

# Integration and evaluation of cutaneous leishmaniasis laboratory diagnosis in the primary health care laboratory network

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## Abstract

**Background:** The lack of an integrated national system prevents the Islamic Republic of Iran from registering and reporting all cases of cutaneous leishmaniasis.

**Aim:** To establish a laboratory network for the improvement of diagnosis and surveillance of cutaneous leishmaniasis in endemic areas of the Islamic Republic of Iran using parasitological and molecular methods.

**Methods:** This descriptive, cross-sectional, pilot study examined 49 laboratories in the 2 endemic areas for cutaneous leishmaniasis in the Islamic Republic of Iran. Samples were taken for identification of the dominant *Leishmania* species from individuals with cutaneous leishmaniasis referred to the laboratories and had not travelled to other endemic regions. Statistical analysis was conducted using SPSS version 25.0. Using the primary healthcare laboratory network, we established a 3-level surveillance system. We compared misdiagnosis, new cases, clinical relapses, treatment resistance, and treatment failure before and after establishment of the network.

**Results:** Network implementation reduced relapse of cutaneous leishmaniasis. After the laboratory training, the average misdiagnosis rate decreased from 49.3% to 4.2% for positive microscopic slides and from 31.6% to 12% for negative slides. Correct diagnosis was significantly higher in the study areas after the intervention.

**Conclusion:** Implementation of a cutaneous leishmaniasis laboratory network can enhance diagnosis, unify diagnostic methods and improve patient care.

Keywords: cutaneous leishmaniasis, laboratory network, capacity building, pilot study, primary health care, Islamic Republic of Iran

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## Introduction

Leishmaniasis encompasses a group of neglected tropical diseases caused by *Leishmania* parasites. According to WHO, leishmaniasis is endemic in at least 98 countries, with 12 million cases, 350 million at risk of infection, and > 20 000 deaths annually (1). There are 3 types of leishmaniasis: visceral, cutaneous, and mucocutaneous, and cutaneous leishmaniasis (CL) is the most prevalent. It affects the skin, causing lesions and ulcers on exposed areas, leaving permanent scars and stigma, or severe impairment (2).

The Islamic Republic of Iran is an area of CL endemicity (2). A recent meta-analysis estimated that the pooled prevalence of CL in the country was 45% (3), indicating that the disease requires attention. Although the Islamic Republic of Iran reports 20 000 new cases of CL annually, the actual number is likely several times higher (4,5). It appears that a significant number of cases of CL in the Islamic Republic of Iran are neither reported nor registered. This demonstrates the lack of an integrated national system that standardizes and unifies

diagnostic tools and treatment plans. Establishment of a laboratory network would standardize and unify diagnostic techniques for CL and aid in differentiating it from other skin diseases. It would also assist in registering and reporting new cases, relapses, treatment failure, and treatment-resistant cases.

This study is believed to be the first effort to establish a laboratory network for CL in the Islamic Republic of Iran and other endemic countries in the Middle East. The study aimed to establish and evaluate a laboratory network to diagnose CL, which will aid in developing a model for improving healthcare system for leishmaniasis in the Islamic Republic of Iran.

## Methods

### Study design and settings

This was a descriptive cross-sectional pilot study conducted from 2014 to 2016 in 2 areas of the Islamic Republic of Iran: Ilam in the west, which is endemic for zoonotic CL, and Mashhad in the east, which is

endemic for anthroponotic CL. We used the primary healthcare laboratory network to establish a surveillance system for active and passive detection of CL during 6 months. The network consisted of 3 levels: sentinel laboratories, university reference laboratories, and national laboratories. The National Health Laboratory was responsible for monitoring the performance of laboratories at all levels and quality control of diagnostic tools (Figure 1). We conducted continuous monitoring of all centres for up to 2 years. The study protocol was approved by the Ethics Committee of Tehran University of Medical Sciences, and the findings were published confidentially with the approval of the authorities (IR TUMS.VCR.REC.1395-373). Figure 2 shows the process of implementation of a pilot cutaneous leishmaniasis laboratory network.

