

PATHOLOGICAL EFFECT OF *TOXOCARA CANIS* EGG DOSES IN EXPERIMENTAL MICE.

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(Received 2 January 2011,Accepted 10 February 2011)

ABSTRACT

In this study we examined the effect of various sensitizing doses of infective *Toxocara canis* eggs and the trapping of larvae in different tissues of the murine host. The level of trapping increased with sensitization egg doses.

Different doses of *Toxocara canis* eggs were given to ten groups each with five mice and then necropsied after different periods.

Experimental toxocariasis in mice sacrificed at (1,2 and 3) weeks post infection showed no lesions in mice given 50, 75 and 100 *Toxocara canis* eggs respectively. Other groups given higher doses of *T.canis* eggs between (125-500 eggs) showed lesions in liver, lungs, eyes and muscles of sacrificed mice after 4 weeks of infection.

INTRODUCTION

The incubation period of toxocariasis varies from one week to months after a child swallows *Toxocara* eggs⁽¹⁾. Toxocariasis is considered an aberrant infection because humans are incidental hosts, and the parasite cannot completely mature in the human body^(2,3). Instead the invasive larvae migrate for months through different organs until they are overcome by the human inflammatory reaction and die. Some larvae are destroyed in the liver and pass onto the lung and are destroyed there⁽⁴⁾. The larvae can survive in tissues for at least nine years and possibly for life of the host⁽⁵⁾. Some *Toxocara* larvae after reaching systemic circulation, may infect distant organs including brain, heart, lungs, kidney, muscles and eyes^(4,6,7,8,9).

Objectives: To evaluate the pathological and histopathological aspects of toxocariasis in laboratory mice. Mice internal organs were examined for pathological evaluation.

MATERIALS AND METHOD

Toxocara canis adults were obtained from intestine of naturally infected local dogs. Intestine were incised, squeezed and the adult female of *Toxocara canis* emerged and were collected, put in (1%) formalin then brought to the laboratory. The freshly recovered female worms were dissected to obtain the uteri using dissecting microscope. The uteri were removed and digested by using the formula (7.5g pepsin powder, 85gms Nacl and 10ml conc.HCL, 1 liter distilled water to liberate eggs). The eggs were washed three times by sedimentation in distilled water and incubated for 40 days at a temperature of 29°C in Petri plates containing 0.1 N H₂SO₄ at a depth of 5 mms. The lots eggs were examined weekly to check for viability and embryonation. The eggs to be ready for infection, were washed three times with normal saline agitated into suspension, when they were fully embryonated⁽¹⁰⁾.

Fifty Albino (Balb/c) 20 males and 50 females mice were used for experimental infection. The mice were 15-25 gm body weight, and 2-6 weeks old maintained in air-conditioned quarters and provided with suitable food and water. These fifty mice were classified into 10 groups each with 5 mice. They were infected orally under light ether anesthesia using 1ml insulin syringe with blunted curved needle covered with polythene fine tube used as stomach tube for egg suspension in broth-gelatin (nutrient broth, 6.4gm. gelatin, 40gm; 1 liter distilled water). Three samples of each egg suspension were placed on slides and examined microscopically; only eggs containing (larva 2) were counted. The mean of these samples were used to estimate the number of eggs per ml of suspension. Suspensions were adjusted by the addition or removal of broth-gelatin to deliver the desired dose to each mouse in a volume of 0.10-0.15 ml. Twenty five mice were given 0.1 ml of sterile distilled water and served as a control group. These mice were kept under same condition of the tested groups.

Before infecting mice, eggs were shaken for 15 minutes at 37°C in 6% sodium hypochlorite solution and then washed several times in distilled water. This process partly removed the outer shell of the eggs and prevented them sticking to apparatus. The following groups of mice were tested :

Group I were given orally 0.10ml of embryonated eggs Suspension contain about 50 eggs /0.1 ml. group II were given eggs suspension of about 75 eggs /0.1

ml. group III were given eggs suspension of about 100 eggs/ 0.1ml. group VI were given eggs suspension of about 125 eggs/0.1ml. group V were given eggs suspension of about 150 eggs/0.1ml. group VI were given eggs suspension of about 200 eggs/0.1ml. group VII were given eggs suspension of about 250 eggs/0.1ml. group VIII were given eggs suspension of about 300 eggs/0.1ml. group XI were given eggs suspension of about 350 eggs/0.1ml. group X were given eggs suspension of about 500 eggs/ 0.1ml.

