Review Article

A Feasibility Study on Using the Facilities of Health Centers for Developing a Laboratory Network on Vectors and Reservoir Hosts of Cutaneous Leishmaniasis in Iran

***Mohammad Reza Yaghoobi-Ershadi[1]; Amir Ahmad Akhavan[1]; Mohammad Reza Shirzadi[2]; Seyedeh Zohreh Hosseini[1]; Oscar Daniel Salomon[3]; Ahmad Ali Hanafi-Bojd[1]; Yavar Rassi[1]**

[1]Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
[2]Communicable Diseases Management Center, Iranian Ministry of Health and Medical Education, Tehran, Iran
[3]National Institute of Tropical Medicine, Puerto Iguazu, Misiones, Argentina

***Corresponding author:** Dr Mohammad Reza Yaghoobi-Ershadi, E-mail: yaghoobi.reza@gmail.com

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Abstract

Background: Cutaneous leishmaniasis is an ancient endemic disease in Iran and continues to be a growing health threat to community development and the environment. This paper explains how to use the facilities of health centers for developing a laboratory network on vectors and reservoir hosts of cutaneous leishmaniasis in Iran.

Methods: A literature search was performed of the relevant multiple databases to include studies on vectors and reservoirs of cutaneous leishmaniasis in Iran. A team of experienced experts was performed. After holding several meetings, field visits and organizing workshops, the activities of laboratories were determined at three levels.

Results: Entomological studies showed that 5 species of the genus *Phlebotomus* and 10 species of the genus *Sergentomyi*a are active in the south, 4 species of the genus *Phlebotomus* and one species of the genus *Sergentomyia* in the central part and 5 species of the genus *Phlebotomus* and 2 species of the genus *Sergentomyia* in the north east. Reservoir hosts were identified in the study areas. The tasks of laboratories were regulated at different levels.

Conclusion: The Iranian Ministry of Health and Medical Education should prioritize the employment of capable persons in the field of Medical Entomology and Vector Control. The survival of this laboratory network depends on hiring and employing interested and persistent people. The universities of Medical Sciences that have the facilities to set up this network will be a very effective partner in the control of the disease in high risk areas. The results can be used in neighboring countries.

Keywords: Laboratory network; Leishmaniasis; Vectors; Reservoirs; Iran

Introduction

Parasitic diseases of the genus *Leishmania* are a huge burden on human health and society. Their incidence is found to be prevalent in some of the poorest countries in the world, but they summon less attention than other infectious diseases like malaria, tuberculosis and AIDS (1). They are categorized as a neglected tropical diseases because, although they cause significant mortality, there is little effort on the part of the global community

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and pharmaceutical industry to invest in research and development of better and innovative therapeutics because lack of sufficient incentives. Also, the disease is found primarily among the poorest of the poor, who have no influence over policy-makers and little access to healthcare and the health-related market (1, 2).

The disease is caused by more than 20 species/subspecies of *Leishmania* protozoa. They are transmitted to humans through the bites of infected Phlebotominae sand flies (3), principally belonging to two genera: *Phlebotomus* (in the Old World) and *Lutzomyia* (in the New World). While *Leishmania* parasites were found in several species of a third genus *Sergentomyia*, which feed on humans, none were up to now incriminated as vectors. An estimated 31 species of *Phlebotomus* and 47 species of *Lutzomyia* are proven vectors of human leishmaniases (4). Sand flies are also known to be the vectors of other human pathogens, such as *Bartonella* spp. (Carrion's disease), and a number of viral agents causing sand fly fever, summer meningitis, vesicular stomatitis and Chandipura virus encephalitis(4). Both cutaneous and visceral forms of leishmaniasis are present in the Eastern Mediterranean Region, making a significant neglected tropical disease there. Regarding cutaneous leishmaniasis, both the anthroponotic form, caused by *Leishmania tropica*, and the zoonotic form, caused by *L. major* with animal reservoir hosts, are endemic in the region.

