



The investigation of the infected hunted mice in Karandish and Kaveh terminals of Shiraz to Leishmaniasis with PCR method in 2015

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ABSTRACT

Leishmaniasis, is one of the common diseases among humans and animals caused by different species of protozoa of the Leishmania genus species and is seen in three forms of cutaneous, mucocutaneous and visceral Leishmaniasis in humans. Leishmaniasis life cycle includes of living on a vertebrate creature and an invertebrate carrier. Humans, dogs and wild canines and a number of small mammals such as rodents or wild rodents, Hyrax (hare) and sloth which is a primate from the bear's family in Central America and some species of marsupials are considered as the main repository of Leishmaniasis. Now cutaneous leishmaniasis is endemic in 88 countries, and its prevalence is estimated around 13 million people. Cutaneous leishmaniasis have been reported in 11 provinces out of 29 provinces in the country and is a health problem, Fars province and Shiraz is one of cutaneous leishmaniasis infection centers and Shiraz is the fourth focus of cutaneous leishmaniasis. Recently some studies on the identification of different species of rodents were conducted in different areas of Fars province. Since Fars province is one of the endemic areas of Leishmaniasis Zeiss and rodents as reservoirs of the disease and a lot of rodents are observed in passenger terminals of Shiraz There is the possibility of the spread of disease in case of the infection of the rodents and transition of the disease to passengers. Therefore 20 mice were hunted by trapping method from Kaveh and Karandish passenger terminal (10 rodents from each terminal). After taking blood sample from each rodent, a smear was provided for microscopic examination. Also, muzzle, ears and feet were sampled and after anatomy, internal organs of the animal such as the liver and spleen were sampled too and the samples were smeared too for microscopic examination. Totally, 50 samples including (ear, muzzle, liver, spleen, feet samples were provided from 10 rodents from each terminal (totally 20 rodents). Then due to the high sensitivity of the samples, DNA and PCR were extracted. In microscopic examination method none of the smears were positive while after PCR method among the 50 samples of the parts of mice bodies which were examined from Karandish terminal of Shiraz, 2 samples (liver) were infected to the major Lishmaniasis and among the 50 samples of the parts of the body of mice which were examined from Kaveh terminal of Shiraz, one sample (spleen) was infected to infantum lishmaniasis. Totally among the 100 samples provided from different parts of rodents (60 samples from 10 rodents from each terminal) 3 percent were positive. Since Fars province is one of the endemic areas of Lishmaniasis and the rodents are considered as the disease reservoir and a lot of rodents are seen in the terminal of Shiraz, and according to the infection of these rodents and transmission of disease to the passengers, there is the possibility of the spread of disease. Therefore more investigations and the implementation of some actions such as the destruction of the rodents' nests can be an effective step in the field of prevention of further spread of the disease.

INTRODUCTION

Leishmaniasis is a common disease among humans and animals caused by different species of protozoa of Leishmaniasis genus [1]. This protozoan is transmitted to humans by the bites of sand fly of phlebotomus genus in the Old World and the New World and exclusively are the reservoir host of domestic and wild animals [1]. The relative orientation of the parasite [offal or skin] causes the classic classification of parasites into three main types of

leishmaniasis, including cutaneous leishmaniasis, visceral and mucocutaneous. [2]. Cutaneous Leishmaniasis is a major health problem in Iran and Fars Province is one of the infection centers in our country. Every year the disease causes lesions in addition to a waste of human energy, medicine, health and economic energy [3].

History

For the first time, Cunningham in 1885 during the French victory over Tunisia, observed protozoa in the exudates of French troops. In 1900, Leishmaniasis in autopsy of a British soldier who had died of fever, and splenomegaly in the area of Dum Dum, near Calcutta in India, observed small things from the prepared smear from spleen aspiration under the microscope, which considered them the cause of the patient death. In 1903 Donovan in medical school discovered Leishmaniasis parasites in the spleen of the patients who had died from intermittent fever and enlarged spleen and because of this, the cause of disease was named Leishmaniasis donovani. Ross also introduced the genus Leishmaniasis in 1903, and Rogers in 1904 was able to kill the parasite and see flagellated forms for the first time. In 1911 Vnyvn raised this possibility that Phlebotomus may be a carrier in Leishmaniasis transmission [4].

