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Review Article

Distribution of *Leishmania* Infection in Humans, Animal Reservoir Hosts and Sandflies in Golestan Province, Northeastern Iran: A Systematic Review and Meta-Analysis

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Abstract

Background: Leishmaniasis is one of the most important parasitic diseases in the Golestan Province, northeastern Iran. In this study, we summarize the results of studies on the disease and its causative agent Leishmania in humans, vectors and reservoirs in the Golestan Province, Iran.

Methods: We retrieved all articles related to leishmaniasis in the Golestan Province, northern Iran from 1994 to 2018 in various databases including PubMed, Scopus and Web of Science. Articles in Persian were retrieved from the IranMedex, SID and Magiran. Reference lists of relevant articles were also hand-searched. Local active researchers in the field of leishmaniasis were contacted to avoid missing any relevant articles. Overall, 54 papers were extracted, later evaluated by two research team members based on inclusion criteria. All analyses were performed using the Stata 14 software. Pooled prevalence was calculated using the metaprop command and a random-effect model. The I^2 statistic was used for measuring heterogeneity of studies.

Results: Ten articles in the province were related to detection of Leishmania species in patients with suspected cutaneous or visceral leishmaniasis. Fifteen articles were dedicated to identification of Leishmania species in disease reservoirs and five articles were about detection of the parasite in sand flies. The pooled prevalence of *L. major* in patients with acute ulcer, wild rodents and sandflies was 83%, 29% and 11%, respectively.

Conclusion: Our findings highlight the need for implementation of control measures among the reservoirs of both cutaneous and visceral leishmaniasis in the Golestan Province, Iran.

Keywords: *Leishmania*; Reservoir host; Sand fly; Systematic review; Iran

Introduction

Leishmaniasis is one of the most important vector-borne diseases transmitted to humans and other animals through bite of infected sandflies (1). It is also the third most important vector-borne parasitic disease after malaria and lymphatic filariasis (2).

There are three main forms of leishmaniases – visceral (also known as kala-azar and the most serious form of the disease), cutaneous (the most common), and mucocutaneous, it is estimated that 700000 to one million new cases of leishmaniasis and some 26000 to 65000 deaths occur annually. Moreover, about 600000 to one million people become infected with cutaneous leishmaniasis and 50000 to 90000 with visceral leishmaniasis (3).

Iran is facing both form of leishmaniasis: cutaneous and visceral and cutaneous leishmaniasis is reported in two forms: zoonotic cutaneous leishmaniasis (ZCL), anthroponotic cutaneous leishmaniasis (ACL) and Visceral leishmaniasi in zoonotic visceral leishmaniasis (ZVL) form. Iran is amongst the six countries where 95% of global cases of cutaneous leishmaniasis occur (3), and each year, more than 22,000 new cases of cutaneous leishmaniasis are reported in Iran, 80% of which are ZCL (4). However, the actual new cases is estimated to be 4-5 fold higher than these reports (5). Golestan Province is amongst the 17 provinces of Iran that are endemic for ZCL (5).
In these areas, *Leishmania major* is the causative agent, while *Phlebotomus papatasi* and wild rodents (*Rhombomys opimus* and *Meriones libycus*) are known as the vectors and reservoir hosts, respectively (5). In ACL foci, *L. tropica* is the agent, while *Ph. sergenti* and dogs and humans are known as the vector and reservoir hosts, respectively (5).
Every year, 100-300 new cases of ZVL are reported in Iran. The disease is sporadic in most parts of the country but has become endemic in the northwestern and southern areas. In these areas, *L. infantum* is the agent, domestic dogs are known as the main and potential reservoir hosts and wild canines (jackal, fox and wolf) have potential role in sylvatic transmission cycle of ZVL (6-8). *Ph. neglectus*, *Ph. kandelakii*, *Ph. keshishiani*, *Ph. perfiliewi transcaucasicus* and *Ph. alexandri* are considered as probable vectors for ZVL (9-13).

Currently, the health system of Iran's approach to cope with leishmaniasis has been mainly established on timely recording and reporting of cases, education, providing free rapid diagnosis and treatment of patients as well as implementation of rodents- and sandflies-targeted control measures.
The Golestan Province is known as the most important foci of leishmaniasis in Iran. ZCL is endemic and visceral leishmaniasis is sporadic in this province, particularly in its northeastern areas. Given the importance of leishmaniasis in this province, numerous studies have been conducted in this area on features of this disease. Therefore, we aimed to summarize results of these studies to contribute to decision-making for prevention, diagnosis, control and treatment of this disease in this area.

Methods

Study area

The Golestan Province (53°57'- 56°23' E, 36°30'-38°08' N) is located northeast of Iran, bordering the Caspian Sea and Turkmenistan. The province has a dry and semi-arid climate in the north and northeastern parts, a temperate climate in the central parts and a mountainous and cold climate in the southern parts. It comprises 14 counties and has an area of 20437.74 Km² (Fig. 1).

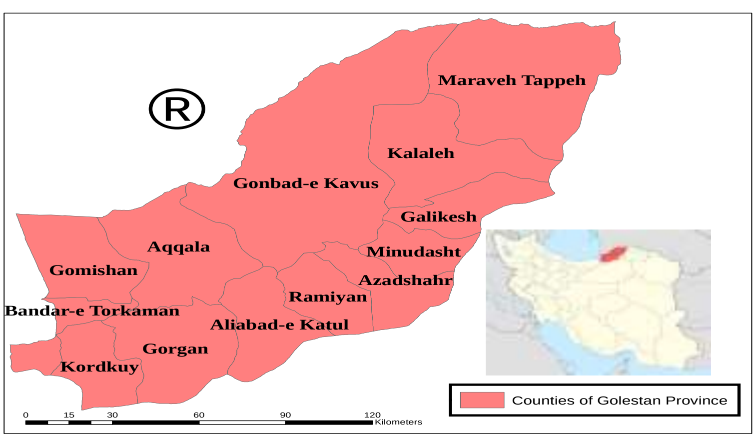

Fig. 1: Location of the Golestan Province and its counties

This study has been designed and performed according to the MOOSE guidelines for meta-analyses and systematic reviews of observational studies.

