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A STUDY ON TOXOCARA SPECIES AND ITS PUBLIC HEALTH IMPORTANCE

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SUMMARY

Toxocariasis is a common zoonotic parasitic disease, causing many problems in human beings especially children. Studying the prevalence of *Toxocara* among dogs and cats in Cairo and Giza revealed infection rates of 14.6% and 5.2% in dogs and cats by faecal examination respectively. Adult worm of *Toxocara* were present in 18.75% and 7.4% of examined intestinal samples of dogs and cats respectively. Soil samples were collected from different public parks proved to be the major source of infection, where *Toxocara* eggs were detected in 13.8% of examined samples. Five available chemical disinfectants varying in composition were tested for their lethal effect against embryonated and non embryonated Toxocara eggs. The results indicated that 5% ammonia solution has a strong ovicidal activity against *Toxocara* eggs, followed by phenolic acid, lime, formalin and betadine solutions. The significance of *Toxocara* infection for human health was discussed.

INTRODUCTION

Toxocariasis is a highly prevalent zoonotic parasitic disease worldwide that affects dogs and cats. The responsible agent: *Toxocara* species. *Toxocara canis* is the major species which in the course of its life cycle infects dogs and *T.cati* infects cats and other mammals including man (Lewis and Maizels, 1993). In Egypt, *T.canis* has been identified from stray dogs by Selim (1967), and *T.cati* was recovered from stray cats by Khalil, et al. (1976). The transmission of infection from the dogs to man seemed to be from contamination of soil around household by *Toxocara* infected dogs. The high resistance of the eggs to

physical and chemical factors is very important in the contamination of the soil as a major source of human infection (Acha and Szyfres, 1980). Eggs of the parasites can readily be recovered from soil samples from private yards, public places, with higher concentrations found near places where dogs aggregate (Jacobs, et al., 1977). Visceral and ocular larval migrans are two distinct clinical syndromes of Toxocariasis in man. The typical patient is the child between the ages of 2-7 years, (Glickman and Schantz, 1981). Using serological techniques, Khalil, (1964) reported that *T.canis* was responsible for causing blindness in 37% of patients examined. Recent diagnostic techniques revealed a possible role of *T.canis* infection in chronic urticaria patients (Ismail and Khalafallah, 2005). Treatment of Toxocariasis is unsatisfactory due to re-infection which makes assessment of response to therapy difficult, so the control of soil contamination is required. Therefore, our present work aimed to study the incidence of *Toxocara* infection in dogs and cats, to indicate the degree of contamination of soil with eggs of the parasites and detection the effect of different disinfectants and detergents on the viability of embryonated and non embryonated eggs.

MATERIALS AND METHODS

1-Faecal examination:-

Faecal samples were collected from 185 dogs and 134 cats, household animals, dogs and cats kept in special care; collected samples were processed using a standard centrifugation floatation method as described by Dada (1979), and then examined microscopically for *Toxocara* eggs.

II-Intestinal examination:-

Small intestines were collected from condemned 64 dogs and 54 cats and examined for *Toxocara* worms; the procured worms were counted and preserved.

III- Soil sampling:-

Soil samples were collected from different localities such as public places, schools playgrounds, and from places where dogs and cats aggregate. A total of 65 soil samples were randomly selected and examined. From each soil sample, 50g were taken and rinsed with water, then sieved through wide mesh sieve and allowed to sediment. The supernatant fluid was discarded and the sediment was suspended in floatation fluid for recovery of *Toxocara* eggs (Gordon and Whitlock, 1939).

IV- Embryonation of the eggs in different soil cultures: -

Three types of soils were prepared, sand, sawdust, and clay soil, from each type of soil; two parts were prepared, dry and moist ones.

About 100g of each soil were heat sterilizd to destroy any contaminating ova before artificial contamination. The viability tests were done under standardized conditions, each soil sample was experimentally contaminated with about 500 unembryonated eggs obtained from uteri of female *T. canis* worms, and kept in clean cups. All samples were monthly examined for a period of 6 months, after that examination is carried out and the obtained eggs were counted.

