Original Article

Comparative Study on the Effectiveness of Coumavec® and Zinc Phosphide in Controlling Zoonotic Cutaneous Leishmaniasis in a Hyperendemic Focus in Central Iran

A Veysi [1], H Vatandoost [1], MR Yaghoobi-Ershadi [1], MH Arandian [2], R Jafari [2], M Hosseini [3], H abdoli [2], Y Rassi [1], K Heidari [4], A Sadjadi [4], R Fadaei [4], J Ramazanpour [4], K Aminian [4], MR Shirzadi [5], *AA Akhavan [1,6]

[1]Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
[2]Esfahan Training and Health Research Center, National Institute of Health Research, Tehran University of Medical Sciences, Esfahan, Iran
[3]Department of Epidemiology and biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
[4]Esfahan Health Center, Esfahan University of Medical Sciences, Esfahan, Iran
[5]Department of Zoonosis, CDC, Ministry of Health and Medical Education, Tehran, Iran
[6]Institute for Environmental Research (IER), Tehran University of Medical Sciences, Tehran, Iran

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Abstract

Background: Zoonotic cutaneous leishmaniasis (ZCL) is an increasing health problems in many rural areas of Iran. The aim of this study was to introduce a new alternative rodenticide to control the reservoirs of ZCL, its effect on the vector density and the incidence of the disease in hyperendemic focus of Esfahan County, central Iran.

Methods: The study was carried out from January 2011 to January 2012. In intervention areas, rodent control operation was conducted using zinc phosphide or Coumavec®. Active case findings were done by house-to-house visits once every season during 2011–2012. To evaluate the effect of rodent control operation on the vector density, sand flies were collected twice a month using sticky traps.

Results: The reduction rate of rodent holes in intervention areas with Coumavec® and zinc phosphide were 48.46% and 58.15% respectively, whereas in control area results showed 6.66 folds intensification. The Incidence of ZCL significantly reduced in the treated areas. Totally, 3200 adult sand flies were collected and identified in the intervention and control areas. In the treated area with zinc phosphide, the density of *Phlebotomus papatasi* was higher in outdoors in contrast with the treated area by Coumavec® which the density of the sand fly was higher in indoors.

Conclusion: Both rodenticides were effective on the incidence of ZCL and the population of the reservoirs as well. Coumavec® seems to be effective on the outdoor density of the vector. This combination of rodenticide-insecticide could be a suitable alternative for zinc phosphide while bait shyness or behavioral resistance is occurred.

Keywords: Rodent control, Zinc phosphide, Coumavec®, Zoonotic Cutaneous Leishmaniasis, Iran

Introduction

Zoonotic cutaneous leishmaniasis (ZCL) is an increasing health problem in many rural areas of Iran, which involves17 out of 31 provinces of the country (Akhavan 2011).

Rhombymos opimus, the great gerbil, and *Meriones libycus,* Libiyan jird, are the main and secondary reservoir hosts of the disease in northeast and central part of Iran (Yaghoobi-

***Corresponding author:** Dr Amir Ahmad Akhavan, E-mail: aaakhavan@tums.ac.ir

Ershadi and Javadian. 1996, Yaghoobi-Ershadi et al. 1996). Due to improvement of reporting system by the Ministry of Health and Medical Education of Iran, natural disaster such as earthquake, urbanization, constructions of buildings near colonies of the rodents , immigration of non-immune people to the endemic areas, a sharp increase of cases have been reported since 2004 (Shirzadi 2010, Saidi et al. 2012).

Phlebotomus papatasi is the main vector of ZCL and *Leishmania major* is the causative agent of the disease in the area. The parasite has been isolated and identified from naturally infected *P. papatasi, P. caucasicus*, *R.opimus, M. libycus* and human in this endemic area (Yaghoobi-Ershadi et al. 1996 and 2001, Yaghoobi-Ershadi 2008, Akhavan et al. 2010a, 2010b, 2010c). In the recent years despite of significance progresses on leishmaniasis studies such as biochemistry and molecular biology of the parasite and host immune response, a little information is available about the effective control operations of the disease. To control the disease a successful leishmanization has been carried out in Iran during complex emergency, but for a very rare rate of immunodeficiency of the recipients, it has been quit except for military personnel in very high risks areas (Nadim et al. 1983). In an intervention study, it was shown that autoclaved *Leishmania major* (ALM) vaccine with BCG has not been protective against ZCL (Momeni et al. 1999). Global efforts to develop an effective vaccine to prevent leishmaniasis so far showed no success (Noazin et al. 2008, 2009, WHO 2010).