All mice were sacrificed after they were infected as follows:

First group after one week post infection. Second group after two weeks post infection. Third group after three weeks post infection. Fourth group after four weeks post infection. Fifth group after five weeks post infection. Sixth group after six weeks post infection. Seventh group after seven weeks post infection. Eighth group after eight weeks post infection. Ninth group after nine weeks post infection. Tenth group after ten weeks post infection.

Mice of the control group were killed and necropsied after 12 weeks. Necropsy was performed to all groups following cervical dislocation and skinning, the carcass musculature was examined with the aid of a dissecting microscope for the presence of lesions. All viscera, liver, spleen, kidneys, lungs and heart were examined for the presence of a milky spot granuloma. The brain and eyes were carefully removed with a small forceps and placed in 10% neutral buffered formalin. The eyes were examined immediately after enucleating, under dissecting microscope for hemorrhages and then sectioned for histopathological examination. The suspected lesion were cut from the whole organ fixed in 10% neutral buffer formalin for histopathological examination.

RESULTS

Ten groups of mice each with five, were infected orally with different inoculants of the infective embryonated eggs of *Toxocara canis* and sacrificed weekly between 1-10 weeks post-infection (Table 1) .

The first group (given 50 embryonated eggs) was sacrificed one week post infection. Autopsy showed absence of any lesion in all mice. Second group (with 75 embryonated eggs) was examined 2 weeks post-infection and the autopsy showed no any lesion in the infected mice.

The third group (with inoculums of 100 embryonated eggs) was sacrificed 3 weeks post infection, and the autopsy showed no lesions in the viscera of all mice examined. Group four (inoculated with 125 embryonated infected eggs) was sacrificed 4 weeks post infection, and the autopsy revealed one mice with a lesion as a small whitish spot 1-2 mms on the surface of liver. Gross finding showed hepatomegaly and irregularity of the surface.

Group five (inoculated with 150 embryonated eggs) was sacrificed (5) weeks post infection, and the post mortem revealed an infection in the liver of one mice. The lesion appeared as a whitish exudative lesion measuring approximately 1-2mm. Group six infected (with 200 embryonated eggs) was sacrificed after 6 weeks post infection, and the post mortem findings revealed small whitish multiple nodule 1-2 mms in right lung of one mice of the group. The seventh group (inoculated with 250 embryonated eggs) was autopsied 7 weeks post infection, and the autopsy revealed an infection in two mice; one mice had a whitish granulomatous lesion in liver while the other had a lesion in the right lung. Group eight (with inoculums of 300 embryonated eggs) was autopsied 8 weeks post infection and revealed a lesion in the liver which was observed as a focal whitish spot in one mice only.

Group nine with inoculums of 350 embryonated eggs was necropsied 9 weeks post infection, and the autopsy revealed two mice out of five were infected. The lesion found in right lung was found as a small whitish or milky spot like lesion in one mice and the second mice had an infection in the muscle of the thigh.

The last infected group was autopsied 10 weeks later after giving inoculums of 500 embryonated eggs, and the results showed three mice out of five were infected, the first mice had a lesion in the liver, and the lung lesion appeared in the second mice, while the eye lesion was detected in the third mice.

Histopathological examination revealed a mild cellular infiltration in localized foci in group four (given 125 infective *Toxocara* eggs) with inflammatory cells, represented by eosinophils and neutrophils (figure 1).

Extensive reaction seen in groups 9 and 10 where 350-500 infective embryonated eggs were given, the granuloma formed revealed a central cellular region of macrophages and epithelioid cells. Granuloma begin to appear in mice sacrificed 4 weeks post infection. In liver with granuloma, there was a congestion of sinusoids, fatty degeneration also have been seen in hepatocytes detected with

fatty degeneration. The granuloma contained a center of closely eosinophils and macrophages surrounded by larger macrophages with pale vesicular nuclei. Multinucleated giant cell was seen.

Table (1) Number of infected mice with embryonated eggs of *Toxocara canis* in ten groups and the site of lesion resulting into granuloma.

