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RESEARCH ARTICLE

Some Visceral Changes in *Mesocricetus auratus* Produced by *Leishmania tropica*

Harith S. Al-Warid*[1, 2], Fawzia A. Al-Shanawi [2]

1. The School of Natural Resources, Department of Fisheries and Wildlife Sciences, University of Missouri, 302 ABNR, Columbia, MO, 65211, USA

2. Biology Department- College of Science-University of Baghdad, Baghdad, Iraq

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Corresponding Author

Harith S. Al-Warid

Abstract

Follow up of viscerlizing induced by 5×10^7 promastigotes of *Leishmania tropica* was screened in golden hamsters along five times intervals (30, 45, 60, 75, and 90) days after infection. In addition of skin lesions seen in the footpads that varied from small 5.63± 0.57 mm to large 8.66±0.4 mm, many visceral changes were noticed in the infected animals. Significant differences were noticed ($p< 0.05$) in spleen weight ratio, spleen length and liver weight comparing with normal non infected group of animals. Our findings indicated that visceral changes in spleen and liver can be produced by *Leishmania tropica* in golden hamsters which may serve as a good model for leishmaniasis.

Introduction

Over 20 species of pathogenic protozoan parasites of the genus *Leishmania* are known to cause a group of disease called leishmaniasis with divers clinical features ranging from self-limiting cutanoues leishmaniasis to visceral disease (Nicholas, *et al*., 2002; Fraga *et al*., 2010). The various species of *Leishmania* are transmitted by sand flies, amastigotes librated from host cells in the insect's gut, transform into promastigotes, which multiply there and finally introduced into a new hosts when sandfly again feed (Kaye *et al*., 2011). With some overlap, visceral and cutaneous leishmaniasis are usually caused by different species of *Leishmania* (Noyes *et al*. 1997).The visceralizing *Leishmania* spp. belong to the *L*. (*Leishmania*) sub-genus, which include *L. infantum* and *L. donovani* in the Old World and *L. chagasi* in the New World. *Leishmania donovani* and *L. infantum*/ *chagasi* usually cause disseminated disease in humans, and in turn most cases of visceral leishmaniasis can be attributed to them. However, there is considerable overlap in clinical manifestations caused by the various *Leishmania* spp., and there are reported cases of cutaneous lesions due to *L. chagasi*/*infantum* and *L. donovani* and cases of visceral disease due to *L. amazonensis* and *L. tropica* (Barral *et al*., 1986; Noyes *et al*., 1997; Rabello *et al*., 2003; Karunaweera *et al*., 2004). *L. tropica* can cause both cutaneous and visceral leishmaniasis in humans. Although the *L. tropica*-induced cutaneous disease has been long known, its potential to visceralize in humans was recognized only recently (Shorabi *et al*., 2013). The BALB/c strain has attracted much attention in recent years since it produces a visceral leishmaniasis which can be used as a model to study human leishmaniasis (Tamnou *et al*., 1981). This paper addresses some features of viscerlizing of *Leishmania tropica* in golden hamsters as a model to study human leishmaniasis.

Materials and Methods

Isolation of the *Leishmania* parasite

Leishmania tropica was isolated from a lesion in the left arm of a 21 years-old male at Baghdad teaching hospital / Baghdad according to method of (Al-Alousi, 1979).

Media

1-Semi –Solid medium: This medium was prepared according to the method of (Alder & Theodor, 1926) and was used for parasite isolation from man .

2- Biphasic Medium: This media was prepared according to the method of (Kagan & Norman, 1970) and used for parasite cultivation for preparing injecting dose.

Animals

Sixty males of Golden hamster (*Mesocricetus auratus*) aged (8-10) weeks were purchased from "The National Centre for Drug Control and Researches / Baghdad / Iraq". Animals were separated into two groups each group contained thirty animals which inoculated as following:

1. Group (1): inoculated with 5×10^7 promastigotes of virulent *L. tropica* isolate / 0.2 ml.
2. Group (2): was considered as control group. Animals in this group were inoculated with 0.2 ml phosphate buffer saline.

All previous animals were inoculated intradermally in the left hind footpad using 1 ml sterile syringe for each animal.