In Karshinskaya Steppes, Uzbekistan, attempts to control of great gerbil using destroying their burrows by plowing or crushing with heavy machines within 2 to 3 km radius of the towns in a three-year period was carried out. The results showed that zonal control of great gerbils was inadequate due to re-invasion of the gerbils to control areas. A massive operation to eradicate great gerbils and their burrows was carried out over an enormous area surrounded by mountain ranges and rivers. The results showed that the operation caused significant reduction in sand flies density and also no cases of ZCL was reported within at least 4 years after the operation (Sergiev 1978, Eliseev 1980). In central Asia a large-scale operation against great gerbils, using poisoned baits eliminated the rodents successfully but had no effect on the density of *P. papatasi* (Dergacheva and Zherikhina 1980). From April to January 1997 a field trail was carried out to control ZCL by destruction rodent burrows and using 2.5% zinc phosphide baits in radius of 500 meters from houses once a month in May, June, July and September in an endemic focus of Iran. The results showed that the control program reduced the incidence of ZCL 12-folds in treated village compared to the control village at the end of the first year of operation and 5-folds at the end of the second year (Yaghoobi-Ershadi et al. 2000). At the same intervention area from 1999 to 2002, the numbers of active burrows were counted in May and October and baited with zinc phosphide, if the rodent hole numbers increased more than 30%. The results showed that changes in the numbers of rodent burrows during the intervention were statistically significant. Furthermore significant difference in incidence rate of ZCL between the intervention and control villages has been shown (Yaghoobi-Ershadi et al. 2005).

Recently some behavioral resistance and/or bait shyness against zinc phosphide among the great gerbil population has been reported from some endemic areas of the disease (personal communication, Esfahan Health Center, Iran). It is necessary to introduce some new effective alternative rodenticides to control the reservoir hosts and subsequently the disease in endemic areas of ZCL in Iran.

The aim of the current study was to introduce a new alternative rodenticide for controlling ZCL in Iran. In this survey the effect of Coumavec® (BATCH NO: COU/204-161, Pro: 07/2010, Exp: 07/2013) a mixture of Coumatetralyl 0.5% and Etofenprox 0.5% on the reservoir host, vector density and the disease incidence were compared with 2.5% zinc phosphide(BATCH NO: Z/T/36, Pro: 10/2010, Exp: 10/2013) bait for the first time in the world.

Materials and Methods

Study area

The present study was carried out in 4 villages (Mazraehshoor, Gishi, Vartoon and Parvaneh-Aliabadchi), 45 to 85 km far from Esfahan City, Esfahan Province, Iran from January 2011 to January 2012. Mazraehshoor (32˚39˙54.84"N/ 52˚08˙07.38"E) and Gishi (32˚29˙24.07"N/52˚21˙47.06"E) were selected as intervention areas for Coumavec® and zinc phosphide respectively; Vartoon (32˚50˙11.48"N/ 52˚06˙45.93"E) and Parvaneh-Aliabadchi (32˚47˙44.36"N/ 51˚58˙27.19"E) were selected as control areas.

The selected areas have desert climate, hot in summer and cold in winter. In 2010, the maximum and minimum mean monthly temperature was 39.1˚ C and -1.6˚ C in July and December, respectively. The total rainfall was 72.2 mm. The minimum mean monthly relative humidity was 7% in July and the maximum was 82% in January.

Rodent control operation

To determine the appropriate bait concentration of coumavec® a trial was conducted under laboratory condition. Poisoned baits were prepared using a mixture of wheat grain and four concentration of Coumavec® (0.03,0.0625, 0.125 and 0.25). Each treatment included 19 great gerbils. Four groups were treated with the poisoned baits of one concentration. The control group only was offered with the wheat grain. The zinc phosphide bait concentration was selected according to the previous studies (Yaghoobi-Ershadi et al. 2000, 2005).

In early April 2011, prior to the beginning of the active season of sand flies, rodent holes were counted in each village. The holes were destroyed by digging. All the activities were conducted in a radius of 500 m from houses around all villages. After 48 hours of the operation, all areas were visited and the opened holes were counted again. In treated areas, the gerbil colonies were baited by Coumavec® or zinc phosphide baits and then closed. Approximately 12–15 gm of the poisoned baits was placed into each burrow at a depth of about 10 cm. The operational areas were revisited after a week and the reopened burrows in treated areas were counted, baited, and closed. Rodent control operations in intervention areas, the village of Mazraehshoor (treated with Coumavec®) and Gihi (treated with zinc phosphide), were repeated once a month in June, July and August. The date of baiting and the number of reopened burrows were recorded. No control operation was done in the village of Vartoon (control), but the numbers of reopened holes were also counted and recorded in each interval visit to compare with intervention areas.