Cutaneous leishmaniasis is one of the priorities in the WHO Eastern Mediterranean Region as it carries a large portion of the global burden. According to data reported in the Global Health Observatory, the Eastern Mediterranean Region reported 69.6% of the total number of cutaneous leishmaniasis cases detected worldwide in 2016 (followed by the Region of the Americas with 28.5% and the European Region with 1.6%). Of the total cases in the Region, over 90% were reported from three countries: the Syrian Arab Republic, Afghanistan and Pakistan, each of which reported more than 10 000 cases. Most of the cases are due to the anthroponotic form of the disease. Regarding visceral leishmaniasis, the Eastern Mediterranean Region carries about 19% of the global burden, with the highest number of cases reported from Sudan and Somalia (5). Cutaneous and visceral leishmaniasis are ancient endemic diseases in Iran and continue to be a growing health threat to community development and the environment in the country. Cutaneous leishmaniasis is endemic in two forms, Anthroponotic Cutaneous Leishmaniasis (ACL) and Zoonotic Cutaneous Leishmaniasis (ZCL). About 20000 cases of leishmaniasis (including ACL, ZCL and Zoonotic Visceral Leishmaniasis) are reported annually but the real figures could be 4–5 folds higher due to underreporting, because of misdiagnosis, lack of active case detection and overlooking the importance of the disease due to the lack of mortality (6) Anthroponotic cutaneous leishmaniasis is still a neglected tropical disease in many parts of the country, while it was greatly reduced in many foci by malaria control measures, many foci remained active in some large and medium sized cities such as Tehran, Mashhad, Neishabur and Sabzevar in the north-east, Shiraz in the south, Kerman and Bam in the southeast, Yazd, Kashan and parts of the city of Esfahan in the central region (7, 8, 9). The parasite is *Leishmania tropica* and the suspected vector is *Phlebotomus* (*Paraphlebotomus*) *sergenti* Parrot 1917. The main reservoir host is the human, but dogs have a role as animal reservoir host and active lesions in dogs have been reported in Tehran, Mashhad, Shiraz and Neishabur (8, 9). Zoonotic cutaneous leishmaniasisis is endemic in many rural areas of 17 out of 31 provinces and is a great health problem in Iran. More than 85% of cases reported in the country are of ZCL form (9, 10). *Rhombomys opimus*, the great gerbil, is the main animal reservoir in foci in the northeast and central part of the country, *Meriones libycus*, the Libyan jird, is considered the principal reservoir host in some parts of the central and south of the country. *Tatera indi*ca, the Indian gerbil, is

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the main reservoir host in the southeast and *Meriones hurrianae*, the desert gerbil, is the reservoir in the southeastern part of Iranian Baluchistan, neighboring Pakistan (11, 12, 13) *Phlebotomus papatasi* Scopoli 1786, the most prevalent among *Phlebotomus* genus, is the only known vector (14, 15, 16, 17).

In 2019, totally 13055 cases of Cutaneous leishmaniasis were reported in the country, 12567 (96.5 %) of which from 18 endemic provinces and 488 (3.5 %) from non-endemic provinces. Most of the cases were from Isfahan Province in the central part of the country with 3127 cases and 59.1 incidence per 100000. But the highest incidence rate was in Semnan Province with 95.9 per 100000 populations (CDC, Iranian Ministry of Health and Medical Education, unpublished data). Fig. 1 shows the trend of reported cases of cutaneous leishmaniasis in Iran in the last 36 years. The incidence of the disease varies between 48/100000 and 15.8/100000 population during 1983–2019 respectively. Whether the incidence has been reduced between these years but, the burden of the disease is still high in some regions. It should be mentioned that the reduction has been due to the implementation of active rodent control operations in ZCL foci and improvement of surveillance and timely treatment of the patients in ACL foci. Iran is facing many challenges-emergencies, crises situations, outbreaks, population displacement, limited funding and weak surveillance, lack of proper management in some provinces, lack of community awareness and some managers-all which may have led to the rising trend in cutaneous leishmaniasis cases in some regions. Even if leishmaniasis was the main public health priority of Iranian Ministry of Health, unfortunately there is not a standard method for the different aspects to study the leishmaniasis, insect vectors in the country, so in this research project the information required on the matter for surveillance and control of sand flies, in order to provide to health authorities in Iran the basis for a program, that could be replicated in the region. This program requires immediate instrumentation because, during the last two decades, the reported cases of leishmaniasis especially that of cutaneous leishmaniasis due to *Leishmania major* have increased in Iran, and it has spread into sites where it did not previously exist, so properly Phlebotomine sand flies have received considerable attention by health authorities in recent years (9). However, the disease needs special entomological surveillance, and to achieve this goal developing a strong laboratory network is required. At the moment, laboratories in many health centers do not have facilities for entomological activities on sand flies and reservoir hosts of cutaneous leishmaniasis, the staffs of health centers are unaware of the matter in different endemic foci of the country, and their duties do not involve clearly the surveillance and control of the disease. Physicians and senior technicians are needed to be trained on leishmaniasis entomology and their awareness on the matter should be increased. Therefore, by conducting this research, the main aspects for developing a laboratory network are stated to support the structuration of this initiative. To obtain a feasible program based on evidences, a search of worldwide information about the topic was performed, followed by consensual workshops with different levels of technical and academic health agents, and visits to the field to assess the actual conditions and capabilities.