Groups of mice	No. of mice in each group	No. of infected mice	%	No. of eggs inoculated	Location of lesion (granuloma)			
					Liver	Lung	Eye	Muscle
Group I	5	0	0	50				
Group II	5	0	0	75				
Group III	5	0	0	100				
Group IV	5	1	20	125	1			
Group V	5	1	20	150	1			
Group VI	5	1	20	200		1		
Group VII	5	1	40	250	1	1		
Group VIII	5	2	20	300	1			
Group IX	5	1	40	350		1		1
Group X	5	3	60	500	1	1	1	
Control group	25							
Total	75	10			5	4	1	1

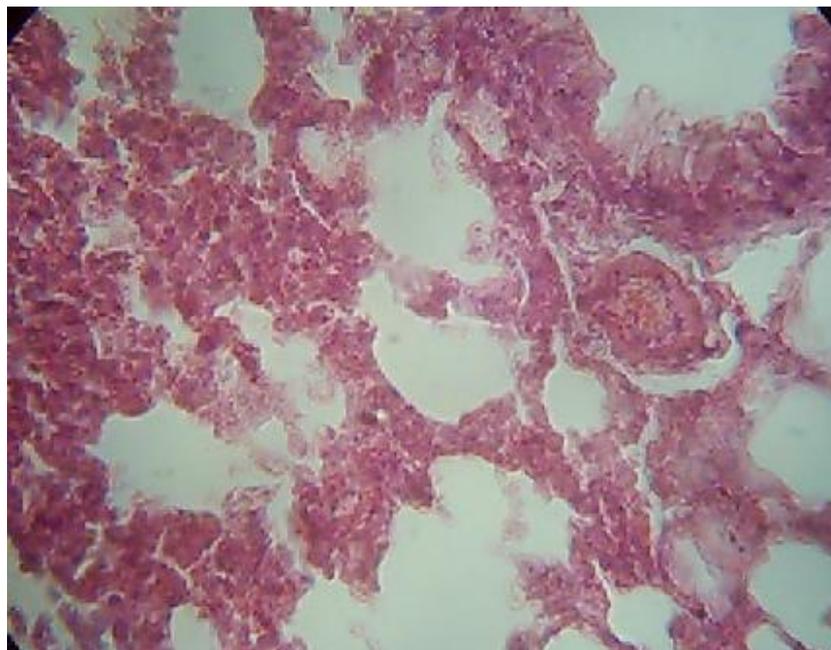


Figure 1: Shows the larva surrounded by inflammatory cells neutrophils, eosinophils, lymphocytes, epithelioid cells .

DISCUSSION

In the present study, ten groups of mice with 5 mice in each group, were infected with various numbers of embryonated *T.canis* eggs (Table 1). The first three groups of mice showed no lesions in various organs examined. The lack of larval trapping in different organs of these groups which received an initial doses of (50, 100 and 125) eggs respectively, might suggest that this level of infection or the number of eggs used was not sufficient to initiate an infection in the recipient mice. These findings are in agreement with results obtained by others^(10,11). No larvae were recovered from the control group at necropsy. In group IV, granulomatous lesions in liver were detected in one mice of each group after (4-5) weeks post infection. It is clear from the present investigation that larvae of *T.canis* can be established in experimental mice. This plateau occurred following a sensitizing dose of 125 and 150 of *T.canis* eggs respectively, administered in both groups. This observation is consistent with the results reported by Parson and Grieve (1990)¹⁰. Furthermore, it was noted that, liver trapping appears to be a phenomenon shared by mice of different genetic background and is not restricted to Balb/c strain⁽¹²⁾. Enlargement of livers in comparison to controls, whitish spots were observed in experimental mice, this is in accordance with the results obtained by others whom reported hepatomegaly with spots and streaks on the surface of the liver⁽¹³⁾. The histopathology of the developing granuloma in larval toxocariasis has been described previously⁽¹⁴⁾. A similar sequence of histological responses was observed in liver tissues from infected mice in this study. In other hosts, similar pattern may arise. *T.canis* juvenile (larva) in liver of a monkey at nine months infection has been reported. These juveniles were found encapsulated by a granulomatus reaction⁽¹⁵⁾.

A matter of concern regarding *Toxocara* larval behavior in experimental and paratenic hosts may arise. It is unclear whether the recovery of larvae from the liver at 4 weeks or more truly represents larval trapping⁽¹⁶⁾, but may, in fact be able to undergo future migration to other sites within the body^(10,17). Residual granulomas devoid of larvae, and larval migration from within hepatic granulomas would suggest the larvae can escape the granulomatus response attendant to infection with *T.canis* larvae⁽¹⁸⁾. Furthermore, it is not clear whether

trapped larvae may be killed with the liver. Granuloma formation may, in fact, act to protect larvae⁽¹⁹⁾.