Thus, leishmaniasis body was found as the cause of cutaneous leishmaniasis and visceral. Cutaneous leishmaniasis disease has long been known in Iran. Abu Ali Sina in the book of law had mentioned a long-term injured named Kheiroone. In Science society's convention, a cutaneous wound called Ghodooh kheiroone, has been mentioned and is almost similar to cutaneous leishmaniasis. In 1965, German doctor Pollack, one of the professors of Darolfonoon medicine school of Tehran wrote a comprehensive description of the cutaneous leishmaniasis and considered it equated with Dokme-Baghdadi and Dokme Halabi. From others who engaged in the study of cutaneous leishmaniasis in Iran; were Nlygan 1913 Gashe 1913-1915- doctor Sheikh 1913. And other researchers did some studies related to epidemiology of sand fly species, clinical features and treatment of the disease and for the first time the existence of kala-azar in Iran was reported by Doctor Yahya Pooya in 1328.

Classification of Leishmaniasis

Leishmaniasis, is a parasite of the family of Trypanosomiasis, Category of Kinetoplastid, phylum of Trypanosomatid and the genus of leishmaniasis including two sub-genus of Leishmaniasis and Viania. Kinetoplast is a kind of mitochondrial DNA including genome materials that are capable of replication and transcription without the need to have nuclear enzymes.

Leishmania genus includes more than 30 species that 21 species and sub-species of it are infective to humans it [9-7]. Although all species and strains of Leishmaniasis are morphologically and structurally similar, but are different in terms of infectiveness, epidemiology and geographical distribution, host [including the original host, vector and reservoir], the enzymes structure [zymodemes] and DNA compounds in kineoplast.[10].

In some categories Leishmania is divided into two groups of the new and old World 1. The Old World Leishmania includes:

- Species causing cutaneous leishmaniasis: L. major, L. tropica, L. aethiopica-
- Species causing visceral leishmaniasis: L. infantum, L. donovani
- Species causing diffuse cutaneous leishmaniasis: L. aethiopica

2. New World Leishmania includes:

- Species causing leishmaniasis: L. mexicana, L. amazonensis
- Species causing visceral leishmaniasis: L. infantum, L. chagasi.

Table 1.1 taxonomy of Leishmania [11]

Kingdom	Protista
Subkingdom	Protozoa
Phylum	Sarcomastigophora
Sub-Phylum	Mastigophora
Class	Zoo mastigophora
Order	Kinetoplastida
Sub order	Trypanosomatina
Family	Trypanosomatidae
Genus	<i>Leishmania</i>

Recently with the creation of several methods to determine Leishmania species like Isoenzyme identification method, analysis of some parts of DNA, using monoclonal antibodies, investigation of the properties of anti gene in promastigote Leishmania phase, it has been shown that the above classification was not complete and Leishmania's genus is as a whole. And now new species of Leishmania and strains have been identified in the world, having 15 separate enzymes and as the number of these enzymes for detection of new strains and the species is increasing, the classification of these parasites is not yet been completely and definitively presented [6].

In the urban leishmanious foci, the dogs are infected like the humans and domestic and wild dogs were found infected in Tehran, Mashhad, Neyshabour, and Bam frequently. Nowadays, the infected person is known nowadays, the infected person is known as the main source of urban kind Leishmania and as soon as sand flies start their activity, they can be easily infected by biting infected person.

Isfahan, Lotf Abad, Torkaman sahara, Varamin, and Shahrod are included in the rural leishmanious foci which in the north and the north east are the main source of Rombimos Epimos . Natanz, a city in Isfahan, Khozestan, east Azarbayejan, and Boushehr are the main source of Merinus Lebicus, Tatra Indica, Persicus, Tatra Indicus respectively.

Literature review

In 2005, Buaki and his colleagues presented some information about leishmaniasis in West Africa. According to their report, the rodents were of the most important reservoirs of the disease in the area, for example Mastomys erythroleucus and Tatera gambiana are the important rodents of the reservoir of disease in Senegal (15). Oliveria and et.al (2005) studied on 123 rodents in Brazil and applied PCR method on the rodents' skin and blood samples after the extraction of DNA, 18% of the blood samples and 4.6% of the skin samples in rodents were positive (16).

