# ABILITY OF *Leishmania donovani* TO CONGENITAL TRANSMISSION IN EXPERIMENTAL BALB/C MICE

Farhan Beden Hachem Albazzoni MuslemAbd-Alrahman Mohammad

Department of Biology , College of science ,University of Basrah, Basrah,Iraq.

(Received 6 December 2018 ,Accepted 31 December 2018)

**Key wards:** *Leishmania donovani,* promastigote stage,Mice.

*Corresponding Author:muslemabd@yahoo.com*

## ABSTRACT

The aim of the current study was to investigate the ability of the transmission of *Leishmania donovani* parasite from pregnant mothers to their embryos in experimental BALB/c mice. After 5 days of copulation males with females,females were injected with $10^{12}$ parasite/ml of promastigote stage through the tail vein, which is grown in NNN-medium.

This study demonstrated parasite ability to congenital transmission from mothers to their embryos by placenta through the appearance of amastigote in tissue sections of liver and bone marrow of infected mice fetusescompared with uninfected fetuses.

## INTRODUCTION

Leishmaniasis is caused by protozoan parasites of more than 20 species. it is transmitted by the sand fly insect that returns to the *Phlebotomus* genus (1).
Leishmaniasis is endemic in 88 countries, including Iraq, and nearly 12 million infected people with more than 350 million are at risk (2) and according to reports of the communicable disease control center at the ministry of health, the disease remains a major cause of morbidity in Iraq (3).

Visceral leishmaniasis is a fatal parasitic disease transmitted by vector-borne caused by a *L.donovani , L.infantum , L.chagasi* complex of parasites and is associated with fever, malaise, anorexia, fatigue, splenohepatomegaly, lymphadenopathy and progressive inhibition of cellular immunity (4). also (5) confirmed the presence of high levels of IgG and IgM in serum of infected individuals with *L.donovani*

compared with serum healthy individuals. while (6) proved the increased infection rate of promastigote phase by inoculation in BALB/c infected adult mice. and according to WHO there are million cases of infection causing more than 50000 deaths case each year (7).

Low & Cookerecorded the first case of kala-azar infection in pregnant fetus that occurred in Africa with an infant appeared signs and symptoms of visceral leishmaniasis at delivery (8). While Banerjirecorded a case of a child infection that born in England,which appeared symptoms and clinical signs that laboratory confirmed as visceral leishmaniasis in the seventh month of age. The mother's child had acquired the disease in the fifth month of pregnancy, which had returned to England from India (9). In addition,Nyakundi*et al*.recorded a case of infection in a male infant in the fourth month was born premature after the sixth month of pregnancy while child's mother was diagnosed as having visceral leishmaniasis during pregnancy (10).

Yadav*et al*. described an 11-month male infant showed symptoms that were later diagnosed as VL., while the of child'smother was infected with the disease when she was pregnant (11), also areported case of VL. a German infant in non-endemic region that potentially transient *L.donovani* congenitally from asymptomatic mother to her child (12).

Haque*et al*. reported the congenital transmission of *L.donovani* in his 25 old lady. She had symptoms of fever in the sixth month of pregnancy and kept the infant with herself because she was breastfeeding him (13). Also, the researchers pointed out that 11 cases of congenital transmission have been recorded since 1926 and this is case number 12 and in most cases the disease appears after the mother become infected during pregnancy (13).

## MATERIALS AND METHODS

### Isolates of *Leishmaniadonovani*promastigote

*L.donovani* isolates were obtained in the promastigote stage from College of science / University of Mustansiriya (DUAA/IQ/2005/MRU15) and transferred to the laboratory by container vails on NNN-medium in refrigerated containers. The cultures

were then immediately renewed after they reached the laboratory on NNN-agar + lock.

**Parasite culture**

The parasite growth is mainly based on NNN-medium according to(14) as solid medium, while the liquid medium or lock solution according to(15).

