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A newly emerged cutaneous leishmaniasis focus in central Iran

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SUMMARY

Objectives: This study was performed to evaluate the epidemiological status of cutaneous leishmaniasis (CL) in the most important endemic foci of Qom Province, central Iran. The city of Qom is the largest center for Shi'a scholarship in the world and is a significant pilgrimage destination.
Methods: During 2006–2011, all suspected CL patients with skin lesion(s) referred to regional health centers of Ghomrood and Ghanavat regions, and all actively detected cases, were examined clinically and parasitologically for CL. Patient information was recorded and patients were categorized based on the number and size of the lesions. Odds ratios (OR) of different risk factors were calculated.
Results: A total of 849 (59.2% male, 40.8% female) confirmed cases of CL were enrolled; the average incidence rate of the disease was 14.9 per 100 000 people. During the study period 2006–2011, the trend in CL incidence showed no sudden variations in the areas studied, except for an outbreak of CL in 2009. *Leishmania major* was identified as the causative agent based on internal transcribed spacer 1 (ITS1) ribosomal DNA PCR analysis. During the study period, the age distribution of CL cases was relatively stable, with the majority (50%) of patients aged 1–25 years. Most cases (n = 468; 55.1%) had a single lesion and 82 (9.6%) patients had four or more lesions (range 1–29). The risk of developing multiple lesions was significantly increased in patients with seasonal jobs (summer workers) (p = 0.023; OR 1.516) and significantly decreased in patients who were affected in winter (p = 0.010; OR 0.398). The risk of developing large-sized lesions (>1 cm) was significantly increased in patients in the age groups >25 years (p = 0.001–0.015; OR 2.5–3.5) and decreased in patients with seasonal jobs (summer workers) (p = 0.005; OR 0.570).
Conclusions: The present data show the importance of CL as a health problem in suburban areas of Qom Province. In order to identify other epidemiological aspects of leishmaniasis in this area, studies on vectors and reservoirs are recommended. Since leishmaniasis caused by *L. major* is typically zoonotic, control measures should focus on rodents as the main reservoirs and *Phlebotomus papatasi* as the main vector. Awareness should be raised in the high-risk populations comprising people with diabetes, young adults (<25 years old), and those who work outdoors during the summer.

1. Introduction

Leishmaniasis is a protozoan parasitic disease caused by *Leishmania* species. It is estimated that in about 100 countries, approximately 350 million people are at risk of acquiring leishmaniasis and 12 million are infected; an estimated two million new cases occur annually.[1] The two most common clinical forms of the disease, cutaneous leishmaniasis (CL) and visceral leishmaniasis (VL), are mainly seen in 14 of the 22 countries of the World Health Organization (WHO) Eastern Mediterranean Regional Office (EMRO) region, including Iran.[2] Self-healing zoonotic CL (ZCL) due to *Leishmania major* and anthroponotic CL (ACL) due to *Leishmania tropica* are two known types of CL that are spread across most parts of Iran. In total, approximately 17 out of the 31 provinces of Iran are endemic foci for ZCL.[3] According to the official reports of the Ministry of Health, the average incidence rate of CL is usually between 20 and 40 cases per 100 000 population.[4] The endemic regions in the central and south-western parts of the country (including Yazd, Semnan, Fars, Ilam, Khoozestan, and Isfahan), with an average incidence of more than 150/100 000 population, have the highest rates of CL.[4] The number of reported CL cases increased from 13 729 in 2002 to more than 24 000 in 2006 and thereafter,[4] and the disease prevalence is increasing and new foci of CL are emerging in Iran.[5–7]

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The province of Qom is located in the center of Iran, 120 km southwest of the capital Tehran, and is the passageway to more than 17 provinces. Each year more than 14 million people travel to or pass through this province; about two million of them are pilgrims who may stay for a while in the city.[8] The city of Qom is currently the largest city for Shi'a scholarship in the world.[9] It is estimated that 10–20% of the total world Muslim population, and more than 38% of the local Muslim population of the Middle East, are Shi'a.[10] Shi'a Muslims consider Imam Ali as the rightful successor to Prophet Muhammad, and the first divinely appointed Imam.[10] There are an estimated 50 000 seminarians in Qom coming from more than 70 countries of Africa, Asia, the Middle East, and other parts of the world.[9] Thousands of immigrants from neighboring countries including Afghanistan and Iraq travel to Iran intermittently and also play a role in importing leishmaniasis to Qom. This huge number of travelers and immigrants make leishmaniasis a priority in health strategy planning for the province.

The most important foci of CL in Qom Province are the villages of Ghomrood and Ghanavat regions, and the first cases of CL were diagnosed in 1999 in an outbreak of leishmaniasis in Ghanavat region.[11] Based on observations, CL cases have constantly been referred from the province to regional health centers during recent years. However, there is no formal report on the endemicity of CL in suburban areas of Qom Province. Since ACL and ZCL have different features with respect to ecology, parasitology, entomology, and clinical characteristics,[12] epidemiological data are needed to establish effective control strategies in the region. This information could help in the integration of the surveillance of leishmaniasis into health system programs, monitoring of leishmaniasis trends during outbreaks, risk assessment for awareness of decision-makers and education of high-risk populations, investigation of fluctuations in vectors/reservoirs, and improving treatment strategies. This study reports the epidemic aspects of CL in Qom Province during recent years.