Helhazar and et.al in 2013 did some studies on the rodents of Sinatra and Symbria of Portugal. They identified the genus and species of the rodents according to their apparent features after collecting 30 rodents of these areas. Some samples from both ears, liver and spleen were provided and after staining by Giemsa method, Real-time PCR was done on the samples. In their study, there were 27 rodents belong to Mus musculus species that in 23% of them Lishmanious parasite was proved. In addition they reported the existence of L. infantum. (17)

Rosi's investigation in 2000-1999 was of the primary researches on rodents as the Leishmaniasis reservoir in Fars province. He and his colleagues studied on the rodents of Arsanjan region in Fars and hunted 59 rodents from this region by trapping method. After cultivation and done of PCR method on samples, it was shown that 6.8% of the rodents were infected to the parasite. (18)

Mohebali and et.al (2004), collected 556 rodents from endemic areas of Iran in different seasons. After identification of the rodents' genus, in 18.6% of the rodents, the existence of Leshmanious parasite was reported through direct observation and RAPD-PCR. There were major Leshmania in four species of the rodents (Rhombomys opimus , Meriones libycus , Tatera indica, Mer. Hurrianae) and for the first time the existence of Toranika Lishmania was reported in the country.(19) Rosi and et.al in 2007, investigated the rodents of Marvdasht region in Fars province. Collecting of 126 rodents, they noted that according to the rodents' apparent features, 75.4% of them are Merones Libycus, 14.3% are Cricetulus migratorius, and 10.3% are Microtus arvalis. After PCR method, 8.4% of Meriones Libycus parasites were introduced as major Lishmaniasis reservoir. (20)

MATERIALS AND METHODS

The required chemical substances are listed in the below table:

Sampling

In the summer of year 94, 20 alive rot or big mouse were hunted from central areas (Karandish terminal located in east north of Shiraz- Kaveh terminal located in east of Shiraz) by trapping method. Then the mice were transferred to the competitive and empirical medicine center of medicine science university of Shiraz and were kept in the cages till the time of sampling.

Sample preparation from hunted rodents

At first the mice of each area were numbered for each district separately and then were anesthetized by ketamine and Zaylozin and were investigated apparently. The heart of animal was blood let and a spear was provided for microscopic investigation from the gained blood. Also some samples were taken from muzzles, ears and feet and the samples were kept in separate containers dishes, mentioning the features. Then the animals bodies were anatomized and internal members of the animal such as liver, and spleen were sampled too and a spear was provided from liver and spleen samples for microscopic investigation. The collected samples were transferred to the parasitology laboratory for doing the investigations.

Table 3.3 the required chemical substances

Chemical substances	manufacturer	Country
Chloroform	Merck	Germany
Alcohol Iso amyl	Merck	Germany
Acetate sodium	Merck	Germany
Ethanol 100%	Merck	Germany
K proteinase	Sina gene	Iran
Tris	Merck	Iran
Gel Red	Gibco	Germany
DNA ladder	Sina gene	Iran
dNTP	Sina gene	Iran
Loading Dye	Sina gene	Iran
Mgcl2	Sina gene	Iran
10X buffer	Sina gene	Iran
Primer	Takapou Zist	Iran
Phenol	Sina gene	Iran
Taq DNA polymerase	Bioflaux	Germany

The prepared spears

The prepared spears from the liver and spleen were stained by Giemsa stain and fixed methanol for microscopic investigation of Lishmania.

Giemsa staining:

Giemsa is one of the stains of Romanovski group and is provided in form of prepared solution. At first concentrated stoke of stain will be diluted with sterilized distilled water in concentration of 1 to 10 and then staining will be done using Giemsa staining in the following way:

1. After the preparation of smear, it was fixed and fully dried using fixed methanol, 1 ml of stain was poured on the lamella which were horizontally placed in trays of staining.
- 2- after 12 minutes the lamella are washed with water and the sedimentations are cleaned with gas.

