Archives of Razi Institute, Vol. 73, No. 4 (2018) 305-310

Original Article

Seroprevalence and Risk Factors of *Neospora caninum* and *Toxoplasma gondii* in Small Ruminants in Southwest of Iran

Gharekhani [1, 2,*], J., Yakhchali [1], M., Esmaeilnejad [1], B., Mardani [3], K., Majidi [4], G., Sohrabi [1,4], A., Berahmat [6], R., Hazhir Alaei [6], M.

1. Department of Pathobiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran
2. Department of Laboratory Sciences, Central Veterinary Laboratory, Iranian Veterinary Organization, Hamedan, Iran
3. Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran
4. Iranian Veterinary Organization, Hamedan, Iran
5. Department of Parasitology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
6. Department of Surgery and Diagnostic Imaging, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran

Received 13 March 2017; Accepted 23 September 2017
Corresponding Author: Gharekhani_76@yahoo.com

ABSTRACT

Toxoplasma gondii and *Neospora caninum* are Apicomplexan intracellular protozoa with global distribution. Small ruminants play an important role as intermediate hosts for *N. caninum* and *T. gondii*, parasites of great public health concern. The main goal of the current survey was to evaluate *N. caninum* and *T. gondii* infection rate in sheep and goats of Khuzestan Province, southwest of Iran, using enzyme-linked immunosorbent assay (ELISA). In this cross-sectional study during February-April 2016, whole blood samples were taken randomly from 735 animals from 37 herds. The animals were reared under the traditional husbandry system in different parts of the province. Among 550 sheep and 185 goats, 37 (6.8%) sheep and 20 (10.8%) goats were seropositive for *N. caninum* and 59 (10.8%) sheep and 37 (20%) goats were seropositive for *T. gondii*. The incidence rates of mixed infection with *N. caninum* and *T. gondii* were 3.2% and 5.4% in sheep and goats, respectively. Seroprevalence rate of *N. caninum* was significantly higher in goats at <1 years of age. There was a significant association between the number of sheep infected with *T. gondii* and abortion (18.2%). Also, a significant correlation was detected between seroprevalence of *N. caninum* and *T. gondii* and mixed infection in goats with a history of abortion. This is the first report of IgG antibody production against *N. caninum* and *T. gondii* co-infection in small ruminants in Iran. Our findings indicated that neosporosis and toxoplasmosis may be responsible for abortion in small ruminants in this region. Therefore, further investigations are needed to improve sanitary strategies in animals' husbandry and launching control programs.

Keywords: *Neospora caninum, Toxoplasma gondii,* Sheep, Goats, Iran

Séroprévalence et Facteurs de Risque de *Neospora caninum* et de *Toxoplasma gondii* chez les Petits Ruminants du Sud-ouest de l'Iran

Résumé: *Toxoplasma gondii* et *Neospora caninum* sont des protozoaires intracellulaires apicomplexes de répartition mondiale. Les petits ruminants jouent un rôle important en tant qu'hôtes intermédiaires de *N. caninum* et de *T. gondii*, parasites extrêmement préoccupants pour la santé publique. L'enquête en cours avait pour objectif principal d'évaluer le taux d'infection par *N. caninum* et *T. gondii* chez les moutons et les chèvres de la province du Khuzestan, au sud-ouest de l'Iran, utilisant un dosage immuno-enzymatique (ELISA). Dans cette étude coupe transversale menée entre février et avril 2016, des échantillons de sang total ont été prélevés au hasard chez 735 animaux de 37 troupeaux. Les animaux ont été élevés selon le système d'élevage traditionnel dans différentes parties de la province. Parmi 550 moutons et 185 chèvres, 37 (6,8%) moutons et 20 (10,8%)

chèvres étaient séropositives pour *N. caninum* et 59 (10,8%) moutons et 37 (20%) chèvres étaient séropositives pour *T. gondii*. Les taux d'incidence des infections mixtes à *N. caninum* et à *T. gondii* étaient respectivement de 3,2% et 5,4% chez les moutons et les chèvres.Le taux de séroprévalence de *N. caninum* était significativement plus élevé chez les chèvres âgés de moins de 1 an. Il y avait une association significative entre le nombre de moutons infectés par *T. gondii* et l'avortement (18,2%). En outre, une corrélation significative a été détectée entre la séroprévalence de *N. caninum* et *T. gondii* et l'infection mixte chez les chèvres ayant des antécédents d'avortement. Il s'agit du premier rapport sur la production d'anticorps IgG contre la co-infection par *N. caninum* et *T. gondii* chez de petits ruminants en Iran. Nos résultats ont indiqué que la néosporose et la toxoplasmose pourraient être responsables de l'avortement chez les petits ruminants de cette région. Par conséquent, de nouvelles enquêtes sont nécessaires pour améliorer les stratégies sanitaires dans l'élevage des animaux et le lancement de programmes de contrôle.