High doses of *T.canis* eggs may start to initiate the lesions in other organs rather than the liver as indicated in groups VI, VII, IX and X. In these groups, the granulomatous lesions start to appear in lungs which indicate trapping of *Toxocara* larvae after escaping from the liver, where the motility of larvae help in this phenomena. Lung manifestation indicated by disseminated pulmonary lesions of visceral larva migration syndrome due to *Toxocara canis* infection has been reported⁽²⁰⁾. Granulomas which are typically found in the liver, may also occur in the lungs⁽²¹⁾. A case of an adult who had acute *T.canis* infection manifested as severe bronchospasm with a right lower lobe infiltrate and hypereosinophilia has been reported⁽²²⁾. The disease then progressed to respiratory failure. Eye infection occurred only in one mice following a high oral dose of 500 *T.canis* larvae per inoculums. The infection occurred at a later time (after 10 weeks post infection). This result is in agreement with findings reported by other workers whom found *T.canis* larvae in the eye of mice after 4 months post infection. the histological examination of the eyes of mice revealed approximately 90% of these eyes were infected, and the larvae observed in the retina⁽²³⁾. A high susceptibility to ocular infection in gerbils other than mice has been reported, and the ocular changes of fundi of these animals after oral infection with embryonated *T.canis* eggs were detected. They observed that vitreous choroidal, retinal hemorrhage and exudative lesions were often present in gerbils rather than mice⁽²⁴⁾. This is consistent with recent report indicated that 55% of infected Mongolian gerbils can exhibit ocular lesions. The migration route of the larvae to the eye is still unclear, however, it is generally accepted that the usual route of entry to the eye is via the blood stream^(24,25). Other migration route of the larvae through the brain to optic nerve and to CSF space, then to the choroids, has also been mentioned⁽²⁴⁾. Ocular toxocariasis is typically a monocular disease of young children and its clinical findings include retinochoroiditis, optic papillitis and endophthlmitis⁽²⁶⁾. In ocular toxocariasis, species differences in susceptibility can be very marked⁽²⁴⁾. Humans are fortunate in that they are far less susceptible to ocular infection. To date, only a small number of nematode larvae have been recovered from eyes affected with the infection⁽²⁶⁾. However it has been established that ocular toxocariasis is caused by *Toxocara canis*, the role of *T.cati* in this condition is still unclear⁽²⁵⁾. It is

perhaps surprising that the prevalence of such a disease in humans should remain in doubt. However, the wide variation in the few estimates of prevalence may well be a reflection of the wide variation in exposure as evidenced by the wide variation in seroprevalence. The present results suggest that ocular invasion in man may also occur in a later stage in the course of the disease. This observation is in accordance with other reports who mentioned that ocular invasion in man may occur at late stage of the disease^(27,28). These reports also stressed that the increasing dose of *Toxocara* eggs increase the mean number of larvae per eye and the percentages of the eye that are infected. It indicates that the severity of the disease in mice is dose dependent. The present investigation showed granulomatous lesion due to the presence of larvae in muscle, after inoculating of mice with high dose of *T.canis* eggs. This is in agreement with a report mentioned the detection of *T.canis* larvae in muscles and other tissues⁽²⁹⁾.

التأثير المرضي لمختلف جرعات بيوض الديدان السهمية في فئران التجارب

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الخلاصة

في هذه الدراسة تم اختبار التأثير التحسسي للجرع المختلفة لبيوض الديدان السهمية وحصر اليرقات في مختلف انسجة الفئران. أن مستوى حصر وتواجد اليرقات يزداد بأزدياد جرعة البيوض التحسسية. النتائج: أن نتائج داء السهميات التجريبي في الفئران المذبوحة بعد اصابتها لفترة أسبوع أسبوعين وثلاثة اسابيع لم تظهر اى افة في الفئران المعطاة جرعة بيوض الديدان السهمية بعدد 50 ، 75 ، و 100 بيضة على التوالي . أما المجاميع الاخرى التي اعطيت جرعة اكثر من بيوض الديدان السهمية والتي تتراوح بين (125-500 بيضة) اظهرت وجود الافات في الكبد، الرئتين، العينين، والعضلات في الفئران المذبوحة بعد أربعة أسابيع من العدوى.

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