Al-Qadisyah Journal for pure Science. Vol.13 No.4 year 2008

The first scientific congress for science col. Which's hold in 26-27 March Year 2008

DETECTION OF *T.canis* ANTIBODY TITER IN HUMAN AT BASRAH CITY

Suzan, A. A. A. Al-Azizz

Department of Microbiology

College of Veterinary Medicine

University of Basrah

A. H. H. Awad

Department of Biology

College of Education

University of Basrah

ABSTRACT

One hundred stool and blood samples were collected from two regions in Basrah city (Garmat Ali and Al-Saraji district) to investigate antibody titers against *T. canis* infection. Stool samples showed that 19 males and 17 females were infected with intestinal parasites. Twenty- eight male and thirty-six females were examined by IHAT with an age ranged from 1-60 years old, which was also divided into two groups: dogs' owners (25) and dogs'non-owners (39). The IHAT test showed that a high positive titer was found in dogs' owners group (88, 40%) with E/S and TES antigens respectively. Age group 1-20 years old showed a high positive value in males (10) as compared with other age groups. High percentage of positive value (61.52%) was found in E/S antigen in group 1-20 years' old males and the lowest (20%) at 21-40 years old females.

TES antigen showed a high percentage of positive value at 1-20 years old females (35.29%) and the lowest at 21-40 years old females (10%).It was concluded that people having contact with dogs had a high antibody titer against *T. canis* larvae.

Part of Ph.D thesis

Al-Qadisyah Journal for pure Science. Vol.13 No.4 year 2008

The first scientific congress for science col. Which's hold in 26-27 March Year 2008

INTRODUCTION

A number of zoonotic diseases are transmitted between domestic pets and human such as *Toxoplasma gondii*, *Echinococcus granulosus*, visceral larva migrans caused by *T. canis* and *T. cati*, and cutaneous larva migrans caused by *Ancylostoma caninum* and *Ancylostoma braziliense* (Schenone, 1987). Human toxocariasis was commonly seen in places where *T. canis* infected stray dogs were high in number (Kaplan *et al.*, 2004). There was a strong correlation between *Toxocara* seroprevalence, life style and infection risk (Difiore *et al.*, 1989). Holland *et al.* (1991) reported that the seroprevalnece was high in developed countries especially in rural areas among children who owner dogs.

Human infection with *T. canis* and *T. cati* was incriminated most frequently in temperate climates. This might be due to many factors including the high frequency of pet ownership, high prevalence of *Toxocara* species in dogs and cats, and the long persistence of *Toxocara* eggs in the environment (Barriga, 1988). Human infection with *T. canis* starts when eggs are ingested by human (accidentally) and hatched in their intestines. The larvae migrated through the blood vessels then reached liver, lungs, brain, spleen, heart, kidney and muscels, then finally reached to the eyes (Garcia, 2001). Soil contamination with embryonated eggs of *T. canis* is the main source of human infection, but in some cases eating unwashed vegetables or uncooked poultry or ingestion tissues of other paratenic hosts like rabbits, pigs and cattle infected with *T. canis* larvae also make a source of human infection (Agudelo *et al.*, 1990; Kazacos and Boyce 1990; Radman *et al.*, 2000). Soil contamination levels were viarible in different regions of the world depending on the number of stray dogs, socioeconomic level and number of unwanted dogs (Papini and Casarosa, 1994). The tendency of some children to eat dirt is a major risk factor for infection (Overgaauw, 1997). The compulsion to eat dirt as a behavior disorder may affect 1-2% of children between the ages 1-6 years old (Schantz, 1989). It was suggested by Wolfe and Wright (2003) that dogs infected with *T. canis* might infect people by direct contact. *T. canis* cause at least three syndromes in human: Toxocaral visceral larva migrans (VLM), ocular larvae migrans (OLM) and covert toxocariasis (CT) (Garcia, 2001). VLM has been reported in human less than 6 years old (Morris and Katerndahl,

