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TOXOCAROSIS AND PARATENIC HOSTS

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SUMMARY

The seroprevalence rates of human toxocarosis figured up to 24.5% versus *Toxocara canis* and 22.1 % versus *Toxocara vitulorum* in some selected localities in Egypt, using enzyme linked immunosorbent assay technique. *Toxocara vitulorum* larvae in experimentally infected mice and rats follow the same manner of migration previously described for *T.canis* with more adaptation to mice than rats as paratenic host. This study clarifies the role of rodents in maintaining *Toxocara* larvae and spread of infection to other host.

INTRODUCTION

Human larval toxocariasis is a serious epidemiological problem in many countries (Barriga, 1988). It is elicited by larvae of nematodes of the genus *Toxocara* parasitizing the intestine of domestic and free-living carnivores.

Toxocara canis, a parasite of canine carnivores, is the probable cause of human toxocariasis although two other species *T.cati (T.mystax)* and *T.vitulorum* are also possible cause of the disease (Soulsby, 1983).

Man being an aberrant host where this round worm is not able to complete its life cycle but causes a spectrum of diseases ranging from no symptoms to eosinophilia, covert toxocariasis, visceral larva migrans or ocular larva migrans (Arambulo and Steele, 1976) .

The zoonotic aspect of *T.vitulorum* is almost lacking although the economic level and poor knowledge of farmers in Egypt are likely to accelerate the transmission of *T.vitulorum* based on the specific life cycle including dormancy of larvae in cows and prenatal or galactogenic transmission.

An important role in maintaining toxocariasis in certain region may be played by small mammals -paratenic hosts of *Toxocara* species. The

occurrence of anti-toxocaral antibodies in small mammals strongly suggests environmental contamination with infective eggs of *Toxocara* species. People living in an environment contaminated with disseminated *Toxocara* species are also exposed to great hazard of infection.

The present study considers the role of *Toxocara* species, namely *T.canis* and *T.vitulorum* from dogs and buffaloes, respectively, in inducing toxocariasis in Egypt.

Also evaluation of the viability, migratory and infectivety of *T. vitulorum* larvae in experimental animal in comparison with the previous known migratory behavior of *T. canis* larvae, where the mouse model has been widely used in the study of toxocariasis largely due to similarities seen between the progression of *T.canis* infection in the mouse and in the human as mentioned by Smith (1991).

MATERIALS and METHODS

Human samples:

204 blood samples were collected from patients with visceral or ocular complains patients who had chronic problems without clear cause as well as workers and attendants closely in contact with animals.

Blood samples were collected from Abu El-Reech Pediatric hospital (80), Institute of Tropical Medicine (60), and private laboratory (19), Ophthalmology Department in KASR EL- Aini Hospital (9), Research Institute of Ophthalmology (Giza) as well as attendants in close contact with animals (18).

Full case history was taken from each patient, includes name, age, locality, socioeconomic state (residence, contact with pet animals, contact with farm animals & history of drinking raw milk), history of pica (in children only) occupation and complain.

Preparation of embryonated *Toxocara* eggs:

Adult *Toxocara vitulorum* (from slaughtered calves in El- bassatin abattoir) & *Toxocara canis* worms (from sacrificed stray puppies) were collected, washed in saline solution (0.85%), the tegument (around the female genital opening) was punctured and eggs in the proximal part of the uterus collected, washed and cleaned, then *Toxocara* eggs spread as single layer at the bottom of clean shallow Petri-dishes (90 mm. diameter) filled to its half with 1% formolsaline. The eggs were left to develop under 28°C in the incubator (Soulsby, 1968). The dishes were aerated, examined under the microscope and the solution was changed every 3 days. Mature eggs containing second larval stage appeared during 17-20 days of incubation.

Experimental animals:

Male and female white Albino Swiss mice 20-25

gram each obtained from Abo-Rawach , Wistar rats 200-250 gram each and New Zealand rabbits 2-2.5 Kg body weight obtained from Department of Animal Hygiene, Management and Zoonoses, Faculty of Veterinary Medicine Cairo University, were used for induction of experimental infection.