V- Disinfectant experiment:-

A number of current disinfectants and chemicals, in the markets were tested for its ovicidal activities against *T. canis* eggs.

1- Lime:-

Fresh calcium hydroxide suspended in water.

2- Ammonium solution:-

Prepared by diluting 33% NH_4OH in water.

3- Formalin solution:-

The product of ADWIC laboratory chemicals. (40% formaldehyde gas in water).

4- Betadine:-

Contains 10% W/V Povidone-Iodine U.S.P (Mundidone).

5- Phenolic acid:-

The last two compounds are manufactured by the Nile Co. for Pharmaceuticals and Chemical industries, Cairo, A.R.E.

Each disinfectant was diluted in water to give a final concentration of 1, 3, and 5% solutions.

For studying the effect of disinfectants on viable unembryonated *Toxocara* eggs, the following steps are taken.

Eggs were taken from uteri of female *T.canis* worms, and counted by Mc Master method. About 50 eggs were suspended in 1ml. from each diluted disinfectant preparation, in a clean centrifuge tube. For each mixture of disinfectant and eggs, 3 different periods (4hrs, 16hrs, and 24hrs) were used for incubation at room temperature. Control tubes containing egg suspension only in saline, were subjected to the same steps. After each specific exposure time, eggs in each tube were immediately washed twice with water and centrifuged at 1000 r.p.m. for 3-5 minutes, after each wash, water was discarded. All tubes were placed in the incubator for two weeks at 26°C for embryonation of eggs, after that microscopical examination was done, and differential counting of eggs to obtain the average percentage of normal and abnormal ones.

Experimental animal:-

About 50 freshly embryonated eggs of *T.canis* were immersed in 1ml. of previously prepared 5% dilution of each disinfectant, and kept for 24 hours. After that, washed with water to eliminate

the disinfectant and resuspended in water. Suspensions of treated and non treated eggs (control) were given to mice per os; five mice were used for each treatment. All mice were sacrificed after 4 hours; Stomach and intestine of each animal were examined for recovery of *T.canis* larvae by using either Baermannisation of washed intestinal wall or submucosal scraping (Slotved et al., 1997).

RESULTS

Ova of *T.canis* were present in faecal samples of dogs showed embryonic development and appear spherical; having deeply pigmented center and rough pitted outer shell, 75x90µm. in diameter (Fig. 1). Whereas those of *T.cati* are smaller, only 65 to 75 µm. in diameter.

Faecal examination revealed *Toxocara* infection 14.6% of the dogs and 5.2% of the cats examined, table (1).

Adult worms of *T.canis* and *T.cati* are large round worms vary in length from 3-10cm. and usually coiled (Fig. 2). Intestinal examination revealed presence of *Toxocara* worms in 18.75% of the dogs and (7.4%) of the cats examined, table (2).

Soil examination revealed the presence of *Toxocara* eggs (Fig. 3) in 13.8% of total examined samples, table (3).

The effect of the soil type on the viability and development of Toxocara eggs was shown in table (4). Twenty per cent of incubated eggs were recovered from clay soil, where 13.4% and 10.6% of eggs were recovered from sawdust soil, and sand soil respectively under moist condition. On the other hand, 4% of incubated eggs were recovered from sawdust soil, where 2.4% and 0% of eggs were recovered from clay soil and sand soil respectively under dry condition.

Viable *Toxocara* eggs showed no morphological changes and appear sub globular, with thick finely pitted shells and measure about 90µ x 75µ with more than one cell embryo or complete developed larvae inside the eggs as shown in (Fig. 4,a-b). Abnormal eggs which affected by the disinfectants showed complete or incomplete destruction, wrinkling, thinning of the wall or shrinkag of protoplasmic mass and degeneration of egg (Fig. 4,c-d-e), or ruptured and release its conten (Fig. 5b).

The effect of different disinfectants on the viabil ty and development of unembryonated *Toxoca* eggs was shown in table (5). The counted nu bers of viable eggs are expressed as percentage total examined eggs. It was clear that ammo solution has a strong ovicidal effect on *Toxoc* eggs, only 11.68% and 20% of total eggs w developed on exposure to 5% solution for 16

4 hours respectively, while no eggs were observed after exposure for 24 hours.

