



EFFECT OF DIFFERENT CONCENTRATION OF IVERMECTIN DRUG ON THE GROWTH AND VITALITY OF EGGS AND ADULT LIVER WORM *FASCIOLA GIGANTICA* IN RABBITS

Nadia Zaidan Khalifa Rashid Al Nuaimi and Jassim Hameed Rahma Al Khuzaie

College of Education for Girls, University of Kufa, Iraq.

Abstract

The present study was conducted for the period from November 2016 to June 2017, which aimed to evaluate the effectiveness of Ivermectin drug on the growth and vitality of the parasite *Fasciola gigantica* inside and outside the body of rabbits. The drug Ivermectin used outside the body in three concentrations (0.10, 0.05, 0.01)% g / ml for both of egg and adults of giant liver worms and inside the body was (10, 5, 1 mg / kg) in the rabbits infected with this parasite.

The results showed that the concentration (0.10) was the most effective in hatching of eggs had rate percentage of hatch eggs was (10.2) and the rate of the percentage for destruction of worms was (91.2) where the signification difference was (1.9) for the weight mean infected liver organ which treated with Ivermectin when compared to the infected rabbits also. The weight mean spleen organ was the signification difference (2.4) when compared with the infected rabbits. The weight mean of kidney organ was the signification difference (1.1) when compared with the infected rabbits. Also, the results of the study within the body showed that dose of 10mg / kg of the ivermectin are the most effective in inhibiting the number of worms in the infected and treatment rabbits when compared to the infected and not treatment rabbits. The total number of worms in control positive of Ivermectin drug (14.8) while it was (12.9, 5.4, 1.1) at concentrations 0.1, 0.05 and 0.10mg / kg respectively. Also concluded that the percentage of *Fasciola gigantica* was decreased when the concentration of Ivermectin increased and increase the percentage rate of destruction worms you when increased the concentration of Ivermectin outside the body, and increase in the weight of infected organ with cercaria in positive control only when compared with the weights of infected organs with cercaria and treatment with different concentrations of Ivermectin.

Key words : *Fasciola gigantica*, Ivermectin drug, rabbits,

Introduction

Fascioliasis is caused by the giant liver worm of the genus *Fasciola*, which occurs in a wide range of mammalia host at over the world (Vazques *et al.*, 2016). The liver worm settles in the bile duct of the and causes several – dangers related to liver health final host (Gajewska *et al.*, 2005).

The incidence rate of Fascioliasis in humans increases in areas where animals are prevalent, such as sheep and cows (Ashrafi *et al.*, 2014). Egypt is one of the regions in the world where hepatitis is endemic (Dietrich *et al.*, 2015). There are several factors that are considered in the spread of the disease (age, sex and reproduction). Studies show that the prevalence of the disease in Ethiopia in young animals was 40% and 42.25% in adult. The prevalence percentage of the disease was 41.43 and

41.38% respectively in both male and female cattle (Biniam *et al.*, 2012). Both types of worms liver *Fasciola gigantica* *F. hepatica* cause several losses.

F.gigantica worm has a length of 75-25mm and a width of 12mm (Karyaand Damle, 2004). Its eggs are large in size, oval and covered and longth 150-190 microns and 70-90 microns width (Markell *et al.*, 1992). Intermediate host is one of the freshwater snails *Lymnaea auricularia* (Anawat *et al.*, 2015), while *F. hepatica* worm is 30mm in length, 13mm width and length egg, 130-150 micron and 63-90 micron the width (Roberts and Janovy, 2000). Its Intermediat host is also freshwater snails, especially *Lymnaea truncatula*. The ivermectin drug is a white powder but tends to yellowing and solubility in water is low (4 micrograms/liter) in the organic and other organic, such as Pro pylenglycol and aromatic

oils is good solubility, Chemically belong ivermectin the insecticide avermectin and derived from soil bacteria *Streptomyces avermitilis* and have a broad spectrum antibacterial and external parasites, because it is considered a medical material has a good spread in most tissues and metabolizes through oxidation pathways in the liver and is removed with waste (Lankas and Gordon, 1989).

Materials and Methods

Animal husbandry

A total of 20 rabbits of the genus *Lepus lepus arabicus* were brought and raised inside the animal house at the College of Education for Girls, University of Kufa. Weighing 1-1.5 kg and was placed in cages furnished with wood sawing and cleaned every two days. The animal house is equipped with heating, At a temperature of 22°C and fed rabbits by giving them food included some types of vegetables and divided into groups according to the requirements of the research.