The lamella are placed in lamella vertically to be dried and then they are used. Molecular investigation of separated samples of the rodents. The DNA extraction of tissue method. In order to extract DNA from tissue, the phenol-chloroform method was used. At first, the tissues were brought out from the -80 degree centigrade freezer to reach to the temperature of the environment. Then 25-30 gr of the tissue were weighted with an exact digital scale and were put in a 2ml micro tube.

150 micro liter of lubricious Buffer (1 cc NaCl 0.1 M + 1 cc Tris 20 Mm +2.5 cc EDTA 25 Mm 150 µl SDS) and 10 micro liter of k proteinase were added to each sample and after doing vortex, the sample was located in 55 degree centigrade ben Mari about 16-20 hours, so that the tissue gets completely lubricious.

- Phenol chloroform is amyl alcohol solution with the ratio of 25:24:1 and the same volume of lubricious buffer was added to each sample
- The micro tube contents got vortex about 10 minutes so that a milky solution will be gained.
- The sample got centrifuged with 12000 rpm in about 5 minutes.
- The top clear liquid was transferred to the new tube 3 molar acetate with pH=5.3 and 0.1 of the volume of clear solution was added to the micro tube.
- 100% ethanol was added to micro tube at the rate of 2 times of the solution.

- The sample was placed in the -20 degree centigrade freezer for about 20 minutes after getting vortex.
- The sample got vortex and was centrifuged for 10 minutes in 4 degrees centigrade and with 12000 rpm, after bringing out from the freezer.
- The micro tube contents were exited gently in a way that the made sedimentations not be exited.
- 1000 micro liters of 70% alcohol were added to the sample and then the sample was put in room cold on the shaker for 15 minutes.
- The sample was centrifuged with 12000 rpm in 4 degrees centigrade for 5 minutes and the micro tube contents were exited gently.
- Then the alcohol was exited and the tube door got opened so that the extra alcohol would be evaporated. 30 micro liters sterile distilled water were added to the sample and vortex was done. The tubes including DNA were kept in 4 degree centigrade refrigerator and for long maintenance in -20 degree centigrade freezer. Due to the use of phenol-chloroform all the above steps were done in sterilized condition and under the laminar hood.

Yekta Tajhizama kit was used for extraction of DNA from some tissues. At first, the tissues were exited from -80 degree centigrade freezer same as before, to reach to the environment temperature. Then 25-30 gr of the tissue were weighted by the exact digital scale and put in the 2 ml micro tube.

1. According to the method of the study, the kit of 200 micro liters of TG1 buffer and 20 micro liters of k proteinase were added to each micro tube and the micro tubes were incubated for 24 hours in 60 degrees centigrade so that the tissue got lubricious.
2. 200 micro liters TG2 buffer were added to each micro tube and the micro tubes were put in 70 degrees centigrade for 10 minutes.
3. 200 micro liters cold absolute ethanol were added to each micro tube and all the micro tube content was transferred to the kit extraction column and was centrifuged for 1 minute with 8000 rpm.
4. The tube placed under the column was removed and a new tube was replaced.
5. 500 micro liters of washing buffer were added to each column and the content was centrifuged for 1 minute with 14000 rpm. This phase was repeated once again.
6. A DNase free micro tube was placed under each column and 30 micro liters of releasing buffer were added to each column.
7. Finally the contents were centrifuged for 2 minutes with 14000 rpm. The micro tube includes DNA that are kept in -20 degrees centigrade.

All the above steps were done in sterilized conditions and under the laminar hood. Both methods were used in this thesis.

The measurement of the concentration and purity of extracted DNA

1-1.5 μ l of the extracted DNA was prepared on the Nano-Drop device and the Values corresponding to the concentration and purity were read from the device. In this simple and exact method, the amount of UV ray absorption was measured by DNA bases. DNA molecules due to the presence of aromatic bases of adenine, thymine, cytosine and guanine have high absorbance in the ultraviolet limitation. Therefore the absorbance measurement of the DNA solutions is a good index for its degree of purity.