Sentinel laboratories

Urban and rural health service laboratories

These laboratories were responsible for collecting and sorting patients' data, and referring new cases, treatment failures, and clinical relapses to the respective district health centre laboratory, as well as sending monthly performance reports.

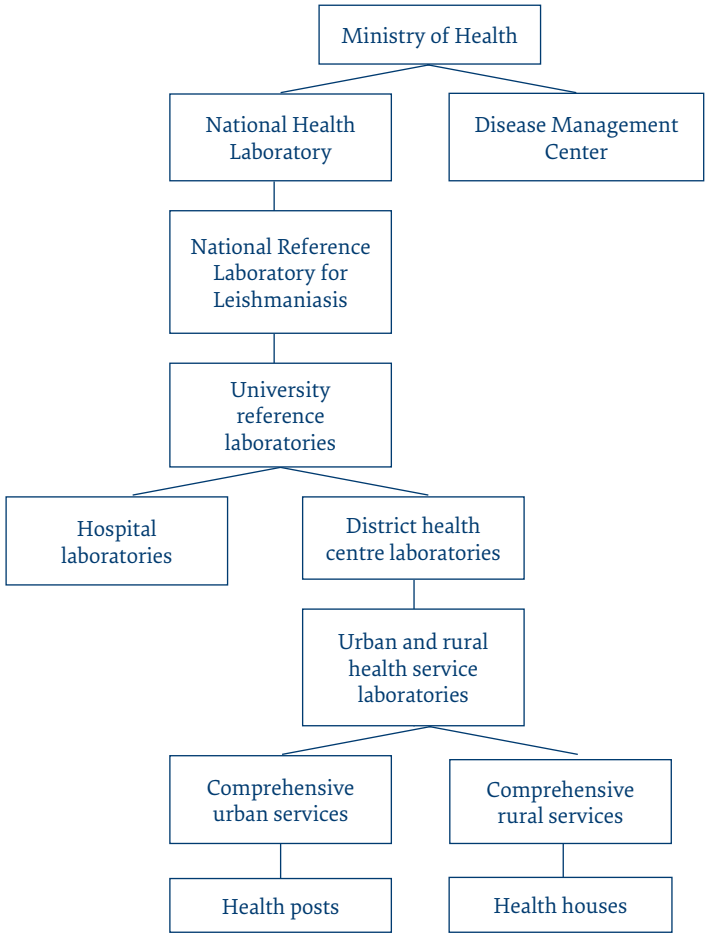
District health centre laboratories

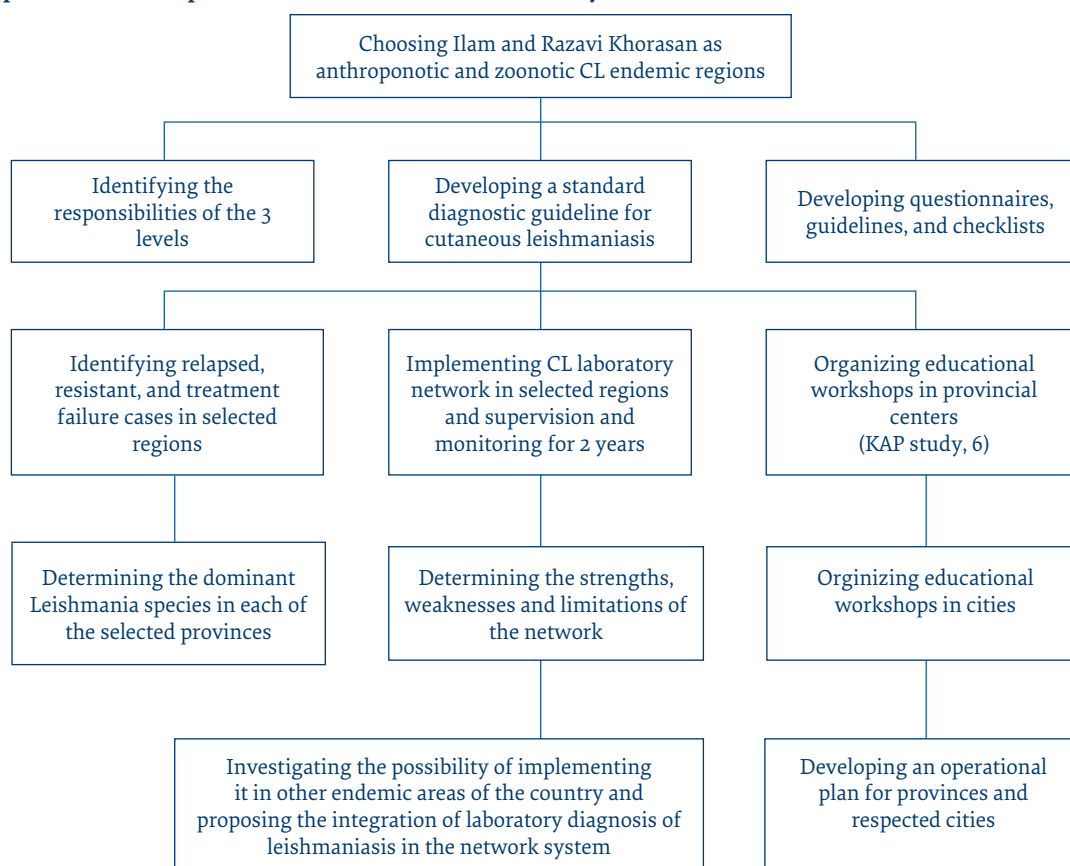
These laboratories played a crucial role in the diagnosis of CL. They were responsible for: preparing microscopic smears from skin lesions; referring clinically suspicious cases with no negative smear tests to higher-level laboratories; preparing smears from treatment failure/recurrent cases; registering smear test results in the laboratory office; sending the results to physicians daily; monitoring sampling procedures; training laboratory personnel in using national guidelines; and sending monthly reports to university reference laboratories.

University reference laboratories

University reference laboratories performed parasitological culture and microscopic examination of *Leishmania* spp., and identified the species using additional molecular (polymerase chain reaction; PCR) or immunological (monoclonal antibody) tests. They were responsible for: training employees of the CL diagnostic laboratories; managing and supervising other laboratories affiliated with the university; recording activities and sending results to the university disease control unit; and quality control. They supervised associated laboratories in outlying cities every 3 months through evaluating 20% of negative and 20% of positive slides in endemic regions

Figure 1 Clinical laboratory network system in Islamic Republic of Iran



