Short Communication

Laboratory Evaluation of a Rodenticide-insecticide, Coumavec®, against *Rhombomys opimus*, the Main Reservoir Host of Zoonotic Cutaneouse Leishmaniasis in Iran

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Abstract

Background: Zoonotic cutaneous leishmaniasis is a growing health problem in many rural areas of Iran. *Rhombomys opimus*, the great gerbil, is the main animal reservoir of ZCL in the northeast and central part of Iran. The aim of the current study was to evaluate the rodenticidal effect of Coumavec® (a mixture of Coumatetralyl 0.5% and Etofenprox 0.5%) on *R. opimus* under laboratory condition.

Methods: Great gerbils were collected from Sejzi rural district, Esfahan Province, Iran. Four groups of 19 great gerbils were treated with the poisoned baits of different concentrations and one group was considered as control. The bating procedure was conducted in three stages: first, second (a week after first) and third (a month after first stage), in each stage baits were offered in 1 day, based on national protocol for rodent control operation in purpose of ZCL control.

Results: The mortality rate for 0.03, 0.0625, 0.125 and 0.25% concentrations in the first stage of baiting were obtained 36.8%, 31.5%, 52.6% and 36.8%, in the second stage 47.3%, 52.6%, 68.4% and 52.6%, and in the third stage 52.6%, 63.1%, 68.4% and 57.8% respectively. The maximum and minimum mortality has occurred in 5-6 days and 31-40 days intervals consequently.

Conclusion: The results of this study showed that, Coumavec® has some rodenticidal effects on *R. opimus* in laboratory condition. For the appropriate rodenticide-insecticide contamination of the rodent body and also considering to the economic issues, we suggest the use of 0.125% concentration for rodent control operation in the field condition.

Keywords: *Rhombomys opimus*, Rodenticide, Coumatetralyl, Zoonotic cutaneous leishmaniasis, Iran

Introduction

Zoonotic cutaneous leishmaniasis (ZCL) is a growing health problem in many rural areas of Iran, which involves 17 out of 31 provinces of the country (Yaghoobi-Ershadi 2012). Rodents, especially those belonging to gerbillinae subfamily have significance role as reservoir hosts of zoonotic cutaneous leishmaniasis (Yaghoobi-Ershadi 2008, Yaghoobi-Ershadi and Javadian 1996).

In addition, different kinds of pathogens like bacteria, rickettsia, viruses, protozoa and helminthes can be transmited by rodents to human and animals (Bell et al. 1988). *Phlebotomus papatasi* is the main vector of ZCL and *Leishmania major* is the causative agent of the disease in the most parts of the country (Yaghoobi-Ershadi 2012). *Rhombomys opimus*, the great gerbil, is the main animal reservoir

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of ZCL in the northeast and central part of Iran (Yaghoobi-Ershadi and Javadian 1996). This gerbil is relatively large and has diurnal activity whereas the most other species have nocturnal activity (Dubrovskiy 1979). A karyotype study revealed that diploid chromosome number for *R. opimus* was 2n=40 (Akhavan et al. 2004). *Rhombomys opimus* is an herbivores rodent and feeds on green dried leaves as well as stems of plants. This species has social life and lives with other members of the colony in their burrows. This species has 1 to 2 and sometimes 3 generations in spring and 1 more generation in summer or fall. They have 5–14 offsprings in each generation. The longevity of this species reaches to 3 years. This long longevity provides appropriate condition for the maintenance of *Leishmania* parasites in the rodent population while sand flies are inactive. Many ecological characters of great gerbils and presence of sand flies, directly or indirectly, effect the leishmaniasis cycle in the nature (Bell et al. 1988). Attempts to control of leishmaniasis in the field condition by rodent control operation have been conducted in some countries including Iran. In central Asia, a large-scale operation against great gerbils through poisoned baits successfully eliminated the rodents (Dergacheva and Zherikhina 1980). Along April to January 1997, a field trial carried out to control ZCL by destruction rodent burrows and using zinc phosphide 2.5% baits in radius of 500 meters from houses in central Iran. The results showed that the control program reduced the incidence of ZCL 12-folds at the end of the first year of the operation and 5-folds at the end of the second year (Yaghoobi-Ershadi et al. 2000, Yaghoobi-Ershadi et al. 2005). Recently some behavioural resistance and/or bait shyness against the conventional rodenticide, zinc phosphide, among the population of great gerbils has been reported from some endemic areas of the disease (unpublished data, Esfahan Health Research Station, Iran). It is necessary to introduce some new, safer and more effective alternative rodenticides to control the reservoir hosts and subsequently the disease in endemic areas of ZCL in Iran.

