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INVESTIGATIONS ON THE WARFARIN RESISTANCE OF SYNANTHROPIC RODENTS

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Summary

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Anticoagulant rodenticides are the most widely used chemical agents for control of harmful rodents, and therefore, the onset of resistance to them poses a serious problem in deratisation practice. The aim of this study was to detect the presence of resistance to warfarin among synanthropic rodents inhabiting animal production facilities. Synanthropic rodents from the black rat (*Rattus ratus*) and house mouse (*Mus musculus* sensu lato) species, caught in animal production facilities in the Stara Zagora region were tested for warfarin resistance. A lethal feeding test with 0.025% warfarin was applied for 28 days in black rats and 21 days in house mice. Out of all tested 117 synanthropic rodents – 41 house mice and 76 black rats, 111 were determined as resistant (94.87 %), and only 6 (5.13%) – as sensitive to warfarin. The results demonstrate a substantial prevalence of warfarin resistance among house mice and black rat populations in animal production facilities in the surveyed region.

Key words: *Mus musculus*, *Rattus rattus*, resistance, susceptibility, warfarin

INTRODUCTION

The implementation of anticoagulant rodenticides in deratisation practice in the late 1940s is a revolutionary step comparable to the introduction of antibiotic treatment of infectious diseases. Compared to the rodenticides used so far, the efficacy of anticoagulants is very high, along with relative ecological safety and low risk for the health of domestic animals and men.

Warfarin is the first anticoagulant implemented in US deratization practice in 1948 and it is still used in a number of countries as a rodenticide. Unfortunately, only a decade later, the first report for appearance of resistance in Norway rats was communicated from Scotland (Boyle, 1960). Later, the resistance among rodents became widespread.

Attempts to counteract the loss of warfarin efficacy were made by synthesizing and implementation of more toxic anticoagulant rodenticides such as diphacinone, coumatetralyl etc., as well as representatives of second-generation anticoagulants. Regrettably, at present rodents are reported to be resistant to all anticoagulant rodenticides used in practice. Moreover, the extent of observed resistance becomes higher and higher, affecting various species of rodents and spreading widely in geographic terms. This alarming trend caused the formation of international working groups attempting to develop strategy for monitoring and management of problems related to resistance in rodents (Telle, 1967; Ophof & Langvelt, 1969; Jakson & Kaukeinen, 1972; Lund, 1972; 1974; Jakson & Ahton, 1980; Greaves *et al.*, 1982; Gill & McNicoll, 1991; Pelz *et al.*, 2005; Ishizuka *et al.*, 2008; Baert Kristof *et al.*, 2012).

In Bulgaria, the information about the presence and spread of resistance to anticoagulant rodenticides among synanthropic rodents is scarce and incomplete. The present study aimed to throw light on the problem by detecting warfarin-resistant subjects among the rodent population inhabiting animal production facilities in the Stara Zagora region, Bulgaria.

MATERIAL AND METHODS

Animals

The tests were performed with wild synanthropic rodents caught in animal production facilities in the Stara Zagora region. The rodents were weighed, sexed and housed in individual cages. A 14-day quarantine period was allowed, during which the rodents were fed pelleted compound feed for pigs and had permanent access to drinking water. Only sexually mature adult animals were included in the experiments, assessed as clinically healthy, non-pregnant and showing normal food intake.

One hundred and seventeen wild synanthropic rodents – 41 house mice (Mus musculus/domesticus) and 76 black rats (Rattus rattus) were tested for resistance to warfarin. The animals belonged to 7 populations inhabiting 5 animal production facilities (Table 1).

