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SEROLOGICAL AND PARASITOLOGICAL INVESTIVATIONS ON TOXOPLASMA INFECTION IN DOMESTIC FOWLS IN IRAN

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ABSTRACT

One hundred sixty two domestic fowls were examined for *Toxoplasma* infection by both indirect haemagglutination test for *Toxoplasma* antibodies and inoculation of their brains intraperitoneally into mice for isolation of the parasite

The overall sero-positive rate (SPR) was 29 percent with the titers ranging from 1:20 to 1:6400. The number of each species tested and their SPR were : 74 hens (27.0%), 35 roosters (37.1%), 25 turkeys (24. 0%), 12 pigeons (33.3%), 8 geese (50.0%) and 8 ducks (0.0%).

Altogether 12 strains (7.4%) of *T.gondii* were isolated from 5 species of the birds. Five strains (6.7%) from the hens,one (2.9%) from roosters, four (16.0%) from turkeys,one (8.3%) from pigeons,one (12.5%) from geese and none from the examined ducks.

In one case *Toxoplasma* was isolated from a seronegative hen and the rest were isolated from the sero-positive fowls. All of the isolated strains were avirulent to mice.

Since in Iran consumption of underdone chicken -kebab is more or less usual, *Toxoplasma* infections in domestic fowls may be taken into account as one of the sources of human infection.

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INTRODUCTION

Infection with *Toxoplasma gondii* in mammals and birds is apparently prevalent all over the world. Results of the previous surveys carried out in man and some animals in Iran also indicate that the natural infections with this intracelular parasite is rather high in this country (7,10,16).

To find out the distribution of *Toxoplasma* infections in domestic fowls, the preliminary serological and parasitological studies were undertaken in Protozoology Unit, School of Public Health , Teheran University of Medical Sciences, and this report presents the findings of this investigation.

MATERIALS AND METHODS

The studied fowls were 74 hens, 35 roosters, 25 turkeys, 12 pigeons, 8 geese and 8 ducks. These fowls were natively bred in the rural areas in different parts of Iran and mostly transfered alive to Teheran, to the poultry shops, where they were slaughtered and sold. For this investigation blood samples were collected at slaughter times in laboratory tubes and the removed heads in small nylon bags and carried to the Protozoology Unit, in a thermos box of ice. Blood sera were separated by centrifugation and kept in - 20 degree centigrade for serological examinations, and the brains of the fowls were inoculated into mice at the day of sampling.

Indirect haemagglutination (IHA) test was used for detection of *Toxoplasma* antibodies according to the methods described by Jacobs and Lund, 1957 (13).

The antigen was prepared from the peritoneal exudate of mice, which had been inoculated intraperitoneally with RH strain of *T.gondii*, 3 to 4 days previously. The exudate was mixed with saline phosphate buffer solution PH 7.2, and after centrifugation the deposit was suspended in 10x volum with distilled water. Then it was frozen and thawed several times. The sheep red blood cells coated with *Toxoplasma* antigen were used in the IHA test.

The prepared IHA antigen was evaluated by indirect fluorescent antibody technique with human *Toxoplasma* serologically positive and negative sera.

The sera were examined in the dilutions of 1:20, 1:100, 1:200 ..... 1:25600 and considered to be positive, when the antibody titers were $\geqslant$ 1: 20.

Isolation of *Toxoplasma* was attemped by the inoculation of the fowls brains intraperitoneally into albino mice according to the procedure of Beverley, 1960(3) as follows:

Brain of the bird was removed aseptically and homogenized with normal saline and a suspension of about 20% was prepared. The suspension of each brain was inoculated into 3 to 4 mice.

A sample of the inoculum was microscopically examined for *Toxoplasma* cysts. Four to five week after inoculation, blood samples from the tails of the inoculated mice were collected in heparinized capillary tubes and after centrifugation the plasma were tesed for *Toxoplasma* antibodies . The seropositive mice were killed and unstained wet films of their brains were examined for *Toxoplasma* cysts. In case no parasite was found, suspension of their brains, liver and spleen were prepared and subpassaged to another group of mice. Whenever any of the inoculated mice became ill or died during the examination, Giemsa stained impression smears of their spleens, livers, lungs as well as unstained wet films of their brains and washed peritoneum were prepared and examined for *Toxoplasma*.

For each group of inoculated mice, nomal mice were used as control.

RESULTS

The distribution of *Toxoplasma* antibodies in the serum samples of 162 different domestic fowls, as measured by IHA test are shown in Table 1, The over-all sero-positive rate (SPR) was 29.0 percent with the antibody titres of 1:20 or higer . Twenty-one out of 47 (44.6%) of the sero-positive birds had a titer $\geqslant$1: 400, and the highest titer level (1:6400) was observed in one hen and one turkey.

