

ERO-EPIDEMIOLOGICAL STUDIES ON TOXOPLASMOSIS AMONG LAUGHTERED ANIMALS AND PEOPLE AT RISK OF INFECTION

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Received : 17- 8- 1997

Accepted : 8- 9- 1997

SUMMARY

A total of 205 blood samples were collected from different farm animals shortly before slaughtering (cows 50, buffaloes 40, sheep 60, goats 30 and calves 25) from the Alexandria abattoirs. In the mean time, a total of 95 blood samples were collected from individuals with expected high exposure rate such as butchers (35) and workers (60) who slaughtered, eviscerated, dressed and skinned such animals. At the same time, a total of 108 blood samples were collected from individuals of normal exposure groups such as poultry breeders (20), laboratory workers (25), nurses (15), housewives (18) and students (30). All blood samples were examined serologically by using Dye test for detection of toxoplasma infection. A total of 35 faecal samples were obtained from stray domestic cats around the abattoirs to investigate the epidemiological role of the cats in the transmission of *Toxoplasma gondii*. From the obtained results it was revealed that seropositive cases in animals (cows, buffaloes, sheep, goats and calves) were 23.5% and in individuals of high risk group (butchers and workers) were 41.1% and other groups of normal

exposure (poultry breeders, laboratory workers, nurses, housewives and students) were 17.6%, and the mature infective *Toxoplasma gondii* oocysts were revealed from cat's faeces with an incidence of 20.0%

The zoonotic and public health importance of *Toxoplasma gondii* was discussed.

INTRODUCTION

Today, toxoplasmosis is considered to be one of the very common zoonotic diseases, that may be acquired congenitally or postnatally. It is caused by an obligatory intracellular protozoan named *Toxoplasma gondii* which infects both humans and animals, and has a world wide distribution. It exists in three basic forms: the trophozoite (proliferative form), the tissue cyst and the oocyst.

One of the most important problems all over the world especially in Egypt is that how the human-beings can get their requirement from animal protein. Much efforts have been achieved

in Egypt for solving this problem under a big programme called "Food security". The study of the zoonotic diseases indicates that food animals may be considered as one of the important sources of infection to man. Man may acquire infection in several ways either transplacentally (Wolf et al., 1941) or by ingestion of tissue cysts in inadequately cooked or raw meat of infected animals (Lord et al., 1975 and Masur et al., 1978) or ingestion of sporulated oocysts from cat faeces (Stagno et al., 1980).

The clinical symptoms in human-beings are usually in the form of abortion in pregnant women (Bentolial et al., 1968) or delivery of child with hydrocephalus, encephalitis, chorioretinitis and severe nervous manifestations (Desmont et al., 1965). In adults, acquired toxoplasmosis is in the form of lymphadenopathy and relapsing fever in the acute cases (Pestre Alexandre et al., 1968).

The domestic cats act as definitive host harbouring the sexual stage of the organisms shedding the oocysts in their faeces, while man, many mammals and birds act as intermediate host harbouring the other two stages of the organism (Hutchison et al., 1970 and 1971).

According to Beaver et al. (1984), toxoplasma cysts remain in a dormant state in the skeletal muscles of domestic animals and become infectious if ingested by man. Therefore, people handling raw meat have a higher risk of infection perhaps by the entry of the parasite through skin abrasions or by ingestion of food contaminated with cat faeces (Beverley et al., 1954).

This study was designed to find out the incidence of toxoplasma infection among slaughtered animals and some people at high risk of infection

MATERIALS AND METHODS

Collectin of samples:

1- Blood samples:

A total of 205 blood samples were collected (about 6-8 ml) from jugular vein of cows (50) buffaloes (40), sheep (60), goats (30) and calves (25) soon before slaughtering at Alexandria abattoirs (El-Max and Amrya slaughter houses). In the mean time, a total of 95 blood samples were collected from a high risk group individuals (about 6 ml of blood from radial vein) including randomly selected butchers (35) and workers (60) in the same abattoir who slaughtered, eviscerated, dressed and skinned the slaughtered animals. In the same time, a total of 108 blood samples were collected from some individuals of normal exposure group as a control group including poultry breeders (20), laboratory workers (25), nurses (15), housewives (18) and students (30), who were also selected randomly from Alexandria province. The blood samples used for serological investigation were collected without anticoagulant in clean and dry centrifuge tubes (15 ml capacity).