**Figure 2 Implementation of a pilot cutaneous leishmaniasis laboratory network**

and all slides in nonendemic regions. They were also responsible for developing an action plan for controlling CL in the areas under supervision of the university, in cooperation with the disease control unit. The action plan included: actively participating in the provincial leishmaniasis committee and reporting on the activities of the laboratory network in the province; holding training workshops to improve the capability of health laboratory experts; and monitoring the performance of subordinates.

## National laboratories

### National Reference Laboratory for Leishmaniasis

The National Reference Laboratory for Leishmaniasis was responsible for diagnostic confirmation, quality assurance, training, research, evaluation of diagnostic products, and collaboration in health laboratory monitoring. The details of these tasks were approved and notified by the National Health Laboratory. The National Reference Laboratory for Leishmaniasis was also responsible for: examining complex samples that were not diagnosed in the first- and second-level laboratories; collaborating on the compilation and revision of national educational standards and checklists; creating an operational plan in cooperation with the Health Reference Laboratory and Infectious Disease

Management Center; designing and collaborating with research projects related to the goals of the network; and cooperating with other scientific institutions abroad and within the country in accordance with the goals of patient care, in close collaboration with the Infectious Diseases Management Center and the National Health Laboratory. Further responsibilities were: evaluation of diagnostic products according to the needs stated by the National Health Laboratory; cooperation with the External Quality Assurance Services programme for health laboratories; quality evaluation of samples from lower-level laboratories upon the request of the National Health Laboratory; participation in the final analysis of laboratory results in annual reports; and submission of a yearly report to the Health Reference Laboratory and Infectious Diseases Management Center detailing past activities and future goals.

