

Field Trial of Autoclaved *Leishmania* Vaccines for Control of Canine Visceral Leishmaniasis in Meshkin-Shahr, Iran

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Summary

To detecting anti leishmania antibodies 152 domestic dogs of Ghourt-Tappeh in Meshkin-Shahr were tested by enzyme-linked immunosorbent assay, indirect immunofluorescence antibody and direct agglutination tests. 119 seronegative dogs without any clinical manifestations were randomly divided into four groups. Group 1 were received autoclaved *Leishmania infantum* (ALi) plus BCG. Group 2 were received autoclaved *Leishmania major* (ALm) plus BCG. Groups 3 and 4 received BCG and normal saline, respectively, as controls. Primary and secondary vaccinations were carried out intradermally. All the dogs were followed up for one year.

The results indicated that administrations of these vaccines plus BCG might effective in protection of dogs against visceral leishmaniasis.

Key Words: canine visceral leishmaniasis, immunization, ELISA, IFA, DA

Introduction

Canine visceral leishmaniasis (CVL) caused by *Leishmania infantum* is endemic in northwest and south parts of Iran (Edrissian *et al* 1998). Dogs are the main sources of infection for human visceral leishmaniasis and too many seropositive dogs are existing in the endemic areas (Bokai *et al* 1998). Since antivectorial measures have been reported unsuccessful (Duan's 1986), treatment of infected dogs are not recommended. Recrudescence following treatment infected dogs with antimonials is common, especially in animals with symptomatic infections (Tesh 1995). Besides in developing countries chemotherapy for infected dogs, due the cost, is not really a feasible approach (Manicianti 1988). In view of the growing public health importance of zoonotic visceral leishmaniasis (ZVL) and the mentioned problems in treatment and controlling it, a new strategy seems necessary. Recent studies (Abranches 1991, Duan's 1989, Pozio 1981, Tesh 1995, Ogunkolade 1988) on

naturally and experimentally infected dogs with *L.chagasi*, *L.infantum* indicate that some of them survive and develop cellular immune response, which probably results in resistance. These findings suggest that a canine vaccine against visceral leishmaniasis is quite feasible. The results of or recent experimental work on vaccination against CVL (Mohebali *et al* 1988) were be successfully.

In the present study the efficacy of autoclaved *L.infantum* (ALi) and autoclaved *L.major* (ALm) vaccines, with natural challenge of promastigotes of *L.infantum*, in domestic dogs was evaluated.

Materials and Methods

Vaccine preparation. ALm vaccine was prepared from the promastigotes of *L.major* (MRho/IR/76/ER, vaccine, Razi Institute, Iran). Promastigotes were grown in RPMI (Gibco, Grand Island, NY, USA) with 15% fetal calf serum (sigma, St. Louis, MO, USA) at 25C. Parasites were harvested on the duration of stationary phase, 16-20 days, by centrifugation at 3200 rpm for 30 min. Promastigotes were washed five times with pyrogen-free phosphate-buffered saline (PBS), pH 7.0-7.2. The sample was divided into small vials, autoclaved at 121C for 15 min and kept at 4C. The amount of protein to estimate number of parasites was measured according to Lowery (1951). ALi vaccine included autoclaved promastigotes of *L.infantum* (Strain MCAN/IR/94/LON 49) was prepared in protozoology section, school of public health, Tehran university of medical sciences according to the same procedure as that used for ALm.

Animal grouping. 152 domestic dogs of Ghourt-Tappeh, Meshkin-Shahr were selected randomly. In order to detecting anti leishmania antibodies three serological methods included ELISA, IFA and DA were carried out. 33 dogs, which were serologically positive with all these tests, excluded. 119 seronegative dogs, randomly divided into four groups as follows:

Group 1 included 22 dogs received ALi (1mg protein/dose)+BCG (400 µg /dose).

Group 2 included 23 dogs received ALm (1mg protein/dose)+BCG (400 µµg/dose).

Group 3 included 7 dogs received BCG (400 µg/dose).

Group 4 included 67 dogs received normal saline.

Dosages choosed based on Mohebali *et al* 1995, Momeni *et al* 1998, Sharifi *et al* 1998 studies. The vaccines were administrated intradermally and 30 day after primary vaccination the boosters injected. All the dogs followed up by 2 mon

intervals on a year. Before the first vaccinations and one year after the second, five ml of blood were taken from each have selected dog.

Challenge test. To determine efficacy of the vaccines the tested dogs were contacted by parasite-infected sandflies. Their antibody titers were evaluated by ELISA, IFA and DA assays.

Results

The results of serological tests after transmission cycle of parasite-infected sandflies, as a natural challenge, showed on table 1. Too many of dogs have been shown seroconversion by ELISA, IFA and DA tests. The $OD > 0.035$ and titers $> 1:320$, $> 1:80$ in above tests, respectively, were considered as positive and indicated of CVL infection.

Table 1. Results of serological tests after natural challenge

Groups	ELISA				IFA				DA			
	Positive*		Negative		Positive**		Negative		Positive***		Negative	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
ALi+BCG	7	31.8	15	68.2	6	27.3	16	27.2	5	22.7	17	77.3
ALm+BCG	7	30.4	16	69.6	10	43.5	13	56.5	5	21.7	18	78.3
Control (BCG)	4	57.1	3	42.9	7	3	4	57.2	7	2	45	67.4
Control (normal saline)	37	55.2	30	44.8	67	42	25	37.3	67	22	45	67.2
Total	55	46.2	64	53.8	61	51.3	58	48.7	34	28.6	85	71.4

* $OD > 0.035$ was considered positive.

