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Comparative Diagnosis of Toxoplasmosis in Egyptian Small Ruminants by Indirect Hemagglutination Assay and Elisa

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Abstract: *Toxoplasma gondii* is a ubiquitous, apicomplexan intracellular protozoan of warm-blooded animals and it is one of the most common parasitic infections in humans. Man can be infected in 3 different ways; ingestion of tissue cysts, ingestion of oocysts, or congenital infection with tachyzoites. The main laboratory diagnosis of *T. gondii* is usually based on microscopy and serological methods including enzyme-linked immunosorbent assay (ELISA), indirect hemagglutination assay (IHAT). In this study the results of the two different main tests used practically in diagnosis of *Toxoplasmosis* were evaluated Blood serum antibody titres against *T. gondii* were detected in 320 sheep and 306 goats reared at Giza region, Egypt. IHAT showed the higher prevalence of toxoplasmosis (47.5 % in sheep and 59.4%in goat) as compared with ELIZA (44 and 55.4%, respectively). Positive samples (titres > 1:80) were investigated one more time after treatment with 2-mercaptoethanol to detect IgM antibodies. In sheep, 152 samples (47.5 %) were positive, with antibody titres ranging from 1:10 - 1:1280, whereas in goats the respective figures were 182 (59.4%) and 1:10 -1:2560. It could be concluded that the IHAT and ELIZA tests are efficient diagnostic tools for detection and selective diagnosis of *Toxoplasmosis*. Also high seroprevalence of *T. gondii* in animals could be indicated that the important for transmission of parasite which is the main source of human infection. through consumption of undercooked animal products.

Key words: Egypt · Goats · Sheep *Toxoplasma gondii* · Epidemiology · Indirect haemagglutination test · ELISA · Seroprevalence

INTRODUCTION

Toxoplasmosis is a widely prevalent zoonosis, caused by the facultative two-host protozoan *Toxoplasma gondii*. *T. gondii* is an extremely widespread and thus successful protozoan with a complex lifecycle involving felines, in which sexual development occurs, as its definitive host. Humans become infected by one of 3 ways: ingesting *T. gondii* tissue cysts (containing bradyzoites) present in the undercooked meat of infected food animals (especially lamb and pork); by ingesting highly infectious oocysts (containing sporozoites) present in water, garden soil, children's sandboxes, etc, contaminated by infected cat feces or through congenital transplacental transmission of rapidly replicating tachyzoites from mothers who become infected during pregnancy (e.g., by changing the cat litter) and pass the infection to the fetus. The definitive hosts of the parasite are domestic and wild cats [1]. Intermediate hosts (all mammals including man) are infected by ingestion of sporulated oocysts, cyst-contaminated meat; milk contaminated by tachyzoites or transplacentarily [2].

Toxoplasmosis is one of these parasitic diseases which cause serious economic losses among sheep industry all over the world, especially at lambing time [3]. Meat from *T. gondii*-infected pigs and sheep and goat milk are shown to be primary sources of infection for men [4,5].

The results from an epidemiological study revealed a statistically significant correlation between seropositivity against *T.gondii* in humans and goat milk consumption [6]. The infection with *T. gondii* is an important cause for abortions, delivery of dead or debilitated offspring [7].

In the diagnostics of toxoplasmosis in animals and men, a number of serological tests are used such as

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indirect haemagglutination test (IHAT), indirect immunofluorescence assay test (IFAT), enzyme-linked immunosorbent assay (ELISA).

Because of the great importance of *T. gondii* as a causative agent of a zoonosis, public health organizations, such as the World Health Organization (WHO), have repeatedly advised the collection of accurate epidemiological data on this parasite. Such data are essential to elucidate the relative importance of the various sources of infection for humans, to control disease and to prevent reduction in quality of human life caused by this parasite. However, only few countries of the world regularly monitor toxoplasmosis in humans and even less countries monitor *T. gondii* infection in animals [8].

Many studies investigate the *T. gondii* incidence in sheep from different regions in Egypt which were 45 % using Sabin Feldman tests [9], 18.30 and 14.8% by using SFT and IHAT, respectively[10], 47 and 50% for ELISA and IFAT, respectively [11], 49.5 and 52% in slaughtered sheep in Tanta abattoir using IHAT and IFAT, respectively [12], 55.9 and 54.1 % with IHAT and IFAT [13] and 37% by ELISA by mean of patent Ag-coated plates from specific kit [14].

Most epidemiological studies on *T. gondii* infections now use different tests for antibody detection. A broad range of serological tests have been developed to detect antibodies to *T. gondii* in humans and animals [15-17]. Serology is widely used in epidemiological surveys, the detected prevalence being various in the different countries [7, 18-20].

The literature about the prevalence of *T. gondii* infection among sheep and goats in Egypt are scarce. The purpose of the present study was to investigate the seroprevalence of *T. gondii* among sheep and goats from different regions in Giza governorate by two different serological tests using local strain.

