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Original Contribution

SEROPREVALENCE OF SHEEP TOXOPLASMOSIS IN NORTH OF IRAN

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ABSTRACT

Toxoplasma gondii is cosmopolitan protozoa infecting many warm-blooded animals including sheep. The aim of this study was to determine the prevalence of antibodies to *T. gondii* from sheep using Indirect Fluorescent Antibody method (IFA) in Golestan Province, north of Iran**.** Sera samples of 764 sheep (650 female and 114 male) were obtained from Golestan Province, north of Iran in 2013. The sera were examined for *T. gondii* antibody (IgG) by IFA, an antibody titer of 1:100 or higher considered as positive. The statistical analysis was performed by chi-square test and logistic regressions were used to analyze the influence of all examined factor (age and sex) on seroprevalence of toxoplasmosis. The antibody titers in positive animals ranged from 1:100-1:1600.The overall seroprevalence of *T. gondii* antibodies in examined sera of sheep was 28.2%. The prevalence of *T. gondii* antibodies in female sheep (29.5%) was higher than male (21%) sheep. With increase in years of age, prevalence of toxoplasmosis raised. No statistically significant difference was observed between male & female sheep and there was relationship between seropositivity and age. High level of *Toxoplasma* infection in examined sheep showed a widespread exposure to *T. gondii* in Golestan Province and revealed high risk of acquiring toxoplasmosis by consumption of raw and undercooked meat of sheep in human population of this region.

Key words: Sero-Prevalence, *Toxoplasma gondii*, sheep, Golestan, Iran

INTRODUCTION

Toxoplasmosis is a zoonotic infection which is considered as an important public health concern for human and also imposes considerable economical losses in veterinary field and animal husbandry. *Toxoplasma gondii*, the causative agent of toxoplasmosis is an obligate intra-cellular protozoan, which is a cosmopolitan parasite (1, 2).

Felids play a major role in epidemiology of this zoonotic disease as final hosts and the parasite can infect a wide variety of warm blooded vertebrates as an intermediate host such as humans and livestock particularly sheep and goats (3, 4). Historically, Hartley et al 1954 and Feldman, Miller 1956 described toxoplasmosis in sheep and goat (5). Sheep become infected by ingesting of sporulated oocysts from food or water sources (6).

Sheep is an important livestock species, especially in developing countries and their products [meat and milk] are used in various parts of the world. Since toxoplasmosis in sheep lead to fetal death, mummification, abortion and neonatal death it causes severe economical losses in the sheep farming industry (7).

Toxoplasmosis in humans occurs either by ingesting sporulated oocysts [from vegetables, fruits, water sources or accidentally] or tissue cysts from uncooked meat. It is estimated that one third of the world population has antibodies against this parasite (8). It is suggested that drinking unboiled, unpasteurized contaminated sheep milk causes toxoplasmosis in humans (9, 10).

Sheep is widely used in Iran for several reasons including meat, milk and dairy products as well as breeding. Hence, sheep products are important sources for *T. gondii* due to transmission to human. Therefore, the current study was designed to evaluate the seroprevalence of *Toxoplasma* infection in sheep using IFA method in Golestan Province, north-east of Iran.

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MATERIAL AND METHODS

SAMPLE COLLECTION

A total of 764 sheep (650 female and 114 male) ranged from one to five years old were collected from Golestan Province, north-east of Iran from April to September 2013. Blood samples were taken from sheep by vein puncture of the jugular vein in tubes without anticoagulant. Sera were harvested following the centrifugation of clotted blood, which were stored at −20°C until assayed.

STUDY AREA

Golestan Province (36°83′93″N 54°44′44″E) is located in the north-east of the country south of the Caspian Sea. It covers an area of 20,380 km² and its population is composed by 1,617,087 inhabitants. Golestan Province enjoys mild weather and a temperate climate most of the year. Geographically, it is divided into two sections: The plains and the mountains of the Alborz range.

SEROLOGICAL EXAMINATION

Antibody against *T. gondii* were examined using Indirect Fluorescent Antibody assay (IFA). Antigen of *T.gondii* Rh strain was prepared by Pasteur Institute of Iran. Anti-sheep conjugated were purchased from Hakimi Company (Tehran, Iran) and is diluted 1:100. Firstly, the collected sera were diluted as following titers: 1:100, 1:200, 1:400, 1:800 and 1:1600 in phosphate-buffered saline (PBS,0.1 M phosphate, 0.33 M NaCl) (PH=7.2). Aliquot of 10 μL from each serum was placed on the well of T. *gondii* slide and incubated in a humidified chamber at 37 °C for 30 min. After washing in PBS, slides were incubated for 30 min in PBS, counterstained with 1% Evans Blue and examined for fluorescein under a light microscope. Besides, positive and negative controls were considered in each test. Antibody titer of >1:100 and greater was considered as positive infected sample.