The maximum of DNA molecule absorption is in wavelength of 260 nm. On the other hand, DNA solutions usually have some protein contamination and the maximum absorption of the protein solutions is due to the presence of aromatic acids such as tryptophan phenylalanine, tyrosine and alanine in wavelength of 280nm. Therefore, the absorption rate in wavelengths of 280/260 is determined. The absorbance is a good criteria for determination of Protein / DNA ratio in a sample and in other words, the purity degree. Therefore, to determine the concentration of dissolved DNA, its absorption should be determined in wavelengths of 260 and 280 nm. A desired and purified DNA sample will have 280/260 absorption ratio in the range of 1.7-2.1 and 230/260 absorption ratio in the range of 2-2.2. The absorption ratio of 260-280 less than the mentioned amount shows protein contamination and the absorption ration of 230-260 less than the mentioned amount shows contamination to some substances including carbohydrate and phenol.

PCR method

PCR is a technique which is used for amplification of a specific area of DNA strands that is called target DNA. Target DNA can be a single gene, or part of a gene or a non-coding areas. Most of PCR methods have the capacity of amplification of DNA with 10 k Bp. Although some techniques have been designed for amplification of 40 kilo base pairs. PCR method involves the following steps:

1. Step 1. Starter phase: It consists of DNA heat denaturation at a temperature of 95-94 ° C for 5-1 minutes. If the reaction is done as hot start, this step can be prolonged. In the Hot start method one of the main compounds of the reaction like Taq polymerase enzyme is added to other compounds when the reaction temperature is high enough (94 degrees centigrade) and the DNA is completely denatured. This method is used to prevent nonspecific products and also primer-dimer and increases the specificity of the reaction.
2. Denaturation phase
3. Hibridation phase (annealing): the temperature of hibridation is often considered about 3-5 degrees under TM primer temperature.
4. Prolongation phase
5. Final prolongation phase

PCR method was done on the extracted DNAs beside minus control during a simultaneous process and in similar condition using amplifier primers. Nucleotide sequence of used primers was as following:

- Forward (Lin4): 5' 5'-GGGGTTGGTGTAATAAGGG-3' 3'
- Reverse (Lin17): 5' TTTGAACGGGATTTCTG 3'

Table 3-4 the concentration and amount of used substances for each sample in PCR

Substance	Amount(micro liter)
Amplicon master mix	12/5
DNA	3
H2O	8/5
Primer forward	0/5
Primer reverse	0/5

3-5 Thermocycler thermal plan for PCR

Step	Cycle	Temperature ©	Time
Primary denaturation	1	95	3 minutes
Denaturation	30	95	30 seconds
Annealing		54	30 seconds
Extension		72	45 seconds
Final extension	1	72	10 minutes

Electrophoresis on agarose gel

PCR products on 1.5 % agarose gel with 100 electrophoresis based pair of ladder marker, and then through use of the UV trans illuminator were observed and photographed.

Preparation of TAE buffer (50X)

To produce one liter TAE buffer (50X), 242 mg Tris powder was dissolved in 500 ml of distilled water. Then 100 ml of Na₂ EDTA.2H₂o (PH = 8) 0.5 and 57.1 ml acetic acid were added to it and eventually its volume reached to one liter.in order to provide gel, the amount of 35 ccTAE (1X) and 0.45 grams of agars powder was put into the microwave to melt well, then 2 micro liters of Gel red were added to it and was poured to the electrophoresis tank which was pre-prepared, then for 30 minutes it was allowed that the gel got ready and cool. The samples were loaded on the gel wells and connected to the electrophoresis devices. Voltage of 85 mV was used.

Examined samples

This study investigates the infection of hunted mice from Karandish and Kaveh terminal s of Shiraz to leishmaniasis through PCR method in year 2015. The information gathering of this study was cross-sectional and descriptive. In this study, 10 mice from Karandish and 10 mice from Kaveh terminal were hunted.

Preparation of samples from hunted mice

The samples were prepared from the muzzle, ears and feet of mice and after the anatomy of each mice, a spear was prepared from the liver and spleen of the mice for microscopic investigation, so that a total of 100 spears (from the ears, muzzle, feet, liver and spleen of 20 mice) for microscopic investigations and 100 samples (samples taken from the muzzle, ears, feet, blood, liver and spleen of 20 mice) for molecular methods.

