



## What is the best animal model for Leishmaniasis studies?

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### ABSTRACT

Leishmaniasis is a zoonotic disease which has worldwide importance and is hard to control and treat. Researchers have not yet developed a protective vaccine for humans in the light of current studies. Various experimental animal models are being used since; i) *Leishmania* has different species and vectors, ii) there are still many clinical, pathological and immunological issues that have to be investigated, iii) new non-toxic medical recipes to have maximum yield in a short time have to be investigated, iv) protective vaccination have to be developed. Mouse, hamster, dog, rodent, and non-human primates are among these animal models. None of them has the same clinical features, pathogenesis and immunology with the disease in human. However, rodents, dogs, and monkeys, which are the last host of the parasite, are among the most preferred models in recent days. Considering the different clinical forms of the disease, it is best to decide which *Leishmania* species to work with which animal. This review is intended to guide the researchers in choosing an appropriate animal model for leishmaniasis studies.

**Keywords:** Leishmaniasis, Animal models, *Leishmania* species, Experimental infection.

### INTRODUCTION

*Leishmania* species are intracellular tissue and blood parasites. These protozoons are transferred to vertebrate hosts from other hosts by vector female sand fly (*Phlebotomus*, *Lutzomyia*) (Mears et al., 2015; Soosaraei et al., 2017). Leishmaniasis is the name of the disease which is caused by this parasite in vertebrate hosts, especially in human and dogs, in other mammals in nature, in rodents and in reptiles such as lizards (İça, 2004). Leishmaniasis is a zoonotic disease which has worldwide importance and is hard to control and treat. It is the third most common vector-borne parasitic disease after malaria and trypanosomiasis (Yaman, 2008). According to the

World Health Organization (WHO) data, about 20 million people are infected with disease in 98 countries worldwide, 350 million people are at risk and about 400 thousand new cases are added to this number every year (Limoncu et al., 2013; Loria-Cervera and Andrade-Narvaez, 2014). The disease is usually seen in tropical and subtropical areas and notification of the diseases is compulsory (Barroso et al., 2015).

There are about 21 known *Leishmania* species infecting people. Among these species *Leishmania tropica* (*L. tropica minor*), *L. major* (*L. tropica major*), *L. aethiopia* and *L. infantum* cause Old World cutaneous leishmaniasis and *L. braziliensis*, *L. mexicana* and *L. peruviana* cause New World

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cutaneous leishmaniasis. *Leishmania donovani*, *L. infantum* ve *L. chagasi* are the causative agents of visceral leishmaniasis (VL). *Leishmania donovani*, *L. infantum*, *L. tropica* and *L. major* are the most common leishmania species in Turkey. *Leishmania donovani* and *L. infantum* cause VL and *L. tropica* and *L. major* cause cutaneous leishmaniasis (CL) (Cassia-Pires et al., 2014; Yazar et al., 2016).

Cutaneous leishmaniasis is an endemic disease in 88 countries including Turkey around the world. *Leishmania tropica* is common in the Middle East, Central Asia and India, while *L. major* is more common in the Northwest of China, India, Pakistan, Africa and Central Asia. Cutaneous leishmaniasis which is more common especially in Southeast Anatolian and east Mediterranean Region in Turkey is also known as oriental boil, Antep boil, Urfa boil or Aleppo boil. Visceral leishmaniasis can be both progressive and lethal or asymptomatic. The reservoir hosts of the disease, which is mainly zoonotic, are domestic dogs and wild canine. Nearly 90% of all cases are seen in Bangladesh, Brazil, Sudan, Nepal and India. The situation is different in India since the disease is anthroponotic because there is no other reservoir than human (Barroso et al., 2015; Yazar et al., 2016; Torres-Guerrero et al., 2017). Moreover, in a study conducted in our country, it was stated that civil war in Syria and immigration from there affected the epidemiology of leishmaniasis (Ozkeklikci et al., 2017).

Animal models such as mice, hamsters, dogs, rodents and primates are used in research on leishmaniasis (Garg and Dube, 2006; Loria-Cervera and Andrade-Narvaez, 2014; Mears et al., 2015). All has distinctive properties and due to the natural and genetic differences from the host, none of them has prognosis as humans. Moreover, the biggest difference between natural and experimental infection is parasite inoculum. While injection of a few hundred metacyclic promastigote is enough in natural infection, inoculation of millions of promastigote that produced in vitro is needed in experimental disease (Garg and Dube, 2006). Hamsters and mice are used in primary tests, dogs in secondary tests, and non-human primates are in tertiary tests used as animal models (Mears et al., 2015).

