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Original Article

Serological Determination of *Toxoplasma gondii* among Sheep (*Ovis aries*) in Guilan Province, Iran

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Abstract

Toxoplasma gondii is one of the most common foodborne protozoan parasite causing congenital infection, abortion, and stillbirth in humans and animals. The temperate and humid climate is one of the most important factors in the high prevalence of *T. gondii*. Sheep are among the important sources of meat production in Guilan province, Iran. Therefore, the consumption of raw and half-cooked meat is one of the major risk factors for *T. gondii* infection. Toxoplasmosis in patients with intact immune systems is usually asymptomatic; however, it but can be life-threatening in patients with a weak immune system (for example, patients with the human immunodeficiency viruses/acquired immunodeficiency syndrome or cancer and transplant recipients). Guilan is divided into three geographical regions of plains with a temperate climatic condition, hillsides with a semi-humid climate, and heights with cold mountainous climate. Climate situations play a role in the prevalence of toxoplasmosis. The present study aimed to investigate the seroprevalence of *T. gondii* infection among sheep in Guilan province, north of Iran. In the current cross-sectional study, a total of 400 sheep sera samples were tested for the determination of immunoglobulin G (IgG) antibody against *T. gondii* using the enzyme-linked immunosorbent assay. The samples were divided into different groups according to the geographical location and animal age. *T. gondii* antibody (i.e., IgG) was detected in 166 sheep (41.5%). The highest frequency of *T. gondii* infection (72.7%; n=56) was observed for the age group of > 4 years; the difference was statistically significant in this regard (P=0.0001) in comparison to that reported for other groups. In addition, the seroprevalence of *T. gondii* was significantly higher in the plains (53.9%) than that of the hillsides and heights (P=0.0001). Consequently, the seroprevalence of *T. gondii* infection in Guilan was high indicating a significant relationship with geographical location and animal age.

Keywords: Toxoplasmosis, Sheep, Enzyme-Linked Immunosorbent Assay

Détermination Sérologique de *Toxoplasma gondii* chez les Moutons (*Ovis aries*) dans la Province de Guilan, Iran

Résumé: *Toxoplasma gondii* est l'un des parasites protozoaires d'origine alimentaire les plus courants, responsable d'infection congénitale, d'avortement et de mortinatalité chez les humains et les animaux. Un climat tempéré et humide est l'un des facteurs les plus importants de la plus forte prévalence de *T. gondii* dans certaines régions. Les moutons sont parmi les sources importantes de production de viande dans la province de Guilan, en Iran. Par conséquent, la consommation de viande crue et mi-cuite est l'un des principaux facteurs de risque

d'infection à *T. gondii*. La toxoplasmose chez les patients dont le système immunitaire est intact reste généralement asymptomatique; cependant, elle peut s'avérer mortelle chez les patients dont le système immunitaire est faible (par exemple, les patients atteints du virus de l'immunodéficience humaine / du syndrome d'immunodéficience acquise ou du cancer et des greffés). Guilan est divisée en trois régions géographiques de plaines avec des conditions climatiques tempérées, des coteaux avec un climat semi-humide et des hauteurs avec un climat montagneux froid. Les conditions climatiques jouent un rôle dans la prévalence de la toxoplasmose. Cette étude visait à déterminer la séroprévalence des infections à *T. gondii* chez les moutons dans la province de Guilan, au nord de l'Iran. Dans le cadre d'une étude transversale, un total de 400 échantillons de sérum de mouton a été testé pour la détermination du taux d'immunoglobuline G (*IgG*) contre *T. gondii* par le biais de test d'immunosorbant lié à une enzyme. Les échantillons ont été divisés en différents groupes selon leur situation géographique et l'âge de l'animal. Des anticorps spécifiques contre *T. gondii* (c'est-à-dire des *IgG*) ont été détectés chez 166 moutons (41,5%). La fréquence la plus élevée d'infection à *T. gondii* (72,7%; n = 56) a été observée pour le groupe d'âge> 4 ans; la différence était statistiquement significative (P=0,0001) par rapport à la séroprévalence rapportée pour les autres groupes. De plus, la séroprévalence de *T. gondii* était significativement plus élevée dans les plaines (53,9%) comparée à celle observée dans les coteaux et les hauteurs (P=0,0001). Par conséquent, la séroprévalence de l'infection à *T. gondii* à Guilan était élevée, indiquant une relation significative avec la situation géographique et l'âge des animaux étudiés.