Dissections and Follow up of *L.tropica* infection:

All animals of the two groups were dissected along five times of follow up (30, 45, 60, 75, and 90) days after infection, the follow up of *L.tropica* infection was done using different parameters:

1- Incubation period from infection date to the appearance of lesion and/or lesions and footpad thickness (Al-Alousi, 1979)
2- Spleen weight ratio (spleen weight (mg) / body weight (gm) (Stauber, 1953)
3- Spleen length (Stauber, 1966)
4- Liver weight (Stauber, 1966).

Statistical Analysis:

Footpad thicknesses, spleen weight ratio, spleen length and liver weight were compared between experimental group and control group and between time intervals for each parameter by the Student's t-test, P value <0.05 was considered statistically significant.

Results

Results showed that the cutanous lesions appeared after 15 days post infection with *L. tropica*, the lesions were reddish and congested in animals inoculated with *L.tropica*, the highest mean of foot pad thickness was 8.66±0.4 mm after 90 days of infection while the lowest foot pad thickness were seen at day 30 of infection which was 5.63± 0.57 mm, significant differences were noticed between infected animals and control group (figure 1), as well as there were significant increasing ($p< 0.05$) of footpad thickness along the different time intervals after infection 30, 45, 60, 75 and 90 days.

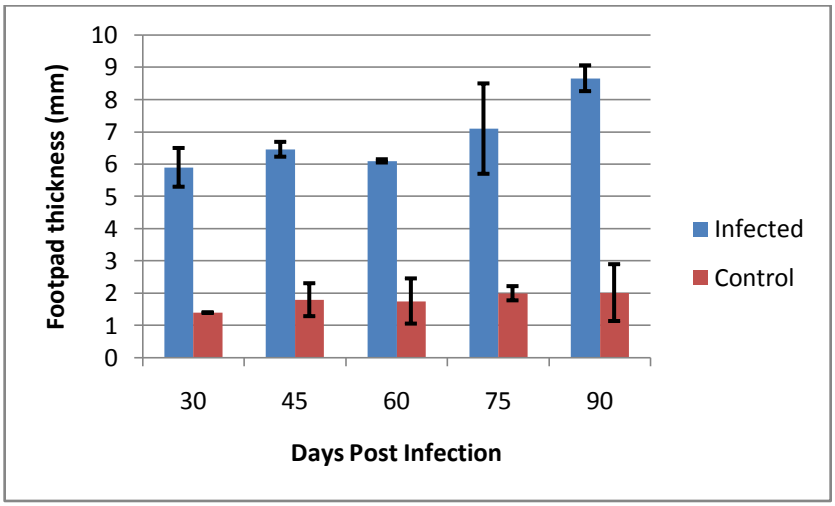

Figure -1: Graph showing the footpad thickness in infected and control groups at five successive peroids.

Animals inoculated with *L.tropica* showed the highest spleen ratio in all intervals of follow up, significant differences ($p < 0.05$) were noticed between animals inoculated with *L.tropica* and control group (figure 2) , the highest mean of spleen weight ratio 5.19±0.05 mg/gm were seen after 90 days of infection, as well as high significant($p < 0.05$) differences were noticed between spleen weight ratio of each time interval, the result of spleen length also showed similar results, animals inoculated with *L.tropica* showed the highest means of spleen length along the five interval times of follow up when compared with the control group, significant differences ($p < 0.05$) were noticed between these two groups (figure 3), as well as significant differences ($p < 0.05$) were noticed between spleen length in each time intervals after infection. The maximum spleen length was recorded after 90 days of infection which was 35.9±2.7 mm.

