



Histopathological Changes of Canine Visceral Leishmaniasis in Experimentally Infected Dogs



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VISCERAL leishmaniasis (VL) is a parasitic disease can parasitize the reticuloendothelial system (RES) of the visceral organs; the amastigote of leishmania parasite invades the visceral organs spleen, liver and bone marrow. This study included experimental intraperitoneal inoculation of stray dogs with a live and infective *Leishmania donovani* promastigote forms produce the histopathological changes of internal organs of infected dogs. The average weight and length of liver and spleen of infected and control dogs were monitored and evaluated, the parasite disseminated to visceral organs liver and spleen, the characteristic histopathological infection was observed. Parasitism and the inflammatory reaction with parasites were noticed in liver and spleen organs, macrophages loaded with intracellular amastigotes were found in tissue of all organs especially in spleen. The histopathological changes in visceral organs of infected animals may be explaining the experimental infection with *L. donovani* strain causes canine visceral leishmaniasis. In conclusion, stray dogs play an essential role in the transmission of visceral leishmaniasis in Iraq; the histopathological changes in visceral organs depend on the virulence factors of strain of *Leishmania* parasite as well as associated with development of inflammatory cells that may be due to host response to the antigenic factors of the parasites.

Keywords: Histopathological, Canine visceral leishmaniasis, Dogs.

Introduction

Leishmaniasis is one of the most important endemic diseases in Iraq and is mostly seen in marshland villages south of Iraq, rural and perurban regions. Visceral leishmaniasis (VL) is more prevalent in the rural areas than in urban one; because of the environmental factors which play an important role in chance of infection [1, 2], the causative agent is *L. infantum* and *L. donovani*, the first proven VL cases since the work of Kulz in 1916 were reported in Iraq [1, 3]. Jackal, dogs and wild canidae are an efficient reservoir host because parasitized fixed macrophages are so abundant in the dermal layer that parasites are readily taken up by feeding sandflies [1]. The infection begins locally in the dermal macrophages at the site of the sandfly bite, and then amastigotes multiply slowly and

may remain more or less quiescent for weeks or months [4]. Enlargement of spleen appears within 10-12 weeks. In the enlarged liver, there is fatty infiltration of Kupffer's cells and the endothelial cells of the blood vessels are invaded by amastigotes [5]. There is gross wasting, the pancytopenia is profound and jaundice. The reticuloendothelial hyperplasia that follows infection with *L. donovani* or *L. infantum* affects the spleen, the liver, the mucosa of the small intestine, the bone marrow, the lymph nodes and the other lymphoid tissues [5, 6]. Stray and feral dogs should be controlled whenever possible, although these actions may not eliminate human disease because of the presence of wild animal reservoirs. The uses of topical insecticides on domestic dogs has reduced the incidence of canine and human visceral leishmaniasis [1, 7].

Material and Methods

Iraqi strain of *Leishmania donovani* (MHOM/IQ/1982/BCR1/AA3) was maintained *in vitro* and *in vivo* on (NNN) diphasic medium and used in this study. Stray dogs were hunted from Basrah marshes areas south of Iraq. Six dogs were used in this study, three animals were injected and the others were as control. The parasites were adjusted to the required concentration of 1×10^7 /0.1 ml for inoculation, each animal was infected intraperitoneally with a 0.2 ml dose of 1×10^7 promastigotes / 0.1 ml three times in one week. Dogs were sacrificed and dissected 9 weeks post infection for histopathological study and monitoring to determine the development of size in spleen and liver for infected and control dogs. Tissue impression smears of liver and spleen were carried out and stained with Giemsa stain to check the amastigotes forms of *Leishmania* by light microscope under oil immersion (objective 100 xs), also aspirate material from these internal organs were cultured aseptically on NNN diphasic medium. Samples of liver and spleen were fixed in 10% formalin solution, the tissues were embedded in paraffin and sections were cut at 5 μ m and stained with Hematoxylin and Eosin (HE) [8]. One-way analysis test was used as differences were recorded as significant whenever probability (p) was less than 0.05 [9].