1987) and individuals following the ingestion of larvae in paratenic hosts (Inoue, 1987). Because *Toxocara* larvae are unable to develop to adult hosts other than dog, a microscopic examination of the stool is useless for the diagnosis of VLM in human (Inoue and Tsuji, 1989). The exact diagnosis of toxocariasis is based upon the identification of larvae in the host tissues. The alternative method is the immunological examinations since it is extremely difficult to detect the infective *Toxocara* larvae in biopsy samples (Yamasaki *et al*., 2000). Children with the clinical syndrome of viseral larva migrans as a result of *Toxocara* species have typical lesions in the liver and other viscera, with granulomatas that contain numerous eosinophils (Kaplan *et al*., 2001)

In most immunological tests, execretory-secretory antigens from 2nd stage larvae of *T. canis* have been used conventionally (De Savigny *et al*., 1979; Jacquier *et al*., 1991). Western blotting (Magnaval *et al*., 1997) and more recently, *Toxocara* check (Akao *et al*., 1997) also have been used, both of which detect immunoglobulin G against TES. Holland *et al*. (1995) reported that boys showed a significant higher seroprevalence than girls, and children in low socioeconomic level had higher seroprevalence than high socioeconomic levels infected with *T. canis* larvae.

Inoue (1987) developed an ELISA test for the detection of antibody against *T. canis* in rats using adult, larval and embryonated eggs extracts as antigens. While, Radman, *et al*. (2000) are pointed out that when experimental animals and human infected with the larvae of *T. canis*, the larvae widely wander in their tissues and finally reach to many organs including brain causing haemorrahage necrosis and granulomatous foci. Bowman *et al*. (1987) showed that ELISA is a good diagnostic test to detect circulating larval antigen, moreover, indirect ELISA was developed for the detection of IgM and IgG antibodies with specific reactivity to the *in vitro* derived antigens of larval toxocariasis. Sensitive immunodiagnostic tests are especially critical for parasitic infections characterised by a low infectious dose, lack of multiplication or eggs production in the hosts, and low concentrations of circulating parasite specific antibody. Specificity is important when other parasites or pathological processes are capable of

producing similar clinical manifestations, such as the case with ocular toxocariasis. Children with visceral toxocariasis typically have pronounced eosinophilia and anti-*T. canis* specific antibody titers in there serum. Patients with ocular toxocariasis are usually a symptomatic and often have lower

concentrations of eosinophils and circulating anti-*T. canis* specific antibody. These differences have been attributed to the level of infection. However, children with visceral toxocariasis are often geophagic or coprophagic and consume many eggs, while children with ocular toxocariasis lack this habit and infected with few eggs (Glickman and Schantz, 1981).

This study was conducted to determine the seroprevalence of *T. canis* infection in the human population suspected (owner, not owner of dogs) in their houses in two different regions at Basrah city.

MATERIALS AND METHODS

Human Samples

One hundred blood and stool samples were collected from people at Garmat Ali district (north station) and Al-Saraji district (south station) at different age groups and from both sexes. Special emphasis has been made on the individuals who are in close association with dogs.

Blood Samples

Three ml of blood was taken from each individual and left to clot at room temperature. The sera were separated and collected in clean vials, then kept at -20 C^0 until used in IHAT.

Stool Samples

Stool samples were collected with clean cups and examined on the same day of collection by direct method and sedimentation method (Hillyer and Apt, 1997).

Indirect Haemagglutination Test (IHAT)

The procedure of Herbert (1967) was used.

RESULTS

Parasitological and IHAT examinations of 100 human faecal samples and corresponding sera samples collected from Garmat Ali and Al-Saraji regions are shown in table (1) and (2). A total of 19 male and 17 female infected with different intestinal parasites were not included in immunodignosis test (Table 1). A total of 28 males and 36 females free of

intestinal parasites in their faeces were tested for *T. canis* antibody titers (Table 2). While , table (3) shows the positive (+ve) (titer ≥40, ≥ 160) and negative (-ve) (titer ≤40, ≤160) of E/S and TES antigens, respectively in peoples having dogs or not in their houses, with a total of positive value (30 and 14) in E/S and TES antigens and 34 and 50 as negative in E/S and TES antigens respectively. A high percentage of positive value was found in people owners of dogs (88%) as compared to not owners of dogs (20.5 in E/S antigen), while in TES antigen the percentage of positive value was 40% and 10.25% in people having or not having dogs respectively (Table 3). A statistical analysis using Chi-Sq test shows a signifecant differences in the percentage of positive results with owners and not owners of dogs with E/S and TES antigens ($P > 0.05$, $X^2 = 0.01$)