On studying the ovicidal effect of different disinfectants in term of the viability and development of embryonated *Toxocara* eggs using mice inoculation, it was observed that no larvae were recovered from mice infected with *Toxocara* eggs after have been treated with 5% ammonia solution. Whereas 6, 8, 14, and 18 larvae (Fig. 6) were recovered from mice infected with *Toxocara* eggs treated with lime, phenolic acid, formalin, and betadine solution respectively, (Table 6).

Table (1): Prevalence of *Toxocara* species eggs through faecal examination.

Host	No. of samples examined	(+ve) samples	% of (+ve) samples
Dogs	185	27	14.6%
Cats	134	7	5.2 %
Total	319	34	10.66%

Table (2): Prevalence of *Toxocara* species through intestinal examination.

Host	No. of samples examined	(+ve) samples	% of (+ve) samples
Dogs	64	12	18.75%
Cats	54	4	7.4 %
Total	118	16	13.56%

Table (3): Prevalence of *Toxocara* species eggs through soil examination.

Number of samples examined	(+ve) sample	% of (+ve) samples
65	9	13.8%

Table (4): Effect of soil type on the viability and development of examined 500 *T.canis* eggs incubated for 6 months.

Type of soil / Type of eggs	Clay soil				Sawdust soil				Sand soil			
	Dry		Moist		Dry		Moist		Dry		Moist	
	+ve	%	+ve	%	+ve	%	+ve	%	+ve	%	+ve	%
Non embryonated eggs	10	2	22	4.4	17	3.4	14	2.8	0	0	11	2.2
Embryonated eggs	2	0.4	78	15.6	3	0.6	53	10.6	0	0	42	8.4
Total	12	2.4	100	20	20	4	67	13.4	0	0	53	10.6

Table (5): Effect of different disinfectants on the viability of *T.canis* eggs.

Disinfectant	Time in hours	Concentration of disinfectants			
		Control (0%)	1%	3%	5%
		% of developed eggs			
Lime	4	85	78.75	51.25	41.25
	16	88	43.75	27.5	21.25
	24	87	35	20	10
Ammonia	4	84	41	33.3	20
	16	87	20	16.65	11.68
	24	85	16.76	8.33	0
Formalin	4	85	58.53	48.78	45.12
	16	87	54.87	42.68	35.36
	24	84	50	41.46	30.48
Betadine	4	88	73.17	69.51	60.97
	16	85	70	63.3	41.66
	24	87	63.3	53.3	33.3
Phenolic acid	4	84	58.33	45	25
	16	88	30	20	13.3
	24	87	25	16.65	3.35

Table (6): Effect of different disinfectants on the viability of 50 embryonated *T.canis* eggs.

Disinfectant	No.of infected mice	No.of (+ve) mice	% of (+ve) mice	Mean No.of detected larvae per mice.	% of lethal rate
Lime	5	1	20 %	6	88%
Ammonia	5	0	0 %	0	100%
Formalin	5	2	40 %	14	72%
Betadine	5	2	40 %	18	64%
Phenolic acid	5	1	20 %	8	84%
Control	5	5	100 %	35	0 %

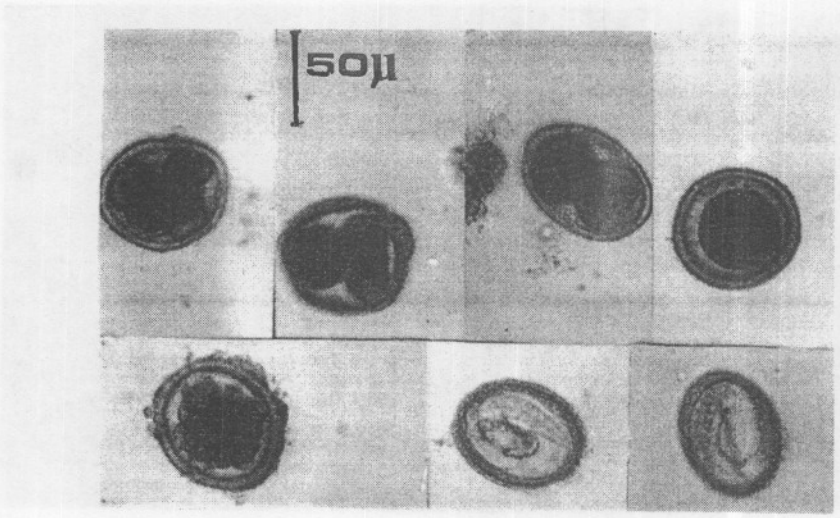

Fig. (1): *Toxocara* eggs recovered from faecal samples of dogs showing embryonic development.

