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## New endemic focus of cutaneous leishmaniasis in Pakistan and future epidemics threats

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## ABSTRACT

**Objective:** To get a clear epidemiological picture of cutaneous leishmaniasis in a new endemic focus, in order to address the most probable epidemic threats and its spread to the neighboring regions.

**Methods:** A total of 870 clinically diagnosed patients were included in the study after taking consent and filling questionnaires from each individual. The field Giemsa-stained slides and cultures were examined microscopically. The data was organized in tables and graphs and analyzed using statistical package SPSS V 21.

**Results:** The ratio of male was higher than females (499:371), cutaneous and mucocutaneous cases were 96.00% and 4.00%, and only 25.20% patient's used bed nets. A total of 57.10% had dirty places near their homes. Similar cases in the neighborhood and in families were 48.50% and 67.10% respectively. Only 87.35 % of Patients in the age range of 1–20 years received treatment. Up to five lesions per individual were noticed and 72.20% of them had symptomatic secondary infections. The lesions were observed on different sites of the body.

**Conclusions:** Leishmaniasis is neglected in Landi Kotal Khyber Agency, the reasons being prevailing security situations and political sensitivity. North Atlantic Treaty Organization forces in Afghanistan are in contact with Afghans and with local people of Landi Kotal for logistic purposes. They may act as carriers in co-spreading of the species. There are potential risks of cross border transmission, with the probability of future epidemics in the surrounding regions.

## 1. Introduction

Leishmaniasis, the third of the most important vector-borne diseases, is caused by a protozoan parasite of the genus *Leishmania*. The parasite is transmitted to human by the bite of sand flies. Leishmaniasis is one of the most neglected tropical diseases with current high worldwide incidence[1,2]. Globally, more than 12 million individuals get infection, about 350 million people are at risk and nearly 2 million new cases are reported annually worldwide according to World Health

Organization[3]. The disease is prevalent in sixteen developed and 72 developing countries. Overall 90% of cases are reported from 3 regions[4]: Sudan/Ethiopia/Kenya, India/Bangladesh/Nepal and Brazil with as many as 0.02 to 0.04 million deaths every year[5].

*Leishmania* infection originates from the bite of sand flies of the *Phlebotomus* spp found in Europe, North Africa, the Middle East, and Asia or of the *Lutzomyia* spp in the Southern USA to Northern Argentina[6,7]. About 30 species, subspecies of sand flies are documented vectors, and interestingly more than 40 additional species are believed to be involved in transmission of the disease, which is either anthroponotic or zoonotic depending on the main reservoir host[8].

In well-identified endemic areas, the incidence is high in children but it decreases after they are over 15 years which is probably due to the acquisition of immunity[9]. In most cases, the infection is confined to households, which is indicative of the short flight range of sand flies, anthroponotic transmission or genetic susceptibility[10,11]. The risk factors for the disease usually include sex pointing to behavioral patterns that increase vector

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All experiments were undertaken according to the Helsinki declaration and approved by the members of ethical committee working under the GSC (graduate studies committee) Quaid-i-Azam University Islamabad, Pakistan. Informed consent was obtained from adults individually and from parents of children included in study.

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exposure, age, house design, construction material and presence of domestic animals[12]. Self-healing of the lesions occurs within 3–9 months[13-15], in case of *Leishmania major* infection whereas lesions due to *Leishmania tropica* heal within 6–15 months[16]. Cutaneous leishmaniasis is prevalent in Khyber pakhtunkhwa, Baluchistan, Sindh and Punjab of Pakistan[17].

The target area of our study has been Landi Kotal in Khyber Agency which is a federally administered tribal region in the Northwest of Pakistan and is located between settled areas of Khyber Pakhtunkhwa Province and the Pak-Afghan border. The community of this area has no or very limited access to health facilities. The reasons are geo-political, cultural, and prevailing security situation. The aim of our study was to get a clear epidemiological picture of the disease in order to address the most probable epidemic threats and its spread to the neighboring regions.