Experimental groups:

Mice:- A total of sixty eight (68) mice were assigned to two groups 34 for each type of infection. Within each group 9 mice were left as control without infection. The other 25 mice in the first group were orally infected each with 500 embryonated eggs of *T. vitulorum*. The other 25 mice in the second group were infected each with 500 embryonated eggs of *T. canis*. The infection was induced through stomach intubation independent of body weight. Within each group, mice were divided into five groups according to time of sacrification which was done at 4, 10, 20, 30 and 60 days post-infection.

Mice in each group were slaughtered and the larval distribution assay was carried out according to the method of Abo-Shehada and Herbert (1985) with little modification. The larval distributions in different organs at different times of scarification were compared and analyzed.

Rats: -Thirty (30) rats were assigned to 6 groups (5 rats/each group), according to time of sacrification which was done at 5,10, 15, 30, 45 and 60 days post-infection. Within each group three rats were infected orally with dose of 3000 embryonated *T. vitulorum* eggs, while two rats were kept as control without infection. All rats in each group were sacrificed, blood samples were collected then larvae were extracted and counted as with mice.

Rabbits: - Twelve rabbits were used in this experiment. They were assigned into two groups (6 / each group). In the first group, 2 rabbits were fed infected mice tissues containing 10 days old *T.vitulorum* larvae. The other 2 rabbits were fed infected rat tissues containing 10 days old *T.vitulorum* larvae. The last 2 rabbits were left as control without infection.

In the second group, 2 rabbits were fed infected mice tissues contain 30 days old *T.vitulorum* larvae. The other 2 rabbits were fed infected rat tissues containing 30 days old *T.vitulorum* larvae. The last 2 rabbits were left as control without infection.

All rabbits were sacrificed at 35 days post-infection, the blood was collected to undergo the ELISA technique and the intestine, liver, lung, spleen, kidney, brain and muscles were examined for the presence of further migrating larvae.

Collection of larvae from tissues: -

This was carried out according to Abo-Shehada and Herbert (1985).

Enzyme linked immunosorbent assay technique (ELISA):

The test was done according to Voller et al. (1976).

RESULTS AND DISCUSSION

Although many cases of visceral larva migrans (VLM) are diagnosed by serologic testing, toxocariasis has been defined as an infection with *Toxocara* species with no attempt to identify the species involved (Nagakura et al., 1990).

In the present study, in order to distinguish between the two species of Toxocara (*T.canis* & *T.vitulorum*) that may infest human being, all collected patientís sera were subjected to parallel examination by using quantitative ELISA technique versus whole *T.canis* and *T.vitulorum* crude antigen. The overall seroprevalence rate of *Toxocara* titer in the examined patient's sera figured up to 24.5 % (50 patients) versus *T.canis* and 22.1 % (45 patients) versus *T.vitulorum* crude antigen, as illustrated in table (1).

The rate of *T.canis* infestation reported here was likewise in agreement with that given by Gueglio et al., 1994 in France, who reported an incidence of 22 %.

The seoprevalence rate of infants infestation with *T.canis* was 38.8 % in Abu- El- Rech pediatric hospital, 18.3 % in Institute of Tropical Medicine, 16.7 % in Research Institute of Ophthalmolo 15.8 % in private laboratory and 11.1 % in wo ers and attendants closely in contact with farm a imals, table (1).

On the other side the seroprevalence rate of p tients against *T.vitulorum* was 16.3 % in Abu-E Rech pediatric hospital, 31.7 % in Institute Tropical Medicine, 16.7 % in Research Ins of Ophthalmology, 31.6 % in private labora & 22.2 % in workers and attendants contact w farm animals, table (1).

From the obtained results, it is clearly evide that, the highest prevalence rate of *T.canis* wa recorded in infants attending Abu- El- Rech pe atric hospital, while the highest prevalence o *T.vitulorum* was recorded in Institute of Tropic Medicine and private laboratory.