Collection and breeding of snails

The snails were collected from the Mishkhab regions in Al-Najaf province transported by plastic containers containing water from the area from which the samples were collected and then transferred to the laboratory at the College of Education for Girls, University of Kufa and placed in the container basins in the tap water which put before 24 hours of disposal from chlorine. *Lymnaea auricularia*, snails according to Mansoorian (2001). Fig. 1 which was placed in glass basins. Fig. 2 container on the *Ceratophyllum* plant collected from the sample collection areas as food for these snails (Al-Ali, 2002). After about two weeks, collected Snail eggs. Fig. 3 were placed in other basins in the laboratory to obtain new generations of snails for the purpose of being infected with the parasite *F. gigantica*.

Collection of *F. gigantica* worms

The livers of infected sheep were collected, fig. 4 from the AL-Najaf massacre and were transported directly by plastic containers to the laboratory at the College of Education for Girls, University of Kufa and isolated parasitic worms fig. 5 of the bile ducts by dissecting the bile ducts of the liver by using a blade. These worms were washed with water several times to remove the impurities and then placed in the Petri-dish and identified based on Lofty and Hillyer (2003).

Collection of parasite eggs

The eggs were removed from the faeces of the animals according to Hillyer (1996) by taking 5g of faeces

of the infected animals and placed in a glass flask (500ml) then tap water was added with mixing by glass rod. Then after filtration by filter papers and then the filter collected in another glass flask and left for 10 minutes to settle. About 10ml of the sediment was kept to be washed again with tap water and then left again to settle for the same time. This process was repeated several times until obtaining a clear solution with transparent color containing the parasite eggs (fig. 6A) and then examined the solution by dissecting microscope under enlarge (100X). The eggs were incubated in the dark at (25°C) with water change every two days and noted the growth of embryos by dissecting microscope based on Soulsby (1982) and quoting (Shlash, 2014). The eggs hatches to miracidia when exposed to severe lighting after 17 days of incubation (Al-Mayah, 2004). These miracidia used to infect the snails (fig. 6B).

Infection of the snails

Total of 400 snails were collected from the new generations of *L. auricularia* and were infected with miracidia parasites of approximately number (20-25) miracidia by using the tissue culture chamber by Pasteur Pipette. After two hours, the miracidia enters into the snail (Al-Ali, 2002). Then the infected snails were put again in the breeding basins and a month later noted the release of cercaria from the snails, which encysted on the flasks wall and water surface (Al-Mayah, 2004) (fig. 7).

7-2 Metacercariae collection : After one month from infection small pieces of nylon were placed on the water surface of the glass basins where metacercaria can be attached and then taken and examined under the dissecting microscope to ensure the presence of metacercaria. The process of metacercaria collected continue by placing nylon pieces for three months to obtain sufficient quantity of metacercaria for infection (Al-Mayah, 2004) (fig. 8).

Experimental infection in rabbits

In this experiment, the rabbits were divided into five groups and each group containing four rabbits. The rabbits were infected by 100 metacercaria in 2ml of distilled water for each rabbit and the metacercaria were given orally with a stomach tube. The first group represented the positive control with metacercaria only and the second, third the and fourth groups were orally given with metacercaria and treated with Ivermectin in three doses (10, 5, 1) respectively. The fifth group is the negative control group fed by the Normal saline solution only. The rabbits were dissected after three months (Al-Ali, 2003).



Fig. 1 :

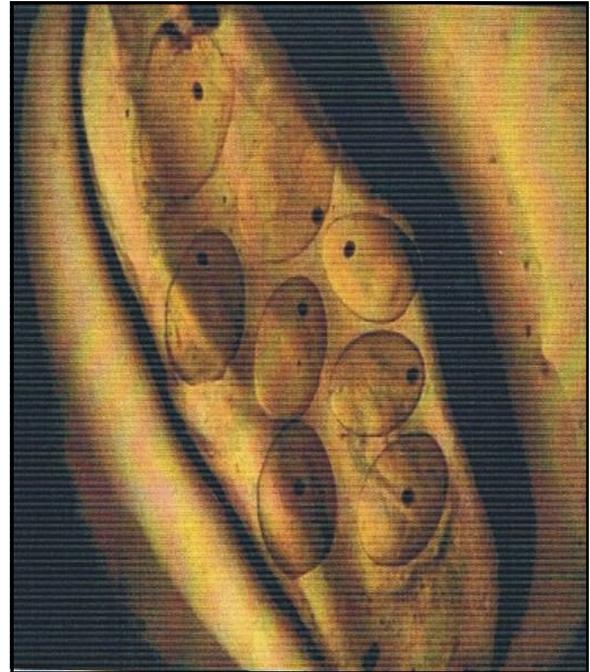


Fig. 3 : *Lymnaea auricularia* eggs.