Materials and Methods

This study was carried out in five high risk provinces during 2019–2020 in Iran.

1) Databases including PubMed, Web of Science, Literature Retrieval System of the Armed Forces, Pest Management Board and also at least more than 40 eligible journal articles on vectors and reservoirs of cutaneous leishmaniasis and its control which are indexed in electronic databases (ISI and Medline) were selected for this review.

1a) Goal: To summarize the experience on surveillance and control program organization on leishmaniasis worldwide;

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2) A team of experienced experts consist of key people from the Tehran University of Medical Sciences and Ministry of Health and Medical Education (MOH) was formed.

2a) Goal: to develop the first blueprint of a program of surveillance and control of leishmaniasis in Iran. The members of the expert team were as follows:

- Four academic members (including Professors and Associate professors of Medical Entomology and Vector Control from Tehran University of Medical Sciences with personal knowledge of participants after more than 40 years of field mission on leishmaniases in Iran.
- Manager of the Zoonotic Department at MOH
- Two senior technicians on leishmaniasis

Measures were carried out by the specialized team as follows:

- Holding a special meeting for analysis of the current situation of leishmaniasis cases.
- Holding more than 10 meetings with the presence of team members on determining the duties of experts at different levels and having a detailed discussion and exchange of views.

2b) To collect relevant information on the current capabilities in the high risk areas

- Field visits to the high risk areas (Provinces of Esfahan, Ilam, Khuzestan, Sistan and Baluchestan and Northern Khorasan. Each one visited once during the months of June-November, 2019–2020
- Holding meetings with the assistant directors of health, disease control managers and zoonotic experts in high risk provinces
- Field visits to the facilities of laboratories at health centers in the provinces under study.

2c) To adapt the blue print to a feasible consensual program to be implemented

- Preparation a list of equipment required for laboratories of cutaneous leishmaniasis vectors and reservoir hosts.
- Determination of duties for entomology senior technicians of health centers in order to detect and reveal the abundance of sand flies and rodent reservoir hosts.
- To identify the collection methods of sand flies and reservoir hosts, mounting and monitoring
- The platform of recording and flowchart of information
- The needs and ways of capacitation "in practice" at different levels of responsibility from professionals to field technicians in the methods of monitoring of surveillance
- The capacitation of decision takers, health, zoonosis and vector agents in outbreak control and integrated vector-borne diseases management.

Results and Program proposal

The results of literature review of the articles on the vectors and reservoir hosts of cutaneous leishmaniasis showed that there is not any network on this issue in the world except for South America. Many studies have been carried out on the control of vectors in different countries with various results (use of residual spraying, spraying rodent burrows, the use of impregnated bed nets and curtains, etc). Although many molecular studies have been conducted on *Leishmania* parasite, unfortunately, studies on reservoir control are very limited and very few. Due to the fact that there is no effective vaccine against cutaneous leishmaniasis, so the control of vectors and reservoirs along with health education by the relevant specialists and allocating sufficient funds, raising awareness of authorities and the community can prevent the spread of the disease.

Results of entomological and reservoir studies in different provinces are as follows:

Khuzestan Province

A total of 4335 sand flies, 23.63% indoors, 76.37% outdoors, collected from Shush County in September 2019. Eight species including *P. papatasi*, *P. alexandri*, *S. sintoni*, *S. squamipleuris*, *S. iranica*, *S. tiberiadis*, *S.*

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dentata and *S. baghdadis* were collected from indoors. Eleven species collected from outdoors comprising *P. papatasi*, *P. alexandri*, *S. sintoni*, *S. tiberiadis*, *S. dentata*, *S. iranica*, *S. baghdadis*, *S. clydei*, *S. squamipleuris*, *S. theodori* and *S. christophersi.*

Out of 22 rodents which were collected in the mentioned county, 8(36.4%), 12(54.5%), and 2(9.1%) were identified as *Nesokia indica*, *Tatera indica* and *Rattus Rattus*, respectively.