MATERIALS AND METHODS

Animal Blood Sera: A total of 320 female sheep (2-5 years) feed *ad libidum* originating from 10 farms and 306 female goats (2-5 years) from 10 settlements in Giza region was tested. A blood sample was obtained (5 ml) from the jugular vein of each animal. After clotting, sera were centrifuged at 5000 for 10 min. and stored at -20°C.

Strain of *T. gondii*: *T. gondii* local strain used for antigen preparation was isolated according to procedures described by [7], after many trials of feeding kittens with meat samples obtained from freshly slaughtered ewes at El-Bassatein abattoir, Cairo, Egypt. The parasite was maintained in the lab as outlined by the procedures of [21].

Serological Tests Used for Detection of *T. gondii* Antibodies: The collected sera were examined serologically for detection of *T. gondii* antibodies using the following two serological tests.

Enzyme Linked Immunosorbent Assay (ELISA): ELISA was performed using the tachyzoite antigen prepared as described by [20].

Indirect Haemagglutination Test (IHAT): Blood sera were tested with the indirect haemagglutination test (IHAT). All positive samples that reacted with titres > 1:80 were tested to detect antibodies [22].

RESULTS

Results of the serological tests for sheep are shown in Table 1. Out of all 320 blood samples, 152 reacted positively (47.5%).

Table 1: Seroprevalence of *Toxoplasma gondii* in sheep using IHAT

			Antibody titre						
Farm no	Sample no	Positive %	1/20	1/40	1/80	1/160	1/320	1/640	1/1280
1	37	16(43.2%)	1	5	7	3			-
2	39	19(48.7%)		1	7	6	3	2	-
3	30	16(53.3%)		3	6	5	2		-
4	29	15(51.7%)	1	3	5	4	1	1	-
5	28	15(53.5%)		2	7	5	1		-
6	30	14(46.6%)		2	9	3			-
7	30	13(43.3%)		2	6	4	1		-
8	35	17(48.5%)	2	3	6	4	1	1	-
9	29	13(44.8%)		2	3	7	1		-
10	33	14(42.4%)	1	3	6	4			-
Total	320	152(47.5%)	5	26	62	45	10	4	-

Table 2: Prevalence of T. gondii antibodies among sheep sera using two serological tests*

	Positive Sero- prevalence		Negative Sero- prevalence	
Test	No	%	No	%
ELISA	141	44.0	211	66.0
IHAT	152	47.5	168	53.5

*No. of samples examined by each test were 320

Table 3: Seroprevalence of Toxoplasma gondii in goat using IHAT

			Antibody titre						
farm	Sample no	Positive %	1/20	1/40	1/80	1/160	1/320	1/640	1/1280
1	32	21(65.6%)	2	6	9	4			-
2	30	19(63.3%)	1	5	8	3	2		-
3	28	16(57.1%)		3	8	3	2		-
4	29	17(58.6%)		6	9	1	1		-
5	35	20(57.1%)	2	5	9	3	1		-
6	29	16(55.1%)	1	4	8	3			-
7	36	22(61.1%)	1	7	10	3	1		-
8	32	19(59.4%)		3	8	4	3	1	-
9	28	16(57.1%)		4	9	3			-
10	27	16(59.2%)	1	5	8	2			-
Total	306	182(59.4%)	8	48	78	28	10	1	-

Table 4: Prevalence of T. gondii antibodies among goat sera using different serological tests*

	Positive Sero-prevalence		Negative Sero- prevalence	
Test	No	%	No	%
ELISA	170	55.5	136	44.5
IHAT	182	59.7	124	41.3

*No. of samples examined by each test were 306

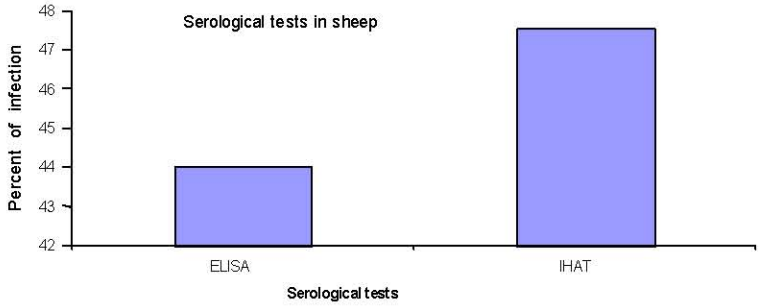

Fig. 1: Incidence of infection with toxoplasmosis in sheep using different serological tests

Also the results of the serological tests for goats are shown in Table 3. Out of all 306 caprine blood samples, 182 have reacted positively (59.4%).

Examination of the 320 serum samples of sheep by ELISA and IHAT revealed that 141(44%) and 152 (47.5%) had antibodies against *T. gondii* respectively, which at the same time was considered the percentage of infection (Table 2 and Fig. 1).

Results of the serological tests in goat are shown in Table 2. Out of all 306 blood samples, 182reacted positively (59.4%).