Blood samples were then labelled with the species, sex, age and the date of collection. The blood samples were left for about one hour for blood clotting. The clot was then separated with a fine loop and the tubes were centrifuged at 3000 r. p. m. for 5 minutes. The supernatant clear fluid

serum) was aspirated with a Pasteur pipette and put in a sterile screw-capped vial and stored at 30°C until used.

1.a. Determination of Toxoplasma antibodies (Dye test):

The technique used was a micro-modification of the original Sabin and Feldman test (Sabin-Feldman, 1948). The principle of this test is to detect antibodies in the serum, these antibodies in the presence of a complement like accessory factor will exert a cytotoxic effect on Toxoplasma parasites. This is demonstrated by adding a vital stain, the alkaline methylene blue where modified parasites remain unstained while normal ones become rounded and stained deeply. Sabin and Feldman dye test is proved to be a very sensitive test and also covers the acute as well as latent Toxoplasma infection.

2. Faecal samples from cats for detection of Oocysts:

A total of 35 faecal samples were obtained from stray domestic cats, randomly taken from different districts around the abattoirs. No preservative was added to the samples, and parasitological examination was carried out on the same day with the minimum of delay for detection of toxoplasma sporulated oocysts by Flotation Concentration Technique (Siam et al., 1978).

Each faecal sample was emulsified into a thick paste and suspended in about 10 volumes of sucrose solution (40% w/v) sucrose in water with 0.8% phenol solution. The suspension was then sieved and centrifuged at 3000 r.p.m. for 10 minutes, 0.5 ml of the top layer was aspirated with

a pipette and immediately mixed with 0.5 ml of aqueous sulfuric acid (H₂SO₄) and then aerated daily at room temperature for 7 days. Sporulated oocysts are then microscopically detected.

RESULTS AND DISCUSSION

The present study emphasized that toxoplasmosis is a true zoonotic disease occurring naturally in man, domestic, wild animals and birds..

The summarized results given in table (1) declared that the incidence of toxoplasmosis in the examined cows, buffaloes, sheep, goats and calves were 4.0, 27.5, 41.7, 33.3 and 0.0% respectively. The highest incidence percentage of seropositive against toxoplasma antibodies by using (Dye test) was observed in sheep as 25 out of 60 samples (41.7%) followed by goats 10 out of 30 (33.3%), buffaloes 11 of 40 (27.5%) and cows 2 out of 50 (4.0%), while all examined sera of calves were sero-negative against toxoplasma antibodies. These results are considered lower than those obtained by Fahmy et al. (1979), Blood and Henderson (1983) and Moussa (1986). These variations may be due to the species, feeding, housing conditions, behaviour, the prevailing climatic conditions and the nature of soil. Moreover, the relative low infection of cows might be attributed to different grazing habit rendering them less susceptible to infection.

The frequency distribution of the titer (Table 1) showed that the incidence of low titers (1/4) was 50.0%, 72.73%, 52.0% and 60.0%, while the mild titers (1/16) were 50.0%, 27.27%, 36.0% and

Table 1. Distribution of Toxoplasma antibody titers among seropositive slaughtered animals examined by Dye test (Sabin & Feldman test)

Animal species	examined animals	No. of cases		Toxoplasma antibody titers					
		No.	%	Low titers 1/4	Mild titers 1/16	Moderate titers 1/64	%		
Cows	50	2	4.0	1	50.0	1	50.0	0	0.0
Buffaloes	40	11	27.5	8	72.73	3	27.27	0	0.0
Sheep	60	25	41.7	13	52.0	9	36.0	3	12.0
Goats	30	10	33.3	6	60.0	3	30.0	1	10.0
Calves	25	0	0.0	0	0.0	0	0.0	0	0.0

Total	205	48	23.4	29	60.42	15	31.25	4	8.33

Table 2. Percentage distribution of Toxoplasma antibody titers among people at high risk group of infection in Alexandria Province.

Occupation	No. of examined	No. of +ve	%	32	64	128	256	512	1024	2048
Butchers	35	19	54.3	3	5	3	2	2	4	-
Workers	60	20	33.3	4	5	4	3	2	2	-
Total	95	39	41.1	7	10	7	5	4	6	-

A titer of = 1:32 was considered as diagnostic

Table 3. Percentage distribution of Toxoplasma antibody titers among naturally exposed human-beings in Alexandria Province.

