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Seropervalence of *Toxoplasma gondii* antibodies in Sheep by Sabin Feldman Dye Test (SFDT) and Latex Agglutination Test (LAT) in Northwest Iran

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ABSTRACT

Toxoplasmosis is a common Zoonotic disease with global distribution caused by an intra cellular protozoan parasite named Toxoplasma gondii .The disease also causes economic losses in sheep almost by abortion. As the climate of Azerbaijan province (Northwest Iran) is suitable for this parasite life cycle, determination of the parasite prevalence has public health importance. The aim of this study was detection of seroepidemiological prevalence of T.gondii in Miyaneh (a county of East Azerbaijan province) sheep. In a Cross-sectional study, 181 sheep blood samples from 9 different regions flocks were obtained from March to May 2011with a sterile venoject from jugular vein. Samples were transferred to the laboratory and serums were separated. Sera were tested for Tg antibodies using Sabin Feldman Dye Test (SFDT) and Latex Agglutination Test (LAT).The seroprevalence of Toxoplasmosis in sheep was 33.7% (61 samples) and 31.5% (57 samples) with LAT and SFDT respectively. From 57 positive samples with SFDT, 45 samples had antibody titers of 1:16, 9 samples 1:64, one sample 1:256 and 2 samples 1:1024. Regarding SFDT as a golden test the sensitivity and specificity of LAT were 80% and 88% respectively. The agreement between two tests was 88%. Positive and negative predictive values of LAT were 75% and 90%, respectively. The results showed that LAT is a very good method for Toxoplasmosis seroepidemiological screening.

Key words: Sheep, Sabin Feldman Dye Test (SFDT), Direct Latex Agglutination Test (LAT), *Toxoplasma gondii*, Miyaneh.

INTRODUCTION

Toxoplasmosis is a common Zoonotic disease with global distribution caused by an intra cellular protozoan. *Toxoplasma gondii* is a parasite of birds and mammals. 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 [7].The disease causes economic losses in farm animals, as intermediate host, by inducing abortion, still birth and fertility reduction especially in sheep. The sexual cycle of the parasite is completed in feline that are considered as final host. Human is infested orally by intaking contaminated foods by cat feces that contain Oocytes or by ingesting the Bradyzoaites of Toxoplasma in raw meat [7, 9, 16, 24, 25 and 26]. 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 [26]. Prevalence of the parasite in farm animals, birds and human is reported widely different [10, 11 and 15]. A prior research in East Azerbaijan (Iran) demonstrated that the parasite exists in sheep [11].Serology and parasitological techniques are used for Toxoplasmosis diagnosis. LAT is a best screening test [5, 11 and 13]. Another test is SFDT that has high sensitivity and specificity [6, 7]. 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 Miyaneh (Northwest Iran) sheep.

MATERIALS AND METHODS

Geographical location

Miyaneh is a city in Southeast of East Azerbaijan Province in Iran, with the altitude of 37° 24' north latitude and 47° 43' east longitude that has 5590 square kilometers area with 350 villages (Fig 1). Most of the inhabitants of Miyaneh district are involved in agriculture and animal husbandry, lives in mud or stone houses, and maintain domestic animals, such as sheep, goats, poultry, and dogs. This city is situated at an altitude of 1100 m above the sea level and the air humidity is moderate however its relative rate varies in different seasons and different hours of a day. Due to the high mountain area, the city has cold winter and mild summer.

Sampling and Testing

Method of this study was Cross-sectional and random sampling. Considering the 95% confidence level and error less than 2 percent, a total of 181blood samples from jugular vein were obtained by a sterile Venoject. Sampling was from 9 different region flocks from March to May 2011. They were transferred to the laboratory and incubated for half an hour at $37^{\circ C}$. 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 $-70^{\circ C}$ until the trial. Sera were tested for *T. gondii* antibodies using Sabin Feldman dye test (Sabin & Fledman, 1984) [8, 23] and latex agglutination test in immunology laboratory of Tabriz University of Medical Sciences. First in $56^{\circ C}$ 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 [8, 23]. For LAT soluble antigen is used. The sera and antigen were mixed on latex and agglutination is considered as positive reaction.