Mots-clés: *Neospora caninum*, *Toxoplasma gondii*, Mouton, Chèvre, Iran

INTRODUCTION

Toxoplasma gondii and *Neospora caninum* are Apicomplexan intracellular protozoa with global distribution (Dubey et al., 2007; Dubey, 2009). Oocyst shedding and congenital transmission of *T. gondii* and *N. caninum* are the major risk factors for spreading these parasites (Dubey et al., 2007). *Toxoplasma gondii* was reported by Nicole and Manceaux (1908) in a hamster-like rodent in Tunis. All felids and canids play a role as final hosts for *T. gondii* and *N. caninum*, respectively. Humans and a wide-range of animals including small ruminants are the intermediate hosts for *T. gondii* (Dubey and Schares, 2011). Toxoplasmosis may cause early embryonic problems, including death, mummification, abortion, and stillbirth (Dubey, 2009). The first report of *N. caninum* was in puppies with congenital encephalomyelitis from Norway in 1984 (Bjerkas et al., 1984). *N. caninum* is a major cause of abortion in cattle and neuromuscular disorders in infected dogs (Dubey et al., 2007). In previous serological studies from Iran, *N. caninum* infection rate was reported 1.5-5.7% in sheep, 6.2% in goats, and 10.6-33% in dogs (Yakhchali et al., 2010; Gharekhani et al., 2014; Gharekhani et al., 2016). Also, *T. gondii* infection was detected in 3.1-72.6% of sheep and 10.6-30% of goats (Sharif et al., 2007). The role of *N. caninum* as a zoonotic agent is still ambiguous (Dubey et al., 2007; Dubey and Schares, 2011). Toxoplasmosis is a common disease in a wide-range of animals, such as livestock (Dubey, 2009). In Iran, toxoplasmosis in sheep and goats plays a potential role in the transmission of the disease to humans through consumption of contaminated meat with *T. gondii* cysts (Sharif et al., 2007). Several laboratory methods, including histopathology, serology, and molecular procedures, have been applied for the detection of *N. caninum* and *T. gondii* infections in animals. Of these, serological examination is an adequate method for epidemiological investigations. Among serological methods, the enzyme-linked immunosorbent assay (ELISA) has the highest sensitivity and specificity for the detection of *N. caninum* and *T. gondii* infections (Dubey et al., 2007; Dubey and Schares, 2011). The present study was aimed to evaluate *N. caninum* and *T. gondii* infection rates in sheep and goats of Khuzestan Province, southwest of Iran.

MATERIAL AND METHODS

Field of study. Khuzestan Province is located in southwest Iran (29°57'N and 47°40'E, 18 m above sea level) with a great potential for agricultural and husbandry expansion. The average maximum and minimum temperature in this region is 30°C and 2°C, respectively; also the average annual rainfall is 240 mm (report of Iranian Aerology Organization). According to the Iranian Veterinary Organization, an average population of 2,600,000 sheep and 1,300,000 goats are reared in the region.

Animal sampling. In this cross-sectional study during February-April 2016, whole blood samples (5 ml by jugular venipuncture) were taken randomly from 550 sheep and 185 goats from 37 herds (Holt and Thrusfield, 1997). The animals were reared under the traditional husbandry methods in different parts of the province. At the beginning of the investigation, sex, age, and history of abortion in female animals were recorded (Table 1).

Serological examination. The sera were separated by centrifuging at 1000 × g for 10 min and stored at -20 °C until laboratory evaluation.

The sera were examined to detect IgG antibody against *Neospora* and *Toxoplasma* using commercially available ELISA kit (ID Screen® Neosporosis and Toxoplasmosis indirect multi-species; ID-Vet company, France) according to the instructions.