The highest seroprevalence rates (versus *T.canis*) recorded in-patients attending Abu- El- Rech pediatric hospital may be ascribed to the nature of this hospital which is specialized for children. Children are the most exposed group to toxocariasis because they commonly play on the ground and put dirt and contaminated objects in their mouth (pica). Moreover, most of the children visiting this hospital are of low socioeconomic level and most of them have a history of contact with pet animals. This explanation coincide with that given by Dubinsky et al. (1994), who declared that rural children were found to have a 3 times

higher seroprevalence than children from urban localities.

Other authors claimed a much higher prevalence of *T.canis* infestation such as Deutz et al. (1996) (33 % in veterinarians). The variation of the seroprevalence results varies greatly from country to another and also varies according to the selected population, study sites, number of examined cases and serological tests used for diagnosis (Glickman & Schantz, 1981).

On the other hand the high seroprevalence rate recorded against *T.vitulorum* among patients in Institute of Tropical Medicine and patients' samples collected from private laboratory may be ascribed to, these patients may have contact farm animals or harboured previous *Toxocara* infection and / or having the habit of drinking raw milk under low socioeconomic level.

The murine model has been widely used in the study of toxocariasis largely due to the similarities seen between the progression of *T.canis* infection in the mouse and in human as mentioned by Smith (1991).

For that the murine model was done to evaluate the ability of *T.vitulorum* larvae in inducing toxocariasis and migration in experimentally infected animals, also to clarify if the larvae of *T.vitulorum* follow the same manner of migration previously described for *T.canis* or not. This in order to prove the ability of *T.vitulorum* to induce toxocariasis as *T.canis*.

The results displayed in tables (2 &3) and Fig. (1 &2), revealed that eggs containing second larval stage of *Toxocara canis* and *T.vitulorum* induce experimental infection in mice in the same manner with slight variation in mean number of larvae per each organ.

Macroscopic changes could be recorded in vital organs of experimentally infected mice. These changes appear in the form of white necrotic foci without changes observed in organs size during the first two weeks post infection. At the 60 th days post infection marked enlargement in size of infected lung, liver and spleen in comparison with that of non infected control mice.

Toxocara larvae were recovered mainly from the brain, muscles, lung and liver. Number of *T.canis* and *T.vitulorum* Larvae recovered from liver was high (260.0 & 230.0 respectively) at the 4 th day post-infection.

This number decreased gradually to (18.0 & 20.0 respectively) at the 60 th day post infection.

From the obtained results it is clearly noticed that there is an indirect relationship between increase in time post infection and number of larvae in liver, while the vise versa of this result was recorded in brain of experimentally infected mice, where

larvae of *T.canis* and *T.vitulorum* were not found in the brain until 4 days post infection, then increased gradually from 10 th day post-infection (8.5 & 6.0 respectively) to (12.2 & 18.0 respectively) at day 60 th post-infection.

Larvae of both species were recovered from spleen and heart during the second and third week of infection. Distribution of larvae in lung and kidney was higher during the first and second week of infection and decreased gradually after that, then no larvae were extracted from spleen, heart and kidney at 60 th day post-infection.

It is worthy to mention that the variation in number of larvae recovered from mice to another one within the same group indicate that, it can't be assumed that individuals within the same dose group and at the same stage of infection will have the same larval burdens in the brain, as numbers seen may vary considerably from individual within the same group (Hay and Aitken, 1984).

The obtained results are in agreement with that mentioned by Srivastava et al. (1988) who confirmed that the migratory behaviour of *T.vitulorum* in mice closely resembles that of *T.canis* of dogs. Small numbers of larvae were recovered from the spleen and the heart in the 4 th, 10 th and 20 th days after infection then disappeared till the end of the experiment.

Migration of *T.canis* and *T.vitulorum* larvae in different organs of experimentally infected mice revealed increase in ELISA mean optical density value of their sera in comparison with non infected group of mice.

The recorded optical density value for both species were increased from day 10 th post-infection (0.652 & 0.690 respectively) to (1.020 & 1.017 respectively) at 30 th day post-infection. This value decreased to (0.758 & 0.626 respectively) at the 60 th day post-infection.