A high number of positive titer (14) was found at age group 1-20 years old females, while a low number (1) was found at age group 41-60 years old males. No positive value was found at age group 21-40 years old females (Table 4). Generally, a high number of positive value was found in people owners of dogs by using E/S as compared to TES antigen (22 and 10) respectively, compared to a value in people having no dogs (8 and 4) in E/S and TES antigens respectively (plate 1, 2). Chi-Sq test showed a significant difference between age groups and antigens in people owner and not owners of dogs ($P > 0.05$, $X^2 = 0.015$). Male from all age groups showed a high percentage of positive value compared to females (33.64% and 27.97% respectively). Age group (1-20) years old males showed a highest (61.52%) positive value (Table 5). Chi-Sq test shows a significant difference between males and females and percentage of positive value ($P > 0.05$, $X^2 = 0.001$).

Al-Qadisyah Journal for pure Science. Vol.13 No.4 year 2008

The first scientific congress for science col. Which's hold in 26-27 March Year 2008

Table (1): Human stool samples examined for infectivity with different intestinal parasites.

Area	Age group (year)	Faecal exam.					
		Male		Female		Total	
		No. exam.	No. inf.	No. exam.	No. inf.	exam.	inf.
Garmat Ali	1-20	10	6	7	2	17	8
	21-40	15	6	7	1	22	7
	41-60	3	0	8	2	11	2
Al-Saraji	1-20	11	2	17	5	28	7
	21-40	8	5	11	7	19	12
	41-60	0	0	3	0	3	0
Total		47	19	53	17	100	36

Table (2): The number of males and females suspected with *T. canis* infection (negative of faecal examination) from Garmat Ali and Al-Saraji regions.

Area	Age group (year)	IHAT					
		Male		Female		T otal	
		No. exam.	No. susp.	No. exam.	No. susp.	exam.	susp.
Garmat Ali	1-20	10	4	7	5	17	9
	21-40	15	9	7	6	22	15
	41-60	3	3	8	6	11	9
Al-Saraji	1-20	11	9	17	12	28	21
	21-40	8	3	11	4	19	7
	41-60	0	0	3	3	3	3
Total		47	28	53	36	100	64

Table (3): Human sera antibody titer tested with E/S and TES of *T. canis* larvae antigens.

Antigen	No. sera exam.	Antibody titer	+ve	-ve	% of positive
E / S	25 (dog owner)	Titer ≥ 40	22	3	88
	39 (not)		8	31	20.5
Total	64		30	34	46.87
TES	25 (dog owner)	Titer ≥ 160	10	15	40
	39 (not)		4	35	10.25
Total	64		14	50	21.87

$P > 0.05$, $X^2 = 0.01$*

Table (5): The percentage of positive titer of human sera sample with E/S and TES larval antigens.

Antigens %	1-20		21-40		41-60		Total %	
	♂	♀	♂	♀	♂	♀	♂	♀
E/S	61.53	47.05	58.33	20	0.0	55.55	53.57	41.66
TES	15.38	35.29	33.33	10	33.33	0.0	25.0	19.44
Total	38.46	41.17	45.83	16	16.66	27.77	39.28	31.88

