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Cutaneous and Visceral Leishmaniasis: Parasites, Vectors and Reservoir Hosts in Endemic Foci of North Khorasan, Northeastern Iran- a Narrative Review

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ABSTRACT

Leishmaniases are tropical diseases caused by various types of intracellular protozoan species of the genus *Leishmania*. The infection is transmitted to vertebrate hosts by the infective bite of female sandflies. Depending on the location of the parasite in mammalian tissues and *Leishmania* species, leishmaniasis presents at least three primary clinical forms: visceral (VL), cutaneous (CL), and mucocutaneous (MCL). Iran is one of the significant endemic areas for CL in Asia. Here, we comprehensively reviewed the literature published from 2009 to 2019 and focused on the distribution, clinical aspects, causatives agents, reservoirs, and vectors of CL and VL in different parts of North Khorasan Province, northeast of Iran. North Khorasan Province in northeastern Iran is one of the principal foci of CL, but VL is just known as an endemic parasitic disease in some areas of this province. Based on a comprehensive literature search on studies published from 2009 to 2019, distribution, causatives agents, reservoirs, and vectors of CL and VL in different parts of North Khorasan Province were determined. Zoonotic cutaneous leishmaniasis (ZCL) due to *Leishmania major* is the main form of CL in this province. The principal animal reservoir hosts of the infection are rodents and *Ph. sergenti* is the most predominant species. VL, caused by *Leishmania infantum*, occurs in some parts of the province, and wild canines were confirmed as reservoirs.

INTRODUCTION

Leishmaniases are zoonotic infectious diseases caused by obligate protozoa of the genus *Leishmania*, among them, roughly 20 species are pathogenic for humans [1, 2].

Natural transmission to mammalian hosts, depending on geographic location, is usually by the infective bite of female *Phlebotomus* or *Lutzomyia* sandflies [1]. Leishmaniasis represents a significant public health problem over a wide geographical area [3]. Currently, the World Health Organization (WHO) recognizes leishmaniasis amongst the nine most important tropical and subtropical diseases occurring in all continents (Africa, Americas, Asia, and Europe) except Oceania [1, 3, 4]. The disease occurs in 98 countries or territories around the world, with 12 million cases at risk and 20,000-40,000 deaths per year [5, 6]. This complex disease is endemic in large areas of the tropics, subtropics, and the Mediterranean basin [2-4]. Leishmaniasis is becoming more common worldwide because of urbanization and vector distribution. Recently, some studies discussed the crucial role of traveling between endemic and non-endemic areas and the possibility of production of new *Leishmania* hybrid [2, 4]. In the Middle East, the most prevalent clinical form is the cutaneous form. About 90% of global CL cases occur in six countries, i.e., Afghanistan, Algeria, Brazil, Peru, Iran (the Islamic Republic of Iran), and Saudi Arabia and Syria [3, 7, 8]. Regarding the parasite species involved and the status of the host immune response, the disease appears with three main clinical manifestations, cutaneous, mucocutaneous, and visceral [1, 3, 4]. As mentioned before, CL is the most widespread form and most cases occurrs in the Americas, the Mediterranean Basin, the Middle East, and Central Asia. In Iran, CL appears in two epidemiological forms: zoonotic cutaneous leishmaniasis (ZCL) due to *L. major* with rodents as reservoir hosts, and anthroponotic cutaneous leishmaniasis (ACL) caused by *L. tropica* with humans and dogs serving as the primary and secondary reservoir hosts, respectively [3]. *Ph. sergenti* and *Ph. papatasi* are the vectors of ZCL and ACL, respectively [3, 9]. CL, with almost 20,000 new annual cases, is one of the most prevalent arthropod-borne parasitic diseases in the country [8, 10, 11]. Both forms are prevalent in rural and urban areas of Iran [7, 8]. *Ph. Papatasi* is the primary vector of *L. major* in rural areas of the country [7, 9]. In Iran, rodents are the leading animal reservoir and source of ZCL. The great gerbil, *Rhombomys opimus* serves as the primary

reservoir host of ZCL. Other species, including *Tatera indica*, *Meriones hurrianae, Meriones libycus, Meriones persicus,* and *Nesokia indica* contribute as reservoirs of ZCL too [7, 10, 12]. In Iran, *Ph. sergenti* has a wide distribution range. This species, with *Leishmania* infection, is prevalent in the northeast, central, south, and southwestern Iran [7, 8].