Fig. 2 : *Lymnaea auricularia* snail.



Fig. 4 : Liver infected with *F. gigantica*.



Fig. 5 : *F. gigantica* worms isolated from the liver of infected sheep.

Ivermectin drug

The drug was presented with different concentrations for use as a drug for laboratory rabbits during the study. The following concentrations were selected (0.01, 0.05, 0.10%) g/ml and the drug was introduced by using the low $c1v1 = c2v2$. Also, the Ivermectin was used as a solution at concentration of (1%).

Table 1 : Effect of Ivermectin concentrations on the hatching of *F. gigantica* eggs *In vivo*.

Percentage for hatching eggs	Number of treated eggs	Time for treatment hours	Concentration of the drug
95.5	100	24	The control
64.2	100	24	0.01
35.4	100	24	0.05
10.2	100	24	0.10

Table 2 : Effect of Ivermectin concentrations on adults of *F. gigantica* *In vivo*.

Percentage of destruction	Number of treated worms	Time of treatment (minute)	Concentration of the drug
5.5	200	30	The control
40.4	200	30	0.01
72.8	200	30	0.05
91.2	200	30	0.10

Table 3 : Weight Mean of infected organs in rabbits treated with cercaria and different concentrations of Ivermectin drug.

Wight mean of kidney (g) M±SD	Wight mean of spleen (g) M±SD	Wight mean of liver (g) M±SD	Rabbits group
11.88±0.02	7.98±0.03	45.47±0.03	Rabbits treated with Saline Solution
13.37±0.1	10.97±0.01	48.41±0.04	Rabbits injection with cercaria only
11.78±0.15	8.83±0.02	45.69±0.06	Rabbits treated with Ivermectin (0.01) with cercaria
12.1±0.12	9.12±0.01	46.5±0.04	Rabbits treated with Ivermectin (0.05) with cercaria
12.7±0.11	10.3±0.03	47.2±0.02	Rabbits treated with Ivermectin(0.10) with cercaria
1.1	2.2	1.9	LSD

Table 4 : The total number of worms in the members of groups of rabbits treated with different doses of Ivermectin after three months of Infection.

Therapeutic efficiency	Total number mean of worms	Number of worms			Treated rabbits group
		Spleen	Kidney	Liver	
0	0	0	0	0	Negative control
	14.8	2.2	5.1	7.5	Positive control
12.83	12.9	1.7	3.5	5.7	Concentration of the drug 0.1%
63.51	5.4	0.5	1.5	3.4	Concentration of the drug 0.05%
92.56	1.1	0	0	1.1	Concentration of the drug 0.10%
	10.2	1.1	2.9	4.2	LSD

Effect of different concentrations of Ivermectin on the vitality and hatching of eggs *In vivo*

About 400 eggs divided into four groups, each group containing 100 eggs, which were divided into four replicates (25 eggs each replicate), each of these eggs were placed in a small beaker with 100ml of distilled water. Then concentrations (0.01%, 0.05%, 0.10%) g / ml of Ivermectin were added to the first three groups,

while the fourth group was considered as a control group treated with distilled water only. The eggs exposed to these concentrations for 24 hours then transferred to distilled water without drug every 48 hours till the end of the experiment and then the eggs were left inside the incubator at 25°C for 17 days and the percentage of hatching eggs was calculated (Al-Mayah, 2002).

Effect of different concentrations of Ivermectin in the adult liver worm outside of *in vivo*

Total of 400 worms were divided into four groups of each group containing 100 worms and divided into 25 worms For each replicated in a small baker containing 100 ml of distilled water. And add three different concentrations of Ivermectin to the first three groups of worms and recorded the loss after 10 minutes of exposure to the different concentrations of the drug and considered these worms dead when they stop moving their body, or oral sucker, or anterior cone (Al-Mayah, 2002).

