Table 1. 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[a]	1906	2	1	6	13	3	0	1931
	2016[b]	1432	4	0	6	16	1	2	1461
Ilam	2014[a]	1556	0	0	2	3	0	0	1561
	2016[b]	1059	0	0	0	1	0	0	1060

[a]*Before implementing laboratory network.* [b]*After implementing laboratory network.*

Table 2. Characteristics of patients with a definite diagnosis of cutaneous leishmaniasis

	Mashhad (N = 96)		Ilam (N = 94)	
Characteristics	n (%)	P	n (%)	P
Age, yr				
<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)	0.002	9 (9.6)	0.1
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)	
Gender			62 (66)	
Male	35 (36.5)	0.314	29 (30.9)	0.001
Female	45 (46.9)		3 (3.2)	
Not reported	16 (16.7)			
Travel history				
No	72 (75)	0.001	92 (97.9)	0.001
Yes	24 (25)		2 (2.1)	
Months				
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	0.001	5 (5.3)	<0.001
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)	
Affected body part				
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)	0.001	3 (3.2)	0.001
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)	
Leishmania species				
L. major	4 (4.2)	<0.001	75 (79.8)	<0.001
L. tropica	82 (85.4)		10 (10.6)	
No DNA	10 (10.4)		9 (9.6)	

Table 3. 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	<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	0.015
		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.*

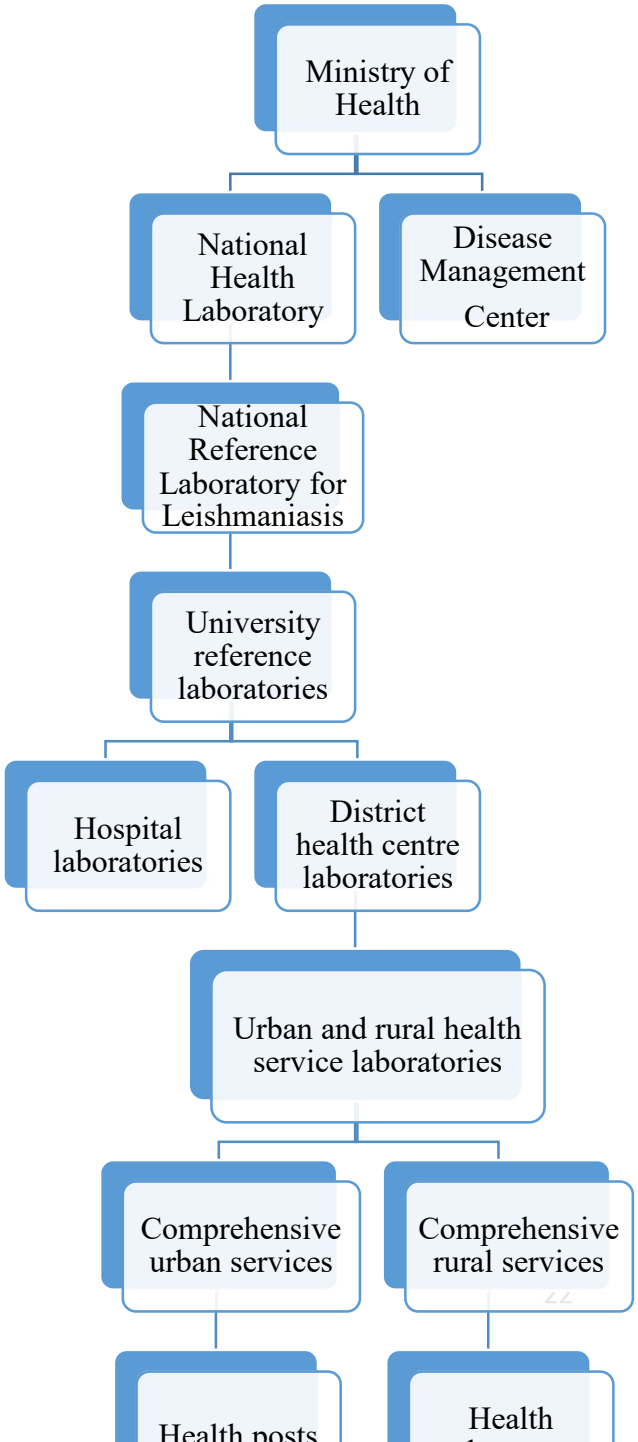
Ministry of Health
National Health Laboratory
Disease Management Center
National Reference Laboratory for Leishmaniasis
University reference laboratories
Hospital laboratories
District health centre laboratories
Urban and rural health service laboratories
Comprehensive urban services
Comprehensive rural services
Health posts
Health

Figure 1. Laboratory network system in Islamic Republic of Iran.