$P > 0.05$, $X^2 = 0.001$

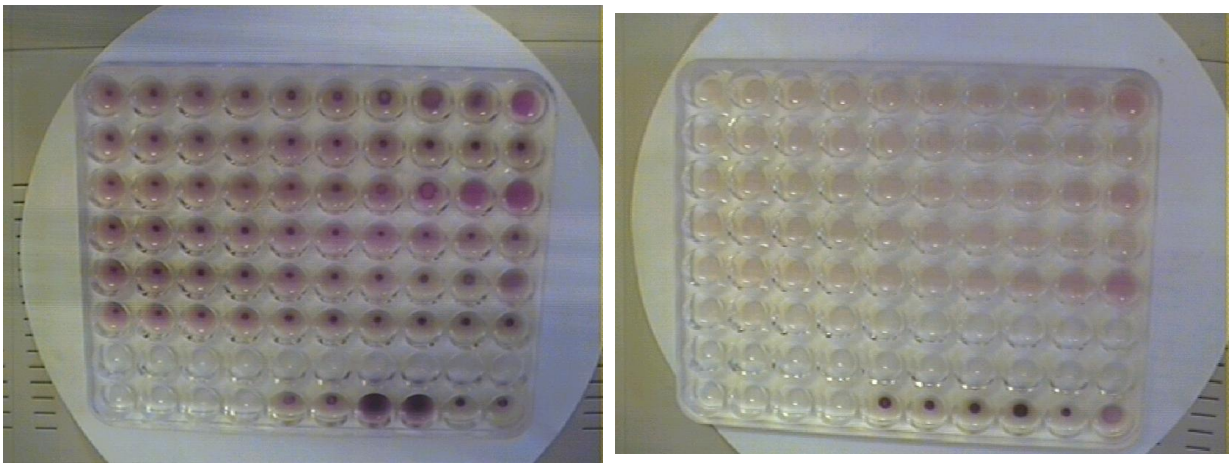
Table (4): IHAT of (64) human sera samples with or without dogs in their houses.

Antigen	Sample	1-20		21-40		41-60		IHAT +ve 1-20		IHAT +ve 21-40		IHAT +ve 41-60		IHAT -ve 1-20		IHAT -ve 21-40		IHAT -ve 41-60	
		♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
E/S	Dog owner	8	7	4	2	1	3	7	6	4	2	0	3	1	1	0	0	1	0
	not	5	10	8	8	2	6	1	2	3	0	0	2	4	8	5	8	2	4
Total		13	17	12	10	3	9	8	8	7	2	0	5	5	9	5	8	3	4
TES	Dog owner	8	7	4	2	1	3	2	3	4	1	0	0	6	4	0	1	1	3
	not	5	10	8	8	2	6	0	3	0	0	1	0	5	7	8	8	1	6
Total		13	17	12	10	3	9	2	6	4	1	1	0	11	11	8	9	2	9

$P > 0.05$, $X^2 = 0.015$*

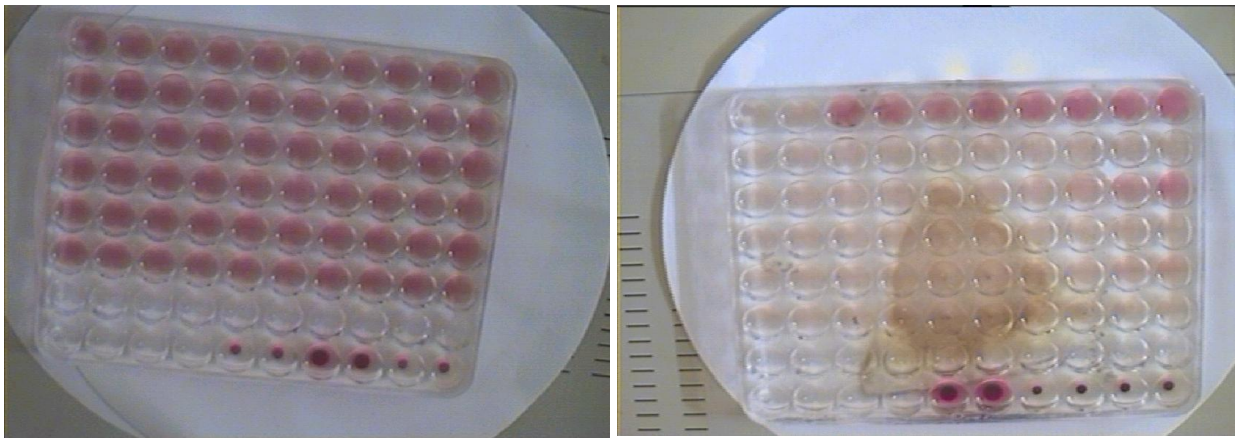
Al-Qadisyah Journal for pure Science. Vol.13 No.4 year 2008

The first scientific congress for science col. Which's hold in 26-27 March Year 2008



A **B**

Plate (1): IHAT of E/S larval *T. canis* antigen with sera from human having (A) and not having dogs (B). (2X).



