

## Original Article

# Application of Flumethrin Pour-On on Reservoir Dogs and Its Efficacy against Sand Flies in Endemic Focus of Visceral Leishmaniasis, Meshkinshahr, Iran

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## Abstract

**Background:** Visceral leishmaniasis (VL) is one of the most important parasitic zoonotic diseases in the world. Domestic dogs are the main domestic reservoirs of VL in endemic foci of Iran. Various methods, including vaccination, treatment of dogs, detection and removal of infected dogs have different results around the world. General policy on control of canine visceral leishmaniasis is protection of them from sand fly bites. The aim of this study was evaluation of pour-on application of flumethrin on dogs against blood-feeding and mortality of field-caught sand flies.

**Methods:** Once every 20 days from May until September 2013, the treated and control dogs were exposed with field caught sandflies for 2 hours under bed net traps. After the exposure time, both alive and dead sand flies were transferred in netted cups to the laboratory. The mortality rate of them was assessed after 24 hours. The blood-fed or un-fed conditions were determined 2 hours after exposure to the dogs under stereomicroscope.

**Results:** The blood feeding index was varied from 12.0 to 25.0 % and 53.0 to 58.0 % for treated and control dogs respectively ( $P < 0.0001$ ). The blood feeding inhibition was 75.0–87.0 % and 41.0–46.0 % for the control and treated dogs ( $P < 0.0001$ ), respectively. The total mortality rate was 94.0–100 % and 19.0–58.0 % respectively for the treated and control groups ( $P < 0.001$ ).

**Conclusion:** Application of pour-on flumethrin on dogs caused 90–100 % mortality until 2.5 month and inhibited the blood-feeding of sand flies.

**Keywords:** Visceral leishmaniasis, Flumethrin pour-on, Sand fly, Control, Iran

## Introduction

Visceral leishmaniasis (VL) is the vector-borne disease and has great importance in public health due to the fatality among children. Although there is an increasing interest to VL but there is not enough approach for control measures. There are various epidemiological features of VL which indicating the needs for defining different control measures (WHO 1990). Kala-azar is a Mediterranean-

form in Iran which the *Leishmania infantum* isolated from VL patients (Mohebali et al. 2005), the sand flies species from subgenera *Larrousius* and *Paraphlebotomus* acts as vectors (Azizi et al. 2006) and the dogs and wild canids are considered as principal reservoirs (Edrissian et al. 1993, Mohebali et al. 2002). Asymptomatic infected dogs are suspected as the main reservoir host for sand

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flies to establish the transmission cycle to humans (Moshfeh et al. 2009). The confirmed endemic foci of VL are distributed in different provinces and districts of Iran including: Ardebil Province, East Azerbaijan Province (Kaleibar, Ahar, Azarshahr districts), Fars Province (Ghir-Karzin, Jahrom) Semnan Province, Bushehr Province (Poshtkooh), Qom Province, Kerman Province (Baft) and Alborz (Savejbolagh district) (Mohebbi 2013). Moreover, the sporadic cases are reported from different parts of Iran annually. The isolated parasite from animal reservoir was identified as *L. infantum* LON49 using biochemical assay (isoenzyme) in Ardabil and Azarbaijan Provinces. The isolated parasite is exactly the same strain which previously isolated from human cases, so it can certainly be considered that the canine family is the most important reservoirs for humans (Mohebbi et al. 2002).

Due to the lack of suitable VL vaccine, the interruption of the transmission cycle seems to be the most effective (Ramezani et al. 2009). At the present, the common control strategies between humans and carnivores VL (ZVL) due to *L. infantum* is limited to early diagnosis and treatment with expensive and potentially toxic pentavalent antimonial drugs (Killick-Kendrick et al. 1997). Treatment of infected dogs with meglumine antimoniate (Glucantime) may be led to decrease of sensitivity of *L. infantum* to these drugs (Gramiccia et al. 1992). Flumethrin is one of the pyrethroids of type II. There is no record for using of flumethrin on dogs as intervention method against control of sand flies in VL foci around the world. Continuous efforts for the control of leishmaniasis and the applicability of these studies will be needed more than ever in the New and the Old World. As surely as the vectors could be spread and transmitted the parasite from dogs to man (Braga et al. 1998). At the present, the methods that can prevent the dogs against visceral leishmaniasis

could not be prevented spreading of infected sandflies (Reithinger et al. 2004). Prophylactic soaps, shampoos and sprays with base of pyrethroids have been used on dogs with different success around the world (Ashford 1989). Yet, any protective measure which could protected completely the dogs against VL, is not introduced (Christopher 1996).