Lethal feeding tests

Lethal feeding tests were conducted as per standard protocols according to EPPO standard: PP 1/198(1) „Testing rodents for resistance to anticoagulant rodenticides (Anonymous, 2005). The tests are based on feeding rodents discriminating dose of an anticoagulant rodenticide,

Table 1. Origin of experimental animals

Origin of animals	Rodent species	sex	number
Site No 1 Stationary animal patient facilities	*Rattus rattus*	M	6
		F	8
	Mus musculus/domesticus	M	9
		F	6
Site No 2 Pig farm	*Rattus rattus*	M	9
		F	7
	Mus musculus/domesticus	M	6
		F	5
Site No 3 Sheep farm	*Mus musculus/domesticus*	M	6
		F	9
Site No 4 Rabbit farm	*Rattus rattus*	M	10
		F	10
Site No 5 City zoo	*Rattus rattus*	M	14
		F	12
		Total:	117

which causes death in 99% of susceptible rodents. Based on numerous research studies, EPPO (European and Mediterranean Plant Protection Organization) has adopted and standardised methods for determination of discriminating doses of anticoagulant rodenticides in the different synanthropic rodent species. For black rats (*Rattus rattus*) discriminating warfarin dose is attained by feeding a poisonous bait containing 0.025% warfarin for 28 days, whereas for house mice (*Mus musculus/domesticus*) – by feeding the poisonous bait with 0.025% warfarin for 21 days.

The 0.025% warfarin baits were prepared using warfarin (analytical standard, Fluka) according to EPPO recommendations: 0.25 g warfarin (technically active substance) was dissolved in 5 mL 1 M sodium hydroxide solution, which was neutralised to pH 7 with adding 1M hydrochloric acid. A sodium chloride solution (0.85% w/v) was then added to a final volume of 50 mL. The poisonous bait was prepared by thorough mixing 50 mL of rodenticide solution with 1 kg compound feed for pigs. The amount of consumed poisonous bait was determined on a daily basis.

After the experimental period, a 21-day monitoring period followed, during which the poisonous bait was replaced by pelleted feed for pigs. The observation of experimental rodents was done twice daily and their health status was recorded. The animals dead during the experimental or monitoring period were necropsied for confirming the presence of haemorrhages. Only dead rodents with haemorrhages in the body were accepted to be warfarin-sensitive. Those who survived the 21-day monitoring period without any manifestations of impaired blood coagulation, were accepted as resistant.

Ethics

The experiments were conduced in strict compliance to regulations for humane treatment of experimental animals in the Republic of Bulgaria and with permission of the institutional Animal Care Committee (permit 41/10.10.2011).

RESULTS

The results of lethal warfarin feeding tests are presented in Table 2.

Warfarin-resistant synanthropic rodents were observed in 100% of all seven surveyed populations.

In house mice, warfarin resistance was established in 100% of tested rodents (41 mice) originating from 3 populations (facilities No. 1, 2 and 3).

The percentage of warfarin-resistant black rats was also high – 100% among the animals inhabiting facility No 1; 95% of the population from facility No. 4; 93.75% among rodents from facility No. 2 and 84.62% of rats from facility No. 5.

Out of all 117 tested synanthropic rodents (*Rattus rattus* and *Mus musculus/ domesticus*) 111 (94.87%) were found to be resistant and only 6 animals (5.13%) – sensitive to warfarin.

DISCUSSION

Warfarin is the first anticoagulant used in deratisation practice and the least toxic among all anticoagulant rodenticides, which makes it the most appropriate and sensitive marker for initial detection of resistant rodents.

The presented results indicate that the resistance to warfarin is widespread both among house mice (*Mus musculus/domesticus*) and black rat (*Rattus rattus)* populations of animal production facilities in the Stara Zagora region – Bulgaria.

Table 2. Lethal feeding tests with 0.025% warfarin in black rats and house mice.

