

Emergence of a new focus of anthroponotic cutaneous leishmaniasis due to *Leishmania tropica* in rural communities of Bam district after the earthquake, Iran

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Summary

OBJECTIVES To describe a new emerging focus of anthroponotic cutaneous leishmaniasis (ACL) due to *Leishmania tropica* in rural areas of Dehbakry county, south-eastern Iran, after the earthquake of 2003.

METHODS House-to-house survey of 3884 inhabitants for active leishmaniasis lesions or scars. The diagnosis was confirmed by smears, cultures and identification of the parasite by polymerase chain reaction (PCR).

RESULTS All age groups were affected, although patients ≤ 10 years of age showed the highest rate of infection ($P = 0.0001$). The overall prevalence rate was 5.3%; 6.3% in females and 4.3% in males. Of 204 cases, 1.8% had active sores and 3.5% had scars, with a significant difference between the sexes ($P = 0.005$). 47% of the lesions were on the face and 77.9% had one lesion. The incidence rose gradually 2004–2005, but grew exponentially 2006–2008. Electrophoresis of PCR products indicated that *L. tropica* was the causative agent.

CONCLUSIONS The current emergence was unexpected in this rural locality, where no previous history of CL was recorded. According to our knowledge this is the first report of a gradually establishing new ACL focus in rural communities after the 2003 earthquake.

keywords anthroponotic cutaneous leishmaniasis, *Leishmania tropica*, epidemiology, Iran

Introduction

Cutaneous leishmaniasis (CL) is an important disease with a wide spectrum of clinical manifestations, posing a public health problem in many parts of the world. It is caused by several pathogenic species of *Leishmania*. 90% of CL cases occur in seven countries: Afghanistan, Algeria, Brazil, Iran, Peru, Saudi Arabia and Syria (Desjeux 2004). Cutaneous leishmaniasis has been present in Bam for decades. Two epidemiological forms of CL occur in Bam district: anthroponotic CL (ACL) is mainly limited to the city, the causative organism is *Leishmania tropica* and the main reservoir is man (Sharifi *et al.* 1997). Zoonotic CL is less common in the communities around the city of Bam. The parasite and the main animal reservoir are *L. major* and gerbils respectively (Sharifi *et al.* 1997, 1998a).

After the massive earthquake of Bam in December 2003 the prevalence of CL cases in the city of Bam increased in school children from 2% in 2005 (Aflatoonian & Sharifi 2006) to about 5% in 2008 (Sharifi *et al.* 2009). The

purpose of this work was to explore the frequency and the magnitude of CL disease in the affected villages. This information is needed for implementation of future control measures and current therapeutic strategy.

Simultaneously, Bam health authorities detected human cases of CL in Dehbakry County, west to the district. According to the health system officials there had not previously been any record of CL from this region. From November 2004 there was an outbreak of CL in central villages of the county. The epidemiological features of the diseases were unknown. Therefore, a survey team was appointed to carry out an epidemiological study in the affected area.

Materials and methods

Study area and population

This study was taken place in the summer of 2008 in villages of Dehbakry county, Bam district, Kerman province. This

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mountainous rural region along the Zagros mountain range lies at altitudes from 1700 to 2000 m. The county consists of 34 villages located 60 km west of Bam city. The climate is moderate, with mild summers (25–35 °C) and winters (–5 to 10 °C), which makes the area attractive for residents of Bam. The population is about 4000. After the earthquake in 2003 nearly 90% of the Bam city infrastructure was destroyed, creating 10 million tons of rubble.

Sampling and examination

A house-to-house census of the entire population was conducted by a physician and two health assistants. Household members were interviewed and examined for the presence of active lesions or scars. A questionnaire was completed for each individual, recording demographic and CL status including sex, age, area of residence, type, number and location of CL lesions and history of contracting the disease.

Smear preparation and culture

Smears were prepared by scraping skin taken from the periphery of active lesions by sterile scalpel blades on glass slides, fixing with methanol and staining with standard Giemsa. Simultaneously, skin scrapings were cultured into tubes containing Novy–Nicolle–McNeal medium at 24 °C for 7 days, then transferred into RPMI 1640 medium (Gibco, UK) containing 15% heat-inactivated fetal calf serum, streptomycin (200 µg/ml) and penicillin (200 units/ml), and incubated at 24 °C. Cultures were checked weekly for promastigotes for up to 4 weeks for further identification of species by polymerase chain reaction (PCR).