Results

The gross finding after autopsy is marked emaciation, enlarged spleen, liver and lymphatic glands, and some times ulceration of the intestine. The characteristic histopathological infection was observed by the formation of parasite granuloma with parasitism and inflammatory reaction with parasites in liver and spleen organs. The average size in spleen and liver according to length and weight of infected and control dogs were shown in Table 1. Statistically there are significant differences between infected and other dogs ($p < 0.05$). The histopathological changes of the liver

and spleen were studied in detail as follows: The examination showed liver cell necrosis with numerous macrophages containing amastigotes forms in liver tissue. Perivascular congestion and perivascular granuloma with inflammatory infiltration surrounding area were seen. Moreover, kupffer cells were hyperplastic and loaded with amastigotes forms of *Leishmania*, also granulomas formation were detected. There was marked atrophy of the hepatocytes with pressure Fig.1 (a,b,c,d). Infected animals showed the spleen was infiltrated with *Leishmania* amastigotes forms and hyperplasia with numerous giant cells. The parasitism was more intense; macrophages loaded with amastigotes form. Intense macrophage proliferation and mild congestion of red pulp was observed. Extensive chronic inflammation reaction and histiocytes heavily infiltrated with *L. amastigotes* were seen. Fig. 2 (a, b, c, d).

Discussion

Leishmania donovani strain inoculated intraperitoneally in stray dogs hunted from south marshes in Iraq. The presence of *Leishmania* especially in the skin of dogs without clinical signs enhances the importance of asymptomatic dogs in the epidemiology of visceral leishmaniasis. So that the dermal layer is more important tissue reservoir of *Leishmania* in infected dogs [3]. The histopathological changes associated with the infiltration of lymphocytes and macrophages containing amastigotes in the liver and spleen were noticed. The diseases are characterized by various degrees of enlargement in internal organs from a barely palpable size to one that extends well below the level of the umbilicus [10,11] and associated with the development of inflammatory cells in visceral organs may be due to host response by the parasitic antigens [2, 12].

Liver weight increased with the increase period of infection to 1107 gm, while in the control group 650 gm in 9 weeks after infection time, the length of spleen increased with the increase

TABLE 1. Mean of liver weight and spleen length in infected and control dogs

Animals	Mean of liver weight (gm)	Mean of spleen length (cm)
Control dogs	650	17
Inoculation dogs	1107	23

($p < 0.05$)

