Compr Health Biomed Stud. 2023 July; 2(1): e146897. https://doi.org/10.5812/chbs-146897.

Published online: 2024 June 12. **Research Article**

The Seroprevalence and Risk Factors of *Toxoplasma gondii* Infection in Sheep in Kohgiluyeh and Boyer-Ahmad Province, Southwest Iran

Marziye Pashmforosh [1], Masoud Foroutan [2], Somayeh Haghi Karamallah [3], Ali Jamshidi [1], Mohamad Sabaghan [1,*]

[1] Behbahan Faculty of Medical Sciences, Behbahan, Iran
[2] Department of Basic Medical Sciences, Faculty of Medicine, Abadan University of Medical Sciences, Abadan, Iran
[3] Student Research Committee, Shiraz University of Medical Sciences, Shiraz, Iran

[*] *Corresponding author:* Behbahan Faculty of Medical Sciences, Behbahan, Iran. Email: sabaghan.m@ajums.ac.ir

Received 2024 March 17; **Revised** 2024 April 28; **Accepted** 2024 May 19.

Abstract

Background: Toxoplasmosis is a zoonotic parasitic disease that affects many different species, including humans. It can cause serious clinical symptoms, including neurological symptoms and abortion. *Toxoplasma gondii*, the causative agent, is particularly concerning due to sheep's extraordinary sensitivity to the disease. It can spread zoonotically through the ingestion of undercooked meat from infected animals. Numerous global investigations have demonstrated that *T. gondii* seroprevalence in sheep can vary greatly based on biological and environmental characteristics.

Methods: To examine the biological and environmental factors that could influence the probability of ovine infection, samples were taken from a subset of sheep kept on 20 farms. The modified agglutination test (MAT) was used to detect antibodies in sera samples from 247 sheep in Kohgiluyeh and Boyer-Ahmad Province, Iran.

Results: Sixty of the 247 sera examined (24.3%) contained *T. gondii* antibodies. Among these, 51 (25.4%) of the ewes and 9 (19.6%) of the rams had antibodies against *T. gondii*. Adult sheep (27.3%) had a greater seroprevalence compared to younger sheep (17.3%). Additionally, there were significant seasonal variations, with wet seasons showing a higher prevalence.

Conclusions: Age, cats' access to water sources, the source of drinking water, and the season were all associated with *T. gondii* seroprevalence.

Keywords: Seroprevalence, *Toxoplasma gondii*,Sheep, Risk Factor

1. Background

Nearly all warm-blooded animals worldwide, including humans, are susceptible to infection by the food-borne intracellular parasite *Toxoplasma gondii* (1). The human population has been widely exposed to *T. gondii* to some extent. In particular, parasite infection can result in death or severe damage to the brain and eyes in patients with immune deficiencies (2-4). Humans primarily become infected with *T. gondii* through the consumption of raw meat, water contaminated with oocysts from the environment, or through vertical transmission (5, 6). *Toxoplasma gondii* can also negatively affect animal growth, development, and reproduction, leading to significant financial losses in livestock husbandry. Livestock often become infected by consuming food and water contaminated with sporulated oocysts (7, 8). Sheep are significant intermediate hosts for *T. gondii*, as the parasite cysts remain dormant within their cardiac and skeletal muscles. Consequently, consuming raw or partially cooked meat is one of the main risk factors for *T. gondii* infection (9). *T. gondii* infection in sheep can produce many non-specific symptoms, such as fever, dyspnea, depression, lethargy, vomiting, diarrhea, chorioretinitis, lymphadenopathy, and even miscarriages and stillbirths (10).

Iran is a country in Western Asia with four distinct climate zones: Hot and dry in the central plateau, cold and mountainous in the west and northwest, warm and humid on the southern coast of the Persian Gulf, and temperate and humid on the Caspian Sea coast in the

north. The frequency of *T. gondii* infection in ruminants varies significantly across the country due to these climate differences (11). While *T. gondii* seroprevalence in sheep has been previously reported from Iran (11-13), we provide comprehensive epidemiological information here, including infection risk variables.

2. Objectives

Therefore, the goal of the current investigation is to conduct a seroepidemiological survey for *T. gondii* infection in sheep bred in Iran's Kohgiluyeh and Boyer-Ahmad province. This study is significant because it identifies the key factors that predispose sheep to *T. gondii* infection, and the findings can be used to prevent and control this parasite in sheep.

3. Methods

3.1. Study Area

The study was conducted in the Kohgiluyeh and Boyer-Ahmad province, located in the southwest of Iran. This province is situated between latitudes 30° and 31° N and longitudes 51° and 52° E. With an average annual temperature of 15°C and an annual rainfall of 900 millimeters, Kohgiluyeh and Boyer-Ahmad experience cold weather conditions (Figure 1).

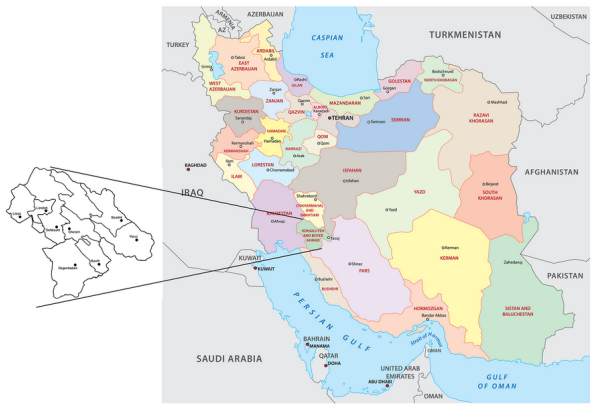

Figure 1. Kohgiluyeh and Boyer-Ahmad province

3.2. Sampling

Over the course of four seasons, blood samples were taken from 247 sheep in 20 selected flocks. Using sterile Venoject tubes, the samples were taken directly from each animal's jugular vein and brought to the laboratory. Priority was given to pregnant female sheep from herds that had previously experienced abortions. Serum samples were separated from 5 mL blood samples using centrifugation at 3000 g for 150 minutes before testing. The samples were then stored at -20°C.