Table 1. Seroprevalence and associated risk factors for *N. caninum* and *T. gondii* in sheep and goats of Khuzestan Province, southwest of Iran

Animals	**Risk factors**	**N (%)**	***N. caninum***		***T. gondii***		***MI***	
			NP (%)	**P-value**	**NP (%)**	**P-value**	**NP (%)**	**P-value**
Sheep	sex:							
	M	163(29.4)	10(6.1)	0.807	14(8.6)	0.349	6(3.7)	0.911
	F	392(70.6)	28(7.1)		46(11.7)		12(3.1)	
	age (year):							
	<1	55(9.9)	5(9.1)		6(10.9)		3(5.5)	
	1-2	69(12.4)	6(8.7)	0.597	8(11.6)	0.974	3(4.3)	0.493
	>2	431(77.7)	27(6.3)		46(10.7)		12(2.8)	
	abortion:							
	+	110(28.1)	12(10.9)	0.094	20(18.2)	0.009#	5(4.5)	0.373
	-	282(71.9)	26(5.8)		40(9)		13(2.9)	
	herd size:							
	<250	180(32.4)	22(12.2)	<0.0001	31(17.2)	0.001	15(8.3)	<0.0001
	250-500	240(43.2)	4(1.7)		23(9.6)		1(0.4)	
	>500	135(24.3)	12(8.9)		6(4.4)		2(1.5)	
	Total	555(100)	38(6.8) CI 95%=4.8-8.8		60(10.8) CI 95%=8.2-13.4		18(3.2) CI 95%=1.8-4.6	
Goats	sex:							
	M	30(16.2)	5(16.7)	0.330	6(20)	1.000	1(3.3)	1.000
	F	155(83.8)	15(9.7)		31(20)		9(5.8)	
	age (year):							
	<1	27(14.6)	7(25.9)		7(25.9)		2(7.4)	
	1-2	38(20.5)	6(15.8)	0.005	6(15.8)	0.602	2(5.3)	0.882
	>2	120(64.9)	7(5.8)		24(20)		6(5)	
	abortion:							
	+	28(18.1)	7(25)	0.022*	14(50)	<0.0001**	6(21.4)	0.001***
	-	127(81.9)	13(8.3)		23(14.6)		4(2.5)	
	herd size:							
	<250	60(32.4)	12(20)	0.016	15(25)	0.456	6(10)	0.150
	250-500	80(43.2)	4(5)		15(18.8)		3(3.8)	
	>500	45(24.3)	4(8.9)		7(15.6)		1(2.2)	
	Total	185(100)	20(10.8) CI 95%=6.4-15.2		37(20) CI 95%=14.3-25.7		10(5.4) CI 95%=2.2-8.6	
Total		740(100)	58(7.8)		97(13.1)		28(3.8)	

F: Female, M: Male, MI: Mixed infection, N=Number of examined animals, NP: Number of seropositive, OR (CI=95%): *OR=3.69 (1.32-10.31), **OR=5.83 (2.46-13.81), ***OR=10.43 (2.73-39.91), #OR=2.25 (1.26-4.03)

Statistical evaluation. Statistical analysis was performed using Chi-square test (χ^2) with confidence interval (CI) of 95% in SPSS version 16. *P*-value less than 0.05 was considered significant.

RESULTS

After serological screening, 37 (6.8%) sheep and 20 (10.8%) goats were positive for *N. caninum* and 59 (10.8%) sheep and 37 (20%) goats were positive for *T. gondii*. The prevalence rates of mixed infection were 3.2% and 5.4% in sheep and goats, respectively. There was a significant difference in *T. gondii* infection between sheep and goats (χ^2=10.287, *P*=0.001). There was no significant difference in *N. caninum* infection between the two examined animal groups (χ^2=3.018, *P*=0.082; Table 1).

The seroprevalence of infection on herd level is presented in Table 2. In sheep, herd size had a significant influence on *N. caninum* (χ^2=19.135, *P*<0.0001), *T. gondii* (χ^2=13.724, *P*=0.001), and mixed infections (χ^2=22.307, *P*<0.0001). Also, the higher seroprevalence rate of *N. caninum* infection was detected in <250 herd size in goats (χ^2=8.228, *P*=0.016). Infections with *N. caninum* and *T. gondii* were not significantly different among animals belonging to different age and sex groups. The incidence rates of abortion in seropositive sheep and goats for *N. caninum* and *T. gondii* were 10.9% and 18.2%, respectively. There was a significant association between seroprevalence of *T. gondii* infection and history of abortion in sheep (χ^2=7.731, *P*=0.009).

Table 2. The seroprevalence of *N. caninum* and *T. gondii* in sheep and goat herds of Khuzestan Province, Iran (N=37)

Herds	No. of seropositive animals (%)		
	N. caninum	*T. gondii*	Mixed infection
Sheep	14 (37.8)	16 (43.2)	8 (21.6)
Goats	12 (32.4)	20 (54.1)	6 (16.2)
Sheep and goats	7 (18.9)	15 (40.5)	3 (8.1)

In goats, a significant association was noted between history of abortion and seroprevalence rates of *N. caninum* (25%, χ^2=6.889,*P*=0.022), *T. gondii* (50%, χ^2=18.559,*P*<0.0001), and both parasites simultaneously (21.4%, χ^2=16.567, *P*<0.001). The prevalence rates of mixed infection with *N. caninum* and *T. gondii* was 3.2% and 5.4% in sheep and goats, respectively. All the animals were of Mehraban race.