Entomological monitoring

To evaluate the effect of rodent control operation on the vector density, an entomological survey was performed. In each village three fixed houses were selected and sand flies were collected by 30 sticky traps (papers impregnated with castor oil) twice a month from the beginning (April) to the end (October) of active season of sand flies. The sticky papers were set before sunset and collected in the following morning. The collected phlebotomines were washed with absolute acetone and preserved in 70% ethanol. Microscopic slides were prepared using Pauri's medium (Smart et al. 1965) and identified by morphologic characters (Theodor and Mesghali 1964, Seyedi-Rashti and Nadim 1992). The same procedure was also done for collecting and identifying sand flies from outdoor resting places.

Human infection

To evaluate the rodent control operation on the incidence of the disease, active case findings were done before and after intervention in treated (Mazraehshoor and Gishi) and control (Vartoon and Parvaneh-Aliabachi) villages by house to house visits in January 2011 and once every season in 2012. In treated villages 150 households and in controls all inhabitants (less than 150 households) were visited. A questioner including identification information of the people, presence or absence of scar (s) or active lesion (s), number of the lesion (s) or scar (s), and traveling history to the other ZCL foci, was filled out for each household. Persons who had experience of traveling to other endemic foci of ZCL were excluded from the study.

Only new cases of ZCL and number of patient with active lesion were recorded on each visit. Yearly incidence of the disease in both treated and control villages were calculated at the end of 2011 and 2012. In the calculation of the disease yearly incidence, the persons with previous scars were excluded from at risk population.

Statistical analysis

The data were analyzed using Stata 11.0 and SPSS 11.5 and graphs were prepared using Excel. Chi-squared and Fisher's exact tests were used to compare the rodent holes changes and incidence of the disease in intervention and control areas. The Kruskal-Wallis non-parametric test was also used to compare the density of sand flies in the areas.

Results

The appropriate bait concentration of coumavec® was 0.125%. Table 1 shows the effect of poisoned baits on rodent holes density in treated and control areas. The treated area of Mazrehshoor (Coumavec® intervention area) was around 212 hectares and the total number of holes before treatment was 11023 (52 per hectare). After 48 hours of destruction the rodent colonies 1296 (6.1 active holes per hectare) of the holes were reopened. Each reopened hole were baited and closed again. After one week of control activity, the number of reopened holes increased to 1557. In June, July and August the number of reopened holes were counted 724, 644 and 668 respectively.

The treated area of Gishi (zinc phosphide intervention areas) was around 193 hectare. The numbers of holes before the treatment were 4729 (24.5 per hectare). After 48 hours of digging the burrows, the numbers of reopened holes were 1682 (8.7 active holes per hectare). The reopened holes were baited and closed. After one week, the number of holes decreased to 600. In June, July and August the number of holes were counted 493, 424 and 704 respectively.

The control area (Vartoon) was aroumd 173 hectare; the number of holes before treatment was 2297 (13.3 per hectare) and 48 hours after destruction 196 (1.1 active holes per hectare) number of these holes were reopened. The reopened holes in this village were not baited or closed. The number of reopened holes increased to 281 after one week. In June, July and August the number of holes increased to 365, 557 and 1306 respectively. The number of holes in each stage in control village showed an increasing trend compared to the intervention areas. The reduction rate of rodent burrows was 48.46% in Mazraehshoor (treated with Coumavec®) and 58.15% in Gishi (treated with 2.5% zinc phosphide). Chi- squared test showed that the decreasing rate of rodent holes between the treated villages with Coumavec® and zinc phosphide were statistically different (P< 0.000001). The rodent burrows in control area (Vartoon) after the rodent control operation increased 6.66 folds in comparison before the operation (Table 1). During May to October 2011, a total of 3200 adult sand flies (2054 from outdoors and 1146 from indoor resting places) were collected. The following three species were collected from indoors: *P. papatasi* (92.5%), *S. sintoni* (7.1%), *P. sergenti* (0.4%). In outdoor resting places *P. papatsi* (95.8%), *S. sintoni* (3.3%), *P. sergenti* (0.5%), *P. ansari* (0.2%), *P. mongolensis* (0.2%) were found. In all places, sand flies started to appear in the late April and disappeared in the late October.