Isfahan Province

A total of 3200 sand flies (35.81%) indoors, (64.19%) outdoors were collected from 6 villages in July 2019 around the city of Isfahan. *Phlebotomus papatasi*, *P. sergenti* and *S. sintoni* were found indoors and 5 species including *P. papatasi*, *P. segenti*, *P. ansarii*, *P. mongolensis*, and *S. sintoni* were found outdoors. *Phlebotomus papatasi* was the dominant species indoors and outdoors.

Totally, 19 rodents were captured using 25 Sherman traps baited with cucumber and dates near gerbil colonies. The great majority were 16(84.2%) *Rhombomys opimus* and 3(15.8%) *Meriones libycus.*

Ilam Province

In total, 1071 sand flies were collected from 4 villages around the city of Mehran in June 2019. *Phlebotomus papatasi*, *P. alexandri*, *S. sintoni* and *S. dentata*, were found indoors and species including *P. papatasi*, *P. caucasicus*, *S. squamipleuris*, *S. clydei*, *S. theodori*, *S. dentata* and *S. tiberiadis* found outdoors. *Phlebotomus papatasi* was a predominant species in both outdoors and indoors. Out of 24 rodents that were caught in three villages, 8(72/7%) and 3(27.3%) were identified as *T. indica* and *N. indica*, respectively.

Sistan and Baluchestan Province

In October of 2019, 644 sand flies collected outdoors and indoors. Three species: *P. papatasi*, *P. sergenti* and *S. sintoni* were collected indoors and 6 species comprising *P. papatasi*, *P. alexandri*, *P. salehi*, *S. clydei*, *S. christophersi* and *S. sintoni* collected outdoors. The most predominant species was *P. papatasi* in both places.

Eight reservoir hosts were captured by Sherman traps around Chabahar and Konarak, identified as *M. hurrianae* (75%) and *T. indica* (25%).

Northern Khorasan Province

An overall 1346 sand flies were collected from 4 villages in Esferayen and Bojnord in August 2019. Four species including *P. papatasi, P. alexandri*, *P. sergenti*, and *S. sintoni* collected indoors and 6 species comprising *P. papatasi*, *P. sergenti*, *P. neglectus*, *P. adlerius group*, *S. sintoni* and *S. sumbarica* collected outdoors. The predominant species in the indoors and outdoors were *P. papatasi* and *S. sintoni*, respectively.

Out of 14 rodents, 6(42.9%), 5(35.7%) and 3 (21.4%) identified as *R. opimus*, *N. indica* and *M. libycus* respectively.

The tasks and activities of laboratories at each level were determined as follows:

a. Periphery levels (County health center laboratory)
b. Province and university levels (Provincial health center laboratory)
c. Country level (National reference laboratory on vectors and reservoirs of cutaneous leishmaniasis)

Peripheral level

The number of peripheral level labs depends on the number of counties in each province. There are 452 counties in Iran (Statistical Center of Iran) and each of them needs one peripheral level lab.

The laboratory of the county health center requires an entomology expert and two field technicians who can play an important role in collection of sand flies and rodents. Peripheral level tasks are as follows:

In order to have the necessary information, at least 3–4 suitable villages will be selected under the supervision of the provincial health center laboratory for entomology (where they could have the capability to keep sand flies colonies) in infected foci of the plain region and the

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same number in the mountainous region of the county. So that the villages are considered as the real representative of the county. Then, from the beginning to the end of each active season, once every two weeks, at sunset, 30 sticky traps (castor oil coated white papers, 20× 35cm) will be installed in fixed indoor places, including bedrooms, living rooms, stables, barns and toilets, and 30 sticky paper traps will be installed in fixed outdoor areas, including on the base of walls, under boulders, caves and rodent burrows. They are collected the next morning, before sunrise and then preserved in 70% ethanol, in which form they are transferred to the laboratory of the county. Indoor locations should be included in three houses, one in the middle of the village, another in the outskirts of the village and the third between the previous two houses to show the actual activity of sand flies.

- Mounting of sand flies in Puri's medium according to the relevant instructions.
- Preparing samples from sand flies blood content in the gut to determine the host blood preference of vectors according to the relevant instructions and sending them to the laboratory of the province.
- Collection of rodents with at least 50 Sherman traps up to a radius of 500 meters from the center of the village, once a season in infected foci, preparing two slides from each rodent earlobe, staining the slides and diagnosing *Leishmania* parasites according to the relevant instructions.
- Separation of skin and skull of collected rodents according to the relevant instructions and sending to the middle level (Provincial centers).
- Record the results on the relevant forms and send it to the coordinator of cutaneous leishmaniasis of the county.
- Sending monthly performance reports regularly to the province level.

