Original Article

Prevalence of *Toxoplasma gondii* in Sheep and Goats in Three Counties of Gilan Province, North of Iran; the More Humid Climate the Higher Prevalence

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Introduction: *Toxoplasma gondii* is one of the most important foodborne pathogens. *Toxoplasmosis* causes abortion and neonatal loss in livestock and imposes significant losses to farming industry. Prevalence of *T. gondii* in sheep and goats was investigated in three adjacent geographical areas within Gilan province in north of Iran. **Methods:** Serum samples were collected from 247 sheep and 155 goats in three counties; Roudsar with humid subtropical climate and average annual precipitation (AAP) of 1400 mm, Masal with sub-humid climate and AAP of 700 mm, and Roudbar with cold semi-arid steppe climate and AAP of 400 mm. The samples were tested for *Toxoplasma* antibodies by using Sabin- Feldman Dye Test. **Results:** The overall prevalence in sheep was significantly higher than goats ($P<0.001$). Moreover, the prevalence in sheep was greatly increased with AAP ($P<0.001$); the prevalence in sheep was 62.2% in Roudsar, 39.3% in Masal and 15% in Roudbar. **Conclusion:** Sheep is the most common source of meat consumed in Iran, as well as many parts of the world, and presents the most danger in foodborne transmission of *T. gondii* to humans. Intensive farm management resulted in decreased prevalence of *Toxoplasma* in poultries and pigs while sheep are raised in small flocks in Iran, as many other developing countries, and extensively exposed to oocysts shed by cats. The information obtained here could have important implications for prevention of *T. gondii* infection in humans as well as reducing the rate of infection, and consequent abortion and neonatal loss in sheep and goats. *J Med Microbiol Infec Dis, 2014, 2 (2): 80-83.*

Keywords: Climate, Iran, Prevalence, Sheep, *Toxoplasma.*

INTRODUCTION

Toxoplasma gondii is a worldwide distributed protozoan parasite that is capable of infecting almost all warm-blooded animals. *T. gondii* is a significant medical problem in pregnancy and immunodeficiency conditions *e.g.,* AIDS, organ transplantation and cancer. It is also a major cause of retinal infection in infected individuals even in immunucompetent adults [1, 2]. Most humans' infections with *T. gondii* are acquired post-natally by consuming undercooked meat containing tissue cysts, food or drink contaminated with oocysts shed by infected cats, or by accidentally ingesting oocysts from the environment [3].

T. gondii is an important zoonotic pathogen and a major cause of reproductive failure associated with abortion and neonate mortality, particularly, in sheep, pigs and goats. These animals are major source of meat in many countries and consequently, are more important in transmission of the infection to humans [3-5].

Diagnosis of *T. gondii* infection is based on serological tests, polymerase chain reaction (PCR), histopathological examination, and isolation of *Toxoplasma* by mouse inoculation [6, 7]. However, most epidemiological studies applied serological tests for evaluating *Toxoplasma* prevalence as they are more convenient to perform and usually present similar performance in comparison to other reference tests. Sabin- Feldman Dye test (DT) is generally regarded as the gold standard for diagnosis of *Toxoplasma* infection, and is one of the most widely used tests for screening *Toxoplasma* infection in ovine and other animals [8, 9].

Prevalence of *T. gondii* infection in livestock varies greatly both within and between countries [3]. The difference in prevalence is related to many factors such as farming operation, environmental humidity and relative distribution of stray cats [3, 4, 10]. Studies showed the prevalence can be as high as 92% and 75% in sheep and goats, respectively [3]. Among livestock, sheep are more affected by congenital *Toxoplasma* infection and consequent abortion and neonate loss [4]. Consumption of undercooked meat, mostly sheep, has made *Toxoplasma* one of the most important foodborne pathogens [11, 12].

Iran is a large country with different climates present across the country. Several studies showed seroprevalence of *Toxoplasma* in human living in north of Iran, with humid sub-tropical climate, was as high as ~70%. However, few studies investigated the prevalence in livestock of this part of country, and no report is available for Gilan province which has the most humid climate. The purpose of the present study was to collect information about prevalence of *Toxolasma* in three adjacent counties of Gilan with great difference in average annual precipitation (AAP); from 400 to 1400 mm.

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The results could have implications for reducing the rate of *Toxoplasma* infection both in ovine and human populations.