### National Health Laboratory

The National Health Laboratory was responsible for: developing and revising operational plans, educational guidelines, and checklists for the laboratory diagnostic network for CL in collaboration with the Center for Disease Management and National Reference Laboratory; monitoring laboratory performance at all levels; holding workshops for regional focal points and laboratory inspectors; implementing the External Quality Assurance Services programme for health; and financially assisting

and equipping laboratories in accordance with the memorandum of agreement.

Determination of dominant *Leishmania* species

Samples were taken from individuals with CL who were referred to health centres and had not travelled to other endemic regions. Samples were free from blood and secondary microbial infection, as much as possible, and were taken from a wide area that included the main foci of leishmaniasis in the selected provinces. Sampling was conducted over the entire year.

Microscopic smears were prepared from suspicious cases by removing material from the edge of skin lesions using a sterile lancet, fixing with absolute methanol, and staining with Giemsa stain.

Nested PCR was used to identify the dominant species. The different species of *Leishmania* were identified by amplification of a high-resolution region of internal transcribed spacer rDNA, as demonstrated previously (9). The PCR protocol is outlined in Table 1.

Assessment tools and outcomes

We had 50 meetings with academics and experts from the Department of Medical Parasitology and Mycology of Tehran University of Medical Sciences, Zoonoses Department of the Center for Communicable Diseases Management, and National Health Laboratory. During these sessions, we developed a diagnostic guideline for CL, and monitoring and evaluation checklists for network implementation. We held training workshops for 41 individuals from Razavi Khorasan Province and 21 from Ilam Province. The design, details of the methodology, and impact of these workshops on knowledge, attitude, and practice of staff have been published previously (10).

We used the census sampling method to include all laboratories in Ilam and Khorasan Razavi. To identify the prevalent *Leishmania* species, we reviewed 192 positive

smears of high quality with a 95% confidence interval and 7.5% margin of error. To evaluate the CL laboratory network, we reviewed 20% of the positive and 20% of the negative samples from each district health centre laboratory before and after implementing the network, using standard microscopic examination methods (11), to estimate the rate of false-positive and false-negative results. We recorded the number of new cases and treatment outcomes (number of relapses, treatment failures, and treatment-resistant cases) throughout the 2-year period before and after network implementation. We used *ITS1* gene and nested PCR using kinetoplast DNA for identification of *Leishmania major* and *Leishmania tropica*, which were circulating in the pilot study areas (6–8,12).

Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics for Windows version 25.0. Quantitative data were reported using mean and standard deviation. Frequency was used to report qualitative variables. To assess the internal consistency of the questionnaire, Cronbach's  $\alpha$  was calculated, and  $> 0.7$  indicated a high level of reliability. We used McNemar's test to compare paired proportions, Fisher's exact test to analyse the relationship between 2 categorical variables, Wilcoxon's test to compare 2 paired samples, and the Kruskal–Wallis test to compare  $\geq 3$  independent groups.  $P < 0.05$  was considered statistically significant.

Results

We studied 15 laboratories in Ilam (10 urban reference laboratories and 5 rural/urban laboratories) and 34 laboratories in Mashhad (19 urban reference laboratories and 15 rural/urban laboratories).