Facility No	Rodent species	Sex	Rodenticide	Experimental period /days/	Monitoring period /days	Number	Dead (sensitive)		Survived (resistant)		daily warfarin intake (mg/kg)								warfarin intake for the entire experimental period (average in mg/kg	
											Sensitive				Resistant					
							n_1	%	n_2	%	mean	min/max	SD	SEM	mean	min/max	SD	SEM	Sensitive	Resistant
1	RR	M	0.025 % warfarin	28	21	6	0	0	6	100	-	-	-	-	36.11	20/50	8.50	2.00	-	1025
		F		28	21	8	0	0	8	100	-	-	-	-	27.32	15/36.88	5.42	1.02	-	765.12
	MM	M		21	21	9	0	0	9	100	-	-	-	-	20.72	15.63/33.75	4.67	1.04	-	431.88
		F		21	21	6	0	0	6	100	-	-	-	-	15.81	10.63/26.25	3.84	0.93	-	343.75
2	RR	M		28	21	9	1^*	11.11	8	88.89	15.62	8.75/31.25	5.88	1.31	12.15	6.88/33.13	5.79	1.16	392.5	341.25
		F		28	21	7	0	0	7	100	-	-	-	-	15.56	8.75/31.88	5.19	1.19	-	439.13
	MM	M		21	21	6	0	0	6	100	-	-	-	-	12.72	8.75/16.25	2.94	0.71	-	246.25
		F		21	21	5	0	0	5	100	-	-	-	-	11.45	7.5/18.13	2.91	0.68	-	222.88
3	MM	M		21	21	6	0	0	6	100	-	-	-	-	18.13	10.63/28.13	6.11	1.48	-	378.13
		F		21	21	9	0	0	9	100	-	-	-	-	21.45	11.25/28.13	7.33	1.83	-	430.63
4	RR	M		28	21	10	1^*	10	9	90	19.2	5/25.63	7.41	1.43	22.07	16.25/25.63	3.11	0.78	546	723.13
		F		28	21	10	0	0	10	100	-	-	-	-	26.88	18.13/38.75	7.32	1.49	-	633.75
5	RR	M		28	21	14	2^*	14.29	12	85.71	16.41	2/25.63	8.25	1.59	26.55	16.25/46.25	9.75	2.03	470.63	823.13
		F		28	21	12	2^*	16.67	10	83.33	14.27	3.0/25.0	6.79	1.28	25.37	14.78/45	8.93	1.86	399.63	796.03
Total:						117	6	5.13	111	94,87										

Legend: Facility No. 1 – stationary animal patient facilities, facility No. 2 – pig farm, facility No. 3 – sheep farm, facility No. 4 – rabbit farm, facility No. 5 – city zoo, RR – black rat (*Rattus rattus*), MM –house mouse (*Mus musculus/domesticus*), N-, N_1-, N_2- number of experimental animals, *- with signs of bleeding diathesis, SD – standard deviation, SEM – standard error of the mean.

Our data are comparable to other reports on the prevalence of resistance. For instance, as early as in 1976, Graves *et al.* have reported a high incidence of warfarin resistance among black rats. The authors have investigated rodents from 41 population and determined 17 of them (41.5%) as warfarin-resistant (Graves *et al.*, 1996). Recent studies in Germany among house mice reported a similar extensity of resistance, as in 29 out of 30 surveyed regions there were resistant subjects and over 90% of tested mice had genetic mutations, responsible for resistance (Anonymous, 2012).

A survey performed by EPPO in 1992 demonstrated that resistance to anticoagulant rodenticides has been reported in 13 countries (43% of EPPO members). Practically, these are all countries where tests for resistance of synanthropic rodents have been conducted (Myllymäki, 1995). The international working group on rodenticide resistance furthermore alarms that the resistance of mice to anticoagulant rodenticides is so broad that sometimes, it is very difficult to find even one susceptible animal.

The commonly encountered resistance to anticoagulant rodenticides necessitates continuous monitoring allowing for a proper choice of rodenticides according to resistance status of rodents. The development and implementation of scientifically justified strategies would also assist the control and restrict the appearance and expansion of the range of distribution of resistant rodent populations in Bulgaria (Anonymous, 2012).

CONCLUSION

Warfarin resistance was widely spread among black rats *(Rattus rattus)* and house mice *(Mus musculus/domesticus)*, inhabiting animal production facilities in the Stara Zagora region, Bulgaria.

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