Phlebotomus papatasi was the common and predominant species in both indoor and outdoor resting places.

The monthly density of *P. papatasi* in both treated areas with Coumavec® and zinc phosphide was compared (Fig. 1 and 2). The outdoor density of *P. papatasi*, the main vector of the disease, in the village treated with Coumavec® was lower than indoors while the density of the sand fly in the village treated with zinc phosphide was higher in outdoors. There was no statistical difference between the density of the vector in indoors and outdoors in intervention and control areas (P> 0.05).

Table 2 shows the yearly incidence of the disease in the treated and control villages. The incidence was calculated at 32.92 and 39.14 per thousand in Mazaehshoor and Gishi (intervention areas) and also 18.40 and 76.19 per thousand in Vartoon and Parvaneh-Aliabadchi (control areas), in 2011 respectively. After intervention, the incidence of the disease reduced to 4.34 and 11.4 per thousand (13.2% and 29. 1% of its original level before the intervention) in Mazaehshoor and Gishi (treated villages) respectively. None-significant difference was observed between reduction rate of the disease incidence between Coumavec® and zinc phosphide treated areas (P> 0.05). As the Tabel 2 shows, the incidence of ZCL decreased in all intervention and control areas but the statistical analysis of the ZCL incidence showed that, the incidence reduction of ZCL in treated areas before and after the intervention were statistically different (P< 0.05) while no statistical differences was observed in the control areas (P> 0.05).

Table 1. Comparison of the number of rodent holes in the intervention and control villages, Esfahan County, Esfahan, Iran, 2011

Place	Treated area (hectare)	May 2011 before treatment	May 2011, 24 hour after burrow destruction	May 2011, One week after first baiting	June 2011	July 2011	August 2011
Mazraehshoor(intervention area with Coumavec®)	212	11032	1296	1557	724	644	668
Gishi(intervention area with zinc phosphide)	193	4729	1682	600	493	424	704
Vartoon(control area)	173	2297	196	281	365	557	1306

Table 2. Comparison of the incidence (per thousand) of zoonotic cutaneous leishmaniosis in the intervention and control villages, Esfahan County, Esfahan, Iran, 2011–2012

	2011		2012	
Name of the village	No. with active leision (s)	Incidence	No. with active leision (s)	Incidence
Mazreahshoor (intervention area with Coumavec®)	8	32.92	1	4.34
Gishi (intervention area with zinc phosphide)	11	39.14	3	11.40
Vartoon (control area)	3	18.40	2	10.92
Parvaneh-Ali abdchi (control area)	8	76.19	5	49.5

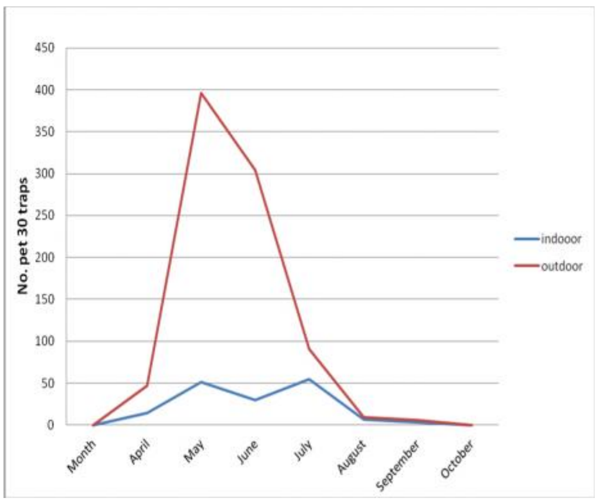

Fig. 1. Monthly fluctuation of *Phlebotomus papatasi* in treated village with Coumavec® (Mazraeshoor), Esfahan County, Esfahan Province, Iran, 2011

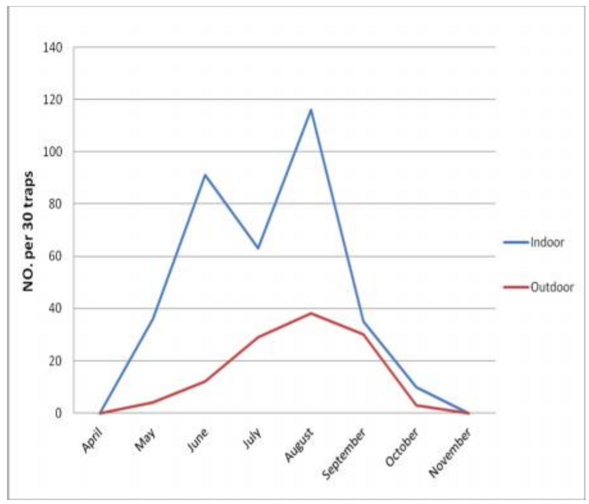

Fig. 2. Monthly fluctuation of *Phlebotomus papatasi* in treated village with zinc phosphide (Gishi), Esfahan County, Esfahan Province, Iran, 2011