It is worthy to mention that the optical density value in negative control mice varied between (0.192 to 0.280) as in tables (2 & 3).

The obtained results came in agreement with Chieffi et al. (1995) who recorded that anti-*Toxocara* antibodies had been found, at least up to the 180 th days post-infection and the significant level in the 30 th day post-infection.

The low optical density value recorded in mice at 60 th day post-infection may be ascribed to that the immune system of experimentally infected mice become exhausted, in addition the infection took the chronic form.

Rats were used as another experimental animal for testing the migrating behavior of *T.vitulorum* and for support the further studies on viability of *T. vitulorum* larvae in rabbits which infected by tissue containing large number of migrating

larvae, since rat able to tolerate higher dose of embryonated eggs than mice.

Testing ability of *T.vitulorum* larvae to migrate in rats as another experimental host than mice was illustrated in table (4) and Fig. (3).

The obtained results cleared that *T.vitulorum* larvae follow the same manner as *T.canis* previously described in the infection of mice, where it can migrate through liver, lung, spleen, heart, kidney, brain and muscle.

Macroscopic changes could be recorded in the vital organs of experimentally infected rats in form of slight enlargement in size of infected lung, liver and spleen in comparison with that of non infected control rats at the 60 th day post-infection.

Migration of larvae induces the same pathological changes and increases the optical density value of the infected rat sera from day 10 th till day 60 th post-infection in comparison with non infected rats. It is worthy to mention that, the experimental infection in rat evidenced marked nervous manifestations, which revealed arrival of *T.vitulorum* larvae to brain of infected rat and induction of nervous damages appearing as nervous manifestations (usually at 10 days post-infection) which were not recorded in mice. Therefore, rats differ from mice in their susceptibility to *T.vitulorum*..

It is necessary to mention that some rats developed apparent signs of disease, emaciation, depression, ataxia, weakness of the hind legs and increase of fighting.

No larvae were recovered from newly born suckling rats of experimentally infected mothers, this cleared that no larval migration from infected mothers (neither transplacental nor transmammary transmission in paratenic host) to their offsprings at the level of this study. This came in agreement with that mentioned by Prociv and Brindley (1986).

Table (5) evidenced evaluation and comparison between viability of *T.vitulorum* larvae obtained from previously infected mice and rats in inducing experimental infection in rabbits.

In this work rabbits were used as animal model to study the infectivety of *T.vitulorum* larvae harbour in rat and mice tissue at different ages post infection by *T.vitulorum* eggs containing second stage larvae.

T.vitulorum larvae in rats and mice tissues after 10 days of migration post-infection when used to induce experimental infection in rabbit, the larvae were able to complete its development and migration in different organs of infected rabbits. As a result of this migration-marked enlargement was observed in spleen, liver and lungs in comparison with non-infected control rabbits. Migration of

these larvae stimulate the immune defense mechanism of the experimentally infected rabbits and increase the serum ELISA optical density value against *T.vitulorum* crude antigen in comparison with that of non infected rabbit at the 35 th day post-infection with tissues containing *T.vitulorum* larvae (of mice and rats origin).

On the other hand, *T.vitulorum* larvae obtained from 30 days mice tissues were still able to induce infection in rabbit after migration in mice tissues and increasing the serum ELISA optical density value against *T.vitulorum* crude antigen at 35 th days post-infection.

On the contrary with that similar rat tissues containing 30 days old migrating *T.vitulorum* larvae failed to induce marked enlargement in organs of infected rabbit or even increase the serum optical density value of the infected rabbit after 35 days post-infection in comparison with that of non infected control rabbit.

During this experiment no larvae could be recovered from the organs or muscles of rabbit, this may be attributed to difficulty of infection of rabbit by large amount of meat in addition to large body size of rabbit. These results are in agreement with that recorded by (Kayes et al., 1985) who found that infection by small infective doses may initiate delayed immune response.

Table (1): Incidence of toxocariasis (anti-*Toxocara* anti-bodies) in the examined patient's sera after further diagnosis of cross reacted patients using ELISA technique.