**Cultivation of *L.donovani*promastigote**

Three to five ml of lock solution was added to the screw tube vials containing sterile NNN-medium and 0.1ml of previous parasite cultures werethen injected, and 0.1ml of gentamycin and 10%Heated Inactivated Fetal Bovine Serum(HIFBS) were added for each vial.The cultures were then incubated in incubator type memmert at a temperature of 24˚c then renewed every 4 days.

**Infection of pregnant mice**

Fifteen female mice were mixed with 5 males for 5 days. They were thenseparated from males and 10 females infected with $10^{12}$parasites suspended in 0.1ml by intravenous injection through tailed vein and left for 4 weeks, while 5 females left as uninfected control group.The fetuses of pregnant females were dissected after delivery and fixed, and the number of pregnant females that lasted to the end of experiment was 4 mice, while the other 6 were not pregnant. Also, the embryos of 3 other pregnant mice were dissected after delivery and fixed as uninfected control group, while the other 2 females were not pregnant, after which the targeted organs (liver , bone marrow) were fixed inside plastic container and added to enough amount of 10% formalin for the purpose of tissue preparation.

**Tissue preparation** (liver , bone marrow)

The Drury *et al*. method was used in tissue preparation while the sections were stained by Haematoxyline-Eosine stain and were loaded with D.P.X. material and cover slide (16). They were then imaged using composite imaging microscope type Leica.

## RESULTS

Histological sections taken from the fetuses of the control mice of liver tissue showed that they were composed of cells with dark nuclei because of their divisional

activity (figure1). In additionthe bone marrow was composed of hematopoiesis tissue, which consists of cells in different stages (figure2).

The current study has demonstrated the presence of*L.donovani*in experimental mice tissues, and this is evident by observation of the amastigote phase in the bone marrow and severe decomposition of blood-producing cells (figure3,4,5). Further the parasite was present in various areas of liver tissue, causing hemorrhage and the accumulation of lymphocyte and kupffer cells (figure 6) and the occurrence of severe decomposition of liver tissue (figure 7), and theamastigote phasefull of the liver cells (figure 8).

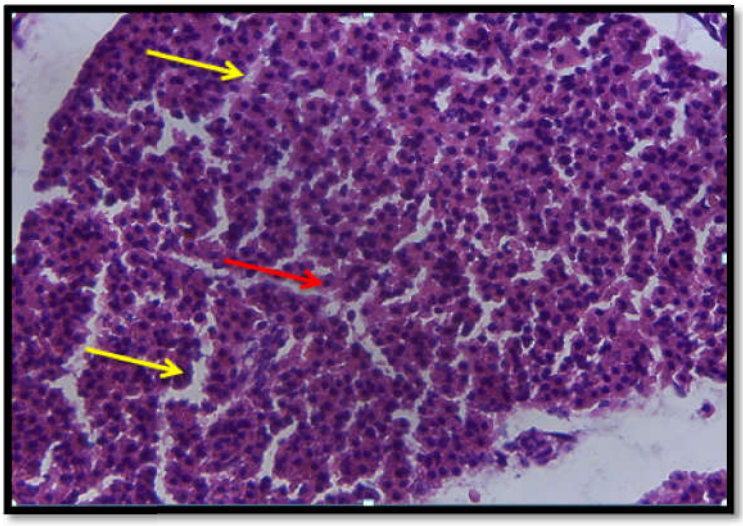

**Figure (1): Transverse section in liver of a mouse fetus showing the hepatic cells and their nuclei (red arrow) and liver sinusoids (yellow arrows) in control group H&E.(10X).**