Mots-clés: Toxoplasmose, Mouton, le test d'immunosorbant lié à une enzyme

Introduction

Toxoplasma gondii is one of the most common zoonotic diseases that can be transmitted through meat around the world (Schluter et al., 2014). Toxoplasmosis is a major contributor to abortion, congenital infection, and stillbirth in humans and animals (Schluter et al., 2014). Toxoplasmosis in patients with intact immune systems is usually asymptomatic; however, it can be life-threatening in patients with a weak immune system (for example, patients with the human immunodeficiency viruses/acquired immunodeficiency syndrome or cancer and transplant recipients) (Abdoli et al., 2016). The cat is the final host, and a wide range of warm-blooded animals, including humans and ruminants, are the intermediate hosts for *T. gondii* (Schluter et al., 2014).

The sexual cycle of *T. gondii* occurs only within the feline intestine, and the produced oocysts are excreted through the host's feces. Sporulated oocysts can resist environmental conditions for 12-18 months (Schluter et al., 2014). Eating foodstuffs, water, vegetables, or even soils contaminated with oocysts is the major source of transmission of the parasite to humans and animals (Schluter et al., 2014). The formation of tissue cysts is also the most important stage of *T. gondii* infection. Humans and felines are infected through the consumption of raw or half-cooked meat containing tissue cysts (Schluter et al., 2014).

Sheep are one of the most important intermediate hosts for T. *gondii* since the parasite cysts lie dormant within the cardiac and skeletal muscles. Therefore, the consumption of raw and half-cooked meat is one of the major risk factors for *T. gondii* infection (Schluter et al., 2014). Iran is located in Western Asia with four main climates, including temperate and humid on the coast of the Caspian sea located in the north of Iran, hot and dry in the Central Plateau of Iran, cold and mountainous in the west and northwest of Iran, and warm and humid on the southern coast of the Persian Gulf. These climatic changes play a major role in changing the prevalence of *T. gondii* infection in ruminants in different regions of the country.

In previous serological studies since 2007 to 2019, due to climate changes in different provinces of Iran, Guilan and Mazandaran provinces had a temperate and humid climate on the coast of the Caspian sea (Sharif et al., 2007; Havakhah et al., 2014). Tabriz, Urmia,

Kurdistan, Kermanshah, Markazi, and Qazvin provinces, Iran, had a cold and mountainous climate (Khezri et al., 2012). Kerman, Jahrom, and Kashan provinces, Iran, had a hot and dry climate in the Central Plateau of Iran (Ahmed et al., 2016; Rasti et al., 2018). Furthermore, Khuzestan, Iran, had a warm and humid climate. According to the published reports, the seroprevalence of *T. gondii* among sheep in Iran is reported within the range of 3.3-36.8%. It is noteworthy that the prevalence of *T. gondii* is associated with climatic conditions; accordingly, in areas with a moderate and humid climate, the prevalence is higher than in hot and dry areas (Sharif et al., 2015).

However, in a study carried out by Havakhah et al. in Iran (2014), the prevalence of toxoplasmosis in sheep was reported only in three cities of Guilan province, and there has been no information about the prevalence of toxoplasmosis across the province. Since sheep are among the major sources of meat production in Guilan province, the current study used the serological investigation of *T. gondii* infection among sheep in Guilan within 2018 to 2019 to identify the prevalence rates of the parasite.

Material and Methods

Study area. Guilan, one of the northern provinces of Iran with a population of about 2,500,000, is located at 37° 27' North latitude and 49° 58' East longitude from the meridian, and its altitude from the sea level varies in different regions (Figure 1). Its area is about 14,711 km^2, and the average annual rainfall is 1,275 mm. Guilan is divided into three regions of plains with a temperate climatic condition, hillsides with a semi-humid climate, and heights with a cold mountainous climate.