A **B**

Plate (2): IHAT of TES larval *T. canis* antigen with sera from human having (A) and not having dogs (B). (2X).

DISCCUSION

Toxocariasis is a public health problem. Human acts as undefinitive hosts in which *Toxocara* larvae will not develop but migrate and survive for a long time.

The human antibody titer in two regions (north and south stations) using IHAT test revealed that the percentage of positive titer was high in people owning dogs with a total percentage of 88% compared to those who are not owners of dogs 40% of E/S antigen and 20.5% and 10.25% in TES antigen (owners and not owners of dogs, respectively).

These two regions are rural with people have low socioeconomic level and many of them have dogs in their houses which means a wide spread environmental contamination with *T. canis* eggs. Also, these communities are attributed to poor standered of hygiene and it is quite clear that people in these regions may expose to the infection and they are suspected to have *T. canis* larvae in their bodies. Furthermore, climatic conditions of Basrah are conductive to keeping *T. canis* eggs alive for a long time. Kincekova *et al.* (1999) pointed out that a high percentage of seropositive titer against *T. canis* infection was found in dog-keeping and puppy-breeding families and the possibility of infection with repeated doses of *T. canis* larvae in human stimulated rise in eosinophilia. Glickman *et al.* (1978) showed that the sensitivity and specificity of *T. canis* infection in human were 78.3% and 92.3%, respectively by using ELISA test. Alderete *et al.* (2003) considered ≥ 160 as a positive titer for human infection with *T. canis* by ELISA test and they showed that the seroprevalence was 38.8% among infected children with the mean age of 9.4 years.Woodruff *et al.* (1981) examined 219 healthy persons aged between 20-40 years old by toxocaral ELISA test and found that 7.3% have antibodies against *T. canis* in Mosul city. The high positive titer in age group 1-20 as compared to the other age groups are explained on the basis that this age group has a high contact with soil and some animals like puppies or kitten, furthermore, some children may eat soil (geophagia) or put their fingers in their mouths. Personal hygiene and behavioral factors like, nail-biting, sucking

fingers and infrequency of daily hand washes could lead to increase *Toxocara* seroprevalence. Moreover, children more than adults have such reasons as frequent contact with contaminated soil, poor hygiene and consuming contaminated food make them more frequently infected with toxocariasis. Rokni *et al.* (2000) reported that most patients with VLM infection had a previous history of contact with cats showed a high peripheral eosinophilia, leukocytosis and high GOT and GPT. A significant increase in seroprevalnce in dog-owner people (5.6%), owners of domestic cattle (9.4%) or cats-owners (10.9%) in contrast with people live in city and do not owne any animals (Kimmig *et al.*, 1991). Knapan *et al.* (1983) reported that children were more frequently infected than adults with *T. canis* larvae and more sever clinical symptoms were found in children of 1-3 years old and young children had closer contact with potentially contaminated soil. Melker *et al.* (1995) pointed out that anti-*Toxocara* titers were more frequently found in old people and they explained that due to *Toxocara* titers lasted for many years. Acero *et al.* (2001) showed that a high prevalence with *T. canis* infection was in children under 5 years old but with the mean titer increasing with age, reaching a strong correlation between positive antibody titers and children not washing hands before eating. A high percentage of seropositive titer 13.33%was found in age group 1-10 years old in patient with VLM infection attended to Nehru hospital in north India (Malla *et al.*, 2002). Kaplan *et al.* (2004) measured anti-*Toxocara* IgG and IgM antibodies by ELISA method in mentally retarded children and they reported that the frequency was 18.8% as compared to healthy children 7.1%.