Results and Discussion

Effect of different concentrations of Ivermectin on the vitality of eggs *In vitro*

The result showed in table 1 that there was a decrease in the percentage of eggs hatching when treated with different concentrations of ivermectin, so the percentage of egg hatching was 64.2%, when treated with

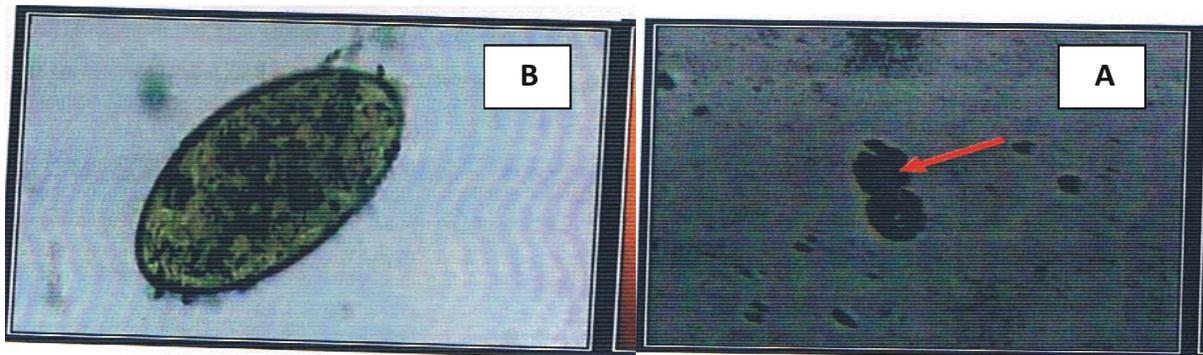


Fig. 6 : Egg and Maracidia of *F. gigantica* parasites.



Fig. 7 : Metacercaria on the walls of the basin.



Fig. 8 : Metacercaria of *F. gigantica* parasites.

concentration (0.01) while 35.4% when treated with concentration (0.05%) and when treated with concentration 0.1%, the percentage was (10.2%) that was the most effective on the hatching of the eggs.

Study of the effect of different concentrations of Ivermectin on the adults of *F. gigantica* in vivo

The results showed as in table 2, there was an increase in the percentage for destruction of adult worms when the concentration of the drug is increased when

compared to the control. The percentage destruction of adult worms when treated with concentration (0.01%) was (40.4%), while when treated with the concentration (0.05%), the percentage of destruction of worms was (72.8%), while the percentage of destruction of worms was (91.2%) at treatment with concentration (0.1%).

Ivermectin lead to the paralysis of the parasite and sometimes kill. This study agree with the study of Turner and Schaeffer (1989) that has been experimentally determined that ivermectin stimulates the release of GABA gamma amino butyric acid from the nerve endings which inhibit and close neuronal stimuli adjacent to the neuron in the parasite or fiber nervous system in the arthropods. Khaled *et al.* (1998) observed that Ivermectin had an effect in inhibition of the average forms of *Leishmania* in the infected host when treated with the drug.

Effect of different concentrations of the Ivermectin on the weight mean of rabbits organs infected with cercaria

The study showed that the weight mean of the organs (liver, spleen and kidney) increased significantly in the group of rabbits injected with cercaria only (positive control) when compared to the negative control (group of rabbits injected with normal saline solution and when treated with different concentrations of the Ivermectin drug (table 3).

During the present study noted that there were significant differences in the weight of infected rabbits compared with infected organs treated with different concentrations of the drug and also noted increased weight, thickness and hardness of the liver in the infected positive control group and treated groups due to the fibrosis that occurs in the liver tissue as a result of infection. This study agree with Kaya *et al.* (2007) and that the liver of calves infected with *Fasciola gigantica* is caused by inflation, resulting in the thickness and darkness of the color (Khogali *et al.* (2008), while