Search strategy

First, the following search terms were determined for finding articles related to leishmaniasis in the Golestan Province from 1994 to 2018: Golestan, Gonbad-e Kavus, Maraveh Tappeh, Turkmen Sahra, Leishmaniasis, Leishmania, Kala-azar and sandfly. Since some of the words may have different spelling, the following search syntax was used: (Leishmanian* OR Kala*azar OR Sand*fl*) AND (Golestan OR Turkemen* OR Gonbad* or Maraveh*).

The search was performed using the following databases: Medline, PubMed, Scopus and Web of Science. Related articles in Persian were retrieved from the SID, Magiran and IranMedex. In addition, the "Iranian Journal of Arthropod-Borne Disease" and the "Iranian Journal of Parasitology" were investigated as key journals. Reference lists of relevant articles were also hand-searched. Finally, we contacted local active researchers in the field of leishmaniasis to avoid missing any articles on leishmaniasis in the Goletan Province.

Selection of articles

The following inclusion criteria were considered for the entry of articles to the study:

1. Articles on Leishmania and its prevalence in humans, vectors and reservoirs.
2. Articles that solely report the results of research conducted in the Golestan Province.

Articles of studies conducted in other parts of Iran or several provinces were entered in the study only if they had reported data related to the Golestan Province separately and clearly. Subsequently, two research team members evaluated the selected articles independently by reading titles, abstracts, and if necessary full texts. Articles that did not meet the inclusion criteria were excluded from the analysis. Articles repeated in two or more databases were excluded. There was a disagreement regarding inclusion/exclusion of an article, but a consensus was reached after holding a group discussion.

Data extraction

Data were extracted from the selected articles using a data extraction form. Quality of the studies was evaluated and scored on a scale of 0 to 10 using the Joanna Briggs Institute's critical evaluation checklist for studies reporting prevalence data. Studies with a quality score of ≥6 were included in the meta-analysis. A table was also designed to present the type, location, date, results and quality of each study.

Subgroup combination

The data were classified into subgroups of prevalence, type of parasite in human, reservoirs and vectors.

Data analysis

Meta-analysis was performed with the Stata software (ver. 14). Pooled prevalence was calculated using the metaprop command and a random-effect model. Moreover, the I^2 statistic was used to measure heterogeneity of studies. If heterogeneity ($I^2 \geq 50\%$) was noted among studies, sensitivity analysis was carried out by removing studies. We calculated the pooled prevalence of *L. major* in humans, reservoirs (rodents) and sandflies using forest plot (at 95% confidence interval).

Results

After searching keywords in Persian and English databases, 235 papers were found in English and 198 in Persian. Overall, 390 papers remain for reviewing their titles and abstracts after reviewing and removing duplicates; and 324 records excluded after title and abstract screening by inclusion criteria, described in methods. The text of 66 papers was carefully investigated and their data entered into the data extraction form. Finally, 34 studies included in qualitative and quantitative study (Fig. 2).