The microscopic investigation of taken samples from the rodents

After staining and investigating of the obtained spears, any suspect samples were not found in spears.

Molecular investigation of samples taken from rodents by PCR:

In this study, PCR method was done after DNA extraction from samples of ear, nose, blood, liver, spleen and foot of each mouse. 2 samples (liver) out of 50 bodies of mice in the Karandish terminal of Shiraz were infected with major Leishmania (Figure 1-4 and 2-4) and the other 48 samples were clean. One sample (spleen) out of 50 samples of body parts of the mice in Kaveh Terminal was infected with Leishmania infantum (Figure 3-4, 4-4) and the other 49 samples were clean.

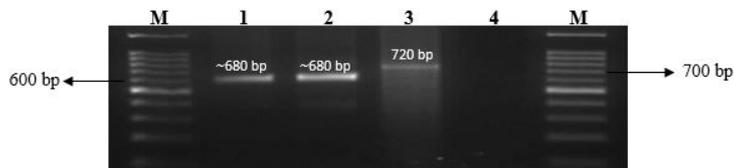


Figure 4-1: PCR of taken samples from the rodents

- M: marker
- 1 and 2: the sample of liver of infected rodents to major leishmania
- 3: spleen sample of infected rodents to infantum leishmania
- 4: negative control

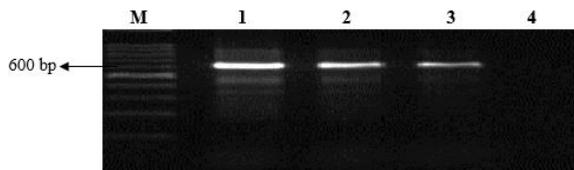


Figure 4-2: PCR on infected liver samples to major Leishmania

- M: marker
- 1: positive control of major Leishmania
- 2, 3: liver samples of infected rodents to major Leishmania
- 4: negative control

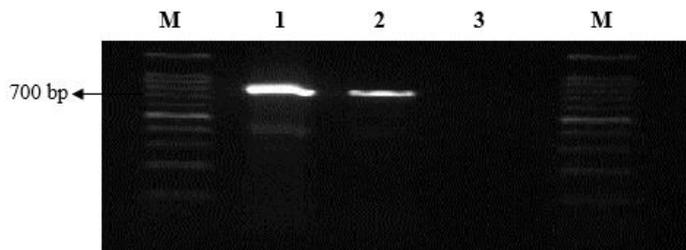


Figure 4-3: PCR on infected spleen sample to Leishmania infantum

- M: marker
- 1: positive control of Leishmania infantum
- 3: negative control

Table 4-1: the amount of infection of taken samples from rodents to leishmania using PCR method

The place of sample collecting		muzzle	ear	foot	spleen	liver	blood
Karandish terminal	The number of infected parts	0	0	0	0	2	0
		0	0	0	0	%4	0
Kaveh terminal	The percent of infected parts	0	0	0	1	0	0
		0	0	0	%2	0	0

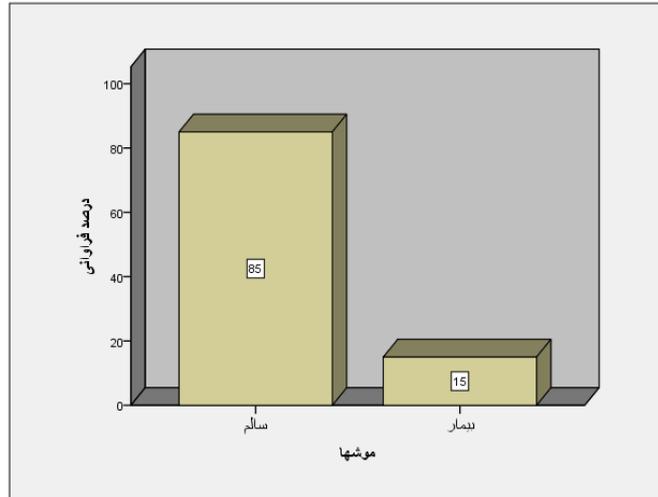


Chart 4-1: the percent of frequency of infected and un-infected rodents

According to the above table and chart, 85% of the rodents were healthy and 15% of them were infected to leishmanai. Therefore the diffusion of leishmania parasite from 3% of infected mice is possible.