Furthermore, Coumavec® has insecticidal effect on the ectoparasites and other blood feeding insects of the gerbils but the aim of the current study was the evaluation of rodenticidal effect of Coumavec® (a mixture of Coumatetralyl 0.5% and Etofenprox 0.5%) on *R. opimus* in laboratory condition.

Materials and Methods

Rodent collection

Active colonies of gerbils were identified in Sejzi rural district (32˚39˙54.84”N/ 52˚08˙07.38”E), Esfahan Province, Iran. Sherman live traps were placed near the rodent holes from January to February 2010. Around 80 to 100 live traps baited with cucumber and sometimes carrot were used in each day. They were set up daily in the early morning to evening in winter. The collected gerbils were transferred to the animal house of Esfahan Health Research Station. Different morphologic criteria of rodents were used for *R. opimus* identification. Captured rodents were identified by valid identification keys (Etemad 1978). Just *R. opimus* species were included in the study. All the animals, which were used in this study, had no experience of exposing to the rodenticides.

Laboratory tests

All experiments were conducted with animals maintained individually in metal cages. Five groups of 19 great gerbils per group were sexed and selected, four groups were treated with the poisoned baits of each concentration and one group was as control. The commercial formulation of Coumavec®, a mixture of Coumatetralyl 0.5% and Etofenprox 0.5% (Levant Overseas Development Ltd, Argenteul, France) which was evaluated in

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these tests, recommended for direct application as a rodenticide dust or for dilution in bait. Poisoned baits were prepared using a mixture of grain and four concentration of Coumavec® (0.03, 0.0625, 0.125 and 0.25%). Sexually mature animals were acclimatized to the laboratory condition at least 7 days. The bating procedure was conducted in three stages: first, second (a week after first) and third (a month after first stage), in each stage baits were offered in 1 day, based on national protocol for rodent control operation in purpose of ZCL control. Along the keeping animals in the animal house, before the tests, they were fed on the standard animal diet (pellet). For providing required water, they were offered carrot. In each stage, the diets were withdrawn and a fresh container, holding 15 gr of poised bait for each rodent, was placed in the cages. The ordinary diet was not resumed until complete consumption of poison bait. The gerbils were then fed on ordinary laboratory diet until the next stage of baiting. Dead animals were examined for internal bleeding and other symptoms of anticoagulant poisoning. Gerbils, which survived during the test period, were maintained on laboratory diet, and those still alive 30 days after third offered poisoned bait, were considered as survived individuals.

Animal ethics consideration

Animal experiments were approved by the Ethical Committee of Tehran University of Medical Sciences, Tehran, Iran.

Data analysing

The data were analyzed using SPSS 11.5 and graphs were prepared using Excel. The cumulative mortality rate of gerbils among the treated and control groups was compared using Chi-squared test.

Results

Totally 95 healthy-mature rodents were used in this investigation. All dead animals were examined for internal bleeding and other symptoms of anticoagulant poisoning (Fig. 1). The results of toxicity test based on concentration in each stage using Coumavec® is shown in Fig. 2. The mortality rate for 0.03, 0.0625, 0.125 and 0.25% concentrations in the first stage of baiting were obtained 36.8%, 31.5%, 52.6% and 36.8%, in the second stage 47.3%, 52.6%, 68.4 and 52.6%, and in the third stage 52.6, 63.1, 68.4 and 57.8% respectively. The maximum and minimum mortality has occurred in 5–6 days and 31–40 days intervals consequently. The maximum rate of mortality for female and male was observed in 0.0625 and 0.125% concentrations consequently (Fig. 3). In spite of more mortality rate in 0.125% concentration, statistical differences between the concentrations have not seen.

The number of dead rodents in the intervals between 1–5, 6–10, 11–15, 16–30 and 31–40 were summarised in Table 1. As the table shows, the maximum and minimum mortality has occurred in 5–6 and 31–40 interval consequently.

The statistical analyses showed that there were no significant differences between four selected concentrations of Coumavec® on the rodent's mortality rate.