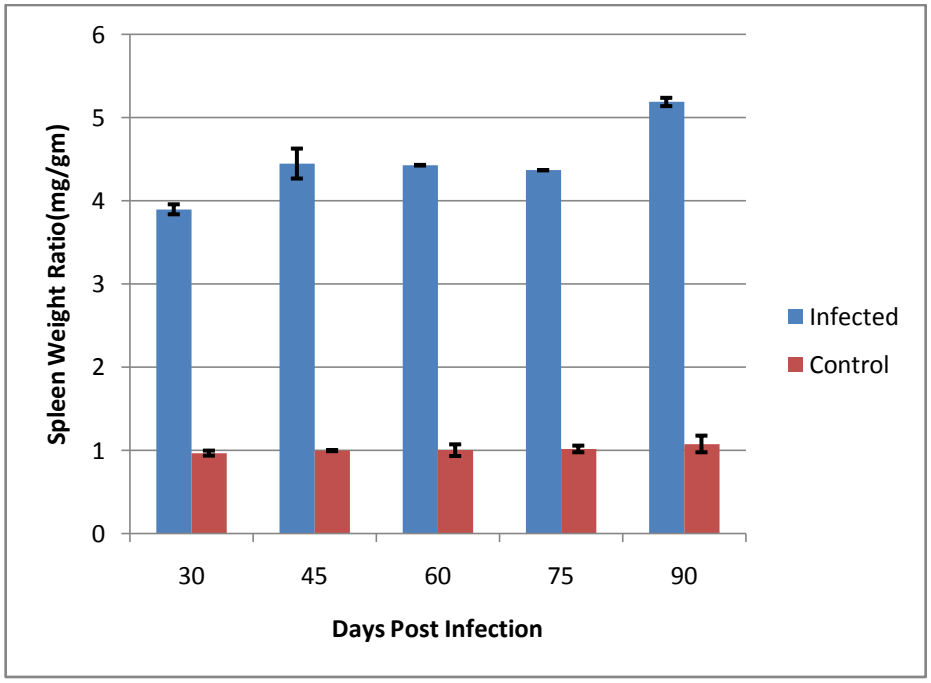

Figure -2: Graph showing the spleen weight ratio in infected and control groups at five successive periods.

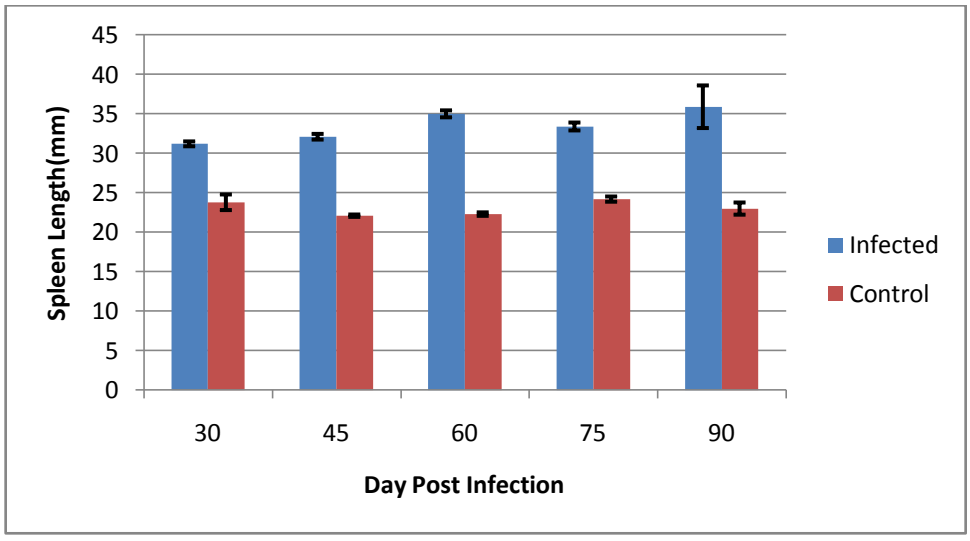

Figure -3: Graph showing the spleen length in infected and control groups at five successive periods.

Animals inoculated with *L.tropica* showed significant ($p < 0.05$) differences in liver weight along all five times of fellow up comparing with normal animals (figure 4), statistical analysis also showed significant differences of liver weight in each time interval, the maximum mean of liver weight was recorded after 90 day of infection 6.33±0.11 gm while it was 5.93±0.079 gm after 30 day of infection.