DISCUSSION

Information on prevalence of sheep and goat neosporosis is important for implementing effective control programs (Bartova and Sedlak, 2012). In Iran, sheep and goats are mostly reared under the traditional farming system with a history of abortion and direct contact with cats and dogs. Additionally, oocyst-contaminated pastures, fodder, and drinking water are considered as potential sources of postnatal infection in animals (Dubey, 2009). In the present study, the findings revealed the occurrence of antibodies against *N. caninum* and *T. gondii* in examined sheep and goats. In previous studies, the range of *N. caninum* infection in sheep and goats varied from 0.45% (UK) to 63% (North Jordan) and from 0% (Taiwan) to 23.6% (Philippines), respectively (Dubey et al., 2007; Dubey and Schares, 2011). In other studies, the titers of IgG against *T. gondii* in sheep were also reported in Pakistan using Latex Agglutination Test (3%) and Kars using ELISA (95.7%) (Dubey, 2009). The *T. gondii* antibody titers in examined goats were reported between 17% in Norway (Stormoen et al., 2012) and 66% in Czech Republic (Bartova and Sedlak, 2012). The differences in findings may be due to the use of different detection methods, cut-off values, study designs, experimental strategies, climatic variations, history of herd contact with cats, and farm management (Gharekhani et al., 2016). Additionally, the positive seroprevalence against *T. gondii* was much higher in herds with less than 250 sheep and more than 500 goats. Thus, herd size was considered as a risk factor for both protozoa infections (Gharekhani et al., 2016). The highest *N. caninum* antibodies were detected in <1 year age group. However, the occurrence of *N. caninum* infection was significantly varied among different age groups. This finding was in accordance with the results of studies performed in west of Iran (Lorestan province), Brazil, Italy, and Spain (Gaffuri et

al., 2006; Ueno et al., 2009; Panadero et al., 2010; Ezatpour et al., 2012). In contrast, in two recent studies, (Cayvaz and Karatepe, 2011; Ghattof and Faraj, 2015), the highest titers of IgG against *N. caninum* were reported 13.8% in sheep of Iraq and 42.3% in sheep of Turkey with the ages of more than 1 and 3 years old, respectively. In our work, the highest seropositivity against *T. gondii* was found in animals aged under 1 year old. In the studies from China and southeast of Iran, significant differences were reported in sheep with age more than 1 year old (Bahrieni et al., 2008; Zhao et al., 2011). Alvarado-Esquivel et al. (2011) reported that seropositivity against *T. gondii* in goats of Mexico had a significant correlation with increased age. This finding was in agreement with the findings of Gharekhani et al. (2014) indicating that seroprevalence is positively correlated with age and number of gestations. Additionally, age-related differences in the occurrence of *T. gondii* infection may be due to exposure to oocysts for longer periods in older animals (Dubey, 2009). The seroprevalence of *N. caninum* and *T. gondii* antibodies was not different between male and female animals, a finding which was also reported by other researchers (Dubey, 2009; Ghattof and Faraj, 2015; Gharekhani et al., 2016). However, there are a few reports indicating that female animals had significantly different titers against *T. gondii* in comparison with male animals (Dubey, 2009). In contrast, Zhao et al. (2011) noted that occurrence of antibodies against *T. gondii* was significantly higher in male goats (15.7%) than females (14.0%) (P=0.023). These differences may be attributed to hormonal changes, nutrition, age, pregnancy, and environment (Alexander and Stimson, 1988).

In the current study, the rate of abortion in seropositive animals was significantly higher than seronegative ones, except for sheep infected with *N. caninum*. This finding was in accordance with the results of other investigations (Dubey et al., 1996). This is the first report on the detection of IgG production against *N. caninum* and *T. gondii* co-infection in small ruminants in Iran. Our findings indicated that neosporosis and toxoplasmosis may be responsible for abortion in small ruminants in this region. Therefore, further investigations are needed to improve sanitary strategies in animals' husbandry and launching control programs.

Ethics

I hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest

The authors declare that they have no conflict of interest.

Acknowledgment

The authors greatly appreciate staff members of Hamadan Veterinary Office for their cooperation in sampling.

References

Alexander, J., Stimson, W., 1988. Sex hormones and the course of parasitic infection. Parasitol Today 4, 189-193.