Fig1. Map of the Northwest region of Iran, Azerbaijan

Statistical analysis

Chi-squared test ($\chi 2$) was used to compare seroprevalence rates (SPR). 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 IBM® SPSS software (ver.17) and the agreement between two tests was calculated by Epi Info software (version 6).

RESULTS

Table 1: Comparative results between LAT& SFDT tests in all sera

LAT	SFDT		
	Positive ( )	Negative (%)	Total(%)
Positive	15(8.3)	46(25.4)	61(33.7)
Negative	109(60.2)	11(6.1)	120(66.3)
Total	124(68.5)	57(31.5)	181(100)

Qualitative results on 181 sheep sera showed that by using SFDT and LAT, 57 (31.5%) and 61 (33.7%) samples respectively, had Toxoplasma antibodies (Table 1). 46 (25.4%) sera were positive but 109 sera (60.2%) were negative by both tests. 15 (8.3%) sera were positive by LAT but negative by SFDT and 11 (6.1%) sera were visa versa. From 57 positive sera by SFDT most of them (45 samples) had antibody titers of 1:16 (Table 2). Regarding SFDT as a golden test, the sensitivity and specificity of LAT were 80% and 88% respectively. There was a good agreement between two tests (88%). The Kappa index of them was 0.402. The positive and negative predictive values of LAT were 75% and 90% respectively.

Table 2: Antibody titers of positive sera by SFDT (57 samples)

No.	45	9	1	2
titers	1:16	1:64	1:256	1:1024

DISCUSSION

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 [17, 27]. This study showed that (33.7%) of the sheep's in Miyaneh were positive for *T. gondii*. Relatively high prevalence of Toxoplasma antibodies maybe due to relative hot and humid climate of this region that preserves the parasite Oocystes [6, 19] and presence of cat as domestic animal anywhere. In the present study, the prevalence of our seropositive Sheep's was similar of other parts of Iran such as: Ghazaei (2006) reported 30% prevalence of Toxoplasmosis in Ardabil province (Northwest, Iran) [10]. Hashemi- Fesharaki (1996) 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 [11]. In a survey by Hamzavi et al. (2007) in Kermanshah (West of Iran) in slaughter house, 22.55% of sheep were positive by indirect immunoflorescent test [12].In another survey by Keshavarz et al. (2007) in Meshkin shahr, 59% of sheep were positive to toxoplasmosis by indirect immune fluorescent test [15]. In other countries there are so many various reports of Toxoplasmosis. Ibrahim et al. (1997) in Egypt reported prevalence of 48.8% in slaughtered sheep [13]. In Saudi Arabia, Amin and Morsy (1997) reported 39% and 28% of Toxoplasma antibodies in sheep and goats sera, respectively [1]. In Indonesia, infestation rate was 47.5% and 9% in goats and cattle, respectively [18]. Pita Gondim et al. (1999) in Brazil reported 18.75% of Toxoplasmosis prevalence in sheep by LAT [21]. Bekele and Kasali (1989) reported 22.9% of prevalence in Ethiopian sheep [2]. In Turkey, Oncel et al. (2005) reported seroprevalence of 66.66% and 65.08% by LAT and SFDT respectively in sheep [21] and Sevgulu et al. (2005) in Sanliurfa province reported 55.66% of prevalence by SFDT [22]. However Babur et al. (2001) reported a range of 7.1- 88.7% of Toxoplasmosis in Turkey sheep [3]. In Serbia 84.5% of sheep were positive in modified latex agglutination test [14]. In researches by Dubey (1985) on various animals sera, he showed LAT had very high sensitivity and specificity like Dye test and is a very good test for screening and sero-epidemiological studies [4, 5]. The results in current study demonstrated high agreement (88%) between LAT and SFDT that is similar with the results of Deasmont & Remington (1980) [8]. So LAT 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 [17]. In this study some reasons of high prevalence of Toxoplasmosis are as following: parasite characteristics, specially the presence of reservoirs like cats, farm animals, wild animals; climate and geography of Miyaneh; suitable temperature and humidity; potentiality of environment for sporulation of Oocytes and more resistance of them in nature; traditional breeding systems and suitable pastures. Since sheep meat has high consumption in this region as kebab and unrefined steak, there is high risk of transmission to human. 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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