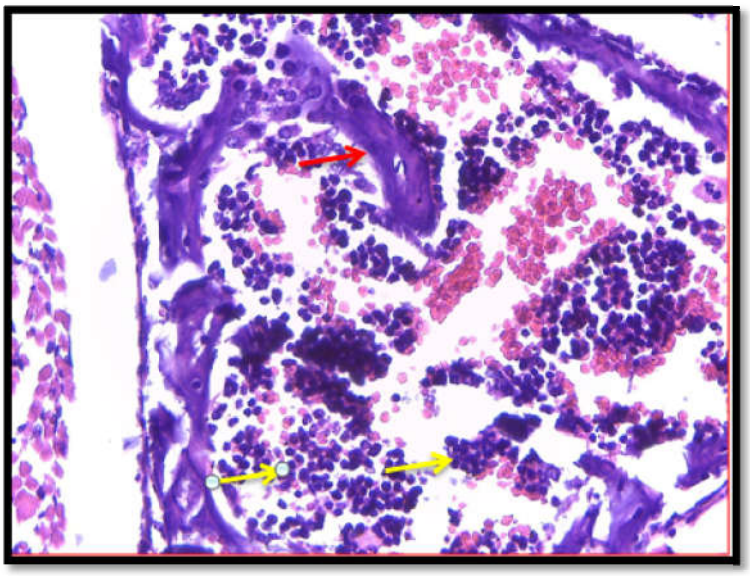

**Figure (2): Transverse section in bone marrow of uninfected mouse fetus shows the types of cells present in the hematopoiesis tissue (yellow arrows) and bone trabecular (red arrow) H&E.(10X).**

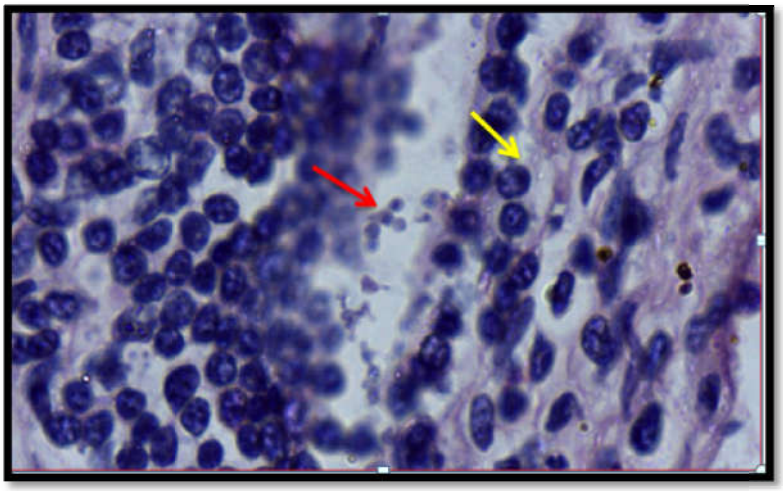

**Figure (3): Transverse section in head of the fetal bone of infected mouse showing the periosteum (yellow arrow) and the presence of the amastigote phase (red arrow) H&E.(100X).**

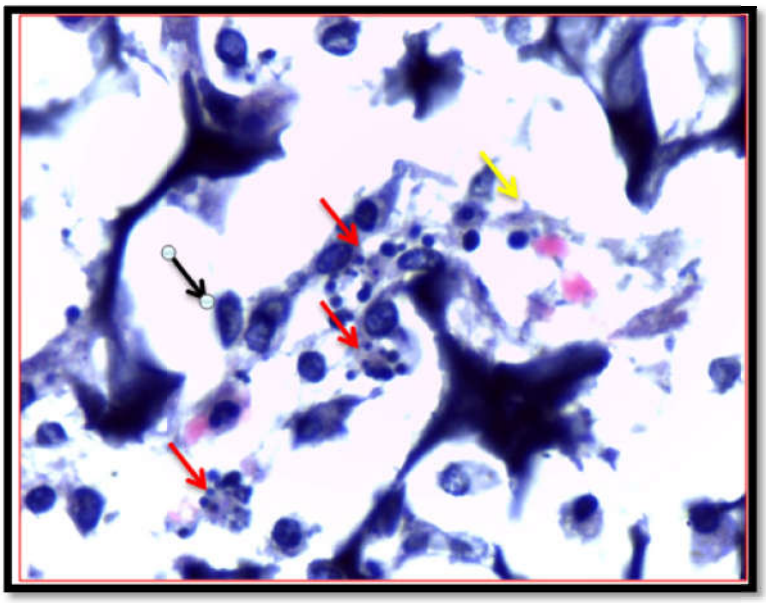

**Figure (4): Transverse section in bone marrow of the mouse fetus shows the spread of a amastigote stage (red arrows) and severe decomposition of blood-producing cells (yellow arrow) H&E.(100X).**