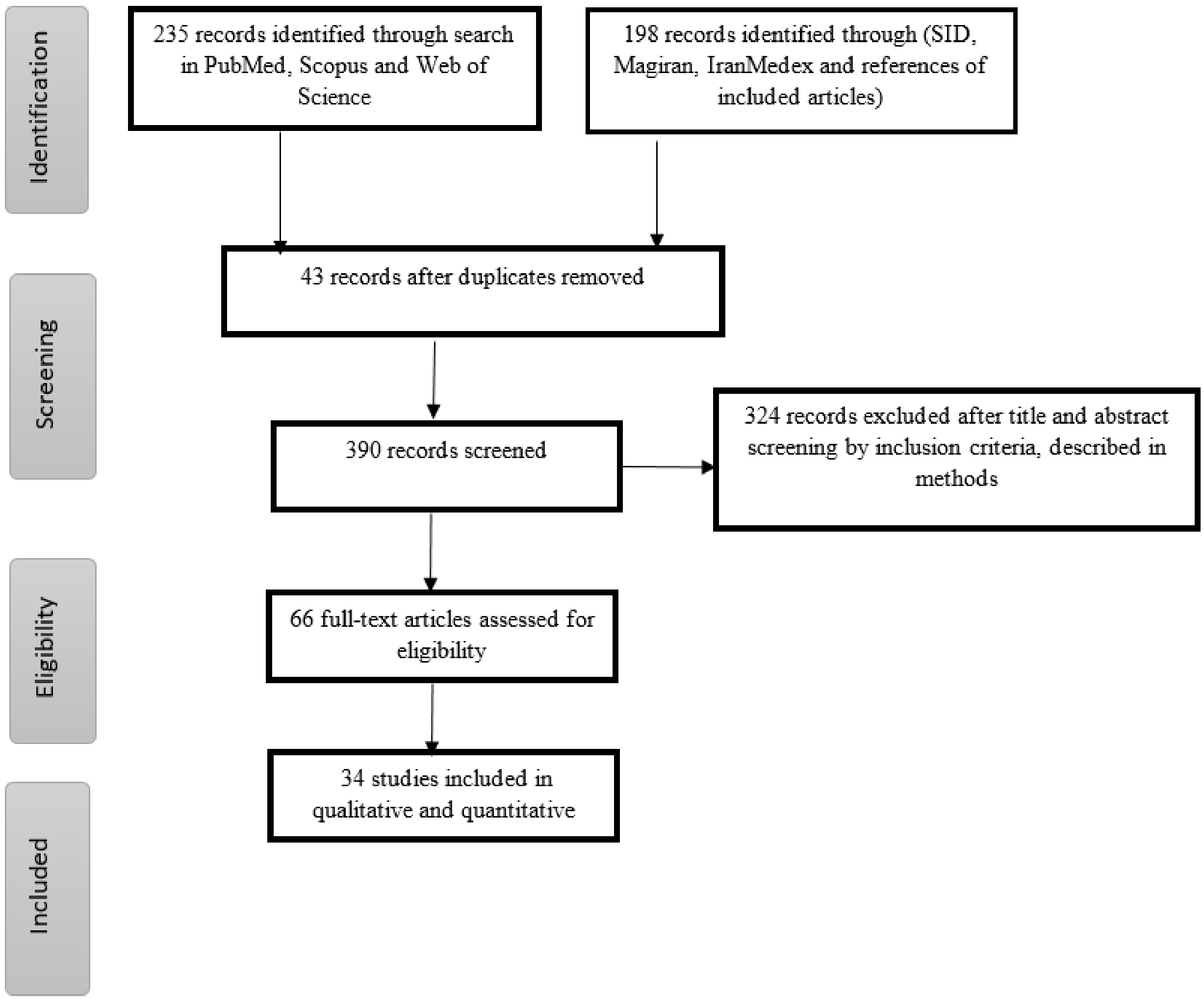

Fig.2: Flowchart describing the study design process

Twelve articles investigated detection of different species of Leishmania in suspected cases of cutaneous or visceral leishmaniasis in the Golestan Province (Table 1) (14-25). In these studies, 2167 patients with acute ulcer and suspected cutaneous leishmaniasis were examined by preparing direct smears from the ulcers, microscopic examination and polymerase chain reaction (PCR) method. In these studies, *L. major*, *L. tropica* and *L. turanica* were isolated from 1561 (72%), 8 (0.7%) and 2 (0.13%) patients, respectively. In Maraveh Tappeh County, 1.3% of the tested humans were positive for *L. infantum* in the serological tests and 2.8% were positive in the PCR method (24). In addition, eight children under five years of age had clinical symptoms and were positive for *L. infantum* (25, 26). We found sixteen articles that investigated reservoirs of Leishmania in the Golestan Province (Table 2). In these studies, *L. major* was detected in *Rh. opimus*, *M. libycus, M. persicus*, and *Hemiechinus auritus*_(22, 27-34), while *L. infantum* was found in dogs, jackals and *Rh. opimus*. Antibodies against *L. infantum* were also detected in *Mus musculus* (24, 35-39), and *L. (Saurolejshmaini) gymnodactyli* was found in lizard (40).

Table 1: Conducted studies in Golestan Province to investigate human infection to leishmania parasites

Author	Year	Region	Method	Num. Sample	Num. Positive	Percentage	Parasite
Mesgarian Et al.	2010	Gonbad-e Kavus	Smear and PCR	35	35	100	*L. major*
Tohidi Et al.	2011a	Golestan	Smear and PCR	63	56	93.6	*L.major*
					7	6.4	*L.tropica*
Tohidi Tohidi Et al.	2011b	Golestan	Smear and PCR	63	56	100	*L. major*
Pagheh Tohidi Et al.	2012	Gonbad-e Kavus	Smear and PCR	303	238	78.5	*L. major*
Mahmoudzadeh-	2012	Golestan	PCR	13	12	92.3	*L. major*
Niknam Tohidi Et al.					1	7.7	*L. tropica*
Baghaei Tohidi Et al.	2012	East of Golestan	Smear and PCR	121	113	93.4	*L. major*
			Smear and PCR		92	76	
Pagheh Tohidi Et al.	2013	Gonbad-e Kavus	Smear and PCR	1398	946	67.6	*L. major*
Bordbar Et al.	2014a	Turkmen Sahra		164	123	75	*L. major*
					2	1.2	*L. major & L. turanica*
Bordbar Et al.	2014b	Turkmen Sahra	PCR	164	123	75	*L. major*
					2	1.2	*L. turanica*
Hezari Et al.	2016	Kalaleh	Smear and PCR	70	38	54.3	*L. major*
Fakhar Et al.	2014	Maraveh Tapeh	DAT and PCR	450	13	2.8	*L. infantum*
Asfaram Et al.	2017	Golestan	Rk39, DAT and PCR	6	6	100	*L.infantum*

L. major: Leishmania major; L. tropical: Leishmania tropica; L. turanica: Leishmania turanica; L. infantum: Leishmania infantum

Table 2: Conducted studies in Golestan Province to investigate reservoir host infection to *Leishmania* parasites

Author	Year	Region	Method	Species	Num. Of investi-gated sample	Num. Positive	Percent-age	Isolated parasite
Parvizi Et al.	1999	Gonbad-e Ka-vus	Smear and Injection to balb/c mouse	*Rh. opimus*	94	35	36.8	*L. major*
Mohebali Et al.	2004	Minoo Dasht	PCR	*Rh. opimus*	27	23	85.5	*L. major*
				M. libycus	1	0	0	-
Rassi Et al.	2008 a	Maraveh Tapeh	PCR	*Rh. opimus*	16	6	37.5	*L.major*
Parvizi Et al.	2010	Gonbad-e Ka-vus	Smear Injection to Balb/C mouse	*Rh. opimus*	27	1	3.7	*L.major*
				M. libycus	12	3	25	
				M. persicus	1	1	100	
Mirzaei Et al.	2011	Gonbad-e Ka-vus and Mara-veh Tapeh	PCR	*Rh. opimus*	227	59	36	*L. major*
						6	2.4	*L. turanica*
						2	0.8	*L. major & unidentified species*
Akhoundi Et al.	2013	Gonbad-e Ka-vus and Mara-veh Tapeh	PCR	*Rh. opimus*	227	59	26	*L. major*
						8	3.5	*L. turanica*
						14	6.2	*L. major & L. turanica*
				M. libycus	19	7	36.8	*L. major*
Hajjaran Et al.	2013	Gonbad-e Ka-vus and Mara-veh Tapeh	PCR	Rh. opimus	124	48	38.7	*L. major*
Mirzaei Et al.	2014	Turkman Sahra	PCR	*Rh. opimus*	227	26	11.4	*L. major*
						7	3	*L. turanica*
						1	0.4	*L. major & L. turanica*
						2	0.8	*L. major & unidentified species*
						1	0.4	*L. infantum*
				M. libycus	19	3	15.8	*L. major*
						1	5.3	*L. major/unidentified species*