New cases and treatment outcomes

In Ilam, the frequency of new CL cases, relapse after systemic therapy, and relapse after topical therapy

Table 1 PCR protocol and primers

	First PCR			Second PCR		
	Duration	Temperature	No. of cycles	Duration	Temperature	No. of cycles
Initial denaturation	5 min	95°C	1	2 min	95°C	1
Denaturation	30 s	95°C	35	15 s	95°C	25
Annealing	45 s	55°C		30 s	60°C	
Extension	45 s	72°C		30 s	72°C	
Final extension	5 min	72°C	1	5 min	72°C	1
External primers: from ITS-rDNA						
<i>Leishmania</i> out Forward (5'-AAA CTC CTC TCT GGT GCT TGC-3')						
<i>Leishmania</i> out Reverse (5'-AAA CAA AGG TTG TCG GGG G-3')						
Internal primers:						
<i>Leishmania</i> in Forward (5'-AAT TCA ACT TCGCGT TGG CC-3')						
<i>Leishmania</i> in Reverse (5'-CCT CTC TTT TTTCTC TGT GC-3')						

ITS = internal transcribed spacer; PCR = polymerase chain reaction.

Table 2 New cases of cutaneous leishmaniasis and treatment outcomes before and after laboratory network implementation

Province	Year	New cases	Treatment failure following topical treatment	Treatment failure following systemic treatment	Relapse following systemic treatment	Relapse following topical treatment	Treatment discontinuation	Drug resistance	Total
Mashhad	2014 <sup>a</sup>	1906	2	1	6	13	3	0	1931
	2016 <sup>b</sup>	1432	4	0	6	16	1	2	1461
Ilam	2014 <sup>a</sup>	1556	0	0	2	3	0	0	1561
	2016 <sup>b</sup>	1059	0	0	0	1	0	0	1060

<sup>a</sup>Before implementing laboratory network. <sup>b</sup>After implementing laboratory network.

decreased following implementation of the network (Table 2). Despite a decline in the number of new cases in Mashhad, relapse, treatment resistance, and treatment failure increased.

### Species of *Leishmania* causing CL

We reviewed 190 positive smears of high quality; 96 samples from Mashhad and 94 from Ilam. In Mashhad, the average age of the individuals from whom smears were obtained was 17.75 years; 45 (46.9%) were women, 35 (36.5%) men, and 16 (16.7%) were unidentified ( $P = 0.314$ ) (Table 3). The age group most frequently affected was 20–30 years, with a frequency of 19.8%. The number of cases differed significantly among the age groups ( $P = 0.002$ ). There were significantly more nontravellers (72, 75%) than travellers (24, 25%) ( $P = 0.001$ ). The season with the most significant number of CL cases was autumn. Ten (10.4%) of the 96 individuals had ulcers, 58 (60.4%) had nodules, and 28 (29.2%) were unreported. The face, cheeks, and hands were the most commonly affected body parts. The dominant species was *L. tropica*, with a frequency of 85.4%.

In Ilam, the average age of the individuals from whom smears were obtained was 23.47 years; 62 (66%) were men, 29 (30.9%) women, and 3 (3.2%) were unidentified ( $P = 0.001$ ) (Table 3). The age group most frequently affected was 20–30 years, with a frequency of 17%. The number of cases in each age group did not differ significantly ( $P = 0.1$ ). There were significantly more nontravellers (92, 97.9%) than travellers (2, 2.1%) ( $P = 0.001$ ). The season with the highest number of CL cases was autumn. The hands and feet were the most commonly affected body parts. The dominant species was *L. major*, with a frequency of 79.8%.

### Misdiagnosis rate before and after network implementation

We assessed smears at baseline (before network implementation), and 6 months (primary assessment), 12 months (secondary assessment), and 18 months (tertiary assessment) after network implementation (Table 3). The average misdiagnosis rate in the 2 studied areas was 49.3% for positive smears and 31.6% for negative smears. After implementing the network, following regular laboratory training, these rates were reduced to 4.2% and 12%, respectively. For ethical considerations, we removed the studied area names from the table. The total rate of correct diagnosis was significantly higher after intervention in both regions: 0.816 versus 0.93 in study area 1 ( $P = 0.015$ ) and 0.358 versus 0.86 in study area 2 ( $P < 0.0001$ ).

## Discussion

This study described the establishment of a laboratory network for the diagnosis of CL and the impact of this network on the misdiagnosis rates for both positive and negative smears. Our findings suggest that implementation of a laboratory network for CL increased diagnostic accuracy and decreased diagnostic errors.