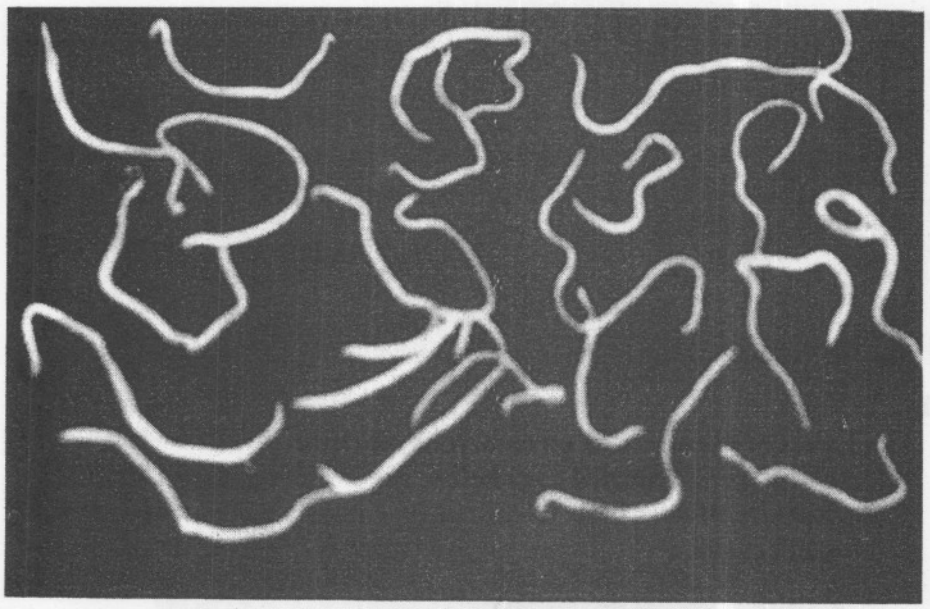

Fig.(2): Adult worms of *Toxocara canis* identified from small intestine of dogs.

Fig.(3): Embryonated eggs of *Toxocara* recovered from the soil.

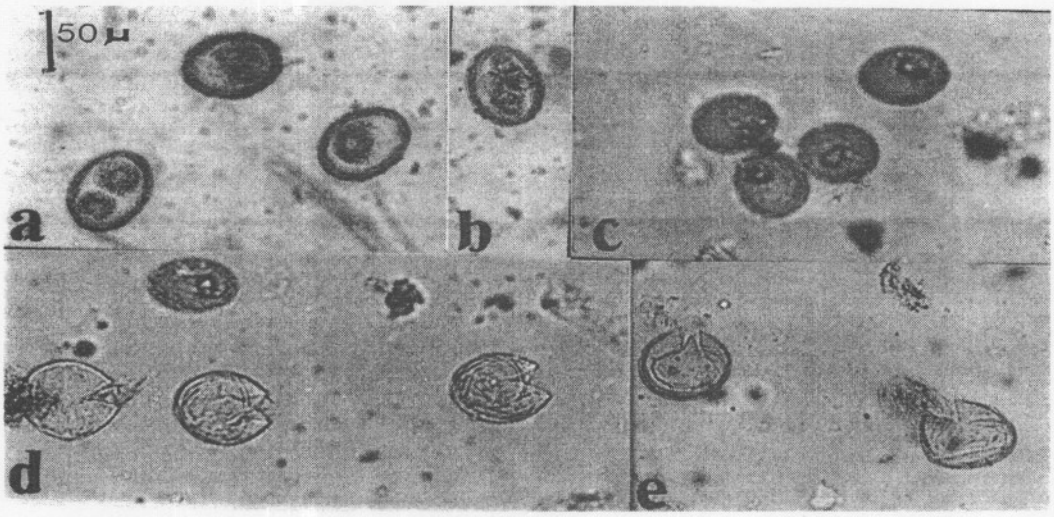

Fig.(4): Effect of disinfectants on *Toxocara* eggs,
(a) Normal unembryonated eggs.
(b) Normal embryonated egg. (c,d,e) Abnormal eggs showing destruction, shrinkage, and degeneration of egg shells.