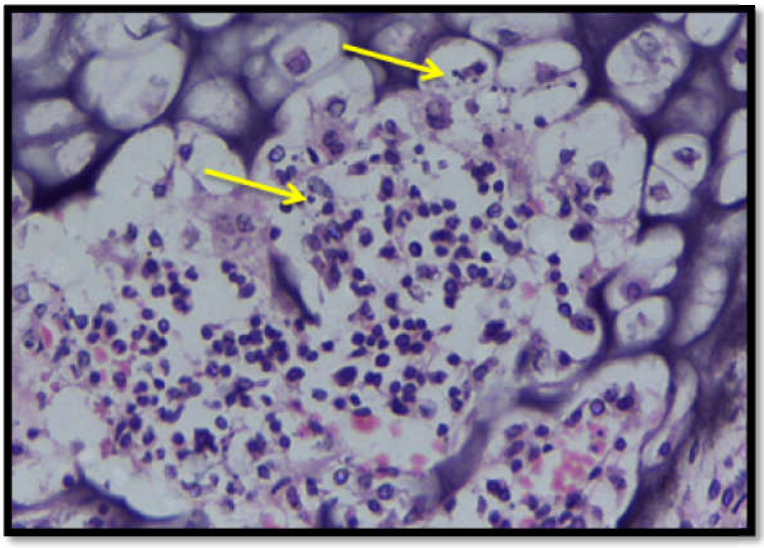

**Figure (5): Transverse section in region of bone transformation and bone marrow demonstrate the presence of theamastigote phase (yellow arrows) H&E.(100X).**

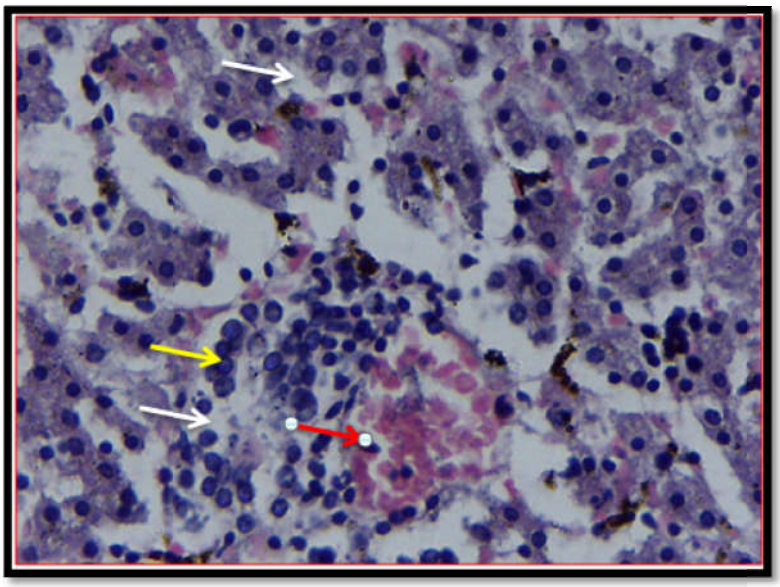

**Figure (6): Transverse section shows bleeding in the infected fetal liver (red arrow) and accumulation of defensive cells (yellow arrow) and the decomposition of liver tissue (white arrows) H&E.(40X).**