Bordbar Et al.	2014 b	Gonbad-e Kavus and Maraveh Tapeh	PCR	*Rh. opimus*	227	59	26	*L. major*
						6	2.6	*L. turanica*
				M. libycus	19	6	31.6	*L. major*
				H. auritus	3	1	33.3	*L. major*
Rouhani Et al.	2014	Turkman Sahra	PCR	*M. libycus*	19	8	42.1	*L. major*
				H. auritus	3	1	33.3	*L. major*
Namroodi & Saberi	2013	Gonbad-e Kavus and Maraveh Tapeh	IFAT	*Mus musculus*	46	6	13	*L.infantum antibody*
Fakhar Et al.	2014	Maraveh Tapeh	DAT and PCR	Dog	50	15	30	*L. infantum*
Namroodi & Saberi	2015	North of Golestan	Elisa, IDvet kit	Dogs (without clinic)	150	23	15.3	*L. infantum*
Namroodi	2015 a	North of Golestan	Elisa, IDvet kit	Jackals	60	5	8.3	*L. infantum*
Namroodi	2015 b	Golestan	PCR	Jackles (killed in road	20	2	10	*L.infantum*
Seyedi Rashti Et al.	1994	Gonbad-e Kavus	Lizard (cryto-podion caspius)	isoenzyme	1	1		*L.(Saurolejshmaini) gymnodactyli*
Mohebali	2004	Minoo Dasht	PCR	*Rh. opimus*	27	23	85.5	*L. major*
Hajjaran	2013	Gonbad-e Kavus and Maraveh Tapeh	PCR	*Rh. opimus*	124	48	38.7	*L. major*

L. major: Leishmania major; L. tropical: Leishmania tropica; L. turanica: Leishmania turanica; L. infantum: Leishmania infantum; L.(Saurolejshmaini) gymnodactyli; Leishmania (Saurolejshmaini) gymnodactyli; Rh. opimus: Rhombomys opimus; M. libycus: Meriones libycus; Meriones persicus: ; H. auritus: Hemiechinus auritus

There were six studies on vectors of the disease in the Golestan Province (Table 3). These studies reported *Ph. papatasi* as the main vector for cutaneous leishmaniasis and *L. major*. Moreover, infection of *Ph. papatasi* with *L. turanica* and *L. closed gerbil* were observed. The three aforementioned parasites were also isolated from *Ph. caucasicus* group (22, 29, 41- 43), and *L. (Saurolejshmaini) gymnodactyli* was found in *S. sintoni* (40).

According to our meta-analysis, the pooled prevalence of *L. major* was 83%, 32.62% and 11% in patients with acute ulcer, rodents and sandflies (Table 4).

Table 3: Conducted studies in Golestan Province to investigate sand fly infection to *Leishmania* parasites

Author	***Year***	***Region***	***Method***	***Species***	***Num. Of investigated sample***	***Num. Positive***	***Percentage***	***Isolated parasite***
Rassi Et al.	2008	Maraveh Tapeh	PCR	*Ph. papatasi*	372	1	0.3	*L.major*
Parvizi & Ready	2008	Gonbad-e Kavus and maraveh Tapeh	PCR	*Ph. papatasi*	52	7	13.5	*L. major*
					20	1	5	*L. turanica*
					20	1	5	*L. closed to gerbili*
				Ph. caucasicus group	8	2	25	*L. closed to gerbili*
Roshanghalb Et al.	2011	Gonbad-e Kavus and maraveh Tapeh	PCR	*Ph. papatasi*	168	18	10.7	*L.major & L. turanica*
Bordbar Et al.	2014	Gonbad-e Kavus and maraveh Tapeh	PCR	*Ph. papatasi*	220	25	11.4	*L. major*
						2	0.9	*L. turanica*
				Ph. caucasicus group	98	10	10.2	*L. major*
						1	1	*L. turanica*
						4	4.1	*L. closed to gerbili*
Sharbatkhori Et al.	2014	Gonbad-e Kavus and maraveh Tapeh	PCR	*Ph. papatasi*	168	17	10.1	*L. major*
						1	0.6	*L. turanica*
				Ph. caucasicus group	38	7	18.4	*L. major*
						1	2.6	*L. turanica*
Seyedi Rashti Et al.	1994	Turkmen Sahra	Isoenzyme	*S. sintoni*	403	60	14.9	*L.(Saurolejshmaini) gymnodactyli*

L. major: Leishmania major; L. tropical: Leishmania tropica; L. turanica: Leishmania turanica; L. closed to gerbil: Leishmania closed to gerbil; L. infantum: Leishmania infantum; L.(Saurolejshmaini) gymnodactyli; Leishmania (Saurolejshmaini) gymnodactyli; Ph. papatasi: Phlebotomus papatasi, Ph. caucasicus group: Phlebotomus caucasicus group; S. sintoni: Sergentomyia sintoni

Table 4: Meta-analysis results of Prevalence of *L. major* in humans, wild rodents and sandflies in Golestan Province

Examined animal	*P value*	I_2	*prevalence*		*Pooled prevalence*
			Min	*Max*	
humans	<0.0001	100	0.54	100	0.81
Wild rodents	<0.0001	89.2	0.27	0.85	0.32
Sand flies	<0.0001	99.5	0.11	0.13	0.11

Studies on the incidence of *L. major* in humans had high heterogeneity (I^2=93.29), which could be due to the difference in the diagnostic methods as well as geographic diversity. For instance, some studies evaluated the incidence rate in a county, while others studied the entire or part of the province (Fig. 3).