MATERIAL AND METHODS

Study area. Three adjacent counties of Gilan province were included in the study; Roudsar with a humid subtropical climate, Masal with a sub-humid climate and Roudbar with a cold semi-arid steppe climate. The AAP is about 1400 mm in Roudsar, 700 mm in Masal and 400 mm in Roudbar.

Serum Samples. Blood samples were obtained from 247 sheep and 155 goats from the three counties. The samples were collected using sterile syringes and tubes, directly from the cervical vein of each animal and transported to the laboratory. Serum samples were extracted from 5 mL blood samples by centrifugation at 2000 g for 10 min, and stored at -20°C prior to testing.

Sabin-Feldman Dye Test. DT is based on methylene blue staining of intact *T. gondii* tachyzoites, whereas parasites killed by complement-mediated lysis do not take up the dye and remain colorless. Live tachyzoites required for the test were prepared from peritoneal fluid of laboratory Swiss mice. The mice were infected with tachyzoites and three days later tachyzoites were collected by repeated flushing of the peritoneal cavity with phosphate-buffered saline (PBS; pH 7.4).

Serum samples were heated at 60°C for 20 min to inactivate preexisting complement, and diluted 1:2 with PBS in a flat-bottomed 96-well plate. Then, live tachyzoites were added, and the plate was incubated for 60 min at 37°C. Methylene blue solution was added to the wells, and the plate was kept in room temperature for 10 min. The DT result was regarded positive if more than 50% of tachyzoites remained unstained under the light microscope at 40 × magnifications.

Statistical analysis. Chi-squared test ($\chi 2$) was used to compare the prevalence among the three counties, and the prevalence in sheep and goats in each county. The differences were considered statistically significant when probability *P*-value was <0.05. The 95% confidence intervals (95% CI) of seroprevalence rates were calculated. Statistical analysis was performed using IBM® SPSS software version 17.

RESULTS

We performed DT on serum samples of 247 sheep and 155 goats collected from three adjacent counties of Gilan province in north of Iran with different climates and annual precipitation. The overall prevalence in sheep and goats was 36.8 and 12.9%, respectively, and showed statistically significant difference (P<0.001) (Table 1). The prevalence in sheep was significantly (P<0.001) higher than goats (62.2% vs. 18.2%) in Roudsar, with AAP of 1400 mm, but the difference (15% vs. 10%) was not significant (P=0.35) in Roudbar, with AAP of 400 mm. No goat sample was collected from Masal County (Table 1).

Interestingly, the prevalence in sheep was greatly different among the three counties, and increased with the AAP (P<0.001); the prevalence in sheep was 62.2% in Roudsar (AAP of 1400 mm), 39% in Masal (AAP of 700 mm) and 15% in Roudbar (AAP of 400 mm). Similarly, the prevalence in goats from Roudsar was significantly (P<0.05) higher than goats from Roudbar (18.2% vs. 10%) (Table 1).

Table 1. Seroprevalence of *T. gondii* infection in sheep and goats in three adjacent counties of Gilan province, north of Iran

County	Climate / AAP	Species	No. of animals tested	No. of positive animals	Prevalence % (95% CI)
Roudsar	Humid subtropical climate / 1400 mm[1]	sheep[2]	37	23	62.2% (45.9-76.69%)
		goat	55	10	18.2% (9.6-30.0%)
Masal	Sub-humid climate / 700 mm	sheep	150	59	39.3% (31.7-47.3%)
Roudbar	Cold semi-arid steppe climate / 400 mm[3]	sheep	60	9.0	15% (7.6-25.8%)
		goat	100	10.0	10% (5.2-17.1%)
Total		sheep[4]	247	91.0	36.8% (31.00-43.00%)
		goat	155	20.0	12.9% (8.3%-18.9%)

[1] Significant difference (P<0.001 for sheep and P<0.05 for goats) between the seroprevalence in the three counties with different climate and AAP

[2] Significantly (P<0.001) higher seroprevalence in sheep than in goats in Roudsar county

[3] No significant difference (P=0.35) between seroprevalence in sheep and goats in Roudbar county

[4] Significantly (P<0.001) higher overall prevalence in sheep than in goats

DISCUSSION

Toxoplasmosis is a global medical problem and causes significant economic and public health burden [12-14]. *T. gondii* was ranked 3 among 14 Foodborne Pathogens in the United States, regarding to the estimated Quality-Adjusted Life Years (QALYs) loss [12]. *Toxoplasma*, together with *salmonella* and *listeria*, were reportedly responsible for about 75% of all deaths due to foodborne disease [12]. Moreover, the estimated cost of illness for *T. gondii* was about 3 billion dollars which accounts for about 20% of all costs imposed by 14 foodborne pathogens in the USA [12]. Clinical importance of *Toxoplasma* infection has been increased as recent publications have linked suicide and schizophrenia to *Toxoplasma* infection [15, 16].