Alvarado-Esquivel, C., Garcia-Machado, C., Vitela-Corrales, J., Villena, I., Dubey, J.P., 2011. Seroprevalence of Toxoplasma gondii infection in domestic goats in Durango State, Mexico. Vet Parasitol 183, 43-46.

Bahrieni, M., Harandi, M.F., Beigzadeh, M., Kamyabi, H., Zia-Ali, N., 2008. Risk factors analysis associated with seropositivity to Toxoplasma gondii in sheep and goats in Southeastern Iran Using Modified Agglutination Test (MAT). Iran J Parasitol 3, 38-43.

Bartova, E., Sedlak, K., 2012. Toxoplasma gondii and Neospora caninum antibodies in goats in the Czech Republic. Vet Med 57, 111-114.

Bjerkas, I., Mohn, S.F., Presthus, J., 1984. Unidentified cyst-forming sporozoon causing encephalomyelitis and myositis in dogs. Z Parasitenkd 70, 271-274.

Cayvaz, M., Karatepe, M., 2011. Seroprevalence of Neospora caninum in goats in Niğde province. Kafkas Univ Vet Fak Derg 17, 935-939.

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

Dubey, J.P., Morales, J.A., Villalobos, P., Lindsay, D.S., Blagburn, B.L., Topper, M.J., 1996. Neosporosis-

associated abortion in a dairy goat. J Am Vet Med Assoc 208, 263-265.

Dubey, J.P., Schares, G., 2011. Neosporosis in animals--the last five years. Vet Parasitol 180, 90-108.

Dubey, J.P., Schares, G., Ortega-Mora, L.M., 2007. Epidemiology and control of neosporosis and Neospora caninum. Clin Microbiol Rev 20, 323-367.

Ezatpour, B., Alirezaei, M., Hassanvand, A., Zibaei, M., Azadpour, M., 2012. The first report of Neospora caninum prevalence in aborted and healthy sheep from Lorestan province of Iran. Asian Pac J Trop Biomed 1, 1-3.

Gaffuri, A., Giacometti, M., Tranquillo, V.M., Magnino, S., Cordioli, P., Lanfranchi, P., 2006. Serosurvey of roe deer, chamois and domestic sheep in the central Italian Alps. J Wild Dis 42, 685-690.

Gharekhani, J., Esmaeilnejad, B., Rezaei, H., Yakhchali, M., Heidari, H., Azhari, M., 2016. Prevalence of anti-Neospora caninum antibodies in Iranian goats. Ann Parasitol 62, 111-114.

Gharekhani, J., Tavoosidana, G., Akbarein, H., 2014. Serological study of Neospora caninum infection in dogs and cattle from west of Iran. Comp Clin Path 23, 1203-1207.

Ghattof, H.H., Faraj, A.A., 2015. Seroprevalence of Neospora caninum in goats in Wasit province, Iraq. Int J Curr Microb Appl Sci 4, 182-191.

Holt, P.E., Thrusfield, M.V., 1997. Tail docking in dogs. Aust Vet J 75, 449.

Nicole, C., Manceaux, L., 1908. Sur une infection corps de Leishman (ou organisms voisins) du gondii: on an infection by Leishman bodies in the gondii. Compt Rend Acad de Sci 147, 763-766.

Panadero, R., Painceira, A., Lopez, C., Vazquez, L., Paz, A., Diaz, P., et al., 2010. Seroprevalence of Toxoplasma gondii and Neospora caninum in wild and domestic ruminants sharing pastures in Galicia (Northwest Spain). Res Vet Sci 88, 111-115.

Sharif, M., Gholami, S., Ziaei, H., Daryani, A., Laktarashi, B., Ziapour, S.P., 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.

Stormoen, M., Tharaldsen, J., Hopp, P., 2012. Seroprevalence of Toxoplasma gondii infection in Norwegian dairy goats. Acta Vet Scand 54, 75.

Ueno, T.E.H., Gonçalves, V.S.P., Heinemann, M.B., Dilli, T.L.B., Akimoto, B.M., de Souza, S.L.P., et al., 2009. Prevalence of Toxoplasma gondii and Neospora caninum infections in sheep from Federal District, central region of Brazil. Trop Anim Health Prod 41, 547-552.

Yakhchali, M., Javadi, S., Morshedi, A., 2010. Prevalence of antibodies to Neospora caninum in stray dogs of Urmia, Iran. Parasitol Res 106, 1455-1458.

Zhao, G.H., Zhang, M.T., Lei, L.H., Shang, C.C., Cao, D.Y., Tian, T.T., et al., 2011. Seroprevalence of Toxoplasma gondii infection in dairy goats in Shaanxi Province, Northwestern China. Parasit Vectors 4, 47.