STATISTICAL ANALYSIS

The data analysis was performed by Chi-Square test using SPSS 18 Chi-Square was used to analyze the associations between seroprevalence and influence of risk factors such as gender and age. The differences in $p<0.05$ were considered statistically significant.

RESULTS

From 650 examined sheep 216 (28.2%) had antibodies against *T. gondii*. The prevalence of seropositive female sheep (29.5%) was higher than male (21%) sheep but no significant difference was observed in the presence of antibodies in females as compared with males. With respect to age, the most and least prevalence rate was observed in 4 and 1 years old sheep, respectively **(Table 2).** 218 (28.2%) of the examined sheep were seropositive at the screening dilution of 1:100, 70 (9.1%) at 1:200, 46 (6%) at 1:400, 32 (4.1%) at 1:800, 12 (1.5%) at 1:1600 **(Table 1).** In addition, 50 (31.2%) out of 160 female sheep which had history of abortion were positive for *Toxoplasma* antibodies.

Table1. *Prevalence of T. gondii antibody and the IFA test titers in examined sheep, Golestan province, Iran.*

Sex	No. (%) examined	No. (%) positive	No. of samples showing the antibody titers at				
			1:100	1:200	1:400	1:800	1:1600
Male	114(14.9)	24(21)	24(21)	8(7)	6(5.2)	6(5.2)	2(1.7)
Female	650(85.1)	192(79)	192(79)	62(9.5)	40(6.1)	26(4)	6(5.2)
History of abortion	160	50 (31.2)	50 (31.2)	22 (13.7)	16 (10)	12 (7.5)	6(3.7)
Total (%)	764(100)	216(28.2)	216(28.2)	70(9.1)	46(6)	32(4.1)	8(1.5)

Table 2. *Prevalence of T. gondii antibody in different age groups of examined sheep, Golestan province, Iran.*

Age (year)	1	2	3	4	5<	Total
No. examined	188	202	184	160	30	764
No. positive	28 (14.8%)	66(32.6%)	52(28.2%)	62(38.7%)	8(26.6%)	216(28.2%)

DISCUSSION

In the current survey, the overall prevalence of *T. gondii* in sheep was 28.2%. The worldwide prevalence of infection rate of sheep ranges from 3% in Pakistan to 95.7% in Turkey. Besides, the average of *Toxoplasm*a infection rate of sheep in world is 31% which is in agreement with finding of present study (2, 11).

The results of this work are in agreement with Youssefi indicated 31.2% of sheep in Babol, North of Iran (12). Bahrieni shown 24.7% of specimens were seropositive for *T. gondii* in Kerman region, south eastern Iran (13). Ghazaei also reported seropositivity of 31% in sheep in Ardabil. Sharif reported 35% of sheep revealed antibody against *Toxoplasman* in Mazandaran province (14). In Kerman Province 35.9% of specimens were infected

with *T. gondii (*15). In Iran, the highest prevalence rate for toxoplasmosis was recorded 95% in Mazandaran province, northern Iran whereas the lowest prevalence rate were 5.2% in northeast of Iran, khorasan province (16).

In two studies in our neighbor countries in Pakistanian and Iraq 44.13% and 36.36% were infected with *T. gondii* (17). Of the total tested sera in Brazil and in Serbia 29.4% and 84.5% were positive for anti-*T. gondii* specific IgG (18, 19).

Toxoplasmosis is an important cause of abortion and stillbirth in sheep on a worldwide basis that leads to considerable economical losses in veterinary field (20). In present study, 50 (31.2%) out of 160 aborted ovine were seropositive for *T. gondii* which revealed probable significant role of this parasite in sheep aborting. In the current study IFA test was used to assay *Toxoplasma* infection in samples. This test for the first time was introduced in 1992 and considered more reliable test rather than other serological diagnostic assays because of its noticeable sensitivity and specificity. The method is relatively simple assay for evaluating the infection of animals and also is particularly useful test for screening a large number of specimens (21, 22).

High seroprevalence of *Toxoplasma* infection in examined sheep in this research may be attributed to some factors including: humid and temperate climate which providing favorable condition for sporolization of Oocytes; the absence of routine treatment against feline toxoplasmosis, considerable cat abundance and exposure to pastures and drinking water contaminated with cats faces.

In conclusion, the results of the present survey indicates a widespread exposure of sheep to *T. gondii* in Golestan Province and there is risk of acquiring infection of human by consumption of raw and undercooked meat of sheep. Furthermore, the probability of economical burdens due to toxoplasmosis in sheep farming industry in this region is high.

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