DISCUSSION AND CONCLUSION

The current project investigates on the infection of hunted mice from Karandish and Kaveh terminals of Shiraz to leishmania by PCR method in year 2015. After sampling from liver, spleen, foot, muzzle, ears of the mice and doing of lamella preparation tests, and staining and PCR it was identified that the spleen of one rodent from among 50 samples related to different parts of 10 mice which were taken from Kaveh terminal and 2 liver samples from among 50 samples related to different parts of mice bodies of Karandish terminal were infected to leishmania.

Iran is one of the countries that has high outbreak of leishmania, so that many studies have been done on leishmania reservoirs in different areas of this country. In a study that has investigated about leishmanai in central areas of Asia and the Middle East, almost 24630 reports of leishmania outbreak in Iran have been recorded between years 2004-2008, that is a big number.

Isfahan, Kerman, Khorasan, Fars, Khuzestan, Ilam, Kermanshah, Baluchistan, and Tehran provinces are of important foci of cutaneous leishmaniasis and infected areas in Iran. According to yearly statistics, rural leishmaniasis, in more than half of the provinces, such as the provinces of Isfahan, Kerman, Khorasan, Fars, Khuzestan, Ilam, Kermanshah, and Tehran and Baluchistan is considered as a health problem. Therefore some investigations about rodents as leishmaniasis reservoir in different areas have been done. The recognition of leishmaniasis parasites through the microscopic observation of stained lamella with Giemsa is usually accompanied with error and its results are not certain.

For exact recognition of this parasite, the separation of parasite, its cultivation, and injection to a sensitive laboratory animal or use of isoenzyme method are needed that implementation of all of these steps is time consuming and expensive and requires parasites multiply in large numbers. In recent years the methods leading on DNA including PCR are used very often in Iran since these methods don't have the mentioned limitations and also the small amount of DNA in infected tissues is possible.

Rosi and et al, in an investigation in 2004 studied the reservoirs and carries of leishmaniasis in urban areas of Marvdasht in Fars province. After preparation of lamella from ear and skin of 126 rodents and implementation of nested-PCR, 8.4 percent of rodents were considered as the major leishmaniasis reservoirs. In our study using PCR method 3% infection was obtained. Nadim and et.al (1986) sampled from skin wounds, blood, liver, spleen, and ear of the rodents and in their study the percentage of infection only with microscopic method was high and the amount of 60% was reported. While in our study no infection from microscopic method was observed.

According to Nicolas and et.al (2002) reports and investigations it is better to do the sampling from ears, foot, liver, spleen for recognition of leishmaniasis, that in the current study in addition of these parts, the muzzle of the rodents were sampled too. In the current study, in addition to preparation of smear from liver and spleen samples, PCR method was used too, because this method can recognize the small amount of parasite due to its high sensitivity, so the unreal negative recognitions will be decreased. (58)

Although the microscopic methods are simple and fast, they have little sensitivity especially on old wounds. In Mehrbani study, microscopic methods, culture, and PCR on foot, ear, spleen, liver, and bone marrow samples of the rodents in different foci of Fars province were compared. The samples were positive in microscopic methods and culturing and 62 samples were positive in PCR method and this difference was statistically significant.

In the current study after smear preparation from liver and spleen, no specious case was observed in the samples while after implementation of PCR, 3% of the obtained samples from 20 rodents were positive.

In Davami et.al study (2004) on 55 rodents of Jahrom in Fars province, it was reported that through PCR method, 14.6% of rodents were infected to Leishmaniasis that the result of the current study is less than that of course needs more population of rodents.

Since Fars province is one of the endemic areas of leishmaniasis and the rodents are considered as the reservoir of the diseases and a lot of rodents are observed in terminals of Shiraz, regarding the infection of these rodents and the transfer of these disease to the passengers is possible. Therefore more investigations and implementation of some actions such as the destruction of rodents' nests can be an effective step for preventing from further spread of the disease. It is obvious that the identification and investigation of epidemiologic aspects of this disease including rodents, and human infection for the purpose of fighting with the diseases and presenting of control solutions and prevention are essential

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