Molecular identification

DNA was prepared from 26 randomly selected clinical isolates. The extraction protocol followed the method described elsewhere (Mahboudi *et al.* 2001).

Ethical Considerations

This work was reviewed and approved by the Ethics Committee of Kerman University of Medical Sciences. Before the survey began, several meetings were held with villagers, community leaders and health authorities to describe the potential benefits and risks. Verbal informed consent was obtained from the suspected CL cases. All patients were treated free of charge with appropriate drugs.

Statistical analysis

The data were statistically analyzed using SPSS software. The chi-square test was used to determine any significant difference between demographic factors and disease prevalence. Statistical significance was defined as $P < 0.05$.

Results

Three thousand eight hundred and eighty four individuals aged 2–97 years (mean 30 years) were physically examined for the presence of active lesions or scars resembling CL (Table 1). All age groups were affected; patients ≤10 years of age showed the highest (9.3%) and 21–30 years the lowest rate of infection (2.9%). Overall, there was a significant difference between ≤10-year-old children and other groups ($P = 0.0001$).

Altogether, the prevalence rate of active infections and scars was 5.3%; 6.3% in females and 4.3% in males. Of 204 cases, 69 (1.8%) showed active sores and 135 (3.5%) scars (Table 2). There was a significant difference in rate of sores and scars between the sexes ($P = 0.005$).

The face (47%) was the most common site of involvement, followed by the hands (42%), the legs (5.8%) and other parts of the body (5.2%). Most CL cases (77.9%) had one lesion and the remaining more than two lesions. The infection rate was 4.1% in local inhabitants, and 1.2% in non-resident individuals ($P = 0.0001$). The first two cases of active lesion occurred in 2004, and another three

Table 1 The prevalence of anthroponotic cutaneous leishmaniasis in different age and sex groups in rural communities in Bam district, south-eastern Iran

Age (years)	Female		Male		Total	
	No. infected /No. examined	Percent	No. infected /No. examined	Percent	No. infected /No. examined	Percent
≤10	34/334	10.1	29/340	8.5	63/677	9.3
11–20	26/402	6.5	21/413	5.1	47/815	5.8
21–30	15/389	3.9	8/402	2.0	23/791	2.9
31–40	20/265	7.5	11/259	4.2	31/524	5.9
>40	25/520	4.8	15/557	2.7	40/1077	3.7
Total	120/1913	6.3	84/1971	4.3	204/3884	5.3

I. Sharifi *et al.* A new focus of anthroponotic cutaneous leishmaniasis in Iran**Table 2** The prevalence of active lesions and scars in rural communities in Bam district, south-eastern Iran

Sex	No. surveyed	Active lesion		Scar	
		No.	Percent	No.	Percent
Female	1913	39	2.0	81	4.2
Male	1971	30	1.5	54	2.7
Total	3884	69	1.8	135	3.5

in 2005, but the majority of cases emerged 2006–2008. The overall age of scars was 1.5 years across sexes.

Of 34 villages surveyed, 22 had no infection, seven had a fairly low infection rate (1%) and the remaining five villages had a higher rate of infection (4.3%). The latter villages are located along the main road connecting the city of Bam and the neighbouring district, Jiroft.

Extracted DNA from promastigotes of 26 isolates displayed a fragment of 800 bp in electrophoresis of PCR products, indicating *L. tropica* as the causative agent (Figure 1).

Discussion

Cutaneous leishmaniasis in Bam had become a focus of interest since 1993 when vaccine trials of single and multiple doses of autoclaved *L. major* vaccine against ACL were conducted (Sharifi *et al.* 1998b). In 2003 an earthquake caused 10 million tons of rubble, creating suitable

conditions for propagation of sand fly vectors. Ever since the epidemiology of CL has been changing with the emergence of new foci and non-healing clinical forms, persisting for a long period of time (Esfandiarpour & Dabiri 2007; Sharifi *et al.* 2010). Hence strict control measures including insecticide spraying, health education and treatment of cases have been a high priority. Nevertheless, the rate of the CL cases has been increasing throughout the district (Sharifi *et al.* 2009).