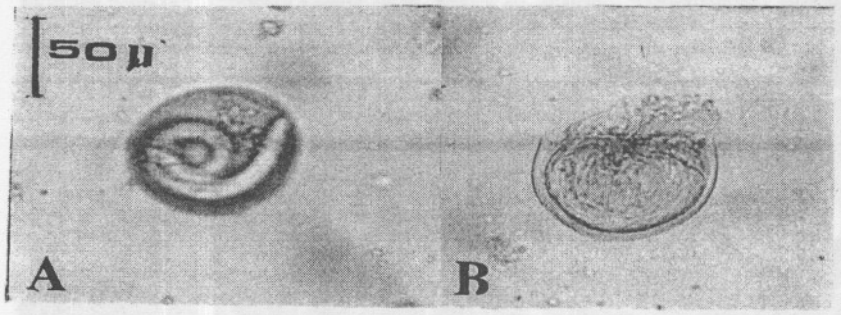

Fig.(5): Effect of disinfectants on embryonated *Toxocara* eggs,
(A) Normal embryonated eggs before exposure to disinfectants.
(B) Rupture of eggs and release of its contents after exposure to disinfectants.

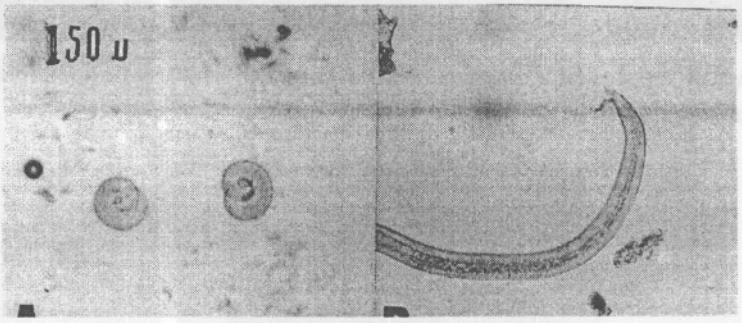

Fig. (6): *Toxocara* larvae after hatching in the intestinal tract of mice.
A) Live coiled larva. **B)** Relaxed, extended larva.

DISCUSSION

Toxocariasis have been described by a number of authors, Glickman and Scantz, (1981), and Omar and Lewis (1993).

Human Toxocariasis caused by *T. canis*, is common in developing and developed countries and lead to visceral and ocular larval migrans (Ahmed et al, 2002). The major hosts of this zoonotic parasite are dogs and cats. Numerous surveys have investigated the prevalence of *Toxocara* infection in these animals, Malloy and Embil, (1978) in Nova Scotia, Canada, Dunsmore et al, (1984) in Australia, Giacometti et al, (2000) in Italy, and Ramirez-Barrios et al, (2004) in Venezuela. These studies revealed heterogeneous results ranged from 5% to 26% among regions for relative prevalence's, and factors involved in parasite transmission.

In the present work, faecal examination revealed the presence of 14.6% *T. canis* and 5.2% *T.cati* infection in dogs and cats respectively in Cairo and Giza. Higher prevalence of *T. canis* 50% identified previously from stray dogs in Cairo and Giza has been reported in Egypt by Khalil et al, (1976a), and although Selim (1967), recorded a lower prevalence of only 8%, all of these infections were in pups. Malloy and Embil (1978) found that stray dogs were infected with *T.canis* at prevalence of 26.6%, and cats infected with T.cati at rate of 25.1% in Nova Scotia.