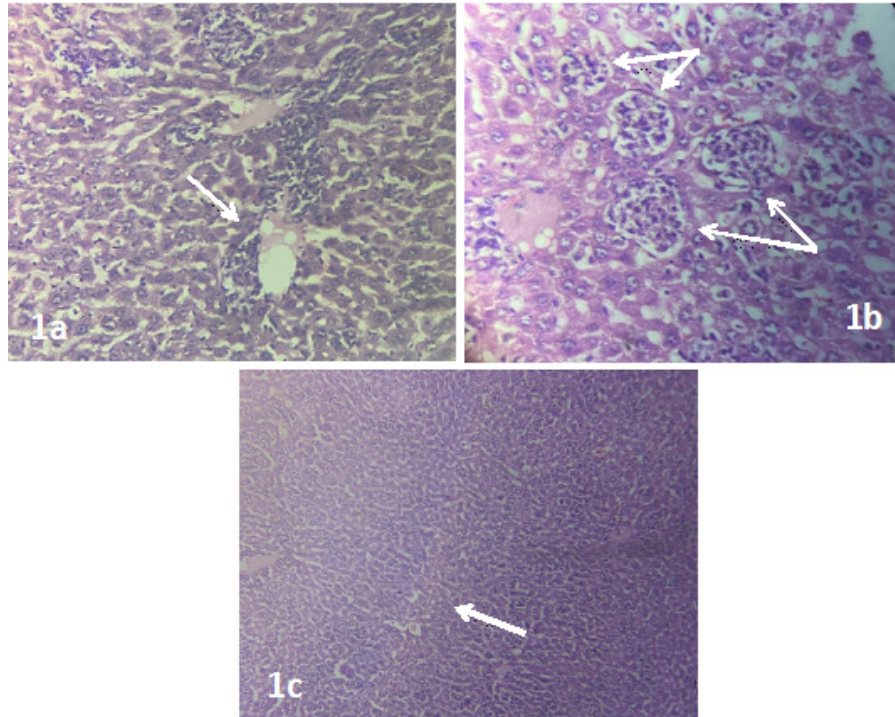


Fig.1. Liver section of infected dog 9 weeks post infection, a- Perivascular congestion and perivascular granuloma with inflammatory area (arrow); HE, 40X. b- Granulomas formation and kupffer cells loaded with amastigotes forms (arrows); HE, 40X. c- Observed a pressure atrophy of hepatocytes (arrow); HE, 10X.

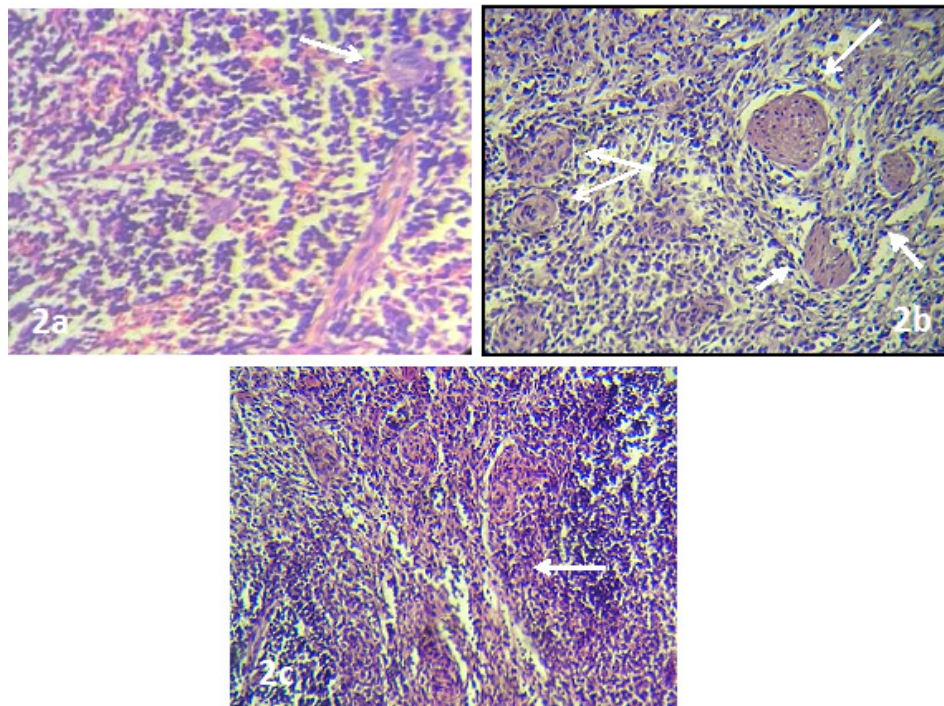


Fig. 2. Spleen section of infected dog 9 weeks post infection, a- Red pulp showing marked intense macrophages proliferation loaded with Leishmania amastigotes (arrow); HE, 20X. b- Diffusely distributed of inflammatory macrophages intensely parasitized with amastigotes forms and histiocytes heavily infiltrated amastigotes of *L. donovani* (arrows); HE, 20X. c- Mild congestion of red pulp (arrow); HE, 20X.

period of infection to 23 cm while in the control group 17 cm. *Leishmania* parasite invasion the reticular syncytium causing reticuloendotheliosis with hepatosplenomegaly, According to present observation, the size of liver and spleen increased with the increase in the infection period. This result is agreement with previous studies [13, 14]. The pathological lesions of the disease depended on the severity of *Leishmania* organism [15]. The main histopathological alteration is hypertrophy and hyperplasia of the monocyte-mononuclear system mostly of liver, spleen of dogs. There is relationship between leishman bodies and inflammatory formation of host to visceral infection [2, 12, 16].

