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Seroprevalence Survey of *Toxoplasma gondii* Antibodies among Sheep in Tabriz District, Northwest Iran

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ABSTRACT

Toxoplasmosis is a zoonosis that affects both animals and humans worldwide. The aim of the present study was to compile initial epidemiological data on the prevalence of Toxoplasma gondii antibodies in Tabriz area (Northwest Iran) sheep. In a Cross-sectional study, 181 sheep blood samples from 17 different regions flocks were obtained with a sterile venoject from jugular vein. Samples were transferred to the laboratory and serums were separated. Sera were tested for Toxoplasma gondii antibodies using Sabin Feldman Dye Test (SFDT).The seroprevalence of Toxoplasmosis in sheep was 56.8% (103 samples) with SFDT. From 103(56.8%) positive samples with SFDT, 61 samples had antibody titers of 1:16, 34 samples 1:64, 7 samples 1:256 and 1 sample 1:1024. The results of age analysis showed an almost two fold higher likelihood of infection in sheep more than one years old than the younger ones [P<0.001]. No significant differences in age were found in sheep. The results showed that Sabin Feldman Dye test is very good method for toxoplasmosis seroepidemiological studies.

Keywords: Sheep, Sabin Feldman Dye test, *Toxoplasma gondii*, Toxoplasmosis, Tabriz.

INTRODUCTION

Toxoplasmosis is a parasitic disease with global distribution caused an intra cellular protozoan [1]. *Toxoplasma gondii* infection is an important issue both in veterinary and in medicine as it is widely prevalent in many species of warm-blooded animals, including birds and mammals, but the primary host is the feline family [2]. Cats are the only definitive host and natural reservoir of infective Oocysts and excrete the resistant Oocyst to environments, but other mammals and birds can develop tissue cysts [3]. Stray cats and dogs in the Iran are becoming a public concern because there is a considerable increase in their number annually [4-8]. This disease is of economic importance in farm animals with regard to animal production, and it has become a public health concern since it leads to abortions and neonatal complications in humans and

Causes of abortion, still birth and fertility reduction especially in sheep. Human is infested orally by in taking contaminated foods by cat feces that contains Oocytes or by ingesting the bradyzoaites of Toxoplasma in raw meat [2, 3, 9, 11-13]. Although clinical signs of Toxoplasmosis in human are not apparent and the disease usually has chronic processes, it may cause still birth, blindness, mental and cerebral disorders of fetus like hydrocephalus, microcephaly and death in congenital infestations [13]. Prevalence of the parasite in farm animals, birds and human is reported widely different [14, 15, 16]. Sheep and goats are widely used as food animals in Iran. There have been a few studies reporting seroprevalence of *T. gondii* in sheep and goats in some parts of Iran [14-19]. A prior research in East Azerbaijan demonstrated that the parasite exists in sheep [15]. Serology and parasitological techniques are used for Toxoplasmosis diagnosis. The tests used include the Sabin-Feldman, the complement fixation, the indirect haemagglutination, the direct agglutination, the indirect fluorescent antibody and the enzyme immunoassay. LAT is a best screening test [15, 20 and 21]. In 1948 a serological dye test was created by Sabin & Feldman, which is now the standard basis for diagnostic tests. [22]. SFDT has high sensitivity and specificity [23]. The demonstration of antibodies by these serological tests just indicates previous infection by *T. gondii.* A laboratory diagnosis defined to toxoplasmosis disease requires the demonstration of high titers of specific antibodies and increasing levels in two serum samples taken 2 to 4 weeks [3]. Regarding the nutritional role of mutton in human diet and the moderate climate of this region that predisposes proliferation of the parasite, determining the epidemiological condition of the parasite has economic and public health importance. The aim of the present study was to compile initial epidemiological data on the prevalence and incidence of *T. gondii* in Tabriz (Northwest Iran) sheep.

MATERIALS AND METHODS

Study area

Tabriz district is located in East Azerbaijan Province in North- western Iran with moderate mountainous climate and is the fourth largest city and one of the historical capitals of Iran and the capital of East Azerbaijan Province. Situated at an altitude of 1,350 meters and has a semi-arid climate with regular seasons. The average annual temperature is 13$^{\circ C}$. Cool winds blow from east to west mostly in summer [24].

Sampling

To determine the seroprevalence of anti *T. gondii* antibodies in sheep in Iran and to investigate related risk factors to the infection, a study was conducted in Tabriz; locate in East Azerbaijan provinces of Iran. For this, 181 serum samples were collected from sheep during a 12 month period from 2009 - 2010 on the 17 villages of Tabriz, located in the East Azerbaijan Province, The study method was descriptive cross-sectional, and the sampling method was multi stage cluster random sampling. 17 villages (cluster) were selected randomly. Based on previous studies, and level of infection of dogs in different areas of Iran, We tested 181 sheep with a 95% confidence level and less than 2% error and studied endemic cases, approximately with ten humans and one dog per study area. The following information was obtained for each sheep using a questionnaire: Owner name, age, gender, hair color, size, habitancy location, and environmental data with local information as well as other distinctive characteristics of each animal owner. Each sheep was assigned a number for identification purposes. From each sheep,

10cc of peripheral blood was taken by a sterile Venoject and dispensed into polypropylene tubes. To prevent lysis of blood samples after 6-10 h, they were transferred to the laboratory and incubated for half an hour at 37°C. After separation of the sera, they were transferred to sterile tubes and centrifuged with the speed of 4000 rpm for 10 minutes (in order to prepare transparent and blood cells free sera). Each sample is transferred to 1.5 ml sterile micro tubes separately. They were labeled and incubated in -70°C until the trial. Sera were tested for *T. gondii* antibodies using Sabin Feldman dye test (Sabin & Fledman, 1984) [22, 25] in immunology laboratory of Tabriz University of Medical Sciences. First in 56°C sera were inactivated for half an hour, the antigens of Toxoplasma was fixed on a clean slides, and various dilutions of sheep sera were added. Sheep serum free of any specific antibody [subsidiary factor] and after 1 hour, alkaline blue due methylene (Ph=11) were added to the slides. Target dilution was the slide that at least 50% of tachyzoaites were not stained and remained colorless [22, 25].