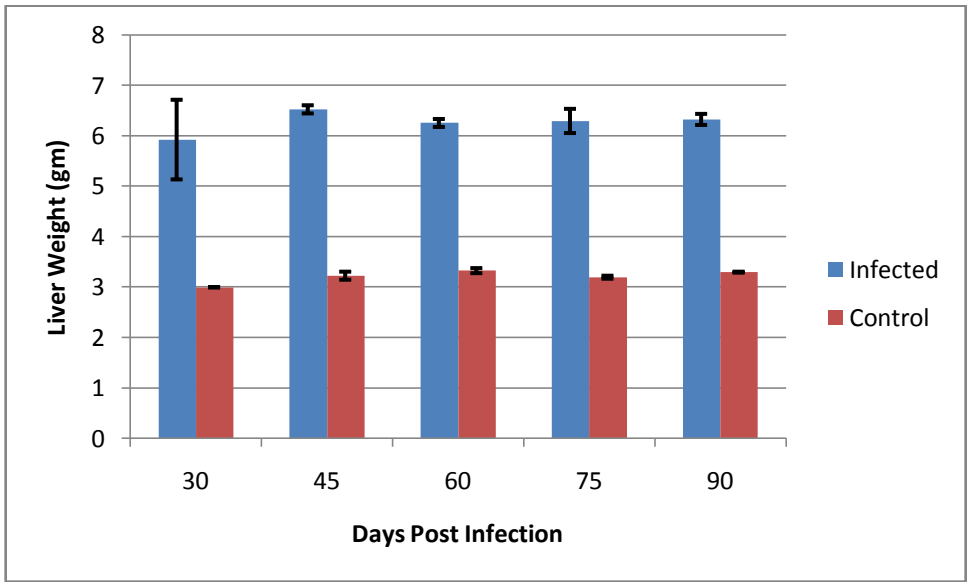

Figure -4: Graph showing the spleen liver weight in infected and control groups at five successive periods.

Discussion

Some manifestations of *L. tropica* infection in golden hamsters are described. These included incubation period, splenomegaly and liver enlargement. Golden hamsters were used in this study because some investigators showed that these animals were the suitable host for *L. tropica* experimental design (Svobodova and Votypka, 2003) although other showed that BALB/c mice may be considered as one of suitable animal models for studying leishmaniasis (Djoko-Tamnou *et al*., 1981) while rats have been reported to be susceptible to *L. tropica* infection with different outcomes ranging from asymptomatic infection at the inoculation site to visceral infection of a specimen caught in Iraq (Aljebori and Evans, 1980).

The outcome of the disease in murine leishmaniasis can be influenced by many factors, including the parasite dose, route and site of inoculation, and developmental stage of the parasite. All these variables were carefully controlled for *L. tropica* in our experiment. The developmental stage of the parasite seems to be critical in this experimental model, so promastigotes (infected stage of *Leishmania* spp.) were harvested in stationary phase, because the promastigotes in stationary phase can be considered to be more virulent than promastigotes in log phase (Sacks, 1989).

The footpads thickness were increased constantly from the first 15 days post infection till the end of follow up period, no reduction of footpad thickness was seen along 90 days follow up , this pattern was the same as reported previously (Lira *et al*., 1998; Mahmoudzadeh-Niknam and McKerrow, 2004). While the results of the current study were quite different from the result of (Mahmoudzadeh-Niknam *et al*., 2007) in mice, they showed that the parasite load in footpad increased up to 1 month, decreased from month 1 to month 4 significantly and remained low from month 4 to month 6 post infections. Our finding illustrated that *L. tropica* caused ulceration in footpad, while the parasite continues to grow in visceral organs, these parasites increased progressively then reached its maximum load at the end of following up period, theses high parasitic load caused enlargement in liver and spleen, which showed significant increasing along the five intervals of following up, these results were similar to the result of (Djoko-Tamnou *et al*., 1981) who recorded numerous parasites in spleen and liver imprints of infected BALB/c mice with *L.tropica* after 4 months follow up, these parasites were accompanied with high spleen index. Our data showed that *L. tropica* infection visceralizes in hamsters and survives efficiently in visceral organs as skin tissues and caused

enlargement in both liver and spleen. The best conclusion from our data is that both skin and visceral organs have to be considered for evaluation of immunization, immunotherapy and/or therapy in this model of *L.tropica* infection.

References

Adler, S. and Theodor, O. (1926): Furthur observation on the transmission of cutaneous Leishmaniasis to man from Phlebotomus papatasi, Ann. Trop. Med. Parasitol., 20: 175-191.

Al-Alousi, R.S. (1979). Investigation into possible existence of more than one strain of *Leishmania tropica* in Iraq, M.Sc. Thesis University of Baghdad.

Aljeboori, T.I.and Evans, D.A. (1980): *Leishmania* spp. in Iraq. Electrophoretic isoenzyme patterns. I. Visceral leishmaniasis, Trans. R. Soc. Trop.Med. Hyg. 74 : 169–177.