During the present study, liver granuloma formation was the main sign of histopathological changes in infected jackals. Many reports demonstrated that granuloma and infiltration induced by the infection with cutaneous strain *L. major* and *L. tropica* [17-19] as well as visceral strain *L. donovani* [14, 20]. The kupffer cells loaded with amastigotes of *Leishmania*, as well as marked liver cells necrosis with inflammatory response were observed in the liver of dog experimentally infected with *L. major*. The nature of the inflammatory cells in the liver was correlated with the number of leishman donovani bodies contained with them [10, 16]. The experimental infection with *L. major* causes dermal leishmaniasis and histopathological changes in internal (visceral) organs [19].

The experimental animal model in this study, *L. donovani* is producing visceral infection with resembling visceral leishmaniasis in the liver and spleen of dogs. The histopathological character of the spleen in experimentally infected with *L. donovani* strain was hyperplasia. The spleen tissue showed a hyperplasia and mild congestion of red pulp with inflammatory cells and giant cells. At that time, the spleen was heavily infiltrated with amastigotes forms of *Leishmania* parasite that could be found inside macrophages. This results in agreement with that described by other reports [3, 12, 14]. Many amastigotes of *L. donovani* out side and containing macrophages. This finding simulates the obtained by other study [3, 15].

The infection with *Leishmania* parasites causes histological changes in visceral organs, when the *Leishmania* strain inoculated intraperitoneal in dogs, it has been shown that the visceral disease develop [21, 22, 23].

The occurrence of visceral infection can be induced by *L. major* (18), *L. donovani* [14, 16, 24] and *L. infantum* [12,25] in different models of animals.

It was concluded that stray dogs play an essential role in the transmission of visceral leishmaniasis in Iraq; the histopathological changes in visceral organs depend on the virulence factors of strain of *Leishmania* parasite as well as associated with development of inflammatory cells that may be due to host response to the antigenic factors of the parasites.

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Conflict of interest

There is no conflict of interest.

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التغيرات المرضية النسيجية لداء اللشمانيا الحشوي الكلبى في الكلاب المصابة تجريبياً

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داء اللشمانيا الحشوي هو مرض طفيلي يصيب خلايا الجهاز الشبكي البطاني للاعضاء الحشوية ، حيث يهاجم الطفيلي الاعضاء الداخلية مثل الكبد ، طحال ونخاع العظم. تضمنت الدراسة الحالية التلقيح التجريبي بالاطوار المسوطة الحية والفعالة لطفيلي *Leishmania donovani* داخل الغشاء البريتوني للكلاب السائبة نتج عنه تغيرات مرضية نسيجية للاعضاء الداخلية للكلاب المصابة . تم مراقبة وحساب معدل ازدياد وزن وطول الكبد والطحال . تم انتشار الطفيلي في الاعضاء الحشوية ؛ الكبد والطحال مع ملاحظة الخصائص المرضية النسيجية للاصابة . تم ملاحظة التطفل والحث الالتهابي لطفيلي اللشمانيا الحشوي في انسجة الكبد والطحال ، الخلايا البلعمية macrophage كانت محملة بالاطوار الداخل خلوية الغير مسوطة amastigotes للطفيلي وكانت موجودة في كل الانسجة المصابة للاعضاء الحشوية خصوصا الطحال . الاصابة النسيجية المرضية للحيوانات المصابة من الممكن ان توضح بان الاصابة التجريبية بعزلة *L. donovani* سببت الاصابة بداء اللشمانيا الحشوي الكلبى. تم الإستنتاج بأن الكلاب المصابة تلعب دور اساسي في نقل داء اللشمانيا الحشوي في العراق ، وان التغيرات النسيجية المرضية في الاعضاء الحشوية تعتمد على عوامل شدة العزلة اللشمانية للطفيلي بالاضافة الى الارتباط مع التطور في الخلايا الالتهابية وذلك من الممكن ان يعزى الى استجابة المضيف للعوامل المستضدة للطفيلي .