Source of sampels	No. of examined patient	positive antibodies in sera of examined patients versus antigens of				positive cases/ each study site	
		T. canis		*T.vitulorum*			
		No.	%	No.	%	No.	%
1- Abu El-Reech Pediatric hospital	80	31	38.8	13	16.3	44	55.0%
2- Institute of Tropical Medicine	60	11	18.3	19	31.7	30	50.0%
3- Ophthalmology department in Kasr-El-Aini	9	0	0.0	0	0.0	0	0.0
4- Research Institute of Ophthalmology	18	3	16.7	3	16.7	6	33.3%
5- Private laboratory	19	3	15.8	6	31.6	9	47.4%
6- Workers & attendants contact to animals	18	2	11.1	4	22.2	6	33.3%
Total/ Overall incidence	204	50	24.5	45	22.1	95	46.6%

Conclusion:

Toxocara vitulorum has zoonotic potential as that previously reported about *T.canis*.

Mice are more suitable host for development and migration of *T. vitulorum* larvae than rats. The elevation of antibody titer (optical density value) versus *T.vitulorum* crude antigen in mice, rats and rabbit infected by rat and mice tissue containing *T.vitulorum* larvae clarifies the significant role of these animals species in the epidemiological cycle of *T.vitulorum* transmission.

Table (2): Distribution of ***T. canis*** larvae and their reflection on Op. D value in sera of experimentall infected mice.

Time of slaughtering post-infection	Mean number of larvae detected in the examined organs of infected animal							positive cases/each study site	
	liver	lung	spleen	heart	kidney	brain	muscle	infected animal	negative control
4 das pi.	260.0	60.0	5.33	3.5	22.5	0.0	0.0	--	
10 days pi.	156.0	110.0	8.33	6.5	18.6	8.5	26.0	0.652	0.280
20 days pi.	90.0	70.0	0.0	4.0	11.5	8.3	52.0	0.792	
30 days pi.	42.0	23.0	0.0	0.0	5.0	12.0	180.2	1.020	0.269
60 days pi.	18.0	12.0	0.0	0.0	0.0	12.2	270.0	0.758	

Table (3): Distribution of ***T. vitulorum*** larvae and their reflection on Op. D value in sera of experimentall infected mice.

Time of slaughtering post-infection	Mean number of larvae detected in the examined organs of infected animal							positive cases/each study site	
	liver	lung	spleen	heart	kidney	brain	muscle	infected animal	negative control
4 das pi.	230.0	45.0	5.0	0.0	30.0	0.0	0.0		
10 days pi.	150.0	92.0	6.0	4.0	27.0	6.0	31.0	0.690	0.210
20 days pi.	110.0	46.0	5.0	2.0	23.0	13.0	72.0	0.833	
30 days pi.	30.0	15.0	0.0	0.0	0.0	12.0	163.0	1.017	0.192
60 days pi.	20.0	10.0	0.0	0.0	0.0	18.0	212.0	0.626	0.230

Table (4): Distribution of *T. vvitulorum* larvae and their reflection on Op. D value in sera of experimentall infected rats.

Time of slaughtering post-infection	Mean number of larvae detected in the examined organs of infected animal							positive cases/each study site	
	liver	lung	spleen	heart	kidney	brain	muscle	infected animal	negative control
5 das pi.	300	452.0	4.5	0.0	13.3	6.0	0.0	---	
10 days pi.	256	365.0	4.3	12.3	11.0	6.0	0.0	0.353	0.262
15 days pi.	180.7	272.3	3.0	8.0	8.3	6.0	22.0	0.453	
30 days pi.	110.3	198.0	0.0	5.0	7.0	4.0	82.5	0.690	0.240
45 days pi.	110.0	176.3	0.0	0.0	4.0	5.5	183.0	0.744	
60 days pi.	82.0	96.0	0.0	0.0	2.0	5.5	233.0	0.600	0.255

p.i = post infection

Table (5): Comparison between the viability of *T. vitulorum* larvae obtained from mice and rats in inducing experimental infection in rabbits.