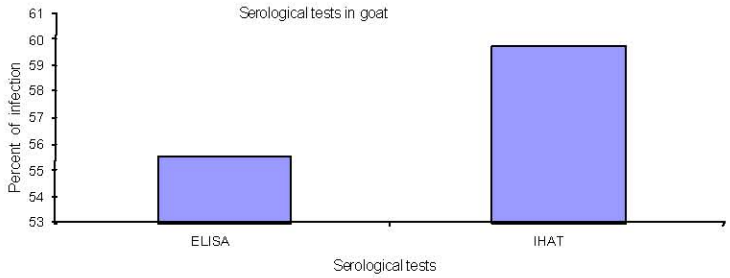

Fig. 2: Incidence of infection of toxoplasmosis in goat using different serological tests

Examination of the 306 serum samples goat by ELISA and IHAT revealed that 170(55.5%) and 182(59.4%) had antibodies against *T. gondii,* respectively, which at the same time was considered the percentage of infection (Table 4 and Fig. 2).

DISCUSSION

Diagnosis of toxoplasmosis by demonstration of *T. gondii* in tissue is too much difficult. Therefore [23] pointed out that the detection of antibody response by serological screening of slaughtered animals appears to be the conclusive tool for proper diagnosis concerning toxoplasmosis public health hazard. The IHAT is simple to perform and is practical when large numbers of sera samples are tested [24]. Enzyme linked immunosorbant assay (ELISA) was used for confirming the results of IHAT.

ELISA is of a great sensitivity, objective, quantitative, low coast and may be automatically adopted, but it need a refinement in the procedures and standardization of the antigen used [25].

A serological survey using IHAT on the prevalence of toxoplasmosis among sheep in Egypt, [26] observed antibody titres from 1:40-1:320.

The results of the present investigation showed the presence of antibodies against *T. gondii* in the tested sheep and goat sera in different settlement of Giza governorate, Egypt. the highest numbers of animals (48and78) reacted with titres of 1:40 and 1:80, respectively. Titres of 1:320 and 1:640 were detected in little number of animals only. In goats, antibody titres were more elevated, whereas78 of seropositive goats exhibited titres of 1:80 and 28, 10 and 1 titres of 1:160, 1:320 and 1:640, respectively. Positive antibody titres were observed in sheep blood sera ranged from 1:20 - 1:640 [27,28]. Also, [26] observed antibody titres in sheep against *T. gondii* (IHAT) ranging from 1:64 to 1:2048. In India, [29] provided evidence that sheep and goats, seropositively reacted for *T. gondii* with antibody titres of 1:10 to 1:2560. The relatively low antibody titres indicated that in most cases, the infection was chronic.

The performed serological survey(IHAT) exhibited a higher prevalence of *T. gondii* among sheep and goats in the region of Giza (47.5 and 59.4%, respectively). Seropositive sheep and goats were detected in all investigated farms and settlements. According to [7,8]. *T. gondii* infection is widely distributed at a worldwide scale, with incidences from 0-100% in the different countries. In the latter,

The detection of *T. gondii* antibodies using ELISA showed that 44% of examined sheep sera and 55,5% of goat sera were sero-positive for *T. gondii* using ELISA at titer $\geq$ 1/100.

Nearly similar results for the incidence of toxoplasmosis in sheep by ELISA were recorded by [24] in Norway (44.3%) and by [14] in sheep from private farm in Fayoum, Egypt (45%). Lower incidences of toxoplasmosis obtained by ELISA were recorded by [23] in young lambs in USA (21.3%) and [25] in Ghana (30.5%).

The difference between the obtained results of serological tests during the present study and those reported by other investigators might be attributed to the host-parasite relationship which depends upon the virulence of *T. gondii* strain; the immune status of the different infected sheep, the age and management of sheep in different localities; and the time of exposure to infection and biology of the parasite This is agreed with that concluded by [23] who added that the prevalence of infection may vary strongly in some country from one locality to another due to difference in certain ecological factors and breeding system in these areas.

Generally the higher infection rate among sheep with *T. gondii* found in this study may be attributed to the feeding habit of sheep which usually graze short grasses and lick soil around them thus are liable to contract the infection with *T. gondii* oocysts. Moreover, stray cats may easily enter to the environment of sheep. This agreed with that obtained by [10, 23, 30].

The observed high seroprevalence of *T. gondii* in sheep and goats is an evidence for environmental contamination with infective oocysts however the wild felids play a more essential role in the epidemiology of toxoplasmosis rather than domestic cats [31].

It could be concluded that the seropositive sheep and goats were harboring *T.gondii* tissue cysts, which is the main source of human infection. Therefore, such animals could be an important source of transmission of the infection to men, through consumption of incompletely cooked meat. Also in the acute stage of the disease those animals shed *T. gondii* tachyzoites in all body fluids, including milk. Similar views have expressed by [32]. The last author proved the release of tachyzoites in the milk of naturally infected goats. Toxoplasmosis in goats is more extensively studied because of its importance for human health, as the consumption of goat milk.

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