Statistical Analysis

Chi-squared (χ^2) was used to compare seroprevalence rates (SPR) relative to gender and age. The differences were considered statistically significant when probability (P) value ≤0.05. The 95% confidence intervals (95%CI) of seroprevalence rates were calculated. Statistical analysis was performed using Epi Info software, version 6.

RESULTS

T. gondii antibodies were detected in 56.8% [103 samples] with SFDT of the 181 sheep blood samples. Qualitative results on 181 sheep sera showed that seroprevalence rate (SPR) of Toxoplasmosis in sheep was 56.8% with SFDT .From 103 positive samples with SFDT, 61 samples had antibody titers of 1:16, 34 samples 1:64, 7 samples 1:256 and 1 sample 1:1024 (*Table.2*). The results of age analysis showed an almost two fold higher likelihood of infection in sheep more than one years old than the younger ones (P<0.001). No significant differences in age were found in sheep.

Table 2: Antibody titers of positive sera by SFDT (103 samples)

titers	1:16	1:64	1:256	1:1024
No	61	34	7	1

DISCUSSION

Many investigations have been conducted on serological diagnosis of *T. gondii* infection in sheep [14-19] cats [6-8], human [4, 18] and other animals in Iran [4, 15, 18, 19, 26, 27].The latex agglutination is a simple, safe and inexpensive test. Findings by researchers on many human serums with this method and in comparison with Dye test show up to 98% agreement between two tests and the antibody titers of this test have compatibility with IFA test and Dye test. Weak false positive reactions in this test are due to non specific IgM [28, 29]. This study showed that [56.8%] of the sheep in Tabriz were positive for *T. gondii*. In the present study, the prevalence of our seropositive Sheepwas similar of other part of Iran [14-18]. Prevalence of Toxoplasma antibodies maybe due to relative climate of this region that preserves the parasite Oocystes [23, 26] and presence of cat as domestic animal anywhere. Ghazaei (2006) [14] reported 30%

prevalence of Toxoplasmosis in Ardabil province [Northwest, Iran]. Hashemi- Fesharaki [1996] [15] reported prevalence of 24.5% and 19.5% in sheep and goats of various parts of Iran by using indirect hemagglutination and latex agglutination tests. In a survey by Hamzavi et al. (2007) [17] in Kermanshah (West of Iran) in slaughter house, 22.55% of sheep's were positive by indirect immunoflorescent test.In another survey by Keshavarz et al. (2007) [16] in Meshkin shahr, 59% of sheep were positive to toxoplasmosis by indirect immune fluorescent test. In northern Iran, a survey by Ghorbani et al. (1983) [30] showed a seroprevalence rate of 32.5% and 17.7% in sheep's and goats, respectively. Hoghooghi Rad et al. (1993) [18] showed seropositivity of 13.8% and 13.1% in sheep and goats, respectively in Ahwaz [southwest Iran]. In other countries there are so many various reports of Toxoplasmosis. Babur et al. (2001) [31] reported arrange of 7.1- 88.7% of Toxoplasmosis in Turkey sheep. Ibrahim et al. (1997) [21] in Egypt reported prevalence of 48.8% in slaughtered sheep. In Saudi Arabia, Amin et al. (1997) [10] reported 39% and 28% of Toxoplasma antibodies in sheep and goats sera respectively. In Indonesia, infestation rate was 47.5% and 9% in goats and cattle respectively. Pita Gondim et al. (1999) [34] in Brazil reported 18.75% of Toxoplasmosis prevalence in sheep by LAT. Bekele et al. [1989] [35] reported 22.9% of prevalence in Ethiopian sheep. In Turkey, Oncel et al. (2005) [32] reported seroprevalence of 66.66% and 65.08% by LAT and SFDT respectively in sheep. Sevgulu et al. (2005) [36] in Sanli urfa province reported 55.66% of prevalence by SFDT. In Serbia 84.5% of sheep were positive in modified latex agglutination test [37]. In researches by Dubey [1985] on various animals sera, he showed LAT has very high sensitivity and specificity like Dye test and is a very good test for screening and seroepidemiological studies [20,38]. So SFDT can be used as a reference test. The little differences in prevalence of Toxoplasmosis in various regions may be due to different diagnostic methods that are used, different climate, cultures and breeding patterns of sheep [28]. In this study some reasons of high prevalence of Toxoplasmosis is as following: climate and geography of Tabriz; parasite characteristics, specially the presence of reservoirs like cats, farm animals, wild animals; suitable temperature and humidity; potentiality of environment for sporulation of Oocytes and more resistance of them in nature; traditional breeding systems and suitable pastures. In conclusion, as the sheep are the main sources of meat in Tabriz as kebab and unrefined steak, there is high risk of transmission to human; results of the present study suggest that consumption of raw or undercooked meat of these animals may be a probable source in the transmission of human toxoplasmosis. So in order to decrease the prevalence of *Toxoplasma gondii* in this region some recommendations are as following: Euthanasia of sauntered cats, modification of contaminated environments, caring hygiene, challenging with animal diseases, education of people to how breed and train their cats and not to eat not well cooked meat.

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