The high positive value which was found in boys of the present study as compared with girls revealed that boys play all the time outside the house making the contact with contamination factors high. Ehrhard and Kernbaum, (1979) related the differences in seroprevalence between boys and girls with *T. canis* infection to the differences in social behavior. While, Buijis (1993)

Al-Qadisyah Journal for pure Science. Vol.13 No.4 year 2008

The first scientific congress for science col. Which's hold in 26-27 March Year 2008

showed that 7% of children and 20% of adults have a high antibodies titer against *T. canis* in Netherlands. Most positive titer cases were boys when examined (100) children aged 1-14 years old at a childrens hospital in Victoria/ Brazil, also, urban children had a high positive titer as compared with rurals (Moreira-Silva *et al.*, 1998). Herrmann *et al*. (1985) reported that serologic surveys on *T. canis* infection in humans of the United States indicated a strong association between *Toxocara* antibody titers and socioeconomic level such as educational attainment and income of the head of the house hold. Furthermore, poor hygiene and sanitation associated with poverty facilitated transmission of *Toxocara* infection.

التعرف على مستوى الأضداد المتكونة تجاه *Toxocara canis* للانسان في مدينة البصرة

أ.م.د.سوزان عبد الجبار عبد العزيز **أ.د.عبد الحسين حبش عواد**

جامعة البصرة كلية الطب البيطري **جامعة البصرة كلية التربية**

فرع الأحياء المجهرية **قسم علوم الحياة**

الخـــلاصـــة

تم جمع مائة عينة دم وبراز من منطقتين في مدينة البصرة (كرمة علي والسراجي) وذلك للبحث عن الأجسام الضد المتكونة ضد طفيلي الـ *Toxocara canis*. عينات البراز بينت أن 19 ذكر و 17 أنثى مصابين بطفيليات معوية. 28 ذكر و36 أنثى فحصت بواسطة فحص التلازن الدموي غير المباشر وبأعمار تتراوح مابين 60-1 سنة، كذلك قسمت هذه الفئات الى مجموعتين رئيسيتين هما: مجموعة تملك كلاباً في بيتها (25) ومجموعة لاتملك كلاباً في بيتها (39). فحص التلازن الدموي غير المباشر وضح ارتفاعاً موجباً في المجموعة التي تملك كلاب (40,88%) ضد المستضدات الإفرازية- الإخراجية E/S والمستضدات الجسمية TES. أظهرت الفئة العمرية 20-1 سنة أعلى قيمة موجبة في الذكور (10) مقارنةً بباقي المجاميع العمرية. أعلى نسبة موجبة كانت 61.52% في المستضدات E/S في المجموعة العمرية 20-1 سنة ذكور واقلها 20% في إناث الفئة العمرية 40-21 سنة. مستضدات TES أظهرت أعلى قيمة موجبة في إناث الفئة العمرية 20-1 سنة (35.29%) واقلها كانت في إناث الفئة العمرية 40-21 سنة (10%). أجمالا يمكن الاستنتاج أن الناس الذين يكونون على اتصال بالكلاب لديهم القدرة على تكوين أجسام ضد تجاه يرقات الـ *Toxocara canis*.

REFRENCES

Acero, M.; Mercedes-Munoz, M.; Florez, A. C. & Santiago – Nicholls, R. (2001). *Toxocara canis*: antibody seroprevalence and risk factors in children. Biomed., 21: 256-263. (English Summary).

Agudelo, C.; Villarreal, E.; Caceras, E.; Lopez, C.; Eljach, J.; Ramirez, N.; Hernandez, C. & Corredor, A. (1990). Human and dogs *Toxocara canis* infection in a poor neighborhood in Bogota. Mem. Inst. Oswaldo Cruz Rio de Janero, 85: 75-78.

Akao, N.; Chu, A. E.; Tsukidate, S. & Fujita, K. (1997). A rapid and sensitive screening kit for the detection of anti-*Toxocara* larval E/S antibodies. Parasitol. Int., 46: 189-195.

Alderete, J. M.; Jacob, C.; Pastorino, A. C.; Elefant, G. R.; Castor, A.; Fomin, A. & Chieffi, P. P. (2003). Prevalence of *Toxocara* infection in school children from the Butanta Region, Sao Paulo, Brazil. Mem. Inst. Oswaldo Cruz Rio de Janero, 98: 593-597.