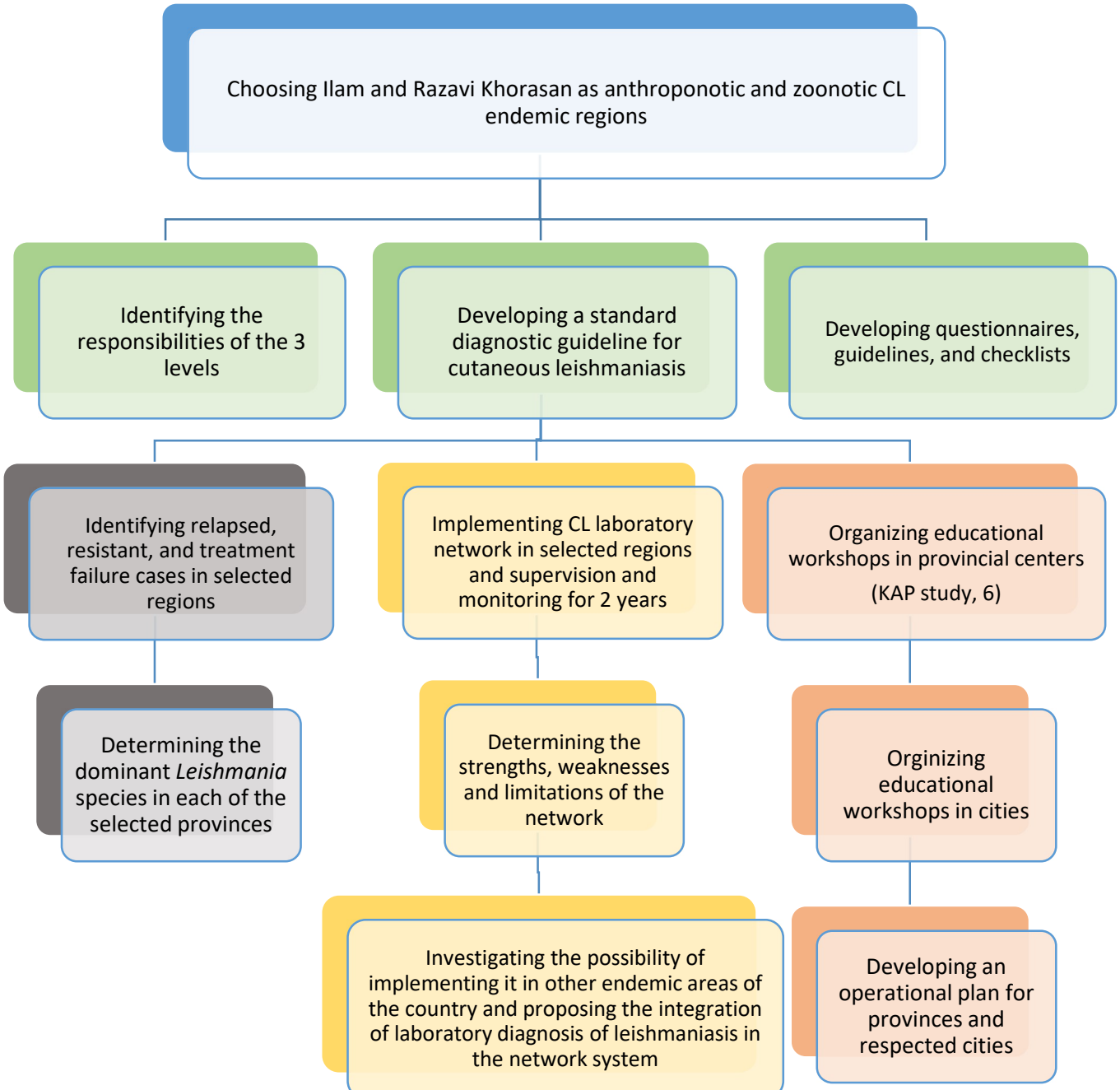
Choosing Ilam and Razavi Khorasan as anthroponotic and zoonotic CL endemic regions
Identifying the responsibilities of the 3 levels
Developing a standard diagnostic guideline for cutaneous leishmaniasis
Developing questionnaires, guidelines, and checklists
Identifying relapsed, resistant, and treatment failure cases in selected regions
Implementing CL laboratory network in selected regions and supervision and monitoring for 2 years
Organizing educational workshops in provincial centers (KAP study, 6)
Determining the dominant *Leishmania* species in each of the selected provinces
Determining the strengths, weaknesses and limitations of the network
Orginizing educational workshops in cities
Investigating the possibility of implementing it in other endemic areas of the country and proposing the integration of laboratory diagnosis of leishmaniasis in the network system
Developing an operational plan for provinces and respected cities

Figure 2. Implementation of a pilot cutaneous leishmaniasis (CL) laboratory network.