Sample collection. In the current study, a total of 400 sheep blood samples obtained from the jugular vein were collected according to the geographical distribution of the sheep population in Guilan province. The samples of pregnant female sheep of the herds with a history of abortion were prioritized in the study. The blood samples were centrifuged at 3,000 rpm for 10 min, and the isolated sera were stored at -20˚C until analysis. According to the climate variations in Guilan province, the samples were divided into three groups of plains, hillsides, and heights based on the geographical location. The sheep were also divided into three age groups of < 2, 3-4, and > 4 years. The study protocol was approved by the Ethics Committee of the Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

Serological detection. The indirect enzyme-linked immunosorbent assay (ELISA) was used for the detection of immunoglobulin G (IgG) antibodies against *T. gondii* in the sera (multi-species ID Screen® Toxoplasmosis Indirect, IDVet, Montpellier, France) according to the manufacturer's instructions. The optical density (OD) was measured at 450 nm with an ELISA automated plate reader (BioTek, USA). The Sample to Positive (S/P) ratio was calculated according to the following formula:

$$S/P\% = \frac{\text{OD sample} - \text{OD negative control}}{\text{OD positive control} - \text{OD negative control}} \times 100$$

The samples presenting an S/P% ≤ 40% were considered negative for *T. gondii*; the samples with S/P% within 40% to 50% were considered doubtful for *T. gondii*; the samples with S/P% ≥ 50% were considered positive for *T. gondii*.

Statistical analysis. The data were statistically analyzed using the Chi-square test by SPSS software (version 24; SPSS, Inc., Chicago, IL).

Results

A total of 400 female sheep with a mean age of 3.4±1.84 years (age range: 1-13 years) were evaluated in the current study. *T. gondii*-IgG antibody was detected in 166 cases (41.5%; n=166). Age was significantly associated with IgG seropositivity with the highest frequency of positive samples (72.7%; n=56) observed in the age group of > 4 years (P=0.0001). The geographical location and IgG seropositivity were also

reported with a significant relationship (P=0.0001) with the highest frequency of the positive samples (53.9%; n=103) observed in the sheep of the plains (Table 1).

With increasing the age from 3 to 4 years and over, the frequency of IgG seropositivity decreased from the plains to heights (Table 2).

Table 1. Seroprevalence and associated risk factors for *T. gondii* infection in sheep of Guilan province, Iran

Risk factor		Seropositive n (%)	Seronegative n (%)	Total	P-value
Age (year)	≤ 2	21 (16)	110 (84)	131 (100)	0.0001
	3-4	89 (46.4)	103 (53.6)	192 (100)	
	>4	56 (72.7)	21 (27.3)	77 (100)	
Geographical location	Plains	103 (53.9)	88 (46.1)	191 (100)	0.0001
	Hillsides	56 (40.3)	83 (59.7)	139 (100)	
	Heights	7 (10)	63 (90)	70 (100)	
Total		166 (41.5)	234 (58.5)	400 (100)	

Table 2. Effect of changes in age and geographical location on serum results

* P<0.05: Statistically significant

Age group	Serum result	Location			Total	P-value
		Plains	Hillsides	Heights		
≤2 years	Positive n (%)	4 (11.1)	15 (30.0)	2 (4.4)	21 (16.0)	0.002*
	Negative n (%)	32 (88.9)	35 (70.0)	43 (95.6)	110 (84.0)	
3-4 years	Positive n (%)	49 (56.3)	35 (42.7)	5 (21.7)	89 (46.4)	0.009*
	Negative n (%)	38 (43.7)	47 (57.3)	18 (78.3)	103 (53.6)	
>4 years	Positive n (%)	50 (73.5)	6 (85.7)	0 (0.0)	56 (72.7)	0.051
	Negative n (%)	18 (26.5)	1 (14.3)	2 (100)	21 (27.3)	