(Ahmeddullah *et al.* (2007) reported that the infected buffalo liver is slightly puffy and pale color which characterized by an increase in size. Arjona *et al.* (1995) indicated that the infection and cyst in the bile duct is caused by the presence of large numbers of worms in the bile duct. These results agree with what is stated by Al-Mayah (2002) and Turner and Schaeffer (1989) with the presence of cirrhosis near and around the bile duct in rabbits, rats and mice infected experimentally with liver giant worm. The infected sheep and cattle are characterized by the expansion of the walls of the bile duct with an increase in thickness (Haridy *et al.*, 1999). The current study also showed an increase in the weight of the spleen and kidney and the spleen is the largest organ of lymph in the production of antibodies specific to parasite antigens and get the increase in weight either by splitting the algal cells. This is consistent with the efficacy of the researcher (Tizard, 1978) who confirmed that the spleen is a case of acute inflammation causing an increase in the white blood cells, especially the cells Eosinophils because these cells are susceptible to parasites and they possess (IgE) antibodies. The concentration increases when worms, which facilitates the process of typhoid and then increase the level of enzymes and lysozymes and then crash parasite. As for the effect of liver giant worm on the weight of kidney also there is a significant difference in infected animals in which necrosis in the kidney that causes the secretion and removal of a substance from the parasite inside the kidney cells during the removal process (Mahdy and Farrag, 2009).

Effect of different concentrations of Ivermectin on the total number of giant liver worms *in vivo*

The results showed that the total number of worms in the groups of treated rabbits with different concentrations of Ivermectin was lower than that rate of infected rabbits. The total number of worms was (12.9) in the treated group with concentration (0.01%) and with therapeutic efficiency (12.83). However in the concentration (0.05%), the total number of worms was (5.4) and with therapeutic efficiency (63.51), while in concentration (0.10%) the total number of worms (1.1) and with therapeutic efficiency (92.56) with significant differences as shown in table 4.

The difference in animals in the sensitivity of infection by these worms depends on several factors such as animal type, strain, age, food type and the nature of the histological structure of the liver as well as the state of immunity. The percentage of infection of domestic rabbits with *F. gigantica* parasites have a percentage

of 100% and consider the sheep are very sensitive to this parasite to be infected (Al-Mayah, 2002; Anderws, 1999). The study found that the liver is the most infected organ by the giant liver worm in laboratory animals. The rate of worms in rabbits' liver for infection and treatment with ivermectin is (14.8) and the treatment of ivermectin has a positive effect on the elimination of the giant liver worm in the infected rabbits with an effect on activating phagocytic activity for pythrombocytes and this agree with Hokelek (2002) where the use of ivermectin in the treatment of the sheep infected with the hepatic water cyst led to a significant decrease in the size of these cysts.

References

- Al-Ali, S. (2003). Antigen preparation for *Fasciola gigantica* Cobbold, 1956 with study of antigenical their efficiency in serological diagnosis of infection in the experimental and natural hosts. *M.Sc. Thesis*, College of Sciences, University of Basra. 79P.
- Al-Mayah, Z. A. (2002). Study the effect of aqueous extract to punica Granatun on *Fasciola gigantica*. *M.Sc. thesis*, College of Education, University of Basra, 102 pp.
- Al-Mayah, S. H. (2004). Studies on serology, biology and control of *Fasciola gigantica* Cobbold, 1856 infection Basra. *Ph.D. thesis*, College of Science, Basrah Univ., 102p.
- Al-Ali, A. A. (2002). Stages of growth and development of *Fasciolas gigantica* and Histopathological changes in the intermediate host *Lymnaea auricularia*. *M.Sc. Thesis*, Coll. Edu, Univ. Basra, 83PP. In Arabic.
- Ahmeddullah, F., M. Akbor, M. G. Haidar, M. M. Hossain, M. Khan, M. I. A. Hossan and I. S. Bund Santa (2007). Pathological investigation of liver of the Slaughtered buffaloes in Barisal district. *Bangl. J. Vet. Med.*, **5** : 81-85.
- Anawat, P., W. Chalobol, R. Amnat and Ci Jong-Yil (2015). Experimental Life History and Biological Charactersthes of *Fasciola gigantica* (digenea:Fasciollidae), **53(1)** : 59-64.
- Arjona, R., J. A. Riancho, J. M. Aguado, R. Salesa and J. Gonzalez-Macias (1995). Fascioliasis in developed countries: a review of Classic and aberrant forms of the disease. *Med. J.*, **74** : 13-23(Abs).
- Anderws, S. J. (1999). The life cycle of *Fasciola hepatica*. In: Dalton, J. P. (eds.) Fascioliasis .CAB Inter. Publi., Dublin city Univ., 1-21 pp.
- Ashrafi, K., M. D. Bargues, S. Oneill and S. Mas-Coma (2014). Fascioliasis : a worldwide Parasitic disease of importance in travel medicine. *Travel Med. Infect. Dis.*, **12** : 636-649.
- Biniam, T., A. Hanna and G. Sisay (2012). Study on coprological Prevalence of bovine *Fasciolosis* in and around Woreta, North Western Ethiopia, **4(7)** : 89-92.
- Dietrich, C. E., A. Kabalioglu, E. Brunetti and Z. Richter (2015).