Our goal in preparing this review is to guide the selection of the most appropriate experimental animal in leishmaniasis studies.

### Mice model

Because of their advantages such as rapid reproduction, ease of maintenance and feeding, mice of different genetic makeup are the most used animal model in leishmaniasis studies. Various mouse species such as BALB/c, C3H/He, CBA, C57BL/6,

CsS16 and 129SvEv are used as models. The promastigote form of the parasite is inoculated into the tail or footpad of the mice to obtain infection (Loria-Cervera and Andrade-Narvaez, 2014; Mears et al., 2015).

The effect of the hydroalcoholic extract obtained from *Echinacea purpurea* was investigated in order to be an alternative treatment with meglumine antimony in BALB/c mice infected with *Leishmania major*. Although the lesions formed in the extract and antimony groups were smaller than the control group, the meglumine antimony was found to be statistically significant in terms of its therapeutic properties (Sarkari et al., 2017).

Experimental infection and lesion formation was observed in *L. tropica*-infected BALB/c. Vector sand flies were fed with inoculation-infected mice and infected vectors were fed with healthy mice to provide infection. In mice, the lesions were detected at the end of 1-6 months, and at the end of one year, *Leishmania* was found to be positive by molecular tests (Svobodova and Votypka, 2003). In a different study, it was reported that the CsS16 mouse model against *L. tropica* is more sensitive than the BALB / c model and is the most suitable model for antileishmanial drug studies (Mears et al., 2015).

*Leishmania amazonensis* related CL in BALB/c type mice was tried to be treated with ursolic acid. Ursolic acid is not toxic to macrophages and removes amastigotes. Lesion size was smaller in ursolic acid treatment compared to miltefosine (Yamamoto et al., 2015).

Eight weeks of BALB/c type mice were infected with *L. amazonensis* and *L. braziliensis*. In the study, the promastigotes were inoculated to the soles of mice in one group, while both the homogenate of the complex salivary glands of the vector and promastigote were inoculated to the other group. Inflammatory reaction was less and lesion size was smaller in the group of mice inoculated with salivary gland homogenates. The study revealed that the vector salivary contains immunomodulator molecules (Franceschini et al., 2014). In another study, different mouse models were infected with *L. amazonensis*. In the BALB/c, C57BL/6 and C57BL/10 mouse models, sensitivity to parasite was high and ulceration and necrotic lesions were observed. In the CBA mouse model, later but very severe disease course was observed. Resistance was observed in DBA and C3H/He models, no lesion was occurred, and very low or no parasitic load was found after 90 days of infection. In BALB/c, C57BL/6 and CBA models, the diseases were in visceral form and in the spleen and liver parasites were detected (de Souza et al., 2018).

The antileishmanial effects of *Lucilia sericata* and

*Calliphora vicina* larvae on BALB / c mice infected with *L. major* were investigated. Although both fly-type larvae reduced lesion size and improved recovery, *L. sericata* larvae treated *L. major* lesions in a statistically significant manner (Sanei-Dehkordi et al., 2016). Polat et al. reported that secretions of larvae obtained by holding 2500 2<sup>nd</sup> and 3<sup>rd</sup> stage sterile *L. sericata* larvae in distilled water during 6 hours had anti-parasitic in vivo (BALB/c mouse) and in-vitro effect on *L. tropica* (Polat et al., 2012). This study has been a good model for human as an alternative and non-toxic treatment and has evoked further studies. Polat and Kutlubay successfully treated the CL caused by sodium stibogluconate resistant *L. major* in human with direct contact of 1<sup>st</sup> stage *L. sericata* larvae and application of the secretions of the larvae that transforming to 3<sup>rd</sup> stage from 2<sup>nd</sup> stage into the lesion (Polat and Kutlubay, 2014).

In studies conducted with the development of parasite antigens, antigen preparations have been shown to have low toxicity on BALB / c macrophages (Ribeiro et al., 2017). The MAX and SP15 vaccines obtained by cloning the proteins in the saliva of the sand fly succeeded. These studies were based on the idea of the suppression of the infection by the antibodies against to the saliva of the sand fly. It has been proved that resistance formation in endemic regions occurs not by the immunity against the parasite but by the suppression of the infection by the antibodies against the sand fly's saliva. In these studies, these vaccines protect the mice significantly against *L. major* infection and ensure that the lesion size is five times smaller (Yaman, 2008).