As shown in Table 2, altogether 12 strains of *Toxoplsama gondii* were isolated from the brains of 5 species of the fowls. All of the isolated strains were avirulent to albino mice. However, one of them, which was isolated from a turkey, killed about 50 percent of the mice within 7 to 10 days, but after 3 passages it became avirulent to the animals. Apparently the causative agent of death was not *Toxoplasma*, and the mortality may have been due to some other pathogenic microorganism . *Toxoplasma* was not observed in the direct microscopic examination of the samples prepared from the brains of the fowls.

If we put all of the fowls together in one group, as shown in Table 3, it seems that the percentage of positive isolation has increased with an increase in *Toxoplasma* antibody titers, taking into consideration that one strain of *Toxoplasma* was isolated from a sero-negative hen.

As shown in Table 4, there are no considerable differences between the SPR found in the fowls from various provinces of Iran with the exception that the birds of Tehran province showed a lower SPR. The positive isolation rates from the fowls collected from Gilan province (11.1%) and also from East and West Azerbaijan provinces (10%) were higher than those of the fowls from other provinces. However, because of the low number of examined birds from each province, accurate interpretation of the results is hard and needs more investigation.

DISCUSSION

Toxoplasma infections have been detected on the basis of parasitological and serological examinations in a wide variety of birds from various parts of the world (1,2,4,5,6,12,14,15,17,18,19).

Although the distribution of *Toxoplasma* infections in mammals including man have been studied in Iran, (7,8,9,10,16),except one isolated case of *T. gondii* from a wild bird (*Milvus migrans*) (10), there is no survey about the infection in fowls in this country.

The results of the present work indicate that *Toxoplasma* infection rate is not low in domestic fowls and the infection rates are more or less similar to infection in mammals. Presumably the source of infection in natively bred domestic birds, that are wandering in the villages and eat anything, should be *Toxoplasma* oocysts, excreted by the infected stray cats, which are usually abundant in the villages of Iran.

One strain of *Toxoplasma* was isolated from the brain of a seronegative hen. This indicated that IHA *Toxoplasma* antibodies may drop to negative in chronicly infected hens. Jacobs et al. 1953, (11) found a fairly rapid drop in the dye test antibody titer in experimentally infected pigeons and they also isolated *Toxoplasma* from the brains of the sero-negative birds. Most cases of human acquired toxoplasmosis occurs either by ingestion of *Toxoplasma* tissue cysts from eating raw or undercooked meat or ingestion of oocysts disseminated in the soil. Fowl meat does not seem to be an important source of human toxoplasmosis, because in most countries fowl meat will be consumed well cooked. However, in Iran consumption of chicken-Kebab, which is more or less under-cooked, is very common. Thus, human infection could be acquired also from eating such kind of under-cooked meat in this country.

As the asymptomatic primary infection during pregnancy may cause congenital toxoplasmosis, which is of great medical importance, the sero-negative pregnant women should aviod consumption of raw or under-cooked meat, including chiken-kebab.

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Table 1- Distribution of IHA antibody titers to *Toxoplasma gondii* in different domestic fowls.

Fowls	No tested	Positive		IHA Reciprocal titers							
		No	%	20	100	200	400	800	1600	3200	6400
Hen	74	20	27.0	2	2	8	4	2	1	0	1
Rooster	35	13	37.1	1	2	3	3	3	1	0	0
Turkey	25	6	24.0	0	1	2	2	0	0	0	1
Pigeon	12	4	33.3	0	1	1	2	0	0	0	0
Goose	8	4	50.0	1	1	1	1	0	0	0	0
Duck	8	0	0	0	0	0	0	0	0	0	0
Total	162	47	29.0	4	7	15	12	5	2	0	2

Table 2- Isolation of *T. gondii* from the brains of domestic fowls by intraperitioneal mice inoculation.

Fowls	No. tested	Positive isolation	
		No.	%
Hen	74	5	6.7
Rooster	35	1	2.9
Turkey	25	4	16.0
Pigeon	12	1	8.3
Goose	8	1	12.5
Duck	8	0	0
Total	162	12	7.4

Table 3- Relation of *Toxoplasma* IHA antibody titers to isolation of the parasite.

IHA Titers	NO. Tested	Positive Isolation	
		NO.	%
Sero-negative	115	1	0.8
1:20 - 1:200	26	4	15.3
1:400 - 1:1600	19	6	31.5
1:3200 - 1:6400	2	1	50.0
Total	162	12	7.4

Table 4- *Toxoplasma* SPR and positive isolation of the parasite in the fowls studied from different provinces of Iran.

Province	No tested	Sero-positive		Positive isolation	
		NO	%	NO	%
Mazandaran	56	17	30.3	3	5.3
Teheran	34	7	20.5	2	5.8
East and West Azerbaijan	30	9	30.0	3	10.0
Gilan	27	9	33.3	3	11.1
Others	15	5	33.3	1	6.6
Total	162	47	29.0	12	7.4

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