Groups of infeted rabbits	Source of larae used to complete their development experimentally in rabbit	Mean ELISA OP.D value of *T. vitulorum* antibodies in rabbit serum at 35 days post infection		Remarks
		Infected rabbit	- Ve control	
Group I Rabbit infected by tissue contain	10 days old migrating *T. vitulorum* ***larvae in Mice*** tissue	0.646	0.221	Successful infection
10 days old *T. vitulorum* larvae	10 days old migrating *T. vitulorum* larvae in **Rat** tisue	0.648	0.242	Succesful infection
Group II Rabbit infected by tissue contain	10 days old migrating *T. vitulorum* larvae in **Mice** tisue	0.755	0.239	Successful infection
30 days old *T. vitulorum* larvae	10 days old migrating *T. vitulorum* larvae in **Rat** tisue	0.57	0.301	Un-infective larvae

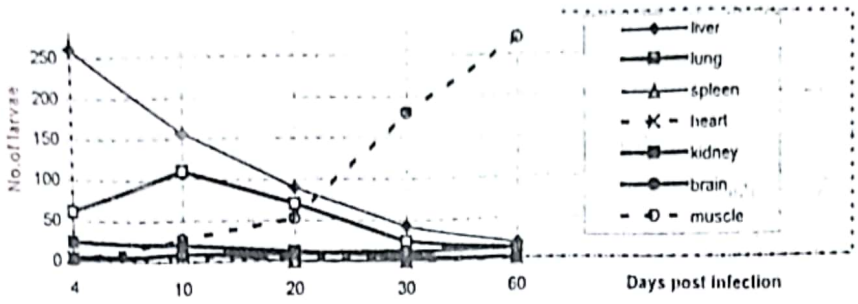

Figure (1): distribution of *T. canis* larvae in different organs of experimentally infected mice (4-60 days post infection)

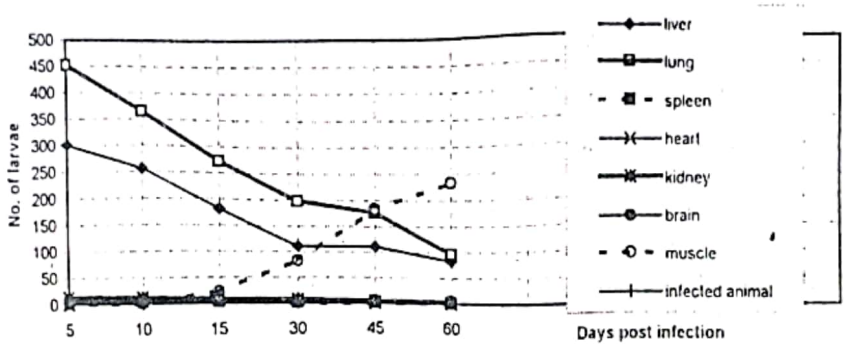

Figure (2): distribution of *T. vitulorum* larvae in different organs of experimentally infected rats (5-60 days post infection)

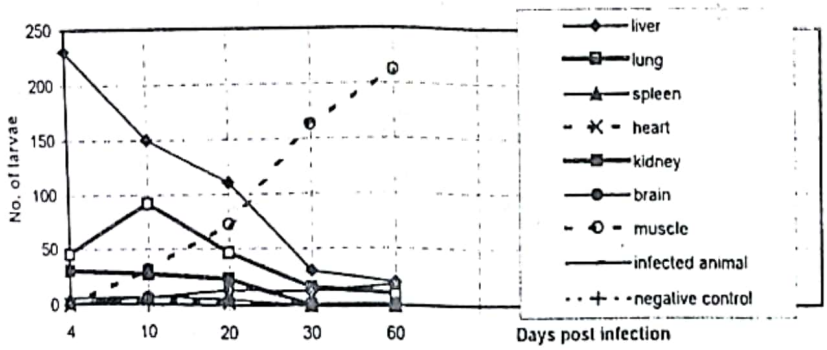

Figure (3): distribution of *T. vitulorum* larvae in different organs of experimentally infected mice (4-60 days post infection)

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