## 2. Materials and methods

### 2.1. Target area of samples collection

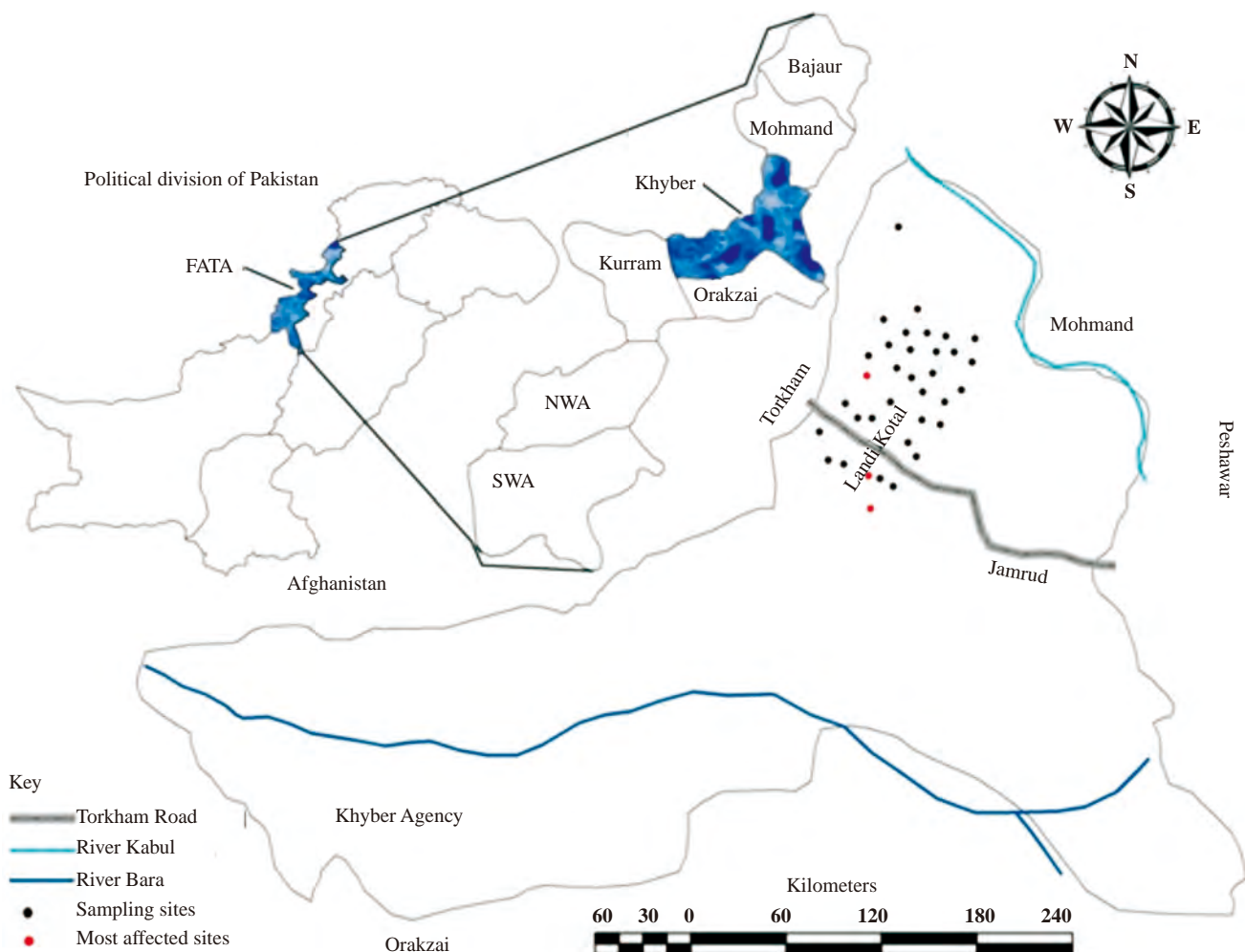
The samples were collected from different villages of Landi Kotal. The area with population of about 144714 is located in 10 km from Afghan border and is a federally administered tribal area.

It is the main route for North Atlantic Treaty Organization (NATO) supply to Afghanistan. The samples were collected from patients visiting General Headquarter hospital, Landi Kotal. Some of the samples were directly collected from patients during visits to the villages (Figure 1). The study was conducted from March 2013 to August 2013.

### 2.2. Samples collection

A total of 870 clinically diagnosed patients were requested for consents to participate in the study. Oral consents were obtained from family members. A structured questionnaire with information about village, gender, age, position of lesion, number of lesions, therapy, use of bed net, hygienic conditions, history of affected family members/neighbors and pain/purulence on or near lesions was filled by every patient.

The samples from clinically diagnosed patients were taken after cleaning the lesions carefully with 70% ethanol. Furthermore, 0.9% saline with a volume of 0.2–0.5 mL was injected into the lesion margin and resucked. A drop of exudates was used for smear preparation and the remaining was transferred to culture media (M199) supplied with 10% fetal bovine serum. The smears on slide were fixed with ethanol and stained with Giemsa stain.



**Figure 1.** Cutaneous leishmaniasis in Landi Kotal.

The dots represent different infected villages. Black dots show low level of prevalence and red indicates a high level of cutaneous leishmaniasis prevalence.