Middle level (Provincial level)

The provincial health center laboratory is the University Reference Laboratory. There are 31 provinces in Iran, and each must have one lab at this level.

- The provincial health center must have a leishmaniasis entomology Lab. With the necessary facilities and equipment. The responsibilities of this level are as follows:
- Identification of mounted sand flies at the species level.
- Calculating the species composition of sandflies and their abundance by species.
- Drawing diagrams related to the monthly activity of sand flies by species in the indoors and outdoors.
- Preparing the distribution map of sand flies by species in the province as reported by rural district and county.
- Dissection of sand flies in order to determine the natural promastigote infection rate during the months of August and September as stated in instructions in county level.
- Computer registration of the results on sandflies and reservoir hosts information based on the relevant instructions and sending it to the national level.
- Technical supervision and quality control of all entomological laboratories of the affiliated counties by examining the mounted samples of sand flies and slides prepared from rodent earlobes and sending feedback.
- Training of the laboratory staff working at leishmaniasis entomology laboratories at the county level.
- Evaluation of vector control operations using standard scientific methods in the event of epidemics and outbreaks.

With the base-line of vector and reservoir relative and absolute abundances, their annual dynamics, and infection rates, any anomalies in the usual patterns provide an early warning system to intensify monitoring and active search of patients, and to make focus studies in order to define precisely the where and when to perform the more effective intervention for prevention or mitigation.

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Country level (National level)

The National Reference Laboratory of vectors and reservoir hosts of cutaneous leishmaniasis is responsible for management, diagnosis, supervising, laboratory, educational and research quality review, and its tasks are as follows:

1- Close cooperation with the Infectious Diseases Management Center in order to better implement the national programme for the control of cutaneous leishmaniasis and the goals of the ministry of health. At any level but also at this level, the programme should also include a network with the medical counterpart.
2- Technical supervision and quality control of all laboratories on vectors and reservoir hosts of cutaneous leishmaniasis in the country, by examining a given percentage of sandflies and rodents collected and identified in the province in order to confirm and send the results as feedback.
3- Climate correlation with abundances and infection rates, and land use change at different space and time scales, including the national level monitoring of changes in the patterns.
4- Isolation of *leishmania* parasites from vectors and reservoir hosts and identification by using molecular methods if necessary. An experienced senior technician will perform this goal.
5- Molecular identification of vectors and reservoirs if needed.
6- Determination the susceptibility level of the main vectors of the disease to the used insecticides by the standard method of the world health organization, if necessary.
7- Determination the host-feeding preference of vectors by advanced diagnostic methods.
8- Necessary recommendations regarding the time of spraying and the type of the insecticide during outbreaks and epidemics of cutaneous leishmaniasis.
9- Carrying out advanced research projects on the ecology of vectors and reservoir hosts and evaluation different methods of vector control and reservoirs in cooperation with the Infectious Diseases Management Center. Eco-epidemiology studies to identify exposure risk and accessibility to health care according to region, gender and age.
10- Preparation of necessary instructions for collection methods and sampling of sand flies and reservoirs and their survey techniques.
11- Launching advanced diagnostic methods for vectors and reservoirs of the disease in provincial laboratories.
12- Participating in analyzing the results of

