rats included in this study were previously determined to be warfarin-resistant in a 28-day feeding test with 0.025 % warfarin according to the OEPP/EPPO method: PP 1/198(1) Efficacy evaluation of rodenticides: "Testing rodents for resistance to anticoagulant rodenticides" (OEPP/EPPO, 1995).

The efficacy of Eradirat in anticoagulant-resistant rodents was evaluated in a series of laboratory feeding choice and no-choice tests of various durations. A one-month rest period was allowed between tests for recovery of experimental animals. By the start of the feeding tests, rodents were weighed, sexed and housed in individual cages with water available *ad libitum*. The *choice* and *no-choice* tests were carried out in accordance with the requirements of Technical notes for guidance in support of Annex VI of Directive 98/8/EC of the European Parliament and the Council concerning the placing of biocidal products on the market: in technical notes for guidance on product evaluation Appendices to Chapter 7 Product Type 14 Efficacy Evaluation of Rodenticidal Biocidal Products (Anonymous, 2009).

For evaluation of the efficacy of Eradirat in field conditions, deratisations of two animal facilities were performed: the rabbit farm at the Agricultural Institute – Stara Zagora, and the City Zoo, Stara Zagora. The sites were of medium density (from 10 to 50 rats per 100 m^2) of black rat populations preliminary determined to be warfarin-resistant. Tests were conducted in compliance with the OEPP/EPPO method: PP 1/114(2) Efficacy evaluation of rodenticides: „Field tests against synanthropic rodents *(Mus* *musculus, Rattus norvegicus, R. rattus)"* OEPP/EPPO, 1998).

RESULTS

For initial evaluation of the palatability and efficacy of Eradirat on warfarin-resistant black rats, a 14-day choice feeding test was conducted (test №1). Standard compound pelleted feed was used as concurrent non-toxic bait. The results from the test are presented in Table 1.

The results demonstrated lack of efficacy of Eradirat, mainly due to the extremely low attractiveness of the preparation for black rats and its resulting low consumption.

The inadequate palatability of Eradirat baits and thus, their low competitiveness vs the alternative food source in this laboratory test suggested a low efficacy of Eradirat in field conditions.

This hypothesis was confirmed by field deratisations carried out in two animal facilities with medium density of warfarin-resistant black rat populations. In both field experiments, no consumption of Eradirat was established, and the efficacy of deratisation (efficacy intensiveness and extensiveness) was zero.

In order to increase the attractiveness of Eradirat pellets, rodent-specific attractants were added by impregnation with a mixture of 3% melted pork lard, 3% powdered sugar and vanilla powder. Then, a new 14-feeding choice test was carried out with thus flavoured pellets (test №2). Standard pig compound feed was once again used as non-toxic bait.

The data demonstrated a higher attractiveness of flavour-enhanced rodenticide, as evidenced from the ten times higher consumption of the flavoured vs the standard Eradirat – the average daily consumption increased from 0.082 to 0.8 g/100 g bw. Nevertheless, the consumption of the rodenticide bait remained 16 times lower than that of the concurrent non-toxic feed. The ingested amount of Eradirat was not sufficient to provoke a lethal effect and the resulting death rate was 0%.

To assess the efficacy of Eradirat in the absence of alternative feed sources, a series of no-choice feeding tests of different duration were carried out. The results from 1-day (test №3) and 3-day (test №4) no-choice tests are given in Table 1.

The results confirmed the extremely low palatability of cellulose-based Eradirat for warfarin-resistant black rats.

In order to make Eradirat pellets more attractive, specific rodent attractants were added through impregnation of pellets with a mix of 3% melted pork lard, 3% powdered sugar and vanilla powder. The flavoured pellets were fed in a new 14-day no-choice feeding test (test №5).

After the 14-day no-choice feeding test with attractant-supplemented Eradirat, the deratisation efficacy was high – total death rate was 90%. The death occurred between the 5th and the 9th day from the beginning of the regular Eradirat intake (by the 7th day on the average).

A noticeable sign of occurring alimentary tract lesions was the change in droppings' appearance. These changes were detected at the earliest by the 2nd day of the test and consisted in: discoloration of black faeces which became black-grayish to light brown; altered shape: from elongated and curved to short and round; altered structure – from thick and homogenous to frail and granular; altered size with a severalfold increase of faecal mass volume (Fig. 1).

The gross anatomy examination of dead rodents revealed signs of general body dehydration (Fig. 2) and severe cae-

Table 1. Lethal feeding tests with Eradirat in warfarin-resistant black rats (*Rattus rattus*).