Congenital *Toxoplasmosis* in farm animals, particularly in sheep, may result in abortion and neonatal mortality. In most countries, *Toxoplasma* comes as the second in prevalence after Chlamydial abortion; and *Toxoplasma* DNA was detected in up to 23% of aborted sheep fetuses [17]. A UK survey showed *Toxoplasmosis* in sheep is responsible for 1- 2% of neonatal losses per annum. Importantly, despite the previous view, some recent studies suggested that about 4%

of sheep with persistent *Toxoplasma* infection may transmit infection to the fetus in subsequent pregnancies [4, 18].

Infected cats, as the definitive host of *T. gondii* [18], shed millions of oocysts in their faces. Ingestion of oocysts, present in contaminated soil and water, is one of the main routes of infection in humans and other animals. Consumption of undercooked meat is also considered a main way of infection. While epidemiological importance of each of these routes of infection might differ among different parts of the world, it seems that both routes significantly contribute to transmission of the infection in most areas [19, 20].

Prevalence of *Toxoplasma* in sheep varied in different parts of Iran. Interestingly, the prevalence has not seemingly decreased during the past decades in Iran [21-23], presumably due to traditional farming industry and large distribution of stray cats. The present study was conducted to evaluate prevalence of infection in sheep and goats from three adjacent counties of Gilan province located in north of Iran. Our results showed the overall prevalence was significantly higher in sheep (P<0.001), as it was the case in animals from Roudsar County with AAP of 1400 mm. While the difference in animals from Roudbar (with a cold semi-arid steppe climate and AAP of 400 mm) was not significant (15% in sheep and 10% in goats) (P=0.35), it might be due to the low prevalence of *Toxoplasma* in this area. The possible reason for higher prevalence in sheep is that sheep crop plants very close to the ground, and have more chance to ingest oocysts shed by cats. In addition, we observed that the prevalence in sheep and goats was significantly increased according to AAP (P<0.001 and P<0.05, respectively). Our results are in agreement with the previous studies showing higher prevalence in more humid climates [10, 24-28]. Sharif *et al.* reported higher prevalence of *Toxoplasma* infection in sheep from western part of Mazandaran province, north of Iran, which has a more humid climate, compared to central and eastern parts of the province. The highest prevalence observed in the present study, 62.2% in sheep from Roudsar, with a humid climate and AAP of 1400 mm, is in accordance with the previous studies reporting high prevalence of 72.6% [29], 59% [30] and 56.8% [31] in sheep in Iran.

In the present study, stray cats are largely distributed in the three counties and farming operation is also quite similar *i.e.* animals are traditionally raised in small flocks. However, it seems that higher environmental humidity favors sporulation and survival of oocysts, thereby increasing the chance of infection, in counties with higher AAP. In fact, the prevalence in humans in Gilan and Mazandaran provinces, with humid sub-tropical climate, is as high as 70% which is much higher than other parts of the country [22].

There is no data available regarding *Toxoplasma*-induced abortion and neonatal loss in sheep and goats In Iran; however, it was shown that the prevalence was higher in sheep experienced abortion**.** As it was reported in other countries [4], *Toxoplasmosis* can impose significant losses to farming industry of Iran, particularly in region with high prevalence.