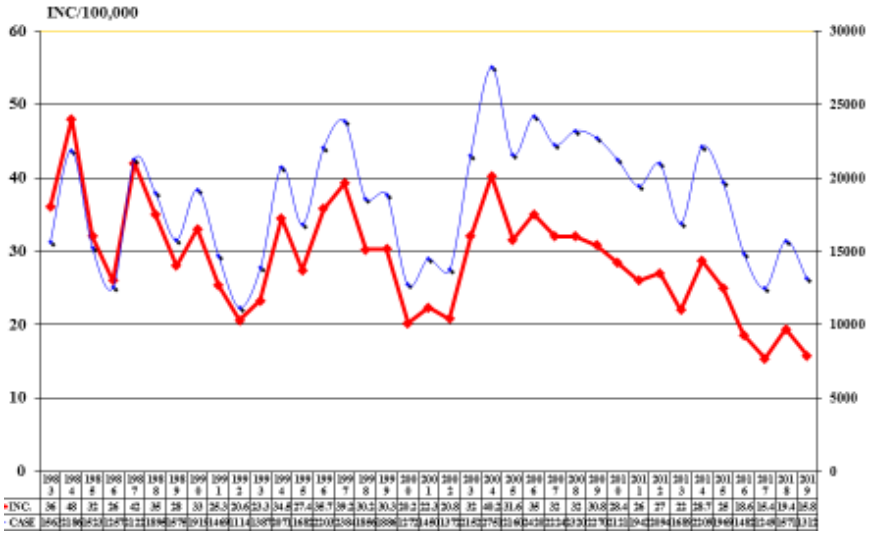

Fig. 1. Trend of cutaneous leishmaniasis Iran 1983–2019

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Table 1. Cutaneous Leishmaniasis cases and Incidence Rate/ 100000 in endemic provinces of Iran, 2019

No	Endemic Provinces	Geographical Zones	CL Number	CL Incidence Rate/100000
1	Semnan	Near north	719	95.9
2	Ilam	West	470	78.7
3	Golestan	North-east	1188	60.9
4	Isfahan	Center	3127	59.1
5	Fars	South-west	2002	40.0
6	North Khorasan	North-east	214	24.0
7	Khorasan Razavi	North-east	1581	23.4
8	Yazd	Center	260	21.4
9	Qom	Near central plateau	293	21.3
10	Khuzestan	South-west	1013	20.7
11	Kerman	South-east	565	17.1
12	Sistan and Baluchestan	South-east	357	12.0
13	Hormozgan	South	160	8.4
14	Kermanshah	West	117	5.9
15	South Khorasan	East	39	4.8
16	Lorestan	West	69	3.8
17	Tehran	North of central plateau	434	3.1
18	Bushehr	South	28	2.3

activities in preparing annual reports.

13- Establishing close relations with prestigious international scientific institutions abroad and inside the country.

14- Organizing of practical and applied training workshops on the control of cutaneous leishmaniasis at different levels for managers, general practitioners, experts and technicians in the country.

15- Periodic visits to provinces and counties in order to monitor executive operations.

16- Organizing of practical and applied entomology workshops of leishmaniasis and its reservoirs at different levels.

Discussion

The results of the search of information indicate the fact, that there are no laboratory networks on vectors and reservoirs of cutaneous leishmaniasis in any country. There is only one Spanish language publication from South America (18) on this issue, which cannot be generalized to our country because of the differences in the geographical and epidemiological situation of the disease between these two regions. Health education is a specialized issue that unfortunately is not considered by the authorities in Iranian health centers, especially since the methods of educating the community have changed in the last two decades and only experts can implement them. In view of the results of this study, it seems that the Iranian Ministry of Health and Medical Education should prioritize the employment of specialized and capable personnel in the field of Medical Entomology and Vector Control so that they can better serve in the field for the country's health system. Because the lack of attention to the existence of a cohesive organization and experienced personnel can pose a serious threat in the future in endemic foci of cutaneous leishmaniasis and other neighboring provinces. It is necessary to set up and use Medical Entomology laboratories in the provinces as it was proposed of the mentioned network as soon as possible while having sufficient facilities and equipment. The chancellors of Medical Sciences Universities and health deputies of the provinces could have an important role in this matter and should pay special attention to setting up the discussed network. If any of the universities of medical sciences in the country have the facilities to set up this network in terms of laboratory equipment and tools and experienced specialists, it will

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undoubtedly be very effective in the control of the disease. Creating a constructive and dynamic competitive environment in this new movement and trying to create links and interaction at different levels (environment-health-social development) among provinces and between jurisdictional levels (from county to national level) can be useful. It should be noted that the sustainability of this laboratory network depends entirely on hiring and employing literate, interested and persistent people. It should be mentioned that the results of this research can be used in Afghanistan, Pakistan, Iraq, Syria, Tajikistan and Turkmenistan which have a similar problem regarding the importance of leishmaniasis as a health issue and lacking a proper national network system to perform integrated management that includes vector, reservoirs and humans. In those senses, it should be mentioned that expert manpower is important to get things done, and the adequate use of already existing or created capacities is essential. It is hoped that in the near future we will see the serious efforts of our colleagues to consolidate a network, and that all respected managers and experts will base their work on activities of the network according to the tasks that have been defined.

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