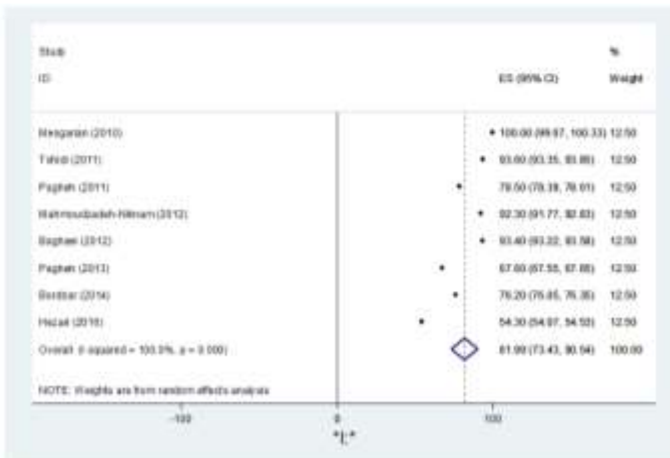

Fig. 3: Meta-analysis of human infection to *L. major*

A high degree of heterogeneity (I^2=0.89) was seen among studies on the pooled prevalence of *L. major* in rodents, which was decreased (I^2=0.21) after removing two studies (30, 39), the pooled prevalence of rodents contaminated with the parasite was 32.62% in the Golestan Province (Fig. 4).

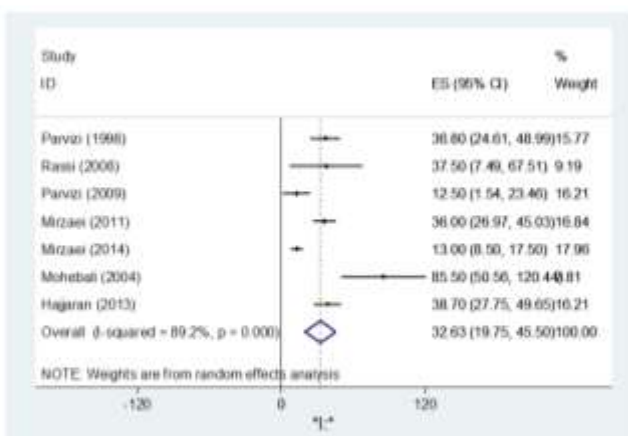

Fig. 4: Meta-analysis of wild rodent's infection to *L. major*

Given the diversity of reservoirs of *L. infantum*, it was not possible to report the pooled prevalence or conduct meta-analysis. Moreover, the I^2 index (92.52) indicated high heterogeneity among these studies.

Of six studies that investigated infection of sandflies with *L. major*, five studies were jointly carried out in the Gonbad-e-Kavus and Maraveh Tappeh Counties and one study was performed in the Maraveh Tappeh County alone. The heterogeneity was high among these studies (I^2=97.07). However, after removing the single study on the Maraveh Tappeh County (29), heterogeneity decreased and the pooled prevalence of *L. major* in sandflies was determined as 11% (Fig. 5).

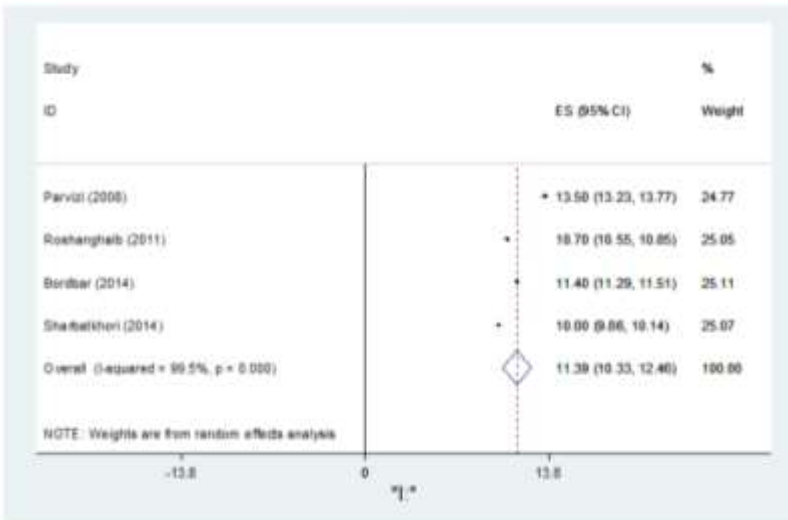

Fig. 5: Meta-analysis of Sand flies infection to *L. major*

Discussion

Overall, 83% of patients with acute ulcer and suspected cutaneous leishmaniasis were infected with *L. major*. This parasite is dominant in this region and endemic in this province. *L. tropica* was found in a small number of patients, particularly in those with a history of traveling to endemic areas of ACL.

In a previous study in this province, *L. turanica* was isolated from patients (21). *L. turanica* as well as *L. gerbilli* and *L.* closed to *gerbili* can infect rodents but not humans. Nevertheless, they can increase stability and persistence of Leishmania in the region (4, 5, 44).