All age groups were affected, but especially children ≤ 10 -year-old due to their higher exposure and susceptibility. Perhaps children are less disciplined in covering up during the nights, when transmission occurs. Why females showed a higher infection rate than males is unclear, but could be due to a variety of environmental and behavioural factors (Desjeux 2001) or genetic or immunological characteristics. The average age of scars was 1.5 years, providing further evidence that the present focus was a new occurrence among a non-immune population, unexpected in this rural locality without a history of CL prior to the earthquake. The increasing CL trend in endemic focus afterward (Sharifi *et al.* in press) and intensified travel from Bam to rural areas may have contributed to CL introduction in Dehbakry County. The recent establishment of new settlements, expansion of villages, development of previously uninhabited areas, poor waste disposal and heaps of construction waste constitute other risk factors (Tayeh *et al.* 1997; Desjeux 2001). Similar factors have contributed to the emergence of new ACL foci in Sudan (El-Safi & Peters 1991), Syria (Tayeh *et al.* 1997), Israel (Jacobson

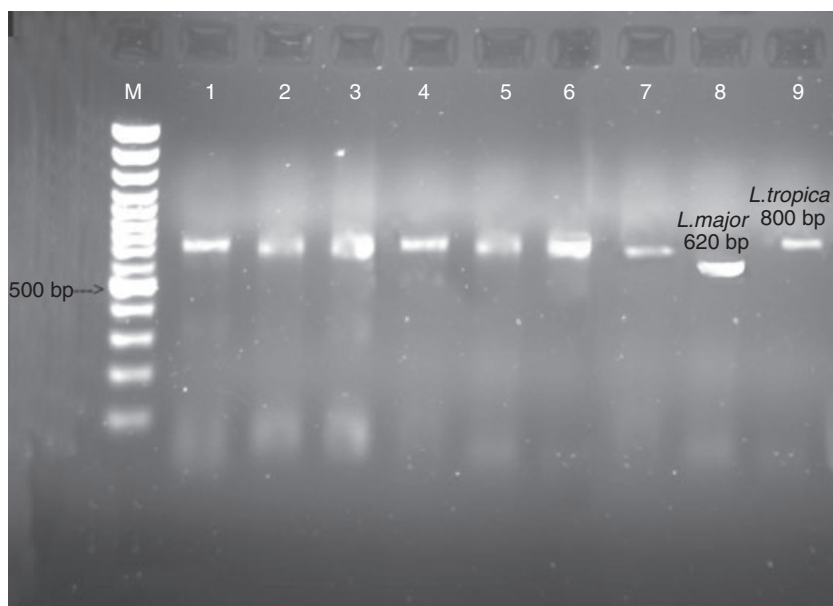


Figure 1 Molecular characterization of anthroponotic cutaneous leishmaniasis isolates by PCR amplification of *L. tropica* kDNA extracted from promastigotes grown *in vitro*. Lane M; ladder marker of 100bp. Lanes 1–7 kDNA extracted from promastigotes of isolates displayed a fragment of 800bp. Lanes 8, 9 are *L. major* (620 bp) and *L. tropica* (800bp) of known isolates used as references.

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et al. 2003), Pakistan (Kolaczinski *et al.* 2004) and Morocco (Ramaoui *et al.* 2008).

In an enquiry from the community leaders two main related factors were identified: (i) movement of rural workers due to large-scale construction projects in Bam, where the infection is endemic, (ii) and transportation of the earthquake's ruins soil from Bam to the countryside. Enrichment and fertility of the agricultural soil and growing crops might transport or dislocate different stages of sand fly vectors. Therefore, the provision and creation of a new suitable environment might have played a role in propagation of the life cycle and transmission of the causative organism.

To our knowledge this is the first report to clearly indicate the gradual establishment of a new focus of ACL in rural areas after the earthquake. The clinical appearance of the lesions was consistent with *L. tropica* kDNA as detected by PCR, providing further evidence that the source of infection is probably the city of Bam, where ACL has been present for decades.

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