2. Materials and methods

2.1. The region

Qom is one of the 31 provinces of Iran and is situated between 50° 06′–51° 58′ E and 34° 09′–35° 11′ N, with an area of 11 237 km^2, covering 0.89% of the total land of the country. Qom Province is located in the central part of the country; it lies 120 km by road southwest of the capital Tehran and has one city, five counties, nine rural districts, and 256 villages[8] (Figure 1). Based on the most recent census of 2011, the province has a population of approximately 1 200 000, with 95.1% residing in urban areas and 4.9% in rural areas.[13] The climate of Qom Province varies from semi-desert to desert conditions; the annual rainfall in the last year was 86.9 mm and relative humidity ranged between 8.5% in June and 89.1% in December. Geographically, the province comprises mountainous areas, foothills, and plains, and in the last year, the minimum and maximum temperatures recorded were −14 °C in December and +47 °C in June.[8]

2.2. Population and sampling

This study was done during April 2006 to November 2011. All suspected CL patients with skin lesion(s) referred to regional health centers of the province were examined clinically and parasitologically for CL. In addition to this passive case detection, monthly routine house-to-house investigations in Markazi District (including Ghanavat and Ghomrood regions) for active detection of possible CL cases was done by trained staff during an outbreak in 2009. Furthermore, from 2006, in accordance with a Ministry of Health instruction, patients under treatment were actively followed up and visited in their homes to ensure treatment courses were fulfilled; possible side effects were recorded and other members of the family were checked for the onset of lesions. The population of the region is among the low income populations; patients would not be able to attend private clinics for CL, and even if they did, they would have to be referred to a health center for treatment services since Glucantime is only distributed in these health centers. Ghanavat and Ghomrood each has one health center. Leishmaniasis is included in the surveillance system, and the staff of these centers are informed of leishmaniasis, so we assume that every case of cutaneous leishmaniasis in the region should be diagnosed.

Patient information including demographic data and clinical history was recorded on specific forms. Using the average number of new cases that occurred each year during the study period as the numerator and the population at risk as the denominator, the average annual incidence rate of the disease was calculated. The size and the number of lesions were recorded at the time of diagnosis. For each lesion, two crossing diameters were measured using a metric caliper and the mean figure was recorded as the size of the lesion. If multiple lesions were present, all lesions were measured and a mean size was calculated and presented.

The skin was sterilized and exudates from the margins of the suspected lesions were taken, fixed with methanol, and stained with Giemsa, then examined under a microscope. Part of the lesion exudate was inoculated into Novy–MacNeal–Nicolle (NNN) medium overlaid with RPMI 1640 (Gibco Invitrogen, Carlsbad, CA, USA). This was incubated at 24 °C for 1 week and examined every day for parasite growth. Another sample was taken and transferred to 2-ml vials containing sterile phosphate buffered saline (PBS) and used for DNA extraction. The disease was diagnosed based on the clinical examination and microscopic observation of intracellular amastigotes in smear or promastigotes in NNN medium.

The most frequent type of treatment was local intra-lesional administration of meglumine antimoniate (Glucantime) followed by systemic Glucantime. The treatment responses and possible side effects in the patients who received standard therapy were recorded. Patients were categorized based on (1) the number of lesions: single lesion or multiple lesions, and (2) the size of lesions: small-sized lesion (<1 cm) and large-sized lesion (>1 cm). Odds ratios (OR) of different variables as risk factors in the outcome of disease were analyzed.

2.3. Molecular identification of parasites by PCR

2.3.1. DNA extraction

Parasite genomic DNA was extracted using the conventional phenol–chloroform procedure.[14] Briefly, 100 μl of each disrupted tissue sample was transferred to a 1.5-ml microtube containing 200 μl of lysis buffer (100 mM Tris–HCl, pH 8; 10 mM ethylene-diaminetetraacetic acid (EDTA), pH 8; 1% sodium dodecyl sulfate (SDS); 100 mM NaCl; 2% Triton X-100) (Sigma, St. Louis, MO, USA) with 20 μl proteinase K (100 mg/ml), vortexed, and incubated at 56 °C for 1 h. Three hundred microliters of phenol–chloroform (1:1) was added, vortexed, and centrifuged at 5000 rpm, 4 °C, for 5 min. The supernatant was transferred to a new microtube and chloroform extraction was performed again. An equal volume of isopropanol and 1/10 volume of 3 M sodium acetate (pH 5.2) was added to the supernatant, incubated at −70 °C for 15 min, and centrifuged at 12 000 rpm for 15 min; the precipitant was then washed with 70% ethanol by centrifugation at 12 000 rpm for 10 min. The pellet was air-dried and resuspended in 20 μl of distilled water and stored at −20 °C until use.

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