Barriga, O. O. (1988). A critical look at the importance, prevalences and control of toxocariasis and the possibilities of immunological control. Vet. Parasitol., 29: 195-234.

Bowman, D. D.; Mika – Grieve, M. & Grieve, R. B. (1987). *Toxocara canis*: Monoclonal antibodies to larval excretory – secretory antigens that bind with genus and species specificity to the cuticular surface of infective larvae. Exp. Parasitol., 64: 458-465.

Buijis, J. (1993). *Toxocara* infection and airway function: An experimental and epidemiological study. Ph. D. thesis, Utrecht University. (Cited from Overgaauw, 1997a)

De Savigny, D. H.; Voller, A. & Woodruff, A. W. (1979). Toxocariasis: Serodiagnosis by enzyme immuno-assay. J. Clin. Pathol., 32: 284-288.

Difiore, M.; Virga, A.; Usticano, V.; Dirosa, S. & Rini, G. B. (1989). Antibodies against *Toxocara canis* in human serum from Western Sicily. Boll. Ist. Sieroter Milan., 68: 93-96.

Ehrhard, T. & Kernbaum, S. (1979). *Toxocara canis* et toxocarose humaine. Bulletin de L'Institut Pasteur., 77: 225-227. (English Summary)

Garcia, L. S. (2001). Diagnostic medical parasitology, 4 th ed., Am. Soc. Med. Press, Washinghton .

Glickman, L. T. & Schantz, P. M. (1981). Epidemiology and pathogenesis of zoonotic toxocaraisis. Epide. Rev., 3: 230-250.

Glickman, L. T.; Schantz, P. M.; Dombroske, R. & Cypess, R. (1978). Evaluation of serodiagnostic tests for visceral larva migrans. Am. J. Trop. Med. Hyg., 27: 492-498.

Herbert, W. G. (1967). Passive haemagglutination. In : Weir, D. (ed.). Handbook of expermintal immunology, Blackwell Sci. Publ. Oxford: 720-744.

Herrmann, N.; Glickman, L. T.; Schantz, P. M.; Weston, M. G. & Domanski, L. M. (1985). Seroprevalence of zoonotic toxocariasis in the United States 1971-1973. Am. J. Epidemiol., 122: 890-896.

Hillyer, G. V. & Apt, W. (1997). Food-borne trematode infections in the Americans. Parasitol. Today, 13: 87-88.

Holland, C.; O'Lorcain, P.; Taylor, M. R. & Kelly, A. (1995). Seroepidemiology of toxocariasis in school children. Parasitology, 110: 535-545.

Holland, C.; O'Connor, P.; Taylor, M. R.; Hughes, G.; Girdwood, R. W. & Smith, H. (1991). Families, parks, gardens and toxocariasis. Scand. J. Infec. Dis., 23: 225-231.

Inoue, H. (1987). Studies on visceral larva migrans: Infectivity of *Toxocara canis* larvae from paratenic host and antibody titers in rats. Hirosh. Med. J., 35: 1417-1429.

Inoue, H. & Tsuji, M. (1989). Studies on visceral larva migrans: Detection of anti - *Toxocara canis* IgG antibodies by ELISA in human and rat sera. Jpn. J. Parasitol., 38: 68-76.

Jacquier, P.; Gottstein, B.; Stingelin, Y. & Eckert, J. (1991). immunodiagnosis of toxocarosis in humans: Evaluation of a new enzyme-linked immunosorbent assay kit. J. Clin. Microbiol., 29: 1831-1835. (Medline)

Kaplan, K. J.; Goodman, Z. D. & Ishak, K. G. (2001). Eosinophilic granuloma of the liver: A characteristic lesion with relationship to visceral larva migrans. Am. J. Surg. Pathol., 25: 1316-1321.

Kaplan, M.; Kalkan, A.; Hosoglu, S.; Kuk, S.; Ozden, M.; Demirdag, K. & Ozdarendeil, A. (2004). The frequency of *Toxocara* infection in mental retarded children. Mem. Inst. Oswaldo. Cruz Rio de Janero., 99: 121-125.