Most cases have been reported from Gonbad-e-Kavus and Maraveh Tappeh Counties, known endemic areas of leishmaniasis (14-19, 21, 23, 45, 46). Human infection with *L. infantum* in the Golestan Province has been reported in two studies (24, 25). Of 450 individuals examined in the Maraveh Tappeh County, 1.3% was seropositive and 2.8% were PCR positive. Given the limited number of studies in this area, we were unable to determine the pooled prevalence in the province. In Iran, 4% and 8.7% of blood donors were positive for *L. infantum* using serological and molecular methods, respectively (47) and in Ethiopia, the pooled prevalence of leishmaniasis was 19% (48).

In studies in the Golestan Province, *L. major* was isolated from *Rh. opimus*, *M. libycus*, *M. persicus* and *Hemiechinus auritus*. Among 1196 *Rh. opimus* tested in this province, 316 (26.4%) were positive for *L. major* and 28 (2.3%) were positive for *L. turanica*. Moreover, *L. major* was detected in 31.8% of *M. libycus* and in a few number of *M. persicus* and *H. auritus* (22, 27-31, 33-34).

In our meta-analysis, the rate of *L. major* infection in rodents was 32.62%. Similarly, in another meta-

analysis in Iran, the weighted prevalence of Leishmania species in rodents was 23% (95% CI=18%-28%) (49).
Six studies investigated reservoirs of *L. infantum* and detected this parasite in dogs (19%), jackals (8.7%) and *Rh. opimus*. In addition, antibodies against *L. infantum* were identified in *Mus musculus* (24, 35-39). The highest rate of *L. infantum* infection was reported from the Maraveh Tappeh County, in a way that 40% of jackals and 30% of domestic dogs were infected with this parasite (24, 37). The Maraveh Tappeh County is bordered by the North Khorasan Province, a well-known endemic area for visceral leishmaniasis in Iran (50). In a systematic review and meta-analysis study in Iran, the rate of *L. infantum* infection in dogs, jackals and wolves was 16%, 10% and 10% respectively (51), which are lower than the rates observed in our study.
Various studies in the Golestan Province have reported *Ph. papatasi* as a main vector of cutaneous leishmaniasis. The rate of *L. major* infection in this species was 11% in our meta-analysis and ranging between 0.3 and 13.5% in other studies in the province (22, 29, 41-43). The rates reported for *L. major* infection in other areas of Iran were similar or lower than the rates observed in our study (52-55).
In addition to *L. major*, *L. turanica* and *L. close to gerbilli* have been also detected in *Ph. papatasi* (22, 29, 41-43). These three parasites have been also isolated from *Ph. caucasicus* and *Ph. mongolensis*, with the highest prevalence observed in the east and northeast of the Golestan Province (46). The rate of infection with *L. major* and *L. closed gerbil* in the *Ph. caucasicus* was 18.4% and 25%, respectively (22, 42-43). However, the rate of *L. major* infection in these species in other areas of Iran is lower than the rates observed in our study (54-55). These findings highlight the irrefutable role of these species as vectors of zoonotic cycle of *L. major* between rodents and secondary vector of this species to humans and stability of these parasites in the country (4-5, 44).
Our findings highlight the need for implementation of control measures among the reservoirs of both cutaneous and visceral leishmaniasis in this area.

Conclusion

Given the high rates of *L. major* infection in rodents and abundance of *Ph. papatasi*, the Golestan Province can be considered an endemic area for cutaneous leishmaniasis. Sporadic visceral leishmaniasis may be present in all counties of the province, particularly in the foothill areas and the Maraveh Tappeh County.

Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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Conflict of interest

The authors declare that there is no conflict of interests.

References

1. World Health Organization (WHO) (2012). A human rights-based approach to neglected tropical diseases. Geneva: World Health Organization; 2012; [Online] Available from: http://www. https://www.who.int/neglected_diseases/Human_rights_approach_to_NTD_Eng_ok.pdf
2. Inceboz T (2019). Epidemiology and Ecology of Leishmaniasis. DOI: http://dx.doi.org/10.5772/intechopen.86359