In the endemic area of the VL foci, it is recommended that all seropositive dogs are eliminated from infected areas. In southern Europe the prevalence rate of VL is about 40 % but the elimination of dogs has not been accepted in these communities. In the most endemic areas, the health policy administration system is based on the elimination of seropositive dogs (Dietze et al. 1998), but due to resource and financial limitations and failure of availability of necessary supplies are not be realized with suitable methods (David et al. 1998). In Europe, the chemical treatment of dogs using antimonial components have been used for dog treatment, but this method is not recommended due to drug resistant of the parasites among dogs. The drug therapy of VL among dogs may led to temporary treatment of infected dogs (Gramiccia et al. 1992, Alessandro et al. 1997) but this drugs do not prevent recrudescence of VL. Important notice that the use of these drugs does not stop the infection of sand flies that feed on infected dogs (Alvar et al. 1994). Application of residual insecticides is advisable for the control of endophagic sandflies in human dwelling, stables, poultries (Marcondes and Nascimento 1995, Jin et al. 2004) but are not applicable for the exophagic sandflies which include most of VL vectors that their resting places is outdoor (Jin et al. 2004).

The present study was carried out in order to evaluate the pour-on formulation of flumethrin on owned dogs in Meshkinshahr district during the activity of sandflies and effects of this method was assessed based on blood-feeding and mortality of exposed sand

flies treated dogs.

## Materials and Methods

Totally 118 dogs of the same race and gender were selected. Due to higher age (over one year) 3 dogs was excluded the study. The blood samples was prepared on filter paper in order to detect the positive cases of VL among selected dogs using Direct Agglutination Test (DAT) which was done in Laboratory of Medical Parasitology, School of Public Health, Tehran University of Medical Sciences, Iran.

Before application of flumethrin, the parazyquantel and albendazole was administered to the dogs in order to protect the research team and it is recommended to the family to bury the dogs' feces up to 48 hours in a sanitary manner. The location of experiments in Mazrae-Khalaf village was selected in front of the house where the cattle stables as well as the nest of sheep dog placed adjacent to it. The bed net size of 2 x 2 x 2 meters was installed and a sheet was spread on top of dogs for collection of knockdown sand flies. The live sand flies were collected using suction tube from indoor and outdoor early morning as well as evening and transferred to the laboratory and fed on 5 % sucrose solution during three successive days. The sugar pad was removed 12 hours before test. At the dusk (19:30 PM) which is synchrony with activity of sand flies, first the control dogs and then the treated dogs were anesthetized with xylazine and transferred to the inside of each net. After this stage, 150 live sand flies were released inside each bed net and the environmental condition including temperature and humidity was recorded. Subsequently the exposed sand flies were transferred to the paper caps covered with net according to physiological conditions eg blood-fed or unfed. The blood feeding condition was

observed immediately after 2 hours after experiments. The mortality rate of sand flies was calculated after 24 hours.

Data were analyzed using paired *t*-test under SPSS ver. 18.

## Results

- 1- Direct Agglutination Test (DAT):** Before pour-on application of flumethrin, titer of the serum in dogs was determined indicating negative. For determination of flumethrin effect on vector, two indicators were considered in relation to blood feeding and viability of exposed sand flies compared to control group.
- 2- Blood feeding index of sandflies:** The blood-feeding index (BFI) of sand flies were 345 out of 620 (55.6%) and 105 out of 614 (17.1%) from 20 up to 80 days post-treatment in control and treated groups (Table 1). The BFI was varied from 56.5 to 53.5 % and from 12.3 to 25 % respectively in control and treatment groups (Fig. 1). The difference between BFI in control and treated groups was significant ( $P < 0.0001$ ).
- 3- The effects of application of pour-on flumethrin on inhibition of blood-feeding (IBF) of sand flies:** Application of pour-on flumethrin during the peak of blood-feeding activity of sand flies were assessed at four interval times post-treatment (Fig. 2). The IBF of sand flies on treated dogs was 75.0 to 87.7 % and on untreated dogs was 46.5–41.3 % respectively. The IBF of sand flies was statistically different between treated and control dogs ( $P < 0.0001$ ).
- 4- Estimation of mortality rate:** The mortality rate of unfed and blood-fed sand flies was studied. The total number of

dead blood-fed sand flies was 115 out of 620 in treated dogs and 100 out of 614 respectively in control and treated groups. The variation of mortality rate in the dead blood-fed sandflies during four interval times was 25–32 % and 15–38 % respectively in control and treated groups (Table 2, Fig. 3).

The mortality rate of dead unfed sand flies was varied from 5 to 51 % and 94.8 to 100 % respectively in control and treated groups. The difference in total mortality rate in control and treated sand flies was significant ( $P < 0.001$ ).