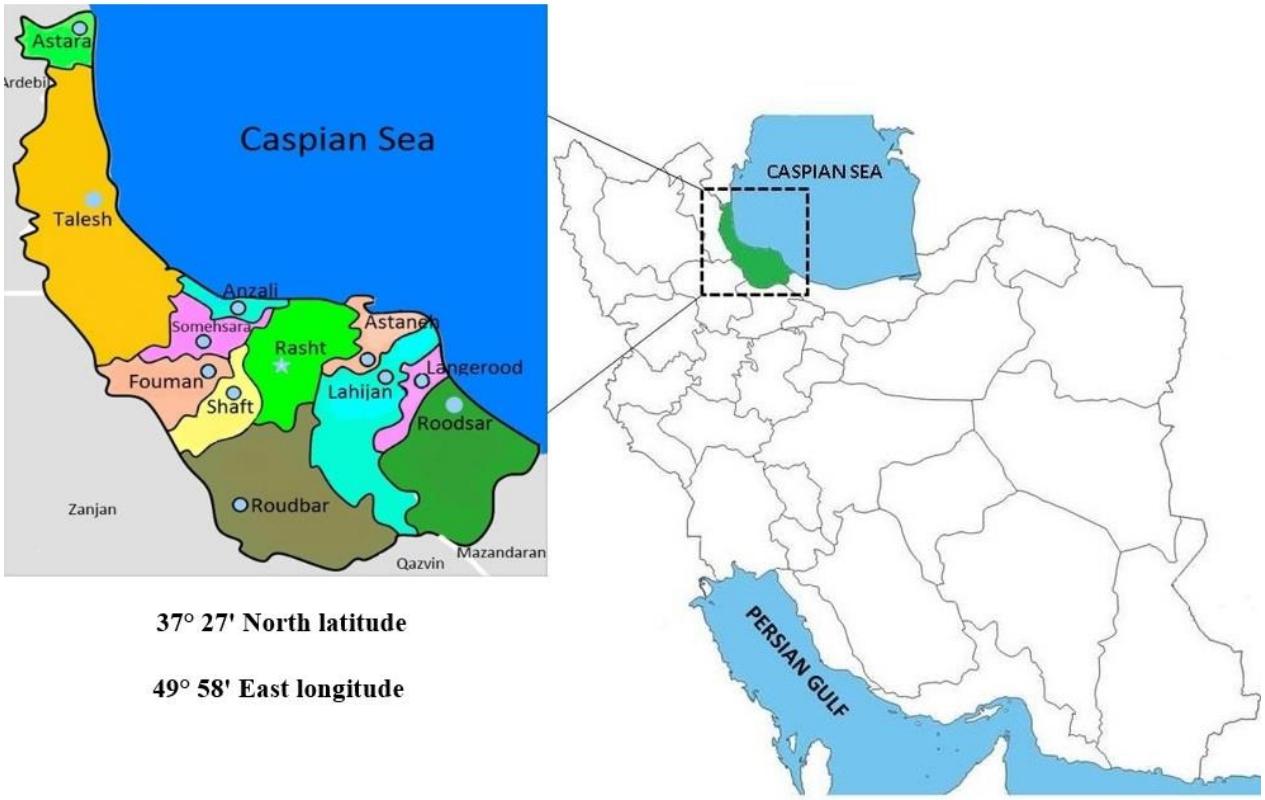

Figure 1. Geographic map of Guilan province, Iran

Discussion

Toxoplasmosis is a common disease among animals and humans around the world and one of the major causes of abortion and stillbirth in sheep (Edwards and Dubey, 2013). Humans, after birth, are infected via the reception of the tissue cysts observed in half-cooked meat, consumption of water and food contaminated with oocysts, or accidentally ingestion of oocysts spread in the environment. Contaminated lambs are among the major sources of *T. gondii* infection in humans and carnivorous animals (Dubey, 2009). These animals are also the intermediate hosts for *T. gondii* (Schluter et al., 2014).

Iran has a wide variety of climates; in the northern regions of Iran, with a moderate and humid climate, the oocysts excreted through felines feces can remain for months or even years in the environment (Zhang et al., 2016; Subedi et al., 2018). Therefore, the highest prevalence of *T. gondii* infection in humans and animals in Iran is reported for the northern region (Sharif et al., 2015; Izadyar et al., 2019) indicating the high importance of this issue in Northern Iran. In previous studies conducted on pregnant women in Northern Iran, the prevalence of *T. gondii* infection was reported within 41.8-75.02% (Foroutan-Rad et al., 2016). In addition, in a serological investigation on

rural individuals in Amol, Northern Iran, the prevalence of this infection was 75.7%. This high prevalence demonstrates that Northern Iran is an endemic area for toxoplasmosis. There was also a significant relationship in the current study between the lamb meat (P=0.015) and raw or half-cooked meat (P <0.001) consumers and presence of *T. gondii*-IgG in their serum (Rostami et al., 2016).