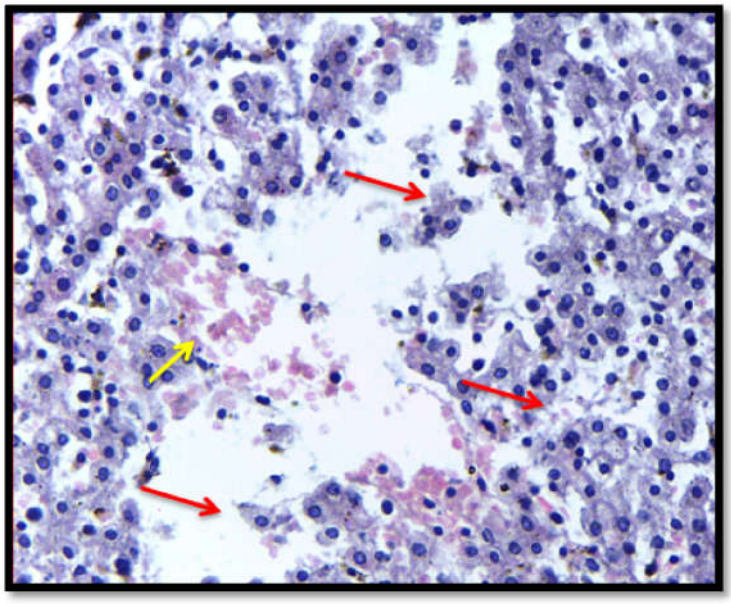

**Figure (7): Transverse section in fetal liver of a mouse infected by congenital transmission shows liver decomposition (red arrows) and bleeding (yellow arrow) H&E.(40X).**

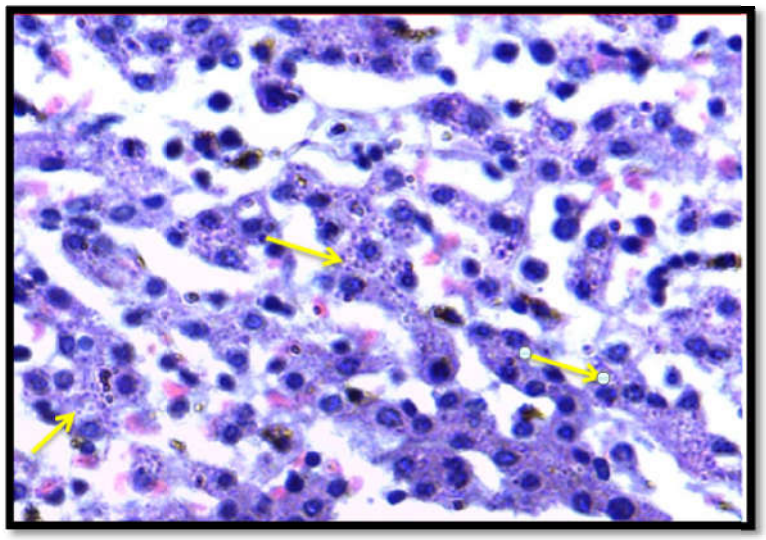

**Figure (8): Transverse section in fetal mouse liver appears to be full of liver cells in large numbers of theamastigote stage (yellow arrows) H&E.(40X).**

## DISCUSSION

The current study demonstrated the ability of *L.donovani* to the transition from pregnant mice to their embryos. The parasite appeared in the liver and bone marrow sections of modern deliveries and this was agreed with (17), who found that 26% of the newborn deliveries were infected through pregnant mothers who were infected with the parasite, while (18) diagnosed the parasite in different organs such as liver, bone marrow, heart, spleen, lymph nodes, kidney and placenta of modern deliveries of infected mothers.