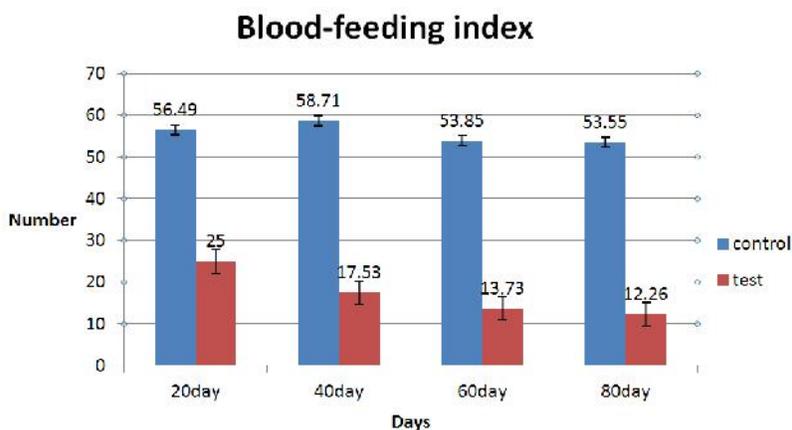
**5- Estimation of the combined effect of flumethrin pour-on:** The combined effect of inhibition of blood feeding and mortality rate of sand flies exposed to treated dogs compared with control group. The combined effect was varied between 94.8 to 100 % and 19.5 to 53.3 % respectively in control and treated groups. The difference of combined effect between four interval times of observation was not significant either treated neither group nor control except for 20 days in control group ( $P < 0.001$ ).

**Table 1.** Blood-feeding index (BFI) of sand flies after application of pour on flumethrin on dogs using bed net trap, Meshkinshahr, Ardabil Province, 2013

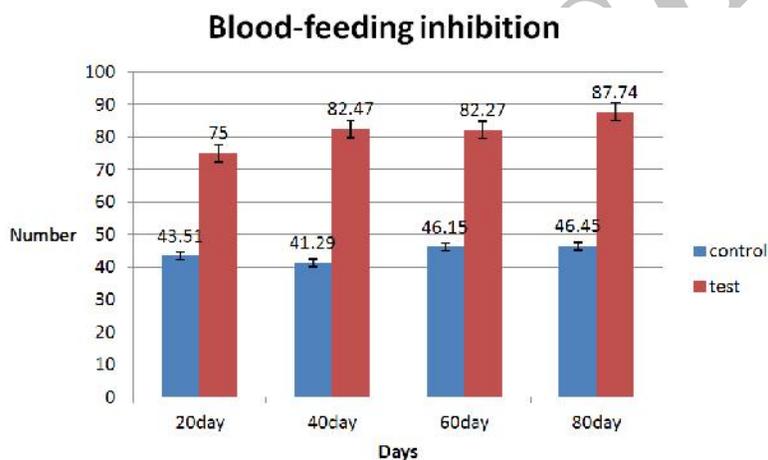
Days after spraying flumethrin	Kind of test	Total Number of sandflies tested	Number of blood-feeding sandflies	Blood-feeding index
20	control	154	87	56.5
	test	152	38	25.0
40	control	155	91	58.7
	test	154	27	17.5
60	control	156	84	53.9
	test	153	21	13.7
80	control	155	83	53.6
	test	155	19	12.3
total	control	620	345	55.7
	test	614	10	17.1

**Table 2.** Mortality rate of blood-fed and unfed sand flies after application pour-on flumethrin on dogs compared to control group using bed net traps, Meshkinshahr, Ardabil Province, 2013

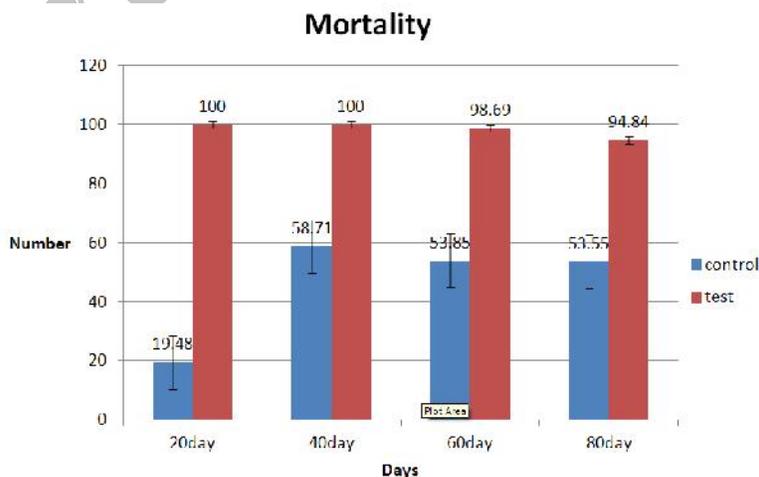
Days after application	Type of test	Number of sand flies tested	Number of dead blood-fed sand flies	Number of dead unfed sand flies	Total number of dead sand flies	Mortality rate
20	Control	154	25	5	30	19.5
	Test	152	38	114	152	100
40	Control	155	30	61	91	58.7
	Test	154	27	127	154	100
60	Control	156	28	56	84	53.9
	Test	153	20	131	151	98.7
80	Control	155	32	51	83	53.6
	Test	155	15	132	147	94.8
Total	Control	620	115	173	288	46.5
	Test	614	100	504	604	98.4