According to previous studies conducted in different parts of the world, the serological prevalence rates of *T. gondii* among sheep are reported as 33.6% in Portugal (Lopes et al., 2013), 15.1-84% in Mexico (Hernández-Cortazar et al., 2015), 8% in South Africa (Hammond-Aryee et al., 2015), 47.8% in Brazil (Rêgo et al., 2016), 26.2% in Pakistan (Ahmed et al., 2016), 22.0% in the USA (Guo et al., 2016), 20.71% in China (Yang et al., 2017), and 42.1% in Northern Iraq (Al Hamada et al., 2019). The prevalence of *T. gondii* infection among sheep in different regions of Iran is within the range of 3.3-38.3% according to the published reports (Rasti et al., 2018). These differences can be attributed to the difference in climates, ages, livestock breeding conditions, and various methods for the detection of the parasite (Izadyar et al., 2019).

In the present study, the serological prevalence of *T. gondii* in sheep in Guilan province was detected at 41.5%. In another study carried out in Mazandaran province, Northern Iran, with a temperate and humid climate, the seroprevalence of *T. gondii* was 35% (Sharif et al., 2007) which was lower than that reported for the present study. In a study conducted by Havakhah et al. (2014) in Guilan province, the prevalence of *T. gondii* infection in sheep was 36.8%, which was lower than that of the present study; however, Havakhah et al. used Sabin-Feldman serologic dye test only in three counties. This could account for the differences reported in the present study, owing to the population distribution of sheep in Guilan province, as the first attempt investigating most regions of the province by the serological method of ELISA, which has a higher sensitivity than the dye test (Balsari et al., 1980).

In addition to the temperate and humid climate, the extensive grazing system of sheep breeding, presence of wild felines, and crop storage facilities that felines have access to are other causes of the high prevalence of *T. gondii* in Guilan province. These findings are similar to the results of a study carried out by Subedi et al. on sheep in Nepal (Subedi et al., 2018). Guilan province was divided into three regions of plains, hillsides, and heights according to the geographical location. In the current study, the highest (53.9%) and lowest (10%) frequency rates of *T. gondii* infection were observed in the plain and height samples, respectively. Furthermore, a significant relationship was observed between the geographical location and frequency of *T. gondii* infection (P=0.0001).

With increasing the altitude from the sea level, the amount of humidity and temperature decreases, and a dry and cold winter dominates the heights of the province reducing the survival rate of oocysts in such climatic conditions; however, in the plains, due to the lower altitude from the sea level and proximity to the sea, the conditions are suitable for the survival of oocytes during winter. Oocytes can survive in moderate and humid climates for up to 18 months (Katzer et al., 2011; Gazzonis et al., 2015).

In a study carried out by Khezri et al. (2012) on sheep in Kurdistan province, Iran, the prevalence of *T. gondii* infection in the southern regions due to hot and humid climate was higher than that of the western regions due to cool and dry climate dominating these regions. In another study performed by Sharif et al. (2007) on the sheep in Mazandaran province, the high prevalence of *T. gondii* infection in the western region was due to the difference in moisture content increasing the number of oocysts, compared to that reported for the eastern and central regions. The aforementioned results are consistent with the findings of the current study.

In the present study, there was a significant correlation between mean age and seropositive (4.2±33.17) and seronegative (2.1±7.2) samples (P=0.0001). Moreover, the highest (72.7%) and lowest (16%) frequency rates of the infection were observed in

the age group of > 4 and < 2 years, respectively. The relationship between the age group and seropositivity was statistically significant (P=0.0001). In previous studies, including Gazzonis et al. (2015) on Northern Italian sheep, Katzer et al. (2011) on Scottish sheep, Hecker et al. (2018) on sheep in Argentina, and Izadyar et al. (2019) on sheep in Qazvin, an increased prevalence of *T. gondii* infection in older animals in comparison to younger ones was consistently reported as observed in the current study (Rahman et al., 2014; Sharif et al., 2015; Izadyar et al., 2019). These results suggest that contamination with *T. gondii* in sheep mostly occur after birth, and horizontal contamination is the main route of infection transmission in herds (Dubey, 2009; Hecker et al., 2018).

Conclusion

A high prevalence of *T. gondii* infection in Guilan province was observed in the present study which was related to the temperate climate and high humidity of the region. Due to climate variations in Guilan province, the prevalence of contamination in the plains of the province was higher than that reported for other regions. It was also shown that as the sheep age increased, the prevalence of *T. gondii* infection also increased, confirming the horizontal transmission of contamination. A high prevalence of *T. gondii* infection was reported in sheep; therefore, in order to improve the management of sheep in this region, it is recommended to develop a comprehensive educational, preventive, and continuous treatment plan in this regard.