We speculate that consumption of undercooked sheep meat is responsible for most foodborne (meat) *Toxoplasmosis* in Iran as well as most countries in the world, since; 1) Pork is not consumed in many countries *e.g.*, Muslim countries or is not the main source of meat, 2) Intensive farm management *e.g.*, confined feeding operations has vastly decreased the rate of infection in pigs and poultries, particularly in developed countries. In contrast, sheep are raised mostly in small flocks, especially in developing countries and are exposed to large amounts of oocysts, and 3) infection rate of other meat-producing animals *e.g.*, goat and cattle is less than sheep. Nevertheless, the risk of infection through consumption of goat products (meat and milk), and backyard-raised chickens probably remains significant in many countries. Regarding the non-decreasing prevalence of *Toxoplasma* infection in sheep in Iran, and perhaps many developing countries, our efforts should be focused on alarming health authorities to implement preventive measure to reduce the risk of infection through consuming undercooked sheep meat. Finally, despite the moderate to high prevalence of *Toxoplasma* in Iran, no information regarding economic and health burden of the disease in the country. Availability of such information could result in development of national guideline for prevention, diagnosis, and treatment of the disease.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

REFERENCES

1. Jones JL, Parise ME, Fiore AE. Neglected parasitic infections in the United States: *toxoplasmosis.* Am J Trop Med Hyg. 2014; 90 (5): 794-9.

2. Commodaro AG, Belfort RN, Rizzo LV, Muccioli C, Silveira C, Burnier Jr MN, Belfort Jr R. Ocular *toxoplasmosis:* an update and review of the literature. Mem Inst Oswaldo Cruz. 2009; 104 (2): 345-50.

3. Tenter AM, Heckeroth AR, Weiss LM. *Toxoplasma gondii:* from animals to humans. Int J Parasitol. 2000; 30 (12-13): 1217-58.

4. Weiss LM, Dubey JP. *Toxoplasmosis:* A history of clinical observations. Int J Parasitol. 2009; 39 (8): 895-901.

5. Dubey JP. *Toxoplasmosis* in sheep--the last 20 years. Vet Parasitol. 2009; 163 (1-2): 1-14.

6. Saadatnia G, Mohamed Z, Ghaffarifar F, Osman E, Moghadam ZK, Noordin R. *Toxoplasma gondii* excretory secretory antigenic proteins of diagnostic potential. APMIS. 2012; 120 (1): 47-55.

7. Montoya JG. Laboratory diagnosis of *Toxoplasma gondii* infection and *toxoplasmosis.* J Infect Dis. 2002; 185 Suppl 1: S73-82.

8. Shaapan RM, El-Nawawi FA, Tawfik MA. Sensitivity and specificity of various serological tests for the detection of

Toxoplasma gondii infection in naturally infected sheep. Vet Parasitol. 2008; 153 (3-4): 359-62.

9. Reiter-Owona I, Petersen E, Joynson D, Aspöck H, Dardé ML, Disko R, Dreazen O, Dumon H, Grillo R, Gross U, Hayde M, Holliman R, et al. The past and present role of the Sabin-Feldman dye test in the serodiagnosis of *toxoplasmosis*. Bull World Health Organ. 1999; 77 (11): 929-35.

10. Dubey JP, Jones JL. *Toxoplasma gondii* infection in humans and animals in the United States. Int J Parasitol. 2008; 38 (11): 1257-78.

11. Boyer KM, Holfels E, Roizen N, Swisher C, Mack D, Remington J, Withers S, Meier P, McLeod R; Toxoplasmosis Study Group. Risk factors for *Toxoplasma gondii* infection in mothers of infants with congenital *toxoplasmosis:* Implications for prenatal management and screening. Am J Obstet Gynecol. 2005; 192 (2): 564-71.

12. Batz MB, Hoffmann S, Morris JG Jr. Ranking the disease burden of 14 pathogens in food sources in the United States using attribution data from outbreak investigations and expert elicitation. J Food Prot. 2012; 75 (7): 1278-91.

13. Torgerson PR, Mastroiacovo P. The global burden of congenital *toxoplasmosis:* a systematic review. Bull World Health Organ. 2013; 91 (7): 501-8.

14. Stillwaggon E, Carrier CS, Sautter M, McLeod R. Maternal serologic screening to prevent congenital *toxoplasmosis:* a decision-analytic economic model. PLoS Negl Trop Dis. 2011; 5 (9): e1333.

15. Pedersen MG, Mortensen PB, Norgaard-Pedersen B, Postolache TT. *Toxoplasma gondii* infection and self-directed violence in mothers. Arch Gen Psychiatry. 2012; 69 (11): 1123-30.