Barral, A., Badaro, R., Barral-Netto, M., Grimaldi Jr, G., Momem, H., and Carvalho, E. M. (1986): Isolation of *Leishmania mexicana amazonensis* from the bone marrow in a case of American visceral leishmaniasis. Am. J. Trop. Med. Hyg., 35(4), 732-734.

Djoko-Tamnou, J., Leclerc, C., Modabber, F., & Chedid, L. (1981): Studies on visceral *Leishmania tropica* infection in BALB/c mice. I. Clinical features and cellular changes. Clin. Exp. Immunol., 46(3), 493.

Fraga, J., Montalvo, A. M., De Doncker, S., Dujardin, J. C., and Van der Auwera, G.(2010):Phylogeny of *Leishmania* species based on the heat-shock protein 70 gene .Infect. Genet. Evol., 10(2), 238-245.

Kagan , I.G. and Norman, L.(1979): Manuel of Clinical Microbiology . Am. Soc. Microbiol. Washington, pp 479.

Karunaweera, N. D.; Pratlong, F.; Siriwardane, H. V. Y. D.; Ihalamulla, R. L. and Dedet, J. P.(2004): Sri Lankan cutaneous leishmaniasis is caused by *Leishmania donovani* zymodeme MON-37. Tran. Roy. Soc. Trop.Med. Hyg, 7(4):380-381.

Kaye, P., and Scott, P.(2011): Leishmaniasis: complexity at the host–pathogen interface. Nature Rev. Microbiol , 9(8):604-615.

Lira, R., Méndez, S., Carrera, L., Jaffe, C., Neva, F., & Sacks, D. (1998): *Leishmania tropica*: The Identification and Purification of Metacyclic Promastigotes and Use in Establishing Mouse and Hamster Models of Cutaneous and Visceral Disease. Exp Parasitol, 89(3): 331-342.

Mahmoudzadeh-Niknam, H., & McKerrow, J. H. (2004): *Leishmania tropica*: cysteine proteases are essential for growth and pathogenicity. Exp Parasitol, 106(3): 158-163.

Mahmoudzadeh-Niknam, H., Kiaei, S. S., & Iravani, D. (2007): Viscerotropic growth pattern of *Leishmania tropica* in BALB/c mice is suggestive of a murine model for human viscerotropic leishmaniasis. Korean J.Parasitol., 45(4): 247-253.

Nicholas, L., Milon, G., and Prina, E.(2002): Rapid differentiation of Old World *Leishmania* species by Light Cycler polymerase chain reaction and melting curve analysis. J. Microbiol.Meth ., 51(3), 295-299.

Noyes, H.; Chance, M.; Ponce, C., Ponce E and Maingon R.(1997): *Leishmania chagasi*: genotypically similar parasites from Honduras cause both visceral and cutaneous leishmaniasis in humans. Exp Parasitol., 85:264–73.

Rabello, A., M. Orsini, and J. Disch.(2003): *Leishmania*/HIV co-infection in Brazil: an appraisal. Ann. Trop. Med. Parasitol . 97.Supplement-1:17-28.

Sacks,D. L. (1998):Metacyclogenesis in *Leishmania* promastigotes.Exp.Parasitol.69:100-103.

Sohrabi, Y.; Havlkova, H.; Kobets, T.; Sima, M.; Volkova, V.; Gerkov, I.; Jarosikova, T.; Kurey, J.; Svobodova, M.; Demant, P. and Lipoldova, M.(2013): Mapping the Genes for Susceptibility and Response to *Leishmania tropica* in Mouse. PLoS. Negl. Trop. Dis. , 7(7):e2282.

Stauber L.A(1953): Some Effect of Host Environment on the course of *Leishmania* in hamster. Ann.N.Y.Acad.Sci ., 56:1064-1069.

Stauber, L.A.(1966):Characterization of strains of *Leishmania donovani*. Exp. Parasotol. 18:1-11.

Svobodova , M. and Votypka, J. (2003):Experimental transmission of *Leishmania tropica* to hamster and mice by the bite of *Phlebotomus sergenti*. Microbs Infect.5:471-474.

Tamnou, J.; Leclerc, C.; Modabber, F. and Chedid, L.(1981): Studies on visceral *Leishmania tropica* infection in BALB/c mice. Clin. Exp. Immunol., 46:493-498.