Authors' Contribution

Study concept and design: M. R. Ch. N. and B. Sh.
Acquisition of data: M. R. Ch. N. and P. Sh.
Analysis and interpretation of data: M. R. Ch. N.
Drafting of the manuscript: M. R. Ch. N.
Critical revision of the manuscript for important intellectual content: M. R. Ch. N.
Statistical analysis: M. R. Ch. N.
Administrative, technical, and material support: M. R. Ch. N.

Ethics

The authors declare that all the ethical standards were respected in the preparation of the submitted article.

Conflict of Interest

The authors declare that they have no conflict of interest.

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References

Abdoli, A., Barati, M., Dalimi, A., Pirestani, M., hoseini shokouh, s.j., 2016. Toxoplasmosis Among Patients with Immunocompromising Conditions: A Snapshot. J Arch Mil Med 4, e41832.

Ahmed, H., Malik, A., Arshad, M., Mustafa, I., Khan, M.R., Afzal, M.S., *et al.*, 2016. Seroprevalence and Spatial Distribution of Toxoplasmosis in Sheep and Goats in North-Eastern Region of Pakistan. Korean J Parasitol 54, 439-446.

Al Hamada, A., Habib, I., Barnes, A., Robertson, I., 2019. Risk factors associated with seropositivity to Toxoplasma among sheep and goats in Northern Iraq. Vet Parasitol Reg Stud Reports 15, 100264.

Balsari, A., Poli, G., Molina, V., Dovis, M., Petruzzelli, E., Boniolo, A., *et al.*, 1980. ELISA for toxoplasma antibody detection: a comparison with other serodiagnostic tests. J Clin Pathol 33, 640-643.

Dubey, J., 2009. Toxoplasmosis in sheep—the last 20 years. Vet Parasitol 163, 1-14.

Edwards, J.F., Dubey, J., 2013. Toxoplasma gondii abortion storm in sheep on a Texas farm and isolation of mouse virulent atypical genotype T. gondii from an aborted lamb from a chronically infected ewe. Vet Parasitol 192, 129-136.

Foroutan-Rad, M., Khademvatan, S., Majidiani, H., Aryamand, S., Rahim, F., Malehi, A.S., 2016. Seroprevalence of Toxoplasma gondii in the Iranian pregnant women: A systematic review and meta-analysis. Acta tropica 158, 160-169.

Gazzonis, A.L., Veronesi, F., Di Cerbo, A.R., Zanzani, S.A., Molineri, G., Moretta, I., *et al.*, 2015. Toxoplasma gondii in small ruminants in Northern Italy - prevalence and risk factors. Ann Agric Environ Med 22, 62-68.

Guo, M., Mishra, A., Buchanan, R.L., Dubey, J.P., Hill, D.E., Gamble, H.R., *et al.*, 2016. A Systematic Meta-Analysis of Toxoplasma gondii Prevalence in Food Animals in the United States. Foodborne Pathog Dis 13, 109-118.

Hammond-Aryee, K., van Helden, L.S., van Helden, P.D., 2015. The prevalence of antibodies to Toxoplasma gondii in sheep in the Western Cape, South Africa. Onderstepoort J Vet Res 82, 01-05.

Havakhah, Y., Esmaeili Rastaghi, A.R., Amiri, S., Babaie, J., Aghighi, Z., Golkar, M., 2014. Prevalence of Toxoplasma gondii in Sheep and Goats in Three Counties of Gilan Province, North of Iran the More Humid Climate the Higher Prevalence. J Med Microbiol Infect Dis 2, 80-83.

Hecker, Y.P., Masson, F.M., Armendano, J.I., Cora, J., Olivares, C.F., Gual, I., *et al.*, 2018. Evaluation of frequency of antibodies against Toxoplasma gondii,Neospora caninum and Sarcocystis spp. and transmission routes in sheep from Humid Pampa, Argentina. Acta parasitologica 63, 416-421.