The BALB/c's used in the CL studies were also used in VL studies caused by *L. donovani* and *L. infantum*. After the infection is established, the parasites in the liver of the mice increase rapidly during the first week. However, organ specific immunity is being developed and infection in liver is being limited. In the spleen the process takes longer. BALB/c mice are good models for VL studies because the disease is chronic in mice. However, the sensitivity of these models to the parasites, uncontrolled lesions and disease progression indicate that they are not a suitable model for CL studies (Loria-Cervera and Andrade-Narvaez, 2014).

### Hamster Model

The Syrian golden hamster (*Mesocricetus auratus*) is a good model for studies of VL. It is frequently used to investigate the symptoms, pathological and clinical features of the disease and the immunity of the host (Loria-Cervera and Andrade-Narvaez, 2014; Eberhardt et al., 2016).

When gold hamsters infected with *L. tropica* were observed, it was found that the Syrian golden hamsters

were not suitable animal models for CL studies. At the end of the study, although *Leishmania* was positive by PCR, there was no lesion in animals (Hanafi et al., 2013). In another study, lesions were observed in gold hamsters experimentally infected with *L. tropica*. Bisabolol obtained from daisy was applied topically and orally to treat these lesions. It was reported that bisabolol had no toxicity and was more effective than meglumin antimony (Corpas-Lopez et al., 2016).

In an in vivo study, *M. auratus* with CL was treated with *L. sericata* larvae. 80-100% decrease and improvement in lesion size was observed 12 hours after treatment with larvae (Arrivillaga et al., 2008). *Lucilia sericata* and *Sarconesiopsis magellanica* (butterfly) larvae were investigated to treat *M. auratus* with *Leishmania panamensis* related CL. In this study, it was reported that *L. sericata* larvae had therapeutic effects (Cruz-Saavedra et al., 2016).

The smell sensitivity of the vectors was investigated in the infected golden hamsters. The scent of the *L. infantum*-infested hamsters were observed to be more attractive for vector sand flies as the infection progressed (Nevatte et al., 2017).

One month old gold hamsters infected with intradermal, intraperitoneal and intracardiac routes using *L. infantum* strains were monitored for 9 months. Symptoms such as loss of appetite, mucocutaneous lesions, weight loss and hair loss were observed in hamster models. This had been reported as a successful animal model for the most similar VL model in human and dogs (Moreira et al., 2016).

Mixed infection was obtained to analyze the humoral response and prognosis against to disease in golden hamsters. Clinical and pathological symptoms had been reported to be much more serious and severe in hamster infected with *L. amazonensis* and *L. infantum* (JL et al., 2017).

An antileishmanial drug, 101R chemotype, was investigated in *L. donovani* infested hamsters and promising results for VL were reported (Tripathi et al., 2017). Immunopathologic studies had also been carried out in golden hamsters infected with the same factor and organ specific immune development had been reported (Rouault et al., 2017). In golden hamsters, especially immunological response and drug studies against VL were carried out (Pandya et al., 2016).

### Dog model

One of the most important reserves of VL is dogs. Disease in dogs is important both for animal health and as a reservoir for humans (İça, 2004; Gönül et al., 2010). Therefore, it is used as successful animal models especially in VL studies (Loria-Cervera and Andrade-Narvaez, 2014).

Intradermal and intravenous inoculation was applied while experiencing experimental infection in dogs. However, intravenous inoculation has been reported to be the best method for clinical disease since parasites spread more rapidly to internal organs (Hosein et al., 2015).

*Leishmania infantum* which leads to VL in dogs is also the cause of zoonotic CL. Hence, it is essential to develop appropriate vaccination in dog models. The importance of this model is emphasized since dogs are both exposed to the natural pathway and are reservoirs (Costa et al., 2013). In another study, it was reported that the incidence of zoonotic CL in humans due to an increase in *L. infantum* infection in dogs is increased, but it is non-linear with zoonotic VL (Kaabi and Zhioua, 2018).

Dogs (race: beagle) experimentally infected with *L. infantum* were observed. Parasites were reported to remain viable for six months in lesions in the bite area (Aslan et al., 2016).

The lesion development, histopathological findings and immunological response in dogs were examined in Beagle dogs intradermally infected with *L. mexicana*. It has been noted that dogs are good models to understand the immunopathology of leishmaniasis caused by *L. mexicana* and to develop drug and vaccines (Cruz-Chan et al., 2014).

Surface antigens of promastigotes in infested dogs with *L. infantum* have been used in vaccination studies. In this phase III study, successful results were obtained and licensed and marketed to the market for canine leishmaniasis. The same study was also undertaken for *L. amazonensis* and promising results were obtained (Petitdidier et al., 2016).