	Sex	Number	Mean body weight (g)	Pre-test period (3 days)		Test period						Death rate		
				Non-toxic pellets		Non-toxic pellets			Eradirat			Number	%	Day
				A_1	B_1	A_2	B_2	C_1	A_3	B_3	C_2			
test №1 choice test (14 days) with Eradirat	M	5	102.5	11.18	10.2-19.14	10.28	9.25-18.14	142.18	0.093	0-0.8	1.52	0/5	0	---
	F	5	90.4	12.85	9.35-16.6	13.25	10.15-18.95	182.36	0.071	0-0.5	0.98	0/5	0	---
	Both	10	95.18	12.35	9.35-19.14	12.26	8.13-20.54	170.55	0.082	0-0.8	1.13	0/10	0	---
test №2 choice test (14 days) with Eradirat + attractants	M	5	99.2	14.07	10.05-22.24	12.14	8.13-20.54	175.18	0.81	0.1-2	11.4	0/5	0	---
	F	5	83.6	15.18	8.94-20.0	13.25	10.15-18.95	183.95	0.78	0.2-1.8	10.9	0/5	0	---
	Both	10	91.4	14.58	9.45-22.24	12.56	8.13-20.54	178.25	0.8	0.1-2	11.15	0/10	0	---
test №3 no-choice test (1 day) with Eradirat	M	5	106.2	13.4	12.3-17.4	---	---	---	0,05	0-0.2	---	0/5	0	---
	F	5	98.6	11.5	7.9-16.8	---	---	---	0	0	---	0/5	0	---
	Both	10	103.5	12.52	7.9-17.4	---	---	---	0.025	0-0.2	---	0/10	0	---
test №4 no-choice test (3 days) with Eradirat	M	5	105.3	14.7	13.5-19.0	---	---	---	0.12	0-0.4	0.28	0/5	0	---
	F	5	96.8	15.5	11.6-19.8	---	---	---	0.08	0-0.6	0.18	0/5	0	---
	Both	10	101.2	15.1	13.5-19.8	---	---	---	0.10	0-0.6	0.24	0/10	0	---
test №5 no-choice test (14 days) with Eradirat + attractants	M	5	106.7	13.5	10.5-18.6	---	---	---	5.60	0.5-17.5	32.8^S 18.5^R	4/5	80	6.4 /5-9/
	F	5	100.5	14.7	11.0-17.5	---	---	---	5.95	1.2- 16.8	36.5^S ---	5/5	100	7.6 /6-9/
	Both	10	101.8	14.2	10.5-18.6	---	---	---	5.77	0.5-17.5	34.02^S 18.5^R	9/10	90	7 /5-9/

Legend: $A_{1,2,3}$- average daily consumption of the bait (g/100 g bw), $B_{1,2,3}$- min/max daily consumption of the bait (g/100 g bw), $C_{1,2}$- average cumulative consumption of the bait for the entire period (g/100 g bw), ^{S}-in warfarin sensitive rats, ^{R}- in warfarin resistant rats.

Fig. 1. Changes in faeces appearance: A – pre-test period; B – test period.

Fig. 2. Body dehydration in dead rats.

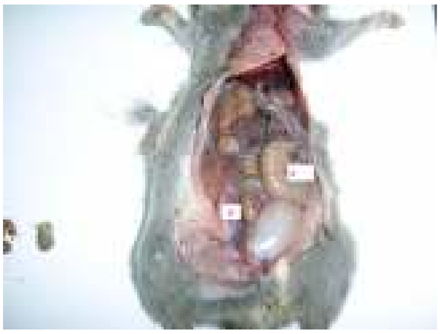

Fig. 3. Severe caecal impaction (A) and large faecal balls in the bowel lumen (B).

cal obstruction with dense faecal masses (Fig. 3A), as well as extremely enlarged hard faecal balls in the bowel lumen (Fig. 3B).

DISCUSSION

The scientific literature data about the efficacy of cellulose-based rodenticides are few and rather controversial.

Jokic (2010) established a good efficacy (70% and 85%) of deratisations with cellulose-based rodenticide Natromouse (a.i. cellulose from powdered corn cobs, Pinus TKI, Slovenia) against common voles *(Microtus arvalis)* in wheat and alfalfa crops at the background of various abundant alternative feeding sources. Having tested this same preparation, the same author's research team reported that it was highly attractive to house mice *(Mus musculus)*, and highly effective (91.66%) against them after deratisation of a feed storage facility. Other researchers have also reported a high efficacy of Natromouse for house mice control in agricultural storage premises (Jokić *et al.*, 2006; 2007; Vukša *et al.*, 2007; Jokić, 2010; Vukša *et al.*, 2010).

Unlike them, Schmolz (2010) observed a very low palatability of two different anonymous cellulose-based rodenticide baits in laboratory choice feeding tests with house mice *(Mus musculus)* and brown rats *(Rattus norvegicus*), resulting in no lethal issues. However, the no-choice tests demonstrated high efficacy of preparations against house mice, with death occurring within 14–21 days after the beginning of tests. On the basis of performed experiments, Schmolz concluded that cellulose-based rodenticides were not appropriate for control of house mice and black rats (Schmolz, 2010).

Possible reasons for the obtained inconsistent results could be the specific taste preferences of the different rodent species, as well as the addition of flavour enhancers to rodenticide baits.

The results of our experiments with black rats *(Rattus rattus)* are exactly the same as those of Schmolz (2010) but not in agreement with the data of Jokić *et al.* (2006, 2007), Jokić (2010) and Vukša *et al.* (2007, 2010). The continuous no-choice tests in black rats with cellulose-based Eradirat were highly effective, but laboratory choice tests as well as the field experiments confirmed convincingly the extremely low palatability of baits, which would inevitably result in low efficacy of deratisation procedures in practice. Our conclusions support the decision of the Canada Pest Management Regulatory Agency to recommend the use of cellulose-based baits only indoor and in premises entirely or partially free of alternative food sources for rodents by reason of their low palatability (Anonymous, 2007).

CONCLUSIONS

On the basis of performed laboratory and field experiments, it could be assumed that the cellulose-based rodenticide Eradirat was extremely unpalatable for black rats (*Rattus rattus)*, and thus inappropriate for use in animal production facilities, where spilled feed is commonly encountered and various alternative food sources are readily available to rodents.

The present investigations outlined the main problems of cellulose-based rodenticide practical application and at the same time, confirmed their potential. They substantiate the performance of more detailed studies in this direction, aimed particularly at increasing the attractiveness of cellulose-based baits for rodents and development of novel, more palatable recipes to ensure the high efficacy of deratisation procedures in practice.

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