A study in Germany described the congenital transmission of visceral leishmaniasis in an infant who has never been present in an endemic area by visceral leishmaniasis, which showed signs of hepatosplenomegaly, bilaterally enlarged cervical lymph nodes, intermittent fever and a rectal temperature of 40˚c. The researchers believe that the disease was transmitted to the infant by mother who acquired the infection during her stay in Spain, which proved serological tests infected with parasite. The researchers explained that the infant infected by transmission of maternal blood during delivery or by the placenta during pregnancy (12), and we believe that the second reason is closest to interpreting the congenital transmission of the parasite in the current study. There was not enough time for transmission of the mother's blood to the fetus because the deliveries dissected directly after delivery.

(19;20;21) noted the transmission of pregnant mother's cells to their fetuses and the transfer of embryonic cells to pregnant mothers. This may be the main cause of parasite transmission from pregnant mothers to their fetuses in the current study. Consequently, the pathways that allow passage of body cells of this size may be sufficient for free amastigote stage to the embryo or through its transmission with the cells containing it.

The congenital transmission of parasites is not limited to *L.donovani* . There are studies confirmed the widespread of congenital transmission of *Trypanosomacruzi*reached to 14000 cases per year, and the researchers explained that congenital infections accompany asymptoms pregnant mothers (22).

Several studies have confirmed the possibility of transmission of parasitic infection to the testis as noted by (23), through the incidence of testicular infection to a child suffering from leukemia, while (24) proved the transmission of infection of *L.infantum* from infected man to his wife where the onset in genital papule followed by hepatomegaly, splenomegaly and enlargement of lymph nodes.The current study has demonstrated the Iraqi isolate of*L.donovani* is able to transmission from pregnant mothers to their embryos through the placenta in experimental mice.

## قابلية طفيلي اللشمانيا الاحشائية *Leishmaniadonovani* على الانتقال الخلقي في الفئران المختبرية سلالةBALB/c

فرحان بدن حاجم البزونيأ.  د. مسلم عبدالرحمن محمد

قسم علوم الحياة ، كلية العلوم ، جامعة البصرة ، البصرة ، العراق.

### الخلاصة

الهدف من الدراسة الحالية التحقق من قابلية انتقال طفيلي اللشمانياالاحشائية *Leishmania donovani*من الامهات الحوامل الى اجنتها في الفئران المختبرية سلالة BALB/c ، بعد مزاوجة ذكور الفئران مع الاناث ، أذ تم حقن الاناث بجرعة $10^{12}$ طفيلي من خلال الوريد الذنبي بالطور امامي السوط للطفيلي الذي نمي على وسط NNN-medium .

أثبتت الدراسة قابلية الطفيلي على الانتقال الخلقي من الامهات الى اجنتها بواسطة المشيمة من خلال ظهور الطور عديم السوط في مقاطع انسجة الكبد ونخاع العظم لأجنة الفئران المصابة مقارنتاً مع الأجنة غير المصابة.

## REFERENCES


**1.Boussaa, S., Kahime, K., Samy, A.M., Salem, A.B. and Boumezzough, A.**(2016). Species composition of sand flies and bionomics of *Phlebotomuspapatasi*and *P. sergenti*(Diptera: Psychodidae) in cutaneous leishmaniasis endemic foci, Morocco. Parasites & vectors,9(1): 60.

**2.Welburn, S., Beange, I., Ducrotoy, M. and Okello, A**.(2015): The neglected zoonoses-the case for integrated control and advocacy. Clinical Microbiology and Infection,21(5):433-443.

**3.World Health Organaization (WHO)** (2003): Communicable diseases who/cds/2003.17).http://www.who.int/infectious-disease-news/IDdocs/whocd2003 .17/1.

**4.Palatnik-de-Sousa, C.B and Day, M. J.**(2011). One Health: the Global Challenge of Epidemic and Endemic Leishmaniasis**.** Paras. Vect. J.,4, pp**:**197-207.

**5.Shani W. S.** (2006) Levels of serum immunoglobulins and complements in patients with visceral leishmaniasis. Bas. J. Vet. Res. Vol. 2 No.5,pp:6-11.