Supplementary Table S1. 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 *Leishmania* out Forward (5′-AAA CTC CTC TCT GGT GCT TGC-3′) *Leishmania* out Reverse (5′-AAA CAA AGG TTG TCG GGG G-3′)						
Internal primers: *Leishmania* in Forward (5′-AAT TCA ACT TCGCGT TGG CC-3′) *Leishmania* in Reverse (5′-CCT CTC TTT TTTCTC TGT GC-3′)						

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

Supplementary Table S2. Checklists

		Yes	No	Improvements needed
Presampling proceedings				
1	Is the patient's identity information accurately recorded in the laboratory?			
2	Is the patient's travel history asked and documented before the sampling?			
3	Is the appropriate device used for sampling according to the instructions?			
4	Is the sampling site disinfected with 70% ethanol before sampling?			
Urban and rural health centre laboratories in nonendemic regions				
5	Is the patient referred to the respective urban health centre laboratory for sampling and microscopic diagnosis in nonendemic regions?			
6	Is the identity information on the referred cases accurately recorded in the laboratory?			
Urban and rural health centre laboratories in endemic regions				
7	Are the required samples collected in the laboratory from various skin lesions?			
8	Have the laboratory personnel ever attended a training session on laboratory diagnosis of leishmaniasis?			
9	Is the sampling process for obtaining samples from patients with multiple lesions done according to the provided instructions?			
10	Are safety principles and use of protective equipment considered during sampling?			
11	Is 70% ethanol used to disinfect the lesion before sampling?			
12	Is flame used to sterilize the sampling device?			
13	Is sampling performed using a sterile vaccinostyle (or a lancet cut and narrowed) or a sterile scalpel with a narrow tip?			

14	Is a proper spread of secretions from the sample prepared on the slide?			
15	Is the patient's profile accurately written on the slide after preparation?			
16	Are the isolated samples in the rural laboratory referred to a higher-level laboratory according to the standard method of sample transfer?			
17	Are the specifications of the prepared or sent samples accurately recorded in the laboratory?			
Urban health centre and university reference laboratories				
18	Is there an appropriate microscope in the laboratory?			
19	Are there any established guidelines for ensuring the quality and accuracy of Giemsa-stained thick smears prior to their use in clinical diagnosis?			
20	Are safety principles and use of protective equipment considered during sampling?			
21	Is the desired level of red and white blood cell staining utilized to control the quality of the Giemsa solution?			
22	Are the specifications of the prepared or transported samples recorded accurately?			
University reference laboratories				
23	Does the laboratory have facilities for parasitology tests (microscopy and culture)?			
24	Does the laboratory have facilities for determining parasite species (additional tests such as polymerase chain reaction or monoclonal antibodies)?			
25	Is the university reference laboratory monitoring the performance of the urban laboratories?			
26	Have the samples been examined, and have the results been communicated to the urban health centre laboratories?			
27	Is culture performed from the lesions of referred patients when required?			

28	Are the activities and results reported to the provincial disease management group?			
29	Are the specifications of the prepared samples accurately recorded?			
Direct visual detection (microscopic tests)				
30	Are there any established guidelines for ensuring the quality and accuracy of Giemsa's solution-stained thick smears prior to their use in clinical diagnosis?			
31	According to the Giemsa type, is it diluted 1:10 with water and adjusted to a pH of 7.2?			
32	Does the laboratory own a pH meter that displays up to 2 decimal places?			
33	Is the pH meter calibrated each time it is used?			
34	Are the slides washed in water with a pH between 7 and 7.2?			
35	Does the purchased dye have a valid expiration date?			
36	Is diluted or filtered dye stored in a dark glass bottle?			
37	Is the diluted dye free of sediment?			
38	Is staining done according to the standard method and instructions?			
39	Are Leishman’s bodies examined using a light microscope with a 10× eyepiece, 100× objective lens, immersion oil, and microscope slides?			
40	Is each slide investigated at least 30 times until finding Leishman’s bodies?			
41	Are Leishman’s bodies well recognized in the positively stained slide?			
42	Will the second or third slide be examined if the sample is negative?			
43	If Leishman’s bodies are not found the first time, will a new blood-free and macrophage-containing sample be taken?			
44	Are at least 30 positive microscopic scans reviewed before negative microscopic results are reported?			
Headquarters monitoring checklist				

45	Are laboratory monitoring and audit documents available based on the specialized checklist?			
46	Are adjustments made in accordance with the results of the existing audit?			
47	Is the documentation from the training workshops and outcome evaluations of the course available?			
48	Are the annual assessment results of 20% positive slides and 20% negative slides of cutaneous leishmaniasis available in the urban health centre laboratory?			
49	Are the test results reported in a timely manner?			

Supplementary Table S3. Topics of the training workshop

First day
• Taking a pretest examination Lectures: • The importance of leishmaniasis care in the country • Current situation of leishmaniasis in the provinces of Khorasan Razavi and Ilam • The principles of leishmaniasis diagnosis and the necessity of implementing a laboratory network • Levelling of leishmaniasis diagnostic services • Action plan for implementation of the leishmaniasis laboratory network • How to take samples from skin lesions, and how to stain samples • Microscopic examination and identification of *Leishmania* parasites • Performing the *Leishmania* skin test on a volunteer and how to interpret it • Types of cultivation environments, performing cultivation, and interpreting the results • Discussion, exchange of opinions, and final summary
Second day
Visiting an urban health centre, examining patients with leishmaniasis, sampling, and taking a post-test examination