- Fascioliasis. *Gastroenterol.*, **53** : 285-290.
- Haridy, F. M., B. B. Ibrahim and T. A. Morsy (1999). Fascioliasis an increasing Zoonotic disease in Egypt. *Soc. Parasitol.*, **29** : 35-48.
- Gajewska, A., K. Smaga - Kozłowska and M. Wisniewski (2005). Pathological changes of Liver in infection of *Fasciola hepatica*. *Wiad. parazytol.*, **51** : 115-23.
- Hokelek, M. (2002). Ivermectin used in percutaneous drug injection method for *The treatment of liver hydatid disease in sheep*. *Gastroenterology*, **122(4)** : 1-8.
- Hillyer, G. V. (1996). Improved diagnosis in human Fascioliasis in :angelico, M. and Rocchi, G. (eds) Infectious diseases and Public health Balaban Publ., Philadelphia, Pp : 314-324.
- Khaled, A. S., Al-Rasheid and A. Tosson (1998). Efficacy of Ivermectin on the Infective of Leishmania Major Promastigote. *J. Egypt. Parasitol.*, **28(1)** : 199-202.
- Kaya, G., E. O. Atesoglu and A. Akca (2007). *Fasciola hepatica* egg-induced Granuloma in a bovine liver a case report. *Medycyna Wet.*, **63** : 175-177.
- Khogali, S. M., R. M. Abdelghafar, F. A. Omer and M. S. Fadel M. S. Ultrasonographic Findings in 25 Zebu Calves with liver Fascioliasis. *J. Ani. Vet. Advan.*, **7** : 77-79.
- Karya karte, R. P. and A. S. Damle (2004). Medical parasitology. Book and Allied (p) Ltd. Pp. 137-142.
- Lankas, G. R. and L. R. Gordon (1989). Toxicology. In : *Ivermectin and Abamectin*. Campbell, W. C., ED. New York, 16-142.
- Lofty, W. M. and G. V. Hillyer (2003). *Fasciola* species in Egypt. *Exp. Pathol. & parasitol.*, **6(11)** : 9-22.
- Mansoorian, A. (2001). Freshwater gastropoda of Khuzestan province, South-west Iran. *Iranian Int. J. Sci.*, **2** : 96-103.
- Markell, E. K., M. Voge and D. T. John (1992). Medical parasitology 7th ed Library of Congress Cataloging in-publication Data, Pp. 196-199.
- Mahdy, K. A. and A. R. Farrag (2009). Amelioration of aluminum toxicity with Black seed supplement on rats. *Toxicologic and Environmental Chemistry J.*, **91(3)** : 567-576.
- Roberts, L. S. and J. Janovy (2000). *Foundation of parasitology*. 6th Edn. J. McGraw Hill Book Co., 256-259 (2000).
- Soulsby, E. J. L. (1982). *Helminthes, arthropods and protozoa of domesticated Animal*. 7th ed., Baillere, Tindall and Cassell, London, Pp: 809.
- Shlash, Shaimaa Abdul Hussein Mohammed (2014). Biological and Pathological Study of the experimental Infection with the Giant Liver Worm *Fasciola gigantica* and evaluate the efficiency of Nigella sativa extract in Rabbits; *M.Sc. Thesis* Kufa Univ.
- Turner, M. J. and J. M. Schaeffer (1989). Mode of action of in: Campbell WC(ed). Ivermectin and Abamectin. New York, U.S.A : pp. 73-78.
- Tizard, I. (1978). *Veterinary immunology* (An introduction). 3rd ed. W. B. Saunders Company, 241-247.
- Vazques, A., M. Lounnas, J. Sanchez, A. Alba, A. Milesi and B. Hurtrez-Boousses (2016). Genetic and infective diversity of the liver fluke *Fasciola hepatica* (Trematoda : Digenea) from Cuba. *The leminthol.*, **14** : 1-7.