Adult worms of *T. cati* were recovered from 17.2% of stray cats in Cairo by Khalil et al, (1976b), and from 11.1% of cats in Behera Governorate by Hasslinger et al, (1988). In our survey, intestinal examination revealed the presence of 18.7% *T. canis* and 7.4% *T. cati* in dogs and cats respectively. However, lower prevalence of only 2.4% *T.canis* was recorded in Cairo by Hasslinger et al, (1993) but they found higher prevalence 27.9% in Beni-Suif. The prevalence varies considerably from one region to another as a function of climate, cultural habits, development of parasiticides and diagnostic procedures (Robertson, et al, 2000).

Since eggs of *T.canis* are not embryonated when passed in faeces of dogs, they are not directly infective for human. Therefore, the presence of numerous burdens of eggs in the soil and become infective under appreciate environmental condition; numerous surveys have proved this point. The contamination of soil and water with eggs of Toxocara from faeces of dogs and cats represent the main source of infection to human. Khalil et al, (1976a), were the first to examine soil samples for Toxocara eggs in Egypt, and recorded a 10% contamination rate in Dakhalia Governorate. Subsequently, Madwar et al, (1986) identified Toxocara eggs in 8.9% of soil samples from Qualubyia with 6.8% in urban and 13% in rural sites being contaminated. The present work recorded a 13.8% contamination rate in soil from Cairo and Giza Governorate. In Britain, Toxoc

ra eggs were found in 24.5% of 800 soil samples from six cities (Borg and Woodruff, 1973). Alcantara et al, (1989), detected the contamination of soil with *Toxocara* eggs in parks and public gardens in Salvador. Guimaraes et al. (2005) found that public parks are settings of more potential risk of *Toxocara* eggs in Brazil; they indicated 17.4% contamination rate.

Experimental contamination of the soil revealed maximum numbers of *Toxocara* eggs were recovered from clay soil 20% , followed by sawdust 13.4% , and sand soil 10.6% after 6 months incubation under moist condition. However, dry sawdust soil showed higher number of eggs 4% , more than clay soil and sand soil 2.4% , and 0% respectively. Sawdust absorb water and keep it for long time more than other types of soils, and this explain the higher numbers of eggs recovered from it. Overgaauw and Boersema (1998), showed that embryonated eggs of *T.canis* were recovered from (20%) of house and kennel dust samples, and from 50% of clay samples, where as Nunes et al (1994), found the highest recovery percentage were observed in soils rich in sand. Eggs of *T.canis* are long-lived, and its resistance to the environmental changes is conferred by the thick shells, and survived composting for at least a year (Pegg and Donald, 1978). However, Dunsmore et al (1984), described rapid disintegration and disappearance of eggs from sand soil over about 6 months during a period of favorable condition for egg survival.

The importance of eradication of *T.canis* eggs in the soil stimulated the investigators to study the ovicidal effect of some chemical preparations which are widely used as disinfectants. The viability of non embryonated *T.canis* eggs was tested by incubation for two week to detect its development and embryonation. The results proved that ammonia has a strong ovicidal activity against *T. canis* eggs, followed by phenolic acid, lime, formalin and betadine, where 0%, 3.35%, 10%, 30.48%, and 33.3% of the total incubated eggs were embryonated after 24hours exposures to 5% solutions of tested disinfectants respectively. The same results were obtained against embryonated *T.canis* eggs using mice inoculation.

However, there is no previous available data concerning the control of *T.canis* eggs in the soil using disinfectants. Some surveys showed the effect of disinfectants on other nematode eggs. Mendez (2004), found that 20% ammonia solution reduced 83% of viable helminth eggs in 2hours contact time. The authors decided that ammonia as a disinfectant which can diffuse through the membrane of highly resistant structures like helminth ova. Capizzi et al (2004), indicated that lime affects ascarid eggs in two ways, pH increase and temperature rise. In Egypt, Rashed et al (2000), obtained similar results using solutions of phenol and formal against eggs and larvae of Haemonchus contortus.

The control of *Toxocara* infection must focus on preventing the contamination by either limiting access to public places by dogs or by stricter enforcement of laws requiring owners to clean up after their dogs. So only improved hygiene and better parental supervision of children playing with puppies at home and private yards can reduce the chance of infection.

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