Discussion

The main measures for ZCL control in Iran are rodent control operation using zinc phosphide bait, impregnated bed nets and curtains with pyrethroids, repellents, indoor residual spraying, health education to the community, and during emergency complex situation leishmanization was also used. Global efforts to develop an effective vaccine to prevent leishmaniasis so far showed no success (Noazin et al. 2008, 2009, Shirzadi 2010, WHO 2010, Saidi et al. 2012).

According to the data released by Zoonosis Department, Ministry of Health, and Medical Education of Iran, the trend of the disease is increasing during the last decade. There are several reasons for this increase which can be summarized as follows: improvement of surveillance system in the country, monitoring of the disease throughout the year, migration of people from non-endemic regions into the disease foci, presence of different reservoirs for ZCL and their migration to non-endemic areas, defect of proper prevention and control operation of the disease and vector control (Shirzadi 2010, Saidi et al. 2012). This evaluation showed that the control operation was effective for decreasing the population of gerbils and incidence of the ZCL in both treated areas. Both rodenticides were effective to control the rodent population. In treated area with Coumavec®, the operation did not show any effect after one week of baiting, while in the area treated with zinc phosphide the number of rodent holes decreased to 35.67%. This different effect of these rodeneicide is attributed to their mode of action, Coumavec® is an anticoagulant, which affects on rodents gradually within 1–2 weeks but zinc phosphide has rapid effect on the rodents. After finishing all stages of rodent control operation, the reduction rate of rodent burrows was 48.46% in the village treated with Coumavec® and 58.15% in the village treated with zinc phosphide. The number of rodent burrows in control area (Vartoon) with no control operation increased 6.66 folds by the end of the study.

Results showed that the number of rodent holes in the intervention areas had decreasing trend while in the control area had increasing trend.

The incidence of the disease decreased in the treated villages, therefore both rodenticides can be effective to reduce incidence of ZCL. The incidence of the disease from 2011 to 2012 showed a decreasing trend in all studied areas (both intervention and control areas) but it is interesting to point that the reduction rates of ZCL in treated areas were significantly different but in control areas non-significant differences were observed.

It seems that although both pesticides are effective on the control of the rodents but Coumavec® is less effective than zinc phosphide. Using these two different rodenticides depends on the field and disease situation. If the population of reservoir host is very high and the aim is reduction their population in short term, the use of zinc phsphide is recommended, but if the aim is keeping the population in low density during a complete control operation Coumavec® seems to be also effective. In the case of requirement of incidence reduction Coumavec® can be an appropriate alternative for zinc phosphid, if necessary.

In a previous study, along April to January 1997 a field trail was carried out to control ZCL by destruction rodent burrows and using 2.5% zinc phosphide baits in radius of 500 meters from houses once a month in May, June, July and September in central Iran. The results of this study showed that, the control program reduced the incidence of ZCL 12-folds in treated village compared to the control village at the end of the first year of operation and 5-folds at the end of the second year (Yaghoobi-ershadi et al. 2000). At the same intervention area, from 1999 to 2002, the numbers of active burrows were counted in May and October and were baited with zinc phosphate if the rodent holes numbers increased more than 30% at the same intervention area. The results showed that changes in the numbers of rodent burrows along the time in the intervention and control villages were statistically significant. Furthermore, significant difference in incidence of ZCL between the intervention and control village has been shown (Yaghoobi-Ershadi et al. 2005).

In Mazrehshoor, the numbers of the captured sand flies in outdoors were lower than indoor resting places. It seems that coumavec® affects the density of *P. papatasi* in outdoors. In contrast, in Gishi the number of collected sand flies in indoors was lower than outdoor resting places. Comparison of *P. papatasi* density trend in control and treated villages showed that rodents control operation has no significantly effect on the *P. papatsi* density.

It is concluded that both rodenticides were effective for controlling ZCL and rodent population as well. Coumavec® could be an appropriate alternative for zinc phophide especially when rodent behavioral resistance or bait shyness is observed.

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