Along with avoiding experimental infection, various medicines and vaccine studies were carried out on natural infected dogs. Preservation levels of the recombinant vaccine and insecticide-impregnated leashes were investigated. It was reported that none of the implementations adequately protected the dogs against VL and was not effective. However, the prognosis was better for dogs using insecticide-impregnated leashes compared to vaccines (Lopes et al., 2018). LBMPL heterologous vaccination studies in leishmaniasis dogs have also been reported to have a strong potential to prevent VL (Roatt et al., 2017).

Since there is not enough studies about CL in dogs which are successful animal models in VL studies, It is too early to make an assessment (Loria-Cervera and Andrade-Narvaez, 2014; Mears et al., 2015).

### Rodent model

Rodents are reservoirs of this disease as well as many vector-borne zoonotic diseases. Different rodents

have been used to reveal the clinical, pathological and immunological course of the disease. Being genetically polymorphic and the final host of the parasite as human leads rodents to be used as an appropriate animal model (Loria-Cervera and Andrade-Narvaez, 2014; Bakirci et al., 2015; Tsakmakidis et al., 2017).

Gerbill (*Meriones unguiculatus*) infected with *L. major* was observed in one of the studies. After the inoculation of the parasite causing zoonotic CL, the disease was converted to the visceral type. Compared to rats and hamsters, gerbils were reported to be more successful animal models since they are more docile and easier to work with (Bakirci et al., 2015).

Hyraxes (*Procavia capensis*) were experimentally infected to study the infection role of vector sand fly. Molecularly diagnosed disease in *L. tropica*-infected hyrax was asymptomatic and no lesions had occurred (Svobodova et al., 2006).

In a study with black rat (*Rattus rattus*), the animals were infected with *L. tropica*. Lesions and symptoms were not observed in this model even though the animals were infective for the sand flies after 24 months (Svobodova et al., 2003).

### Primate model

Non-human primate models are commonly used to test and evaluate potential anti-leishmanial compounds. These models used in the last phase of the experimental work include monkey species such as macaque, mandrill and baboon (Mears et al., 2015).

Vervet (spp: *Chlorocebus pygerythrus*), sykes (*Cercopithecus albogularis*) and baboon-type monkeys were infected with *L. major* and reported that they all had sensitivity to the disease. In particular, vervet and sykes-type monkeys had similar symptoms to CL in the human body. Vervet and sykes were reported to be successful models for CL studies (Mears et al., 2015).

Cutaneous leishmaniasis, especially from *L. major*, is an important disease that needs vaccine development worldwide (Freidag et al., 2003). *Leishmania major*'s attenuated strains and pressure-inactivated promastigotes had been applied as two different vaccination protocols in rhesus monkeys (*Macaca mulatta*). Although both vaccination protocols were safe for primates, they did not prevent lesions in the vaccination area (Amaral et al., 2002). Rhesus monkeys were infected by varying doses of promastigotes and lesion dimensions were measured. Previously infected monkeys were found to have smaller lesions despite the high dose of promastigote (Freidag et al., 2003).

Vervet monkeys (spp: *Chlorocebus aethiops*), have

been vaccinated after infected with *L. donovani*. It has been reported that the active agents used in the study may be used as leishmania vaccine due to low Th2 cytokine and high IgG2 antibody response (Mutiso et al., 2012). In vaccination studies with vervet monkeys infected with *L. donovani*, Montanide ISA 720 (MISA) was found to be a safe, protective against infection and an agent for immunological response (Mutiso et al., 2012; Mutiso et al., 2012).

## CONCLUSION

There is only one common goal: to treat Leishmaniasis with the least toxicity and to be protected from the disease. With the animal models used, new drugs have been tried and vaccine have been tried to be developed. Most of the mouse, hamster and rodent models used in CL studies have migrated to the visceralized form of the disease or no lesions have been observed. However, *L. tropica*-CsS16 may be a suitable choice. Other mouse models, hamsters and rodents are thought to be not suitable models for CL. The most suitable models for *L. major* are vervet, sykes and rhesus-type monkeys. Golden hamsters and dogs in *L. infantum* studies and vervet-type monkeys in studies with *L. donovani* should be first choice models. It should not be forgotten that in all models the infection may develop symptomatically or asymptotically in some way. The clinical features of the infection in human are not the same in animal models. So far from setting up new search for a new model, the immunopathological progression and immunological response in the natural hosts of the parasites should be clarified. As a result, we think that dogs, both natural hosts and reservoirs, should be preferred as models.

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