3. World Health Organization (WHO), (2019). Leishmaniasis. [Online] Available from: https://www.who.int/news-room/fact-sheets/detail/leishmaniasis
4. Karimi A, Hanafi-Bojd AA, et al (2014). Spatial and temporal distributions of phlebotomine sand flies (Diptera: Psychodidae), vectors of leishmaniasis, in Iran. *Acta Trop*, 132: 131–139.
5. Yaghoobi-Ershadi MR (2012). Phlebotomine Sand Flies (Diptera: Psychodidae) in Iran and their Role on *Leishmania* Transmission. *J Arthropod Borne Dis*, 6(1): 1–17.
6. Mohebali M (2013). Visceral leishmaniasis in Iran: Review of the Epidemiological and Clinical Features. *Iran J Parasitol,* 8(3): 348–358.
7. Mohebali M, Arzamani K, Zarei Z, et al (2016). Canine Visceral Leishmaniasis in Wild Canines (Fox, Jackal, and Wolf) in Northeastern Iran Using Parasitological, Serological, and Molecular Methods. *J Arthropod Borne Dis*, 10(4): 538–545.
8. Mohebali M, Moradi-Asl E, Rassi Y (2018). Geographic distribution and spatial analysis of *Leishmania infantum* infection in domestic and wild animal reservoir hosts of zoonotic visceral leishmaniasis in Iran: A systematic review. *J Vector Borne Dis*, 55(3):173-183.
9. Seyedi Rashti MA, Sahabi Z (1995). *Phlebotomus (Larroussius) keshishiani*, Shchurenkova, another vector of visceral leishmaniasis in Iran. *Iran J Public Health*, 24(1-2):25-30.
10. Azizi K, Rassi Y, Javadian E, et al (2006). *Phlebotomus* (*Paraphlebotomus*) *alexandri*: a probable vector of *Leishmania infantum* in Iran. *Ann Trop Med Parasitol*, 100(1):63-8.
11. Azizi K, Rassi Y, Javadian E, et al (2008). First detection of *Leishmania infantum* in *Phlebotomus* (*Larroussiuss*) *major* (Diptera: Psychodidae) from Iran. *J Med Entomol*, 45(4):726-31.
12. Sanei Dehkordi A, Rassi Y, Oshaghi MA, et al (2011). Molecular detection of *Leishmania infantum* in naturally infected *Phlebotomus perfiliewi* transcaucasicus in Bilesavar district, northwestern Iran. *Iran J Arthropod-Borne Dis*, 5(1):20-7.
13. Rassi Y, Abai MR, Oshaghi MA, et al (2012). First detection of *Leishmania infantum* in *Phlebotomus kandelaki* using molecular methods in northeastern Islamic Republic of Iran. *EMHJ*, 18: 387-392.
14. Mesgarian F, Rahbarian N, Mahmoudi Rad M, et al (2010). Identification of *Leishmania* species isolated from human cutaneous leishmaniasis in Gonbad-e-Qabus city using a PCR method during 2006-2007. *Tehran Uni Med J*, 68(4): 250-256.
15. Tohidi F, Borghaei A (2011). Cutaneous leishmaniasis parasite identification via PCR in the Infected Areas in Golestan Province. *Knowledge and Health*, 6(2): 26-31.
16. Tohidi F, Ghaseminejad P, Saedi E, Rostami M (2011). Determination species of cutaneous leishmaniasis by its-PCR in the locations of infection Golestan Province in Iran. *Trop Med Int Health*, 16(1): 234-235.
17. Pagheh AS, Fakhar M, Mesgarian F, et al (2012). Detection and Identification of Causative Agent of Cutaneous Leishmaniasis Using Specific PCR. *J Mazandaran Uni Med Sci*, 21(1): 85-92.
18. Mahmoudzadeh-Niknam H, Ajdary S, Riazi-Rad F, et al (2012). Molecular epidemiology of cutaneous leishmaniasis and heterogeneity of *Leishmania major* strains in Iran. *Trop Med Int Health*, 17(11): 1335-44.
19. Baghaei A. Parvizi P, Amirkhani A, et al (2012). Identification of *Leishmania* using microscopic and molecular methods in suspected patients of Cutaneous Leishmaniasis by targeting ITS-rDNA gene, Golestan Province, Iran (2009-10). *J Gorgan Uni Med Sci*, 14(3): 72-81.
20. Pagheh AS, Fakhar M, Mesgarian F, et al (2013). Incidence Trend of Rural Cutaneous Leishmaniasis in Gonbad-e-Qabus City (Golestan, Iran), during 2009-2012. *J Mazandaran Uni Med Sci*, 23(104): 27-33.
21. Bordbar A, Parvizi P (2014). High infection frequency, low diversity of *Leishmania major* and first detection of *Leishmania turanica* in human in northern Iran. *Acta Trop*, 133: 69-72.
22. Bordbar A, Parvizi P (2014). High density of *Leishmania major* and rarity of other mammals' *Leishmania* in zoonotic cutaneous leishmaniasis foci, Iran. *Trop Med Int Health*, 19(3): 355-363.
23. Hezari F, Niyyati M, Seyyed Tabaei SJ, et al (2016). Frequency of Cutaneous Leishmaniasis and Species Identification in Suspected Individuals from Golestan Province, Northern Iran in 2014. *Iran J Public Health*, 45(10): 1348-54.
24. Fakhar M, Asadi Kia A, Gohardehi S, et al (2014). Emergence of a new focus of visceral leishmaniasis due to *Leishmania infantum* in Golestan

Province, north-eastern of Iran. *J Parasit Dis*, 38(3): 255-259.