Unlike many other infectious diseases, the incidence of CL is increasing globally, including in the Islamic Republic of Iran (1,2,4). Also, the number of visceral leishmaniasis epicentres has increased significantly (1). The reasons for these increases include: indiscriminate development of large cities in the form of satellite towns; an increase in wild rodents as disease reservoirs; increased number of sandflies; movement of people, and settlement of nonimmune groups in contaminated areas; and settlement of infected people in areas susceptible to becoming epicentres of the disease (13,14).

Currently, there is no proven and cost-effective way to control CL (14,15). There is a mistaken belief among the general population that epidemics of this disease, like other infectious diseases, can be quickly controlled. However, even with appropriate and costly measures, control can take several months to a year. Treatment of CL is time-consuming and associated with numerous complications (16,17). The disease is more prevalent among poor people, and producing new treatments by pharmaceutical companies is costly. Therefore, there is no motivation to manufacture drugs that are safer and easier to use.



**Table 3 Characteristics of patients with a definite diagnosis of cutaneous leishmaniasis**

Characteristics	Mashhad (N = 96)		Ilam (N = 94)	
	n (%)	P	n (%)	P
<b>Age, yr</b>		0.002		0.1
<5	7 (7.3)		11 (11.7)	
5–10	13 (13.5)		9 (9.6)	
10–15	12 (12.5)		2 (2.1)	
15–20	10 (10.4)		9 (9.6)	
20–30	19 (19.8)		16 (17)	
30–40	3 (3.1)		10 (10.6)	
>40	3 (3.1)		11 (11.7)	
Not reported	29 (30.0)		26 (27.7)	
<b>Gender</b>		0.314		0.001
Male	35 (36.5)		62 (66)	
Female	45 (46.9)		29 (30.9)	
Not reported	16 (16.7)		3 (3.2)	
<b>Travel history</b>		0.001		0.001
No	72 (75)		92 (97.9)	
Yes	24 (25)		2 (2.1)	
<b>Months</b>		0.001		<0.001
March	7 (7.3)		1 (1.1)	
April	7 (7.3)		2 (2.1)	
May	9 (9.4)		2 (2.1)	
June	2 (2.1)		0	
July	0		5 (5.3)	
August	0		4 (4.3)	
September	16 (16.7)		8 (8.5)	
October	11 (11.5)		4 (4.3)	
November	14 (14.6)		22 (23.4)	
December	1 (1)		12 (12.8)	
January	0		15 (16)	
February	1 (1)		6 (6.4)	
Not reported	29 (30.2)		14 (14.9)	
<b>Affected body part</b>		0.001		0.001
Malleolus	1 (1)		0	
Arm	5 (5.2)		13 (13.8)	
Auricle	2 (2.1)		0	
Cheeks	10 (10.4)		0	
Chin	6 (6.3)		0	
Face	17 (17.7)		9 (9.6)	
Fingers	4 (4.2)		1 (1.1)	
Forearm	6 (6.3)		11 (11.7)	
Forehead	1 (1)		0	
Leg	1 (1)		3 (3.2)	
Hand	9 (9.3)		38 (40.4)	
Feet	3 (3.1)		18 (19.1)	
Nose	2 (2.1)		1 (1.1)	
Wrist	1 (1)		0	
Trunk	0		8 (8.5)	
Thigh	0		3 (3.2)	
Head	0		3 (3.2)	
Neck	0		3 (3.2)	
Elbow	0		1 (1.1)	
Eye	0		1 (1.1)	
<b>Leishmania species</b>		<0.001		<0.001
<i>L. major</i>	4 (4.2)		75 (79.8)	
<i>L. tropica</i>	82 (85.4)		10 (10.6)	
No DNA	10 (10.4)		9 (9.6)	

New methods for rapid diagnosis of CL have not yet been implemented in the Islamic Republic of Iran. As a result, patients are occasionally referred to university reference laboratories for CL when they can no longer be treated. In addition to increasing the accuracy and precision of laboratory diagnosis of CL, standardization

and unification of diagnostic methods, and the establishment of a unified and active care system in endemic and nonendemic areas would contribute to the improvement of disease management.