VL, the most severe systemic form of the disease, is reported from many countries in Asia, Africa, the Mediterranean Basin, the Middle East, Central Asia, South America, and Central America [1]. VL occurs in two types; anthroponotic VL and zoonotic VL or Mediterranean form, caused by *L. donovani* and *L. infantum*, respectively. The zoonotic form is endemic in countries of the Mediterranean region, including Iran [3]. Mediterranean form of VL with domestic dogs *(Canis familiaris)* as the principal reservoir hosts has been reported sporadically in all geographical zones of Iran, but the majority of VL cases are from northwestern and southern areas where about 100–300 new cases appear annually [1, 13-16]. This form, also known as infantile VL, occurs mainly (99% of the cases) in children of ≤ 12 years of age, and most VL cases in Iran are observed in this group as well [5, 13, 14, 17]. The causative agent of VL in Iran is *Leishmania infantum.* Domestic dogs (*Canis familiaris*) are primary reservoir hosts for this Mediterranean form of VL [13, 14]. Six sand fly species have been reported infected and are considered as the probable vectors of VL in the country. The species *Phlebotomus kandelakii*, *Phlebotomus perfiliewi transcucasicus,* and *Phlebotomus tobbi* in northwestern of the country, and *Phlebotomus major* s.l., *Phlebotomus keshishiani* and *Phlebotomus alexandri* in southern parts are the probable or proven VL vectors [13, 14]. The vectorial capacity of *Ph. tobbi* for *L. infantum* is suspected, but further research is needed [18].

This work is a comprehensive literature review of the studies published from 2009 to 2019 and focuses on the distribution, clinical aspects, causatives agents, reservoirs, and vectors of CL and VL in different parts of North Khorasan Province, northeast of Iran. This review includes related articles in the Medline/Pub Med, ISI, Scopus, and Google Scholar databases and Iran's databases, including IranMedex, SID, and Magiran. This review provides the latest information on CL and VL in North Khorasan province and can help in planning future strategies and developing control programs in this endemic region.

North Khorasan is one of the three provinces that separated following the division of Khorasan in 2004. Located in northeastern Iran, This province, covers an area of about 28434 $km^{2,}$ with a population of 867,727. It is mountainous and the climate is temperate with cold winters. North Khorasan shares a long border with Turkmenistan in the north. This province with Bojnord city as the capital includes eight counties of Shirvan, Esfarayen, Maneh and Samalqan, Raz and Jargalan, Jajarm, Faruj, and Garmeh (figure 1).