25. Asfaram S, Pagheh A, Fakhar M, et al (2017). Case Series of Visceral Leishmaniasis (kala-azar) in Mazandaran and Golestan Provinces, North of Iran. *J Mazandaran Univ Med Sci*, 26(144): 373-381.
26. Qorban M, Cheraghali F, Sofizadeh A, et al (2017). Kala-azar in Maraveh Tapeh County, Golestan Provice, Iran: Seven case reports. *J Gorgan Uni Med Sci*, 19(2): 98-103.
27. Parvizi P, Javadian E, Rassi Y, et al (1999). A Study on Vector and Reservoir Host of Cutaneous Leishmaniasis in Turkamansahra, Golestan Province, North-East of Iran. *Pathobiol Rea*, 3-4 (2): 125-130.
28. Mohebali M, Javadian E, Yaghoobi-Ershadi MR, et al (2004). Characterization of *Leishmania* infection in rodents from endemic areas of the Islamic Republic of Iran. *East Mediterr Health J*, 10(4-5):591-9.
29. Rassi Y, Sofizadeh A, Abai MR, et al (2008). Molecular Detection of *Leishmania major* in the Vectors and Reservoir Hosts of Cutaneous Leishmaniasis in Kalaleh District, Golestan Province, Iran. *J Arthropod Borne Dis*, 2(2): 21-27.
30. Parvizi P, Hedayati M (2010). *Leishmania* infections in rodents, reservoir hosts of zoonotic cutaneous leishmaniasis in Turkemen Sahara.*J Guilan Uni Med Sci,* 18(72): 30-8.
31. Mirzaei A, Rouhani S, Taherkhani H, et al (2011). Isolation and detection of *Leishmania* species among naturally infected *Rhombomis opimus*, a reservoir host of zoonotic cutaneous leishmaniasis in Turkmen Sahara, North East of Iran. *Exp Parasitol*, 129(4): 375-80.
32. Akhoundi M, Mohebali M, Asadi M, et al (2013). Molecular characterization of *Leishmania* spp. In reservoir hosts in endemic foci of zoonotic cutaneous leishmaniasis in Iran. *Folia Parasitol (Praha)*, 60(3): 218-224.
33. Hajjaran H, Mohebali M, Abai MR, et al (2013). Natural infection and phylogenetic classification of *Leishmania* spp. Infecting *Rhombomys opimus*, a primary reservoir host of zoonotic cutaneous leishmaniasis in Northeast Iran. *Trans R Soc Trop Med Hyg*, 107(9):550-7.
34. Rouhani S, Mirzaei A, Spotin A, et al (2014). Novel identification of *Leishmania major* in *Hemiechinus auritus* and molecular detection of this parasite in *Meriones libycus* from an important foci of zoonotic cutaneous leishmaniasis in Iran. *J Infect Public Health*, 7(3): 210-217.
35. Namroodi S, Saberi M (2013). Serological survey of *Leishmania infantum* in wild rodents of Turkemen Sahra, Golestan province, North-East Iran. *Int J Molecul Clin Microbiol*, 2: 300-302.
36. Namroodi S, Saberi M (2015). Seroepidemiology of *Leishmania infantum* in rural dogs in Golestan Province, Iran. *Mljgoums*, 9(2): 97-102.
37. Namroodi S (2015). Serological Survey of *Leishmania infantum* in Jackals in Golestan Province, Iran (2011-2013). *J Mazandaran Uni Med Sci*, 24(120): 25-9.
38. Namrodi S (2015). Molecular detection of *Leishmania infantum* in road-killed carnivores from North of Iran, Golestan Province. *Int J Molecul Clin Microbiol*, 5(2): 585-590.
39. Mirzaei A, Schweynoch C, Rouhani S, et al (2014). Diversity of *Leishmania* species and of strains of *Leishmania major* isolated from desert rodents in different foci of cutaneous leishmaniasis in Iran. *Trans R Soc Trop Med Hyg*, 108(8): 502-12.
40. Seyedi Rahti MA, Agh-Atabay MD, Mohebali M (1994). Natural promastigote infection of *Sergentomyia sintoni*, its seasonal variation and reservoir hosts in Turkemen Sahara, Iran. *Iran J Public Health*, 23 (1-4): 41-50.
41. Roshanghalb M, Parvizi P (2012). Isolating and Determining *Leishmania major* and *Leishmania turanica* in *Phlebotomus papatasi* in Golestan Province. *J Mazandaran Uni Med Sci*, 21(1): 74-83.
42. Parvizi P, Ready P (2008). Nested PCRs and sequencing of nuclear ITS-rDNA fragments detect three *Leishmania* species of gerbils in sandflies from Iranian foci of zoonotic cutaneous leishmaniasis. *Trop Med Int Health*, 13(9): 1159-71.
43. Sharbatkhori M, Spotin A, Taherkhani H, et al (2014). Molecular variation in *Leishmania* parasites from sandflies species of a zoonotic cutaneous leishmaniasis in northeast of Iran. *J Vector Borne Dis*, 51(1): 16-21.
44. Yaghoobi-Ershadi MR, Javadian E (1996). Epidemiological study of reservoir hosts in an endemic area of zoonotic cutaneous leishmaniasis in Iran. *Bull World Health Organ*, 74(6): 587-90.

45. Sofizadeh A, Vatandoost H, Rassi Y, et al (2016). Spatial Analyses of the Relation between Rodent's Active Burrows and Incidence of Zoonotic Cutaneous Leishmaniasis in Golestan Province, Northeastern of Iran. *J Arthropod Borne Dis*, 10(4): 569-576.
46. Sofizadeh A, Rassi Y, Hanafi-Bojd AA, et al (2018). Distribution and ecological aspects of sand flies (Diptera: Psychodidae) species in Northeastern Iran. *Asian Pac J Trop Med*, 11(9): 526-533.
47. Foroutan M, Dalvand S, Khademvatan Set al (2017). A systematic review and meta-analysis of the prevalence of *Leishmania* infection in blood donors. *Transfus Apher Sci*, 56(4): 544-551.
48. Assefa A (2018). Leishmaniasis in Ethiopia: A systematic review and meta-analysis of prevalence in animals and humans. *Heliyon,* 4(8): e00723.
49. Foroutan M, Khademvatan S, Majidiani H, et al (2017). Prevalence of *Leishmania* species in rodents: A systematic review and meta-analysis in Iran. *Acta Trop*, 172: 164-172.
50. Arzamani K, Fazeli R, Shirzadi MR, et al (2014). Visceral Leishmaniasis in North Khorasan Province, Iran. *J Zoonoses*, 1(1): 47-53.
51. Shokri A, Fakhar M, Hosseini Teshnizi S (2017). Canine visceral leishmaniasis in Iran: A systematic review and meta-analysis. *Acta Trop*, 165: 76–89.
52. Vahabi A, Rassi Y, Oshaghi MA, et al (2016). Detection of *Leishmania major* DNA within wild caught *Phlebotomus* papatasi and species composition of sand flies in endemic focus of cutaneous leishmaniasis, in western Iran. *J Parasit Dis*, 40(1): 69-74.
53. Saghafipour A, Vatandoost H, Zahraei-Ramazani AR, et al (2017). Epidemiological Study on cutaneous leishmaniasis in an Endemic Area, of Qom Province, Central Iran. *J Arthropod-Borne Diseases*, 11(3): 403-413.
54. Rassi Y, Abai MR, Javadian E, et al (2008). Molecular data on vectors and reservoir hosts of zoonotic cutaneous leishmaniasis in central Iran. *Bull Soc Pathol Exot*, 101(5): 425-8.
55. Rassi Y, Oshaghi MA, Azani SM, et al (2011). Molecular Detection of Leishmania Infection Due to *Leishmania major* and *Leishmania turanica* in the Vectors and Reservoir Host in Iran. *Vector Borne Zoonotic Dis*, 11(2): 145-150.