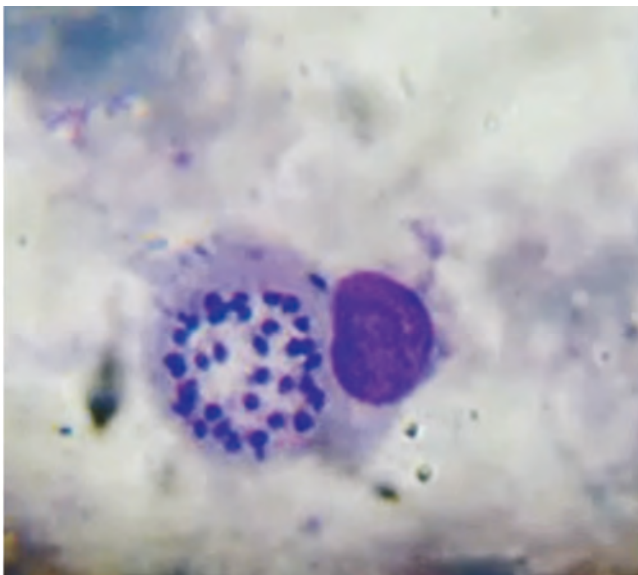
### 2.3. Laboratory method

The field Giemsa stained slides were examined microscopically. The data were organized in tables and graphs and analysed by statistical package SPSS V 21.

## 3. Results

### 3.1. Cutaneous lesions

All 870 slides were positive for leishmaniasis and had intramacrophagic amastigotes (Figure 2). During this study, two types of lesions were observed, the cutaneous and muco-cutaneous. The former type was found in 96% (835) patients, while the later was in only 4% (35) (Figure 3). The cases of muco-cutaneous type were greater in female children than in male. These lesions affected either nose or lips of patients.



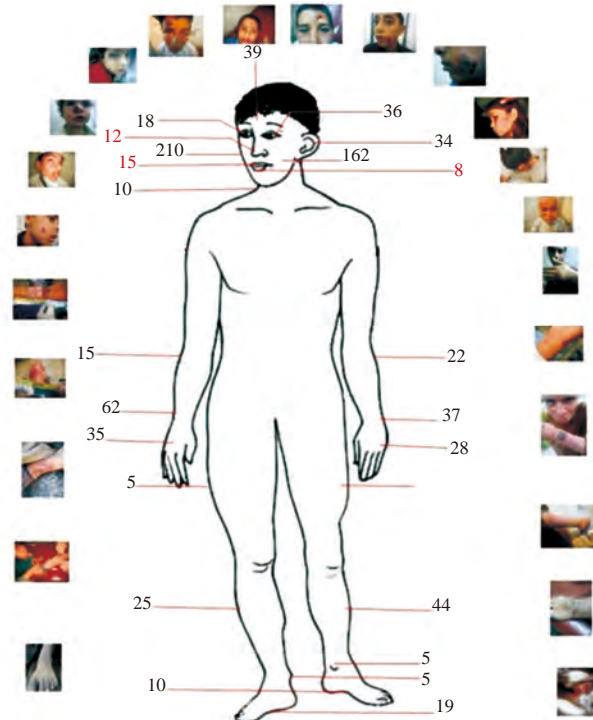
**Figure 2.** The intramacrophagic amastigotes in blood smear of cutaneous leishmaniasis patients at 100× magnification.



**Figure 3.** Muco-cutaneous lesion on lower lip (a), upper lip (b), nose (c), cutaneous lesions on multiple sites of face (d), ear (e) and on both feet (f).

The infected individuals had various number of lesions on different positions. Among them, 74.7% (650) had one single lesion, 17.4% (151) had two lesions and only six individuals suffered from 5 lesions each. Cutaneous lesions are usually developed on the exposed

parts of the body which is accessible to mosquitoes for blood meal. The highest incidence 62.5% (544) was on the facial and neck region followed by hands with 19.4% (169). Patients with lesions on their legs were 12.4% (108) while lesions on other parts (abdomen, back side of the body, shoulders, etc.) of the body were only 5.6% (49) (Figure 4).



**Figure 4.** The sites of body where cutaneous lesions were observed. Each number on labeled sites shows the repetition of lesion at the same site in different individuals. Blue numbers indicate cutaneous while red demonstrate muco-cutaneous lesions. Lesions on dorsal sites of the body are not shown.

### 3.2. Gender and age-wise lesion distribution

The infection in male population was higher (57.4%) as compared to female population (42.6%). In addition, 87.35% (760) infected individuals were below 20 years old. Among this group, 57.47% (500) were male and 42.53% (370) were females (Table 1).

**Table 1**

The distribution of lesion in different age range of both genders. [n (%)].