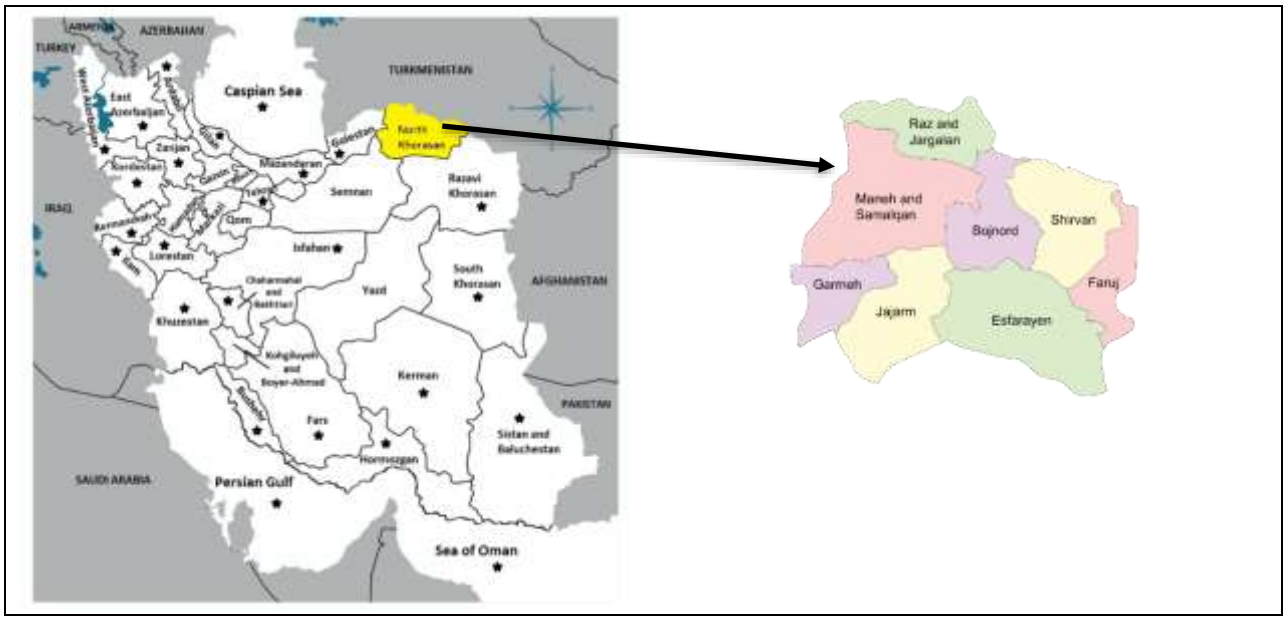

Fig. 1. Map of North Khorasan Province of Iran

Cutaneous Leishmaniasis in North Khorasan Province

a. **Distribution.** From 2005 to 2008, 1453 CL patients were recorded in North Khorasan Province, with more than one-third of the cases occurring in 2005. Most patients were adult men ≥ 15 years old. The Jajarm county, was the most active focus of CL in 2005, while the least cases were from the Faruj county in the same year. The dramatic increase in the number of cases was attributed to the displacement of the population [19] (Table 1).

CL cases occurred throughout the year, mainly in autumn from September to November. Between 2009 and 2011, a total of 485 unevenly distributed CL cases appeared in North Khorasan Province. Most cases (73.8%) were from Esfarayen (n=198) and Jajarm (n=160).

Table 1. Distribution of 1453 CL cases in different counties of Northern Khorasan Province from March 2005 to September 2008 (14)

District	3/2005-3/2006		3/2006-3/2007		3/2007-3/2008		3/2008-9/2008	
	n	%	n	%	n	%	n	%
Bojnord	75	13.5	45	11.8	54	12.3	12	15.4
Esfarayen	235	42.3	90	23.6	150	34.2	16	20.5
Jajarm	218	39.3	214	56.2	196	44.6	31	39.7
Shirvan	22	4.0	22	5.8	24	5.5	12	15.4
Maneh and Samalqan	4	0.7	4	1.0	6	1.4	2	2.6
Faruj	1	0.2	6	1.6	9	2.1	5	6.4
Total	555	100.0	381	100.0	439	100.0	78	100.0

About 60.2% of patients were men, and 39.8% were women. Human cases were more among inhabitants of small rural settlements or those with travel history to endemic areas of Iran. A control program against the disease was performed in the area [20] (Table 2).

Between 2006 and 2013, a total of 2831 CL cases occurred in the province, with most patients (38.7%) from Esfarayen. Most human cases were within the age group 16-30 years, and the majority were men who resided in the nearby villages [21]. Some surveys revealed that most records of CL were from Garmeh, Jajarm, Bojnord, and Esfarayen [19, 22]. Moreover, other studies in Esfarayen county showed that this city was the most crucial center for ZCL due to *L. major* [20, 23, 24].

Table 2. Distribution of 485 CL patients in different counties of Northern Khorasan Province between 2009 and 2010 (17)

District	2009		2010	
	n	%	n	%
Bojnord	30	13.3	35	13.5
Esfarayen	109	48.2	89	34.3
Jajarm	87	38.5	73	28.2
Shirvan	0	0	36	13.9
Maneh and Samalqan	0	0	13	5
Faruj	0	0	13	5
Garmeh	0	0	0	0

b. **Clinical manifestations.** Most of the cutaneous lesions were on the face and hands and rarely on lower limbs. Most patients had one lesion, but a few numbers exhibited two or three different skin ulcers [19-21].

c. **Causative agents.** The identification of causative agents in different foci using molecular tools revealed *L. major* in vectors and rodent reservoirs in North Khorasan Province [25, 26]. The parasite species identification in humans was not available, but the clinical and epidemiological data suggested *L. major* as the causing agent of ZCL [7, 24, 27]. In 2017 for the first time, a case of disseminated cutaneous leishmaniasis (DCL) in an opium abuser due to *L. major* was reported in the province, and leishmanial was identified using a PCR-based diagnostic assay [28]. Recently, *L. tropica* species, as well as *L. major*, were detected in CL cases in Esfarayen and Jajarm cities using PCR-RFLP analysis [29].