16. Torrey EF, Bartko JJ, Yolken RH. *Toxoplasma gondii* and other risk factors for schizophrenia: an update. Schizophr Bull. 2012; 38 (3): 642-7.

17. Pereira-Bueno J, Quintanilla-Gozalo A, Pérez-Pérez V, Alvarez-García G, Collantes-Fernández E, Ortega-Mora LM. Evaluation of ovine abortion associated with *Toxoplasma gondii* in Spain by different diagnostic techniques. Vet Parasitol. 2004; 121 (1-2): 33-43.

18. Buxton D, Maley SW, Wright SE, Rodger S, Bartley P, Innes EA. *Toxoplasma gondii* and ovine *toxoplasmosis:* new aspects of an old story. Vet Parasitol. 2007; 149 (1-2): 25-8.

19. Tenter AM. *Toxoplasma gondii* in animals used for human consumption. Mem Inst Oswaldo Cruz. 2009; 104 (2): 364-9.

20. Petersen E, Vesco G, Villari S, Buffolano W. What do we know about risk factors for infection in humans with *Toxoplasma gondii* and how can we prevent infections?. Zoonoses Public Health. 2010; 57 (1): 8-17.

21. Ghorbani M, Hafizi A, Shegerfcar MT, Rezaian M, Nadim A, Anwar M, Afshar A. Animal *toxoplasmosis* in Iran. J Trop Med Hyg. 1983; 86 (2): 73-6.

22. Mostafavi SN, Jalali Monfared L. *Toxoplasmosis* Epidemiology in Iran: A Systematic Review. Journal of Isfahan Medical School. 2012; 30 (176) [In Persian].

23. Babaie J, Amiri S, Mostafavi E, Hassan N, Lotfi P, Esmaeili Rastaghi AR, Golkar M. Seroprevalence and risk factors for *Toxoplasma gondii* infection among pregnant women in Northeast Iran. Clin Vaccine Immunol. 2013; 20 (11): 1771-3.

24. Alvarado-Esquivel C, Estrada-Malacón MA, Reyes-Hernández SO, Pérez-Ramírez JA, Trujillo-López JI, Villena I, Dubey JP. Seroprevalence of *Toxoplasma gondii* in domestic sheep in Oaxaca State, Mexico. J Parasitol. 2013; 99 (1): 151-2.

25. Assmar M, Amirkhani A, Piazak N, Hovanesian A, Kooloobandi A, Etessami R. [*Toxoplasmosis* in Iran. Results of a seroepidemiological study]. Bull Soc Pathol Exot. 1997; 90 (1): 19-21.

26. Sharif M, Gholami Sh, Ziaei H, Daryani A, Laktarashi B, Ziapour SP, Rafiei A, Vahedi M. Seroprevalence of *Toxoplasma gondii* in cattle, sheep and goats slaughtered for food in Mazandaran province, Iran, during 2005. Vet J. 2007; 174 (2): 422-4.

27. Caballero-Ortega H, Palma JM, García-Márquez LJ, Gildo-Cárdenas A, Correa D. Frequency and risk factors for *toxoplasmosis* in ovines of various regions of the State of Colima, Mexico. Parasitology. 2008; 135 (12): 1385-9.

28. Sharif M, Sarvi Sh, Shokri A, Hosseini Teshnizi S, Rahimi MT, Mizani A, Ahmadpour E, Daryani A. *Toxoplasma gondii* infection among sheep and goats in Iran: A systematic review and meta-analysis. Parasitol Res. 2014; 114 (1): 1-16.

29. Hamidinejat H, Goraninejat S, Ghorbanpoor M, Nabavi L, Akbarnejat F. Role of *Toxoplasma gondii* in abortion of ewes in Ahvaz (South-West Iran). Bull Vet Inst Pulawy. 2008; 52 (3): 369-71.

30. Keshavarz H, Mohebali M, Shahnazi V, Zareei Z. Frequency of *Toxoplasma* Infection in Livestock of Meshkin Shahr, by Immuno Fluorescent Antibody Test and it's Health Importance. Med J Tabriz Uni Med Sci. 2007; 29 (2): 115-8.

31. Khanmohammadi M. Seroprevalence Survey of *Toxoplasma gondii* Antibodies among Sheep in Tabriz District, Northwest Iran. Scholars Research Library, Annals of Biological Research. 2011; 2 (6): 484-8.