Age (years)	Male	Female
1–10	317 (36.43)	226 (25.97)
11–20	134 (15.40)	83 (9.54)
21–30	30 (3.44)	34 (3.90)
31–40	5 (0.57)	16 (1.83)
41–50	4 (0.45)	6 (0.68)
51–60	7 (0.80)	6 (0.68)
61–70	3 (0.34)	1 (0.11)

### 3.3. Use of mosquitoes net

The use of bed net was recorded and 74.8% (651) did not use bed net and only 25.2% (219) used ordinary mosquitoes net. The area

under investigation is socio-economically low and is, therefore, unhygienic. The residential as well as guest sitting places are made up of clay and stones, which provide shelter for sand flies. About 57.1% (497) of the infected individuals lived in unhygienic conditions while 42.9% (373) of the patients have no flies' possible breeding sites near their homes. Cowsheds and dried cow dung provide ideal conditions for oviposition and breeding of sand flies (Figure 5).



**Figure 5.** The dung cakes pasted on the walls near the houses (a), waste discharged in front of houses made up of clay (b and c) and the dumpy water places in the surrounding of living places (d).

### 3.4. Play grounds risk

The high incidence 48.5% (424) of leishmaniasis in teenage males is evident from the fact that they gather in the evening in the so-called designated play grounds. Such places are usually surrounded by rubbish, dry cow dung or animal sheds where sand flies get shelters (Figure 6).



**Figure 6.** The play ground between houses where dung cakes and other waste things are present.

### 3.5. Cutaneous leishmaniasis in family members

Leishmaniasis cases in the individuals' families were also recorded. It was found that 77% (374) of the families had one, 103 families had two, 10 families had three, 39 families had four and 58 families had five infected members.

### 3.6. Treatment history

The available treatment for leishmaniasis is the sub-cutaneous administration of sodium stibogluconate, amphotericin B, pentamidine and paromomycin. Thermotherapy is also practiced and is effective in the treatment of cutaneous leishmaniasis[18]. Among the infected individuals, 31.1% (271) were never exposed to any treatment, only 26.6% (231) were subjected to chemotherapy, about 27.4% (238) patients were subjected to thermotherapy and 14.9% (130) have experienced both. The chemotherapy could not result in significant improvement.

### 3.7. Secondary infection

A total of 27.8% (242) patients did not complain about any pain or purulence in and around the lesion whereas 72.2% (628) of the infected individuals had pain and/or purulence in their lesions indicating secondary infection.

## 4. Discussion

The muco-cutaneous infection may be due to the introduction of the new world species resulting from cross border movement, which is a documented risk factor. In 1997, the migrants from Kabul was the source of anthroponotic cutaneous leishmaniasis outbreak in an Afghan refugee settlement in northwestern part of Pakistan[19]. In future, there is a possibility that people of this region involved in the NATO supply may also become a source of introducing old world cutaneous leishmaniasis in NATO forces, which may be transmitted to their respective countries.

The highest incidence of lesions was noted on the exposed surfaces of the body which were accessible for mosquitoes biting that's why most of the infected individuals have lesion on their faces or hands and feet.

The main reason for the difference of infection gender wise is the social norms of this area, where male members can move freely outside and female members are confined to their homes. The prevalence of the disease is greater in individuals with ages from 1 to 20 years and decreases in adults due to acquire immunity[20].

Commonly available bed nets are not sufficient for prevention from sand flies because of their small size. The risk can be reduced if fine mesh bed nets or nets impregnated with an insecticide such as permethrin or deltamethrin[21].

The environmental and unhygienic conditions in Landi Kotal have provided the favorable conditions for the survival of the vectors. It has adversely affected the region and may easily spread to other villages in the vicinity. Most of the infected individuals have same cases in their family members. It's because of the unawareness about the transmission of disease and short flight range of sand flies, which put the whole family at risk[22].

Those infected patients who have received chemotherapy or thermotherapy remain ineffective. The possible reason for this ineffectiveness could be the quality of drugs available locally or the unhygienic conditions of the patients which also lead to secondary infections in the lesion[23]. Previous studies have shown

that the possibility of secondary bacterial infections should be taken into consideration while diagnosing and treating cutaneous leishmaniasis lesions[24]. Ignoring of secondary infection of cutaneous leishmaniasis may prolong the treatment duration with the probability of no recovery.

The results indicate the low socio-economic conditions of the people in this region. The various risk factors observed in this study facilitate vectors population and thus affect transmission dynamics. Being potential carriers of the disease, the NATO forces, Afghans and people of Khyber Agency, are in constant touch for logistic purposes which may support cross-border transmission of old world *Leishmania*. The region needs proper attentions in terms of preventive and control measures at individual and community level. The potential risk for future epidemics in the surrounding regions is predicted with the possibility of the co-spreading of the disease.

### Conflict of interest statement

We declare that we have no conflict of interest.

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