** $> 1:80$ titres were considered positive.

*** $> 1:320$ titres were considered positive.

The results of serological and parasitological tests on five infected dogs after a transmission cycle of VL summarized on table 2. Before killing the infected dogs,

Table 2. Results of serological and parasitological tests in five infected dogs

Groups	n	Clinical Manifestations	Splneme-galy	Serological exam	Parasitological exam	
					Direct	Culture
ALi+BCG	1	+	+	+	+	+
ALm+BCG	1	+	+	+	+	+
Control (Normal saline)	3	+	+	+	-	-

two blood samples were taken from each dog. These samples were tested by ELISA and DA. The spleen, liver and popliteal lymph nodes of all dogs were cultured in a

Novy-Nicol-Mac Neal (NNN)+Liver infusion broth tryptose (LIT) medium and checked twice a week for six. The results following necropsy confirm the transmission of *L. infantum* during and after vaccination.

Table 3 shows the results of DA test on blood of 499 children under 12 years old, which live in that village, before and one year after the dog vaccinations. Of those 18 (7.8%) and 16 (5.9%) were showed anti leishmania antibody (>1:3200).

Table 3. Results of DA test in children (<12 yrs) before and one year after dogs vaccination

Genus	Before dogs vaccination				After dogs vaccination			
	No.	%	>1:3200		No.	%	>1:3200	
			No.	%			No.	%
Male	123	53.48	9	3.91	151	56.13	15	9.9
Female	107	46.52	9	3.91	118	43.86	1	0.9
Total	230	100	18	7.82	269	100	16	5.94

Discussion

The first vaccine trial against CVL was carried out by Monjour (1988) that used a *L. infantum* synthetic antigen was injected in 393 seronegative dogs in south of France. The dogs were followed for two years and no differences in rate of infection between vaccinated and unvaccinated groups were seen. Other trials using merthiolated ultrasound disrupted promastigotes of *L. braziliensis* vaccine+BCG were carried out on dogs by Genaro (1996).

The efficacy of autoclaved leishmania vaccine against CVL was evaluated by experimental challenge of promastigotes of *L. infantum* and *L. major* (Mohebbali *et al* 1998). It showed that ALi and ALm vaccines could protect dogs against active promastigotes. There was a significant difference ($P<0.05$) between vaccinated and unvaccinated groups.

In this study based on leishmania infection rate in control and treat groups the two vaccines plus BCG were effective, 22-24.5%, in protection of dogs against VL. In spite of vaccination, the serological and parasitological examinations and dissection of five dogs were indicated that active transmission of CVL infection persisted in Ghort-Tappeh. Similarity, there was no significant difference ($P<0.05$) in children before and after vaccination of dogs. The results confirm natural transmission between dogs, as source, and human especially children below 12, which sensitive to VL infection.

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References

- Aranches, P.(1991). An experimental model for canine visceral leishmaniasis. *Parasite Immunology* 13:235-550.
- Bokai, S., Mobedi, I., Edrissian, G.H. and Nadim, A.(1998). Seroepidemiological study of canine visceral leishmaniasis in Meshkin-Shahr, northwest of Iran. *Archives of Razi Institute* 48&49: 41-46.
- Duan's.(1989). Vaccination trials against canine visceral leishmaniasis. *Parasite Immunology* 11:397-402.
- Edrissian, G.H., Nadim, A., Alborzi, MD. and Ardehali, S.(1998). Visceral Leishmaniasis; the Iranian experience. *Archives of Iranian Medicine* 1(1): 22-26.
- Genaro, O.(1996). Immunization of dogs as reservoir control for human disease. Paper presented at the symposium on clinical trials of killed leishmania major vaccines. 16 April 1996, Jordan.
- Lowry, O.H.(1951). Protein measurement with folin phenol reagent. *Journal of Biological Chemistry* 193:265-75.
- Mancianti, F.(1988). Studies on canine leishmaniasis control. I evaluation of infection of different clinical form of canine leishmaniasis following antimonial treatment. *Transactions of the Royal Society of Tropical Medicine* 82:266-267.
- Mohebalı, M., Fallah, E., Jamshidi, S. and Hajjran, H.(1998). Vaccine trial against canine visceral leishmaniasis in Islamic Republic of Iran. *Eastern Mediterranean Health Journal* 4(2): 234-238.
- Mohebalı, M., Javadian, E. and Hashemi-Fesharki. R.(1995). Trial of non-living crude vaccine against zoonotic cutaneous leishmaniasis. *Medical Journal of Islamic Republic of Iran* 8: 211-215.
- Momeni, A.Z., Jalayer, T. and Emamjomeh, M.(1998). A randomized, double-blind, controlled trial of killed *L.major* vaccine plus BCG against zoonotic cutaneous leishmaniasis in Iran. *Vaccine* 17: 466-472.
- Monjour, L.(1998). Immunization of dogs with *Leishmania infantum*. derived vaccine. *Veterinary Parasitology* 23:33-37.

Ogunkolade, B.W.(1988). Immunization of dogs with a *Leishmania infantum*, derived vaccine. *Veterinary Parasitology* 28:33-41.

Pozio, E. (1981). Leishmaniasis in Tuscany (Italy). *Acta Tropical* 38:383-393.

Sharifi, I., Fekri, A.R., Aflatonian, M.R., Khamesipour, A. and Nadim, A.(1998). Randomized vaccine trial of single dose of killed *Leishmania major* plus BCG against anthroponotic cutaneous leishmaniasis in Bam, Iran. *The Lancet* 351:1540-1543.

Tesh, R.(1995). Control of zoonotic visceral leishmaniasis is it time to change stratiges. *American Journal of Tropical Medicine* 52:287-92.