The results of our pilot study showed that implementation of a leishmaniasis laboratory network, which included national guidelines for the validation of diagnostic methods, led to consistency in providing uniform training for all laboratory personnel and facilitated diagnosis and referral of patients. Network implementation resulted in accurate identification and reporting of CL, treatment failure, relapse, and treatment resistance. This is important because laboratory confirmation is essential given the time, money, and complications involved in treating CL. Accurate and prompt diagnosis leads to complete registration of cases, decreased prevalence, reduced scar size and disease complications, and a drop in mortality rates.

To identify the dominant species of *Leishmania*, we used molecular methods of nested PCR and DNA sequencing. Previous studies have identified the predominant CL species in different regions of the Islamic Republic of Iran, with *L. major* the most prevalent in Ilam (18,19) and *L. tropica* the dominant species in Mashhad (20–22). Our findings are consistent with these studies. Although *L. major* was the predominant species in Ilam, *L. tropica* was also reported, and although *L. tropica* was the dominant species in Mashhad, *L. major* was also documented. These findings highlight the importance of developing a consistent approach for identifying and managing each *Leishmania* species rather than focusing only on the most prevalent in each region. A standardized national programme should be established to regularly investigate and monitor all relevant *Leishmania* species. This programme could be administered by universities and research institutes under the supervision of the Infectious Diseases Center and the National Health Laboratory.

The reduction in misdiagnosis rates observed in our study highlighted the importance of providing professional skill and experience-based training to laboratory personnel to improve diagnostic accuracy and ensure the success and progress of quality improvement initiatives.

The main limitation in our study was turnover of laboratory staff. Future steps should include: (1) development of a strategic plan for CL diagnosis and assisting universities to develop short- and long-term plans to establish a national laboratory network for CL diagnosis; (2) estimating the financial, human, and laboratory equipment resources required to establish a nationwide laboratory network; and (3) communication with international organizations to exchange information and gain insight into best practices for establishing and maintaining a successful laboratory network.

## Conclusion

Our study revealed that implementation of a laboratory network for CL increased the accuracy of laboratory diagnosis, unified diagnostic methods, and improved the care system for management of CL.

**Table 4 Misdiagnosis rates before and after laboratory network implementation**

Study area	Diagnosis	Reported	TRUE	Error rate (percentage)	κ coefficient	P*	P**
1	Before intervention	Negative	18	14	22.2	0.496	<0.0001
		Positive	79	65	17.8		
		Total	97	79	18.5		
	Primary assessment after intervention	Negative	19	15	21	0.768	<0.0001
		Positive	81	78	3.7		
		Total	100	93	7		
	Secondary assessment after intervention	Negative	3	3	0	—	—
		Positive	53	52	1.9		
		Total	56	55	1.8		
	Tertiary assessment after intervention	Negative	79	76	3.8	—	—
		Positive	10	10	0		
		Total	89	86	3.4		
2	Before intervention	Negative	20	12	40	0.498	0.027
		Positive	136	44	67.6		
		Total	156	56	64		
	Primary assessment after intervention	Negative	45	33	27.7	0.611	<0.0001
		Positive	155	140	3.7		
		Total	200	173	13.3		
	Secondary assessment after intervention	Negative	0	0	0	—	—
		Positive	10	9	10		
		Total	10	9	10		
	Tertiary assessment after intervention	Negative	46	34	26.1	—	—
		Positive	61	58	4.9		
		Total	107	92	14		

\*Inter-rater reliability. \*\*Comparison before and after intervention.

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**Competing interests:** None declared.

## Intégration et évaluation du diagnostic de la leishmaniose cutanée dans le réseau de laboratoires de soins de santé primaires

### Résumé

**Contexte :** La République islamique d'Iran ne dispose pas d'un système national intégré permettant d'enregistrer et de signaler tous les cas de leishmaniose cutanée.