**Fig. 1.** Blood-feeding index (BFI) of sand flies after application of pour on flumethrin in dogs using bed net traps, Meshkinshahr, Ardabil Province, 2013



**Fig. 2.** Inhibition of blood-feeding rate (IBF) of sand flies after application of pour-on flumethrin on dogs, in bed net trap, Meshkinshahr, Ardabil Province, 2013



**Fig. 3.** Mortality rate of sand flies after application pour-on flumethrin on dogs using bed net traps, Meshkinshahr, Ardabil Province, 2013

## Discussion

In the North West, of Iran *Ph. kandelakii*, *Ph. perfiliewi*, *Ph. tobbi* and at the southern *Ph. keshishiani*, *Ph. alexandri*, *Ph. major* are the responsible for the transmission of the disease (Nadim et al. 1992, Sahabi et al. 1992, Rassi et al. 1997, 2000, 2005, 2012, Azizi et al. 2006, 2008, Oshaghi et al. 2009). According to the census of Iranian Center for Control of Disease, during the 1988 to 2005, the total number of recorded cases of VL in the country was 2056 cases which 624 of them (30%) related to Ardabil Province (Mohebbali 2013). In a seroepidemiological study to determine the prevalence of VL between the years of 2002 to 2005, the more positive cases related to the northwest, especially Ardabil Province (Mohebbali et al. 2006). In the foci of VL in Iran, the domestic dogs act as the the main reservoir (Mohebbali et al. 2002).

At the present, the control approach of VL due to *L. infantum* is reserved to early detection and treatment of the patients with expensive and potentially toxic of pentavalent antimonial drugs. In some countries, where the disease is endemic situation, the main control strategies has been established for indoor residual spraying as well as reservoir population control (infected dog culling). In Brazil, in spite of residual spraying of indoor places and annually elimination of the dogs, the trend of ZVL has been increased over the past 20 years (Marcelino et al. 1998). Methods for the elimination of dogs was questionable due to availability of facilities as well as social acceptance (Reithinger et al. 2004). Treatment of *Leishmania*-infected dogs with anti-*Leishmania* drugs was not effective due to recrudescence of VL (up to 74.0%) (Mohebbali et al. 2002). While waiting for a vaccine against *Leishmania*, the new ways switch to interrupt the VL transmission, by passing the dogs from livestock deltamethrin impregnated bath (David et al. 1998, Changfa et al. 1994, Guanghua et al. 1994) as well as

topical application of pyrethroid lotions. Epidemiological effects of topical application of insecticide on dogs, depends on not only reducing the number of sand flies blood-fed on dog, but also survival rate of sand flies infected with *Leishmania* (Reithinger et al. 2001). The main way for the control of VL is protection human and domestic reservoir from bite of sand flies (Poli et al. 1997).

Despite the nature of VL epidemiology which the special species of sand flies are responsible for transmission of the disease from infected dogs to others including human. Nevertheless due to specific behavior of the vectors, control of VL is based on control of reservoir population. In this relation, different methods including vaccinations, treatment of dogs, separation of infected dogs has been used with different results in the VL foci around the world (Ashford et al. 1998, Reithinger et al. 2001).

In the recent decade, the application of deltamethrin impregnated collars has been successfully used with high social acceptance as prevention measure for the dogs in different countries. The latter method has the repellency and killing effects against sand flies and so protected the dogs against VL (Ramezani et al. 2009). By application of pour-on flumethrin, the feeding rate of sand flies on treated dogs was 3.3 folds lower than control. The inhibition of feeding rate on treated dogs was 1.8 fold higher than controls respectively. The difference between total mortality of treated and control groups was highly significant ( $P=0.0001$ ).

In this study, the immediate mortality rate of sand flies was 5 fold more than control group during 2 hours exposure time. The delayed mortality rate of sand flies was known as a best indicator for the evaluation. The combined effect of pour-on flumethrin was 1.3 more than control group. The delayed mortality rate in blood-fed sand flies

was 94.8 % compared to 27.5 % in control group. There was no mortality of sand flies between unfed sand flies in control group.

## Conclusion

It seems the age of dogs is an important factor for pour-on application of flumethrin. The field observation showed high efficacy of flumethrin in younger dogs than elder. It also appears that inhibition of blood feeding or combined effects of flumethrin is prior to killing effect. According to the results, this method could be a good alternative for control of sand flies of VL with high social acceptance by dog owners.

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