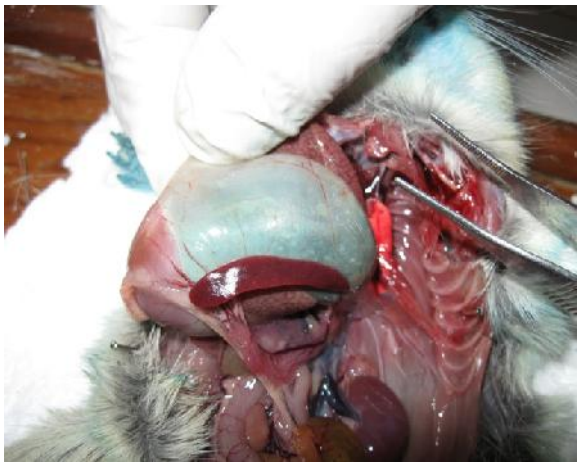

Fig. 1. Internal bleeding of a *Rhombomys opimus* treated by Coumavec®

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Table 1. Mortality time of the tested rodents with Coumavec® under laboratory condition in each stage of the baiting

Concentration	Total	1–5	6–10	11–15	16–30	31–40
0.03	19	8 (41.1%)	0 (0%)	1 (5.2%)	1 (5.2%)	0 (0%)
0.625	19	6 (31.5%)	1 (5.2%)	2 (10.5%)	3 (15.78%)	0 (0%)
0.125	19	6 (31.5%)	4 (21%)	2 (10.5%)	0 (0%)	1 (5.2%)
0.25	19	6 (31.5%)	3 (15.7%)	0 (0%)	1 (5.2%)	1 (5.2%)
Control	19	0	0	0	0	0

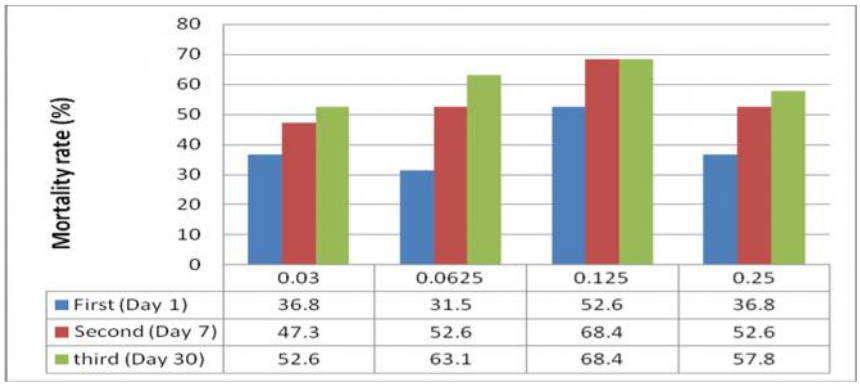

Fig. 2. Mortality rate of the tested rodents with Coumavec® in laboratory condition in each stage of the baiting

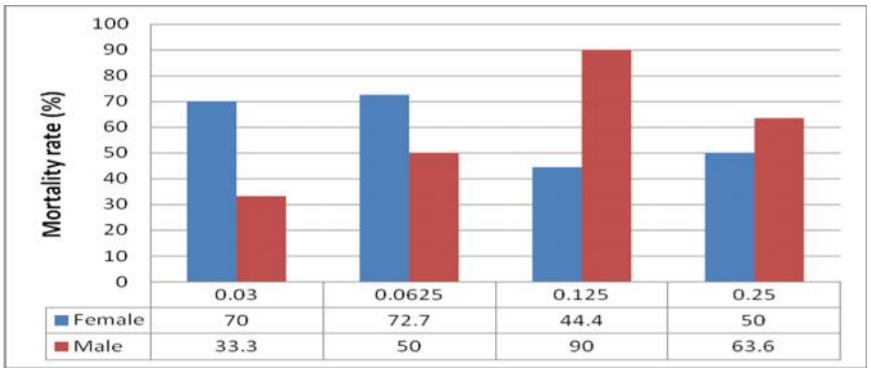

Fig. 3. Mortality rate of the tested rodents with Coumavec® in laboratory condition based on sex

Discussion

The results showed that maximum mortality occurred along 1–5 days after first baiting, slightly more than 40 percent for 0.25, 0.125, and 0.0625% and over 40 percent for 0.03% concentrations. It is suggested that the later baiting stages did not have dramatic effects on the mortality. The complete mortality was not obtained for all concentration as well. The maximum mortality was slightly more than 68% and the minimum was almost 52% occurred in 0.125 and 0.03% concentration, consequently. In addition Gill and Redfern (Gill and Redfern 1983) showed that complete mortality with Coumatetralyl (15 days feeding, in 0.0375% concentration) against *Meriones shawi* were not obtained.

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All concentrations have an increasing mortality trend, as prepared baits offered in each stage, the mortality increased consequently. In dissected dead animals, the expected organs were checked. Bleeding mostly occurred in stomach and after that extent to the intestine, spleen and liver.

Therefore, all concentrations can be used in the field operations but in view of the fact that this pesticide is an anticoagulant-insecticide mixture, for the appropriate rodenticide-insecticide contamination of the rodents' body and also considering to the economic issues, this article suggests 0.125% concentration for rodent control operation in the field condition.

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