Hernández-Cortazar, I., Acosta-Viana, K.Y., Ortega-Pacheco, A., Guzman-Marin, E.d.S., Aguilar-Caballero, A.J., Jiménez-Coello, M., 2015. Toxoplasmosis in Mexico: epidemiological situation in humans and animals. Rev Inst Med Trop Sao Paulo 57, 93-103.

Izadyar, N., Nikfarjam, B.A., Rastaghi, A.R.E., Alizadeh, S.A., Heydarian, P., Saraei, M., 2019. A serologic study on Toxoplasma gondii infection in slaughtered sheep and goats in Qazvin Province, Iran. Trop Anim Health Prod 51, 1289-1293.

Katzer, F., Brülisauer, F., Collantes-Fernández, E., Bartley, P.M., Burrells, A., Gunn, G., *et al.*, 2011. Increased Toxoplasma gondii positivity relative to age in 125 Scottish sheep flocks; evidence of frequent acquired infection. Vet Res 42, 121-121.

Khezri, M., Mohammadian, B., Esmailnia, K., Khezri, O., 2012. Toxoplasmosis in sheep from Kurdistan province, Iran. Afr J Microbiol Res 6, 3989-3992.

Lopes, A.P., Dubey, J.P., Neto, F., Rodrigues, A., Martins, T., Rodrigues, M., *et al.*, 2013. Seroprevalence of Toxoplasma gondii infection in cattle, sheep, goats and pigs from the North of Portugal for human consumption. Vet Parasitol 193, 266-269.

Rahman, M., Azad, M.T., Nahar, L., Rouf, S.M., Ohya, K., Chiou, S.P., *et al.*, 2014. Age-specificity of Toxoplasma gondii seroprevalence in sheep, goats and cattle on subsistence farms in Bangladesh. J Vet Med Sci 76, 1257-1259.

Rasti, S., Marandi, N., Abdoli, A., Delavari, M., Mousavi, S.G.A., 2018. Serological and molecular detection of Toxoplasma gondii in sheep and goats in Kashan, Central Iran. J Food Saf 38, e12425.

Rêgo, W.M.F., Paula, N.R.O., Vitor, R.W.A., Silva, R.A.B., Diniz, B.L.M., Sousa, M.M., *et al.*, 2016. Risk factors for Toxoplasma gondii infection in goats and sheep raised in the State of Piauí in northeast Brazil. Small Rumin Res 141, 17-23.

Rostami, A., Seyyedtabaei, S.J., Aghamolaie, S., Behniafar, H., Lasjerdi, Z., Abdolrasouli, A., *et al.*, 2016. SEROPREVALENCE AND RISK FACTORS ASSOCIATED WITH Toxoplasma gondii INFECTION AMONG RURAL COMMUNITIES IN NORTHERN IRAN. Rev Inst Med Trop Sao Paulo 58, 70-70.

Schluter, D., Daubener, W., Schares, G., Gross, U., Pleyer, U., Luder, C., 2014. Animals are key to human toxoplasmosis. Int J Med Microbiol 304, 917-929.

Sharif, M., Gholami, S., Ziaei, H., Daryani, A., Laktarashi, B., Ziapour, S., *et al.*, 2007. Seroprevalence of Toxoplasma gondii in cattle, sheep and goats slaughtered for food in Mazandaran province, Iran, during 2005. Vet J 174, 422-424.

Sharif, M., Sarvi, S., Shokri, A., Hosseini Teshnizi, S., Rahimi, M.T., Mizani, A., *et al.*, 2015. Toxoplasma gondii infection among sheep and goats in Iran: a systematic review and meta-analysis. Parasitol Res 114, 1-16.

Subedi, S., Sharma, B., Singh, S., Bindari, Y.R., 2018. Sero-prevalence of Toxoplasma gondii in sheep in different geographical regions of Nepal. Vet Anim Sci 5, 7-9.

Yang, Y., Feng, Y., Yao, Q., Wang, Y., Lu, Y., Liang, H., *et al.*, 2017. Seroprevalence, Isolation, Genotyping, and Pathogenicity of Toxoplasma gondii Strains from Sheep in China. Front Microbiol 8, 136-136.

Zhang, N., Wang, S., Wang, D., Li, C., Zhang, Z., Yao, Z., *et al.*, 2016. Seroprevalence of Toxoplasma gondii infection and risk factors in domestic sheep in Henan province, central China. Parasite 23, 53.