Kazacos, K. R. & Boyce, W. M . (1990). Basylisascaris larva migrans. J. Am. Vet. Med. Assoc., 195: 894-903.

Kimmig, P.; Naser, K. & Frank, W. (1991). Seroepidemiological surveys of human toxocariasis. Zl. Hyg. Umwe., 19: 402-422. (English Summary).

Kincekova, J.; Reiterova, K. & Dubinsky, P. (1999). Larval toxocariasis and its clinical manifestation in childhood in the Slovak Republic. J. Helminthol., 73: 323-328.

Knapan, F. V.; Leusden, J. V. & Conijn-Van Spaendonk, M. A. (1983). Human toxocariasis, diagnosis and incidence in the Netherlands. Tijdschr. Diergenees. Skd., 108: 469-474.

Magnaval, J. F.; Glindo, V.; Glickman, L. T. & Clanet, M. (1997). Human *Toxocara* infection of the central nervous system and neurological disorders: A case control study. Parasitology, 115: 537-543.

Malla, N.; Aggarwal, A. K. & Mahajan, R. C. (2002). A serological study of human toxocariasis in North India. Nat. Med. J. Ind., 15: 145-147.

Melker, H. E.; Peet, T. E.; Berbers, G. A.; Akker, R.; Knapen, F. V.; Schellekens, J. F. & Coneyn – Van Spaendonck, M. A.(1995). Pilot-study for the PIENTER – project. Report N. 213675004. Nat. Ins. Publ. Heal. Env. Bilth. Netherlands, 37-38.

Moreira-Silva, S. F.; Leao, M. E.; Mendoca, H. F. & Pereira, F. E. (1998). Prevalence of anti-*Toxocara* antibodies in a random sample of in pateints at a children's hospital in Victoria, Espirito Santo, Brazil. Rev. Inst. Med. Trop. Sao Paulo, 40: 259-261.

Morris, P. D. & Katerndahl, D. A. (1987). Human toxocariasis. Review with report of a probable case. Postgrad. Med., 81: 263-267.

Overgaauw, P. A. M. (1997). Prevalence of intestinal nematodes of dogs and cats in the Netherlands. Vet. Quar., 19: 14-17.

Papini, R. & Casarosa, L. (1994). Observations on the infectivity of *Baylisascaris transfuga* eggs for mice. Vet. Parasitol., 51: 283-288.

Radman, N. E.; Archelli, S. M.; Fonrouge, R. D.; Guardis, M. V. & Linzitto, R. O. (2000). Human toxocariasis: Its seroprevalence in the city of La Plata. Mem. Inst. Oswaldo Cruz Reo de Janero, 95: 281-285.

Rokni, M.; Massoud, J. & Mowlavi, G. (2000). Report of 10 cases of visceral larva migrans in Iran. Iran J. Publ. Health., 29: 61-66. (English Summary).

Schantz, P. M. (1989). *Toxocara* larva migrans now. Am. J. Trop. Med. Hyg., 41: 21-34.

Schenone, H. (1987). Human parasitoses which may be caused by or transmitted by domestic pets in Chile. Bol. Chil. Parasitol., 42: 16-23.

Wolfe, A. & Wright, I. P. (2003). Human toxocariasis and direct contact with dogs . Vet. Rec., 152: 419-422.

Woodruff, A. W.; Watson, J.; Shikara, I.; Al-Azzi, N. S.; Hadithi, T. S.; Al-Adhamy, S. B. & Woodruff, P. W. (1981). *Toxocara* ova in soil in the Mosul district, Iraq and their relevance to public health measures in the Middle East. Am. Trop. Med. Parasitol., 75: 555-557.

Yamasaki, H.; Araki, K.; Chooi Lim, P. K.; Zasmy, N.; Mak, J. W.; Tai, R. & Aoki, T. (2000). Development of a highly specific recombinant *Toxocara canis* second stage larva excretory – secretory antigen for immunodiagnosis of human toxocariasis. J. Clin. Microbiol., 38: 1409-1413