Occupation	No. of examined	No. of +ve	%	Titer (Reciprocal)						
				32	64	128	256	512	1024	2048
Poultry breeders	20	5	20.0	2	1	1	1	1	-	-
Laboratory workers	25	4	16.0	1	2	-	-	1	-	-
Nurses	15	5	33.3	1	1	-	2	1	-	-
House wives	18	2	11.1	-	1	-	-	1	-	-
Students	30	3	10.0	1	1	-	-	1	-	-
Total	108	19	17.6	3	7	1	3	5	-	-

A titer of = 1:32 was considered as diagnostic

Table 4. Incidence of Toxoplasma sporulated oocysts among examined stray domestic cats.

Total No. of examined cats	+ve cases		-ve cases	
	No.	%	No.	%
35	7	20.0	28	80.0

10.0% among seropositive cases of cows, buffaloes, sheep and goats respectively. Moderate titers (1/64) were recorded with 12.0% and 10.0% among seropositive cases of sheep and goats respectively. Frequency distribution of titers showed that the sum of low and mild titers was greater than moderate titers (Table 1). These results were in agreement with those recorded by Elifaat et al. (1977).

In view of data illustrated in table (2) it can be noticed that the results of the present work indicated that people handling raw meat (at high risk of infection) such as butchers and workers (Table 2) had a higher risk of infection with toxoplasmosis (41.1%). This was in agreement with Reinman et al. (1975), whose results are higher than those obtained by Hanna et al. (1977); These variation may be due to locality, the prevailing climatic conditions, the technique used for detection of toxoplasma antibodies and the occupation of the examined human-beings.

The results achieved in the present investigation revealed that the incidence of toxoplasmosis in people of naturally exposed group was found to be 17.6%. The high prevalence of toxoplasma antibodies among nurses and laboratory workers may be due to their contact with some pathological materials e. g. sputum, cerebrospinal fluid (C. S. F.) and vaginal discharge that may contain viable organisms (W. H. O., 1980). The relatively high incidence of toxoplasma antibodies among poultry breeders was in consistent with McCulloch et al. (1963). The frequent presence of cats in poultry farms with possible seeding of the floor with toxoplasma oocysts may explain the

high frequency of toxoplasma antibodies among the employees of these farms (Table 3). Ruiz and Frenkel (1980) found that soil contaminated with cat faeces would contribute to the prevalence of toxoplasmosis.

The present investigation emphasized the role of the domestic cats in the transmission of *Toxoplasma gondii* both to our domestic animals and man in contact. Recovery of the mature sporulated infective oocysts from the faeces of domestic cats around the abattoirs indicated that this animal species is the definitive host. As shown from table (4), the incidence of *Toxoplasma* oocysts among cats was found to be 20.0% This finding was nearly similar to that reported from Czechoslovakia (17.0%) Rasin (1973) and in Costa Rica (23.2%) Ruize and Frenkel (1980). The variation in the incidence might be attributed to the mode of keeping cats. In the present study, the faecal samples were obtained from stray cats which are used to defecate in the open and always sprinkle some earth on their excrements, besides the exposure of their faeces to the environmental conditions that might adversely influence the survival of the oocysts.

From the afore-mentioned results and discussion, it can be concluded that toxoplasmosis in man is likely to be occupational as recommended by Blood and Henderson (1983). So, handling of infected meat in the slaughter-houses was considered as a means of infection to man. On the other hand, farm animals are also considered to be an important source of human infection. Moreover, serological surveys for toxoplasmosis

among farm domestic and wild animals are going on in all Egyptian provinces to locate the animal reservoirs of the disease and to give a clear picture of the epidemiology of toxoplasmosis in Egypt. Almost all countries of the world share in the problem of toxoplasmosis and its harmful effect on man.

For the prevention of toxoplasmosis in man, the following instructions and suggested recommended points must be taken into consideration which include: Adequate cooking and freezing killed toxoplasma in meat and meat-products, periodical examination of the indoor cats serologically and parasitologically, cats should never be fed uncooked meat and they must be nutritionally supplemented with only dry canned or boiled food, meat for consumption should be cooked until the colour changes (66°C) which is sufficient to kill the toxoplasma cysts in the muscles of beef, mutton and pork. Also freezing generally kills *Toxoplasma gondii*, Flushing and disposal of cats faeces or contaminated sand used for cats and application of efficient control measures for the eradication of cockroaches, flies and other invertebrate vectors to prevent the mechanical transmission of infection to food.

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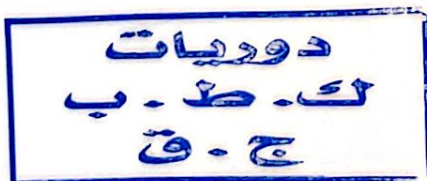
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