**6.Al-Bayati N. S., Anwar S. A., Mahmood O. I.** (2017) Purification of metacyclicleishmanialpromastigotes by using peanut lectin agglutinin test. Bas. J. Vet. Vol.16, No.2, pp:184-192.

**7.World Health Organization (WHO).**(2010). First WHO report on neglected tropical diseases: Working to overcome the global impact of neglected tropical diseases. WHO, Geneva.

**8.Low G.C., Cooke W.E.**.(1926) A congenital infection ofkalaazar. Lancet.:1209-11.

**9.Banerji D**.(1955) Possible congenital infection of kala-azar. J Indian Med Assoc ;24:433-5.

**10.Nyakundi P.M., Muigai R., Were J.B.O., *et al*.**, (1988) Congenital visceral leishmaniasis: case report.Trans, R. Soc. Trop. Med. Hyg.;82:564.

**11.Yadav T.P., Gupta H., Satteya U., *et al*.**,(1989) Congenitalkala-azar. Ann. Trop. Med. Parasitol.;83:535 – 7.

**12.Boehme C., Hain U., Novosel A., EichenlaubS.,Fleischmann E., Loscher T.(**2006)Congenital visceral leishmaniasis [letter].Emerg Infect Dis;12(2):359-360.

**13.Haque A., Ekram A.R., Sharmin L.S., Belaluddin M, Salam M.A.**(2010) Congenital Visceral Leishmaniasis.Pak J Med Sci;26(2): 485-487.

**14.Kang ,I. G. and Norman, L**.( 1970) Manual of Clinical microbiologyMicrobiol.,Washington,USA.

**15.Meredith, S.E. ; Kroon, N.;Sondorp ,E.; Seaman, J.; Goris, M.; Ingen, C.; Oosting, H. and Schoon, G.** (1995):Leish-kit, a stable direct agglutination test based on freeze dried antigen for serodiagnosis of visceral leishmaniasis.J.Cli.Microbiol.,33:1742-1745.

**16.Drury, R. A. B**. (1967).Cariltons histological technique. 4thEd. Oxford Univ. Prentice-Hall,New York.110.

**17.Masucci M., De Majo M., Contarino R.B., Borruto G., Vitale F.,Pennisi M.G.** (2003) Canine leishmaniasis in the newborn puppy. Vet. Res.Commun. 27 Suppl. 1: 771-774.

**18.Rosypal A.C., Troy G.C., Zajac A.M., Frank G., Lindsay D.S.** (2005)Transplacental transmission of a North American isolate of *Leishmaniainfantum* in an experimentally infected beagle. J.Parasitol. 91: 970-972.

**19.Maloney S., Smith A., Furst D.E., Myerson D., Rupert K., Evans P.C., Nelson J.L.**(1999) Microchimerism of maternal origin persists into adult life. J. Clin. Invest.; 104:41-7.

**20.Srivatsa B., Srivatsa S., Johnson K.L., Bianchi D.W.**.(2003) Maternal cell microchimerism in newborn tissues. J. Pediatr.; 142:31-5.

**21.Nelson J.L., Gillespie K.M., Lambert N.C., Stevens A.M., Loubiere L.S., Rutledge J.C., Leisenring W.M., Erickson T.D., Yan Z., Mullarkey M.E., Boespflug N.D., Bingley P.J., Gale E.A**..(2007) Maternal microchimerism in peripheral blood in type 1 diaberes and pancreatic islet beta cell microchimerism. Proc. Natl. Acad. Sci. USA; 104:1637-42.

**22.Bern C., Martin D.L, Gilman R.H.**(2011). Acute and congenital Chagas disease. Adv. Parasitol. 75:19–47.

**23.Kapila K., Prakash M.B., Mehrota R., Vermar K**. (1994)Testicular leishmaniasis in a boy with acute lymphoblastic leukemia. ActaCytol 38: 878-879.

**24.Symmers W.S.** (1960) Leishmaniasis acquired by contagion: a case of marital infecation in Britain. Lancet 1: 127-132.