**Objectif :** Mettre en place un réseau de laboratoires afin d'améliorer le diagnostic et la surveillance de la leishmaniose cutanée dans les zones d'endémie en République islamique d'Iran à l'aide de méthodes parasitologiques et moléculaires.

**Méthodes :** La présente étude pilote transversale et descriptive a été menée auprès de 49 laboratoires situés dans les deux zones d'endémie de leishmaniose cutanée en République islamique d'Iran. Dans le but d'identifier les espèces dominantes de *Leishmania*, des échantillons ont été prélevés auprès de personnes touchées par la maladie qui n'ont pas voyagé vers d'autres régions endémiques. Ces échantillons ont ensuite été envoyés aux laboratoires. Une analyse statistique a été réalisée à l'aide du logiciel SPSS version 25.0. Le recours au réseau de laboratoires de soins de santé primaires nous a permis de mettre en place un système de surveillance à trois niveaux. Nous avons comparé

les erreurs de diagnostic, les nouveaux cas, les rechutes cliniques, la résistance au traitement et son échec, avant et après la création du réseau.

**Résultats :** La mise en œuvre de ce réseau a permis de réduire le nombre de rechutes pour la leishmaniose cutanée. Après la formation en laboratoire, le taux moyen d'erreurs de diagnostic a diminué, passant de 49,3 % à 4,2 % pour les lames microscopiques positives et de 31,6 % à 12 % pour les lames négatives. Le nombre de diagnostics corrects était significativement plus élevé dans les zones étudiées après l'intervention.

**Conclusion :** La mise en place d'un réseau de laboratoires pour la leishmaniose cutanée peut permettre d'améliorer le diagnostic, d'en unifier les méthodes et d'optimiser les soins dispensés aux patients.

## دمج التشخيص المختبري لداء الليشمانيات الجلدي وتقييمه في شبكة مختبرات الرعاية الصحية الأولية

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### الخلاصة

الخلفية: نظراً إلى عدم وجود نظام وطني متكامل في جمهورية إيران الإسلامية، يتعذر تسجيل جميع حالات داء الليشمانيات الجلدي والإبلاغ عنها.

الأهداف: هدفت هذه الدراسة إلى إنشاء شبكة مختبرات لتحسين تشخيص داء الليشمانيات الجلدي وترصده في المناطق المستوطنة به في جمهورية إيران الإسلامية باستخدام وسائل التشخيص الطفيلي والجزيئي.

طرق البحث: فُحصت هذه الدراسة التجريبية الوصفية المقطعية 49 مختبراً في المنطقتين المستوطنتين بداء الليشمانيات الجلدي في جمهورية إيران الإسلامية. وقد أخذت عينات لتحديد أنواع الليشمانيات السائدة من الأفراد المصابين بداء الليشمانيات الجلدي المحليين إلى المختبرات ولم يسافروا إلى مناطق مستوطنة أخرى. وأجري تحليل إحصائي باستخدام الإصدار 25.0 من برنامج SPSS. وباستخدام شبكة مختبرات الرعاية الصحية الأولية، أنشأنا نظام ترصد ثلاثي المستويات، وقارنا التشخيص الخاطئ، والحالات الجديدة، والانتكاسات السريرية، ومقاومة العلاج، وفشل العلاج، قبل إنشاء الشبكة وبعده.

النتائج: قلَّ تنفيذ الشبكة من انتكاسات مرضى داء الليشمانيات الجلدي. وبعد التدريب المختبري، انخفض متوسط معدل التشخيص الخاطئ من 49.3 % إلى 4.2 % بالنسبة للشرائح المجهرية الإيجابية، ومن 31.6 % إلى 12 % بالنسبة للشرائح السلبية. وصار التشخيص الصحيح أعلى كثيراً في مناطق الدراسة بعد التدخل.

الاستنتاجات: يمكن أن يؤدي تنفيذ شبكة مختبرات داء الليشمانيات الجلدي إلى تعزيز التشخيص وتوحيد طرق التشخيص وتحسين رعاية المرضى.

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