d. **Animal Reservoirs .**In 2014, a study focused on ZCL in Esfarayen county, revealed *L. major* in *R. opimus* (one of the main reservoirs of ZCL in Iran) by molecular methods [25, 27]. Also, *M. libycus* showed to serve as a secondary reservoir host of *L. major* [25]. Faunistic studies identified other rodent species, including *M. persicus, M. libycus, Mus musculus, and N. indica* in North Khorasan Province, but *Leishmania* parasites were not detected [22].

e. **Sandflies Vectors .**The sand fly fauna is very diverse in the east of the country. In North Khorasan Province, reports of the sand fly fauna and the species composition indicated *Ph. sergenti* and *Ph. papatasi* as the most prevalent species in this area [24]. Up to now, no reports of *Ph. sergenti* infection with *leishmania* parasite is available from the province, but the reports showed this sandfly as the most predominant species [26, 30].

Visceral leishmaniasis in North Khorasan Province

a. **Distribution .**A study on human VL in North Khorasan showed that the annual cases were on the increase and illustrated this region as a significant focus of VL in the country. From 1990 to 2010, about 160 VL cases in infants, children, and adults were recorded. Most cases (90.6%) were children under the age of 10, and the majority of the patients (74.4%) lived in rural areas. Some districts such as Shirvan, Bojnord, Maneh, Samalqan, and Raz va Jargalan were defined as endemic foci in the province [31] (Table 3).

A serological survey using the direct agglutination test (DAT) on 4608 sera of healthy inhabitants in eight settlements showed 38 seropositive individuals suggesting a low endemicity [32]. Since then, only one report on VL in this area was published that identified wild canines as VL reservoirs [33].

Table 3. Distribution of 160 VL patients in different districts of Northern Khorasan Province during 1990-2010 (15)

District	N	%
Bojnord	58	36.25
Shirvan	56	35
Maneh & Samalqan	29	18.1
Raz va Jargalan	14	8.75

b. **Clinical manifestations.** The clinical files of the VL patients in different parts of the province from 1990 to 2010 indicated that most patients presented with clinical manifestations including fever, splenomegaly, anemia, hepatomegaly, and weakness [31]. However, another study showed VL antibodies in healthy individuals living in the province [32].

c. **Causative agents.** No data on the identification of the parasites in human cases is available, but the application of serological tests was against *L. infantum* antibodies [32]. In 2018, an atypical case of DCL in a 26-year-old HIV-positive man was reported. The patient who was the first case of a *Leishmania*/ HIV coinfection in the province showed a mixed *L. infantum/L. major* infection by molecular tools [34].

d. **Animal Reservoirs.** Wild canines showed to be part of the sylvatic transmission cycle of VL in different areas of North Khorasan. In a study, among the wild animals examined for VL agents, four foxes and seven jackals were positive by serology, and one fox and one jackal exhibited parasite by microscopy and culture. The causative agent, *L. infantum* (Accession No.KM350534), was confirmed by sequence analysis of α-tubulin and GAPDH genes. Also, domestic dogs showed to serve a reservoir host of *L. infantum*-caused VL in different foci of the province [33].

e. **Sandflies Vectors.** In 2012, PCR amplification followed by sequencing of minicircle kinetoplast (k)-DNA, ITS1, and cathepsin B-like cysteine protease E/F genes identified *L. infantum* in two female *Phlebotomus kandelakii* captured in the Shirvan county of Khorassan [35]. Recently, some proven or suspected vectors of VL in Iran, including *Ph. caucasicus*, *Ph. alexandri* and *Ph. major* were reported from the study area [36].

CONCLUSIONS

This review covers a decade of research on CL and VL in North Khorasan Province. Our study provided information on CL and VL in this province as an active focus for leishmaniasis in northeastern Iran. The review shows that ZCL is endemic in various parts of the province, and *R. opimus* is the primary reservoir host of the disease. *Ph. sergenti* and *Ph. papatasi*, proven vectors of ZCL and ACL, have been identified in this area. VL in this province is in the Mediterranean form, similar to other parts of the country. Dogs and wild canids are the primary reservoir hosts for *L. infantum*. Our survey showed that leishmaniasis is a health concern in North Khorasan Province, and this province is one of the highly endemic areas in Iran. Therefore, appropriate measures such as health education, rodent and vector control, proper disposal of garbage are required to control this disease in this endemic region.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

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