3.3. Determination of Antibodies Against T. gondii

Specific IgG antibodies to *T. gondii* were examined using the Modified Agglutination Test (MAT) as described by Desmonts and Remington (14). The Department of Parasitology at Ahvaz University of Medical Sciences in Iran provided the *T. gondii* RH strain tachyzoites, which were grown in mice in cell culture before being separated and preserved in formaldehyde solution (15). In addition to negative and positive controls, sera were tested on 96-well plates with U-shaped bottoms (25 µL of prepared mix antigen + 25 µL of serum) using two-fold serial dilutions from 1: 10 to 1: 160. An antibody titer of ≥ 1: 20 was deemed positive (13). Every test included both positive and negative controls, which were examined using the identical serum sample dilutions.

3.4. Risk Factors

Two categories of risk factors were examined to correlate the prevalence of *T. gondii* infection with potential risk factors: Those related to the biological traits of the animals (age and sex) and those related to environmental conditions (access of cats to feeding facilities, access of cats to water supplies, season, and source of drinking water) (16).

3.5. Statistical Analysis

The statistical analyses were conducted using SPSS software version 22 in Chicago, IL, USA. The researchers utilized the chi-square test and logistic regression to assess the association between several variables: Age (sub-adult and adult), sex (male vs. female), season (wet vs. dry), and abortion with infection. Differences were considered statistically significant when $P < 0.05$.

4. Results

4.1. Seroprevalence

Sixty of the 247 sera examined (24.3%) contained *T. gondii* antibodies. Among the 247 samples, there were 201 (81.38%) ewes and 46 (18.62%) rams. Table 1 reveals

that 51 (25.4%) of the ewes and 9 (19.6%) of the rams had antibodies against *T. gondii*.

Table 1. Risk Factors Associated with Biological and Environmental Characteristics of Animals

Variables	Positive	Negative	Seroprevalence (%)	P-Value	95% CI	OR
Age						
≤ 1 year	13	62	17.3	0.001	0.17-0.64	0.33
> 1 years	47	125	27.3		1.76-5.11	3.15
Gender						
Female	51	150	25.4	0.052	0.63-3.09	1.40
Male	9	37	19.6		0.32-1.58	0.72
Source of drinking water						
Surface	47	111	29.7	0.0001	1.25-4.89	2.48
Underground	13	76	14.6		0.21-0.79	0.40
Access of cats to water supplies						
Yes	43	78	35.5	0.0001	2.16-7.60	4.05
No	17	125	11.9		0.13-0.46	0.25
Access of cats to feeding facilities						
Yes	27	78	25.7	0.758	0.64-2.05	1.43
No	33	109	23.2		0.49-1.57	0.87
Season						
Wet	36	81	30.7	0.035	1.09-3.55	1.96
Dry	24	106	18.4		0.28-0.92	0.51

4.2. Environmental and Biological Risk Factors

Age, cats' access to water sources, source of drinking water, and season were all associated with risk factors for *T. gondii* seroprevalence (Table 1). Adult sheep had a greater seroprevalence (27.3%) compared to younger sheep (17.3%). Additionally, females had a higher seroprevalence than males. Seasonal variations were also significant, with wet seasons showing higher susceptibility. On 25.7% of sheep farms, cats were seen even in the feed store. The area's cats' freedom of movement also impacted the contamination of animal drinking water, with farms using surface water to water sheep showing a higher seroprevalence (29.7%).

5. Discussion

Toxoplasmosis is a prevalent infectious disease affecting both humans and animals worldwide. It is one of the main reasons why sheep abort and give birth to stillborn offspring (17, 18). Humans can become infected after birth by eating meat containing tissue cysts, drinking water or eating food contaminated with oocysts, or accidentally ingesting oocysts dispersed in the environment. Contaminated lambs are one of the main sources of *T. gondii* infection in humans and carnivorous animals (1, 19). Additionally, *T. gondii* uses these species as intermediate hosts (9).

This study showed that 24.3% of the sheep in Kohgiluyeh and Boyer-Ahmad were positive for *T. gondii*. Several studies on *T. gondii* infection in sheep have been conducted in many countries, and the prevalence in sheep was reported as 51.8% in Egypt (20), 26.2% in Pakistan (21), 36.2% in Nepal (22), 85% in Brazil (23), 59% in Senegal (24), and 53.5% in Romania (25). According to published research, the frequency of *T. gondii* infection among sheep in various regions of Iran ranges from 3.3% to 38.3% (26). Comparing the results of the present study to earlier studies in Iran revealed that the prevalence of our seropositive sheep was similar to other parts (27-29). Variations in prevalence can be explained by factors such as climate, age, animal breeding conditions, and different parasite detection techniques (30). It is widely known that sporulated oocysts can endure for up to 12 months in a favorable environment (humidity and temperature) (31).

Research linking *T. gondii* seropositivity to the biological and environmental traits of animals indicates that age, cats' access to water sources, the source of drinking water, and season can all affect an animal's susceptibility to *T. gondii* infection. High seroprevalence levels have been found in sheep that are female and older than one year. An increased prevalence of *T. gondii* infection in older animals compared to younger ones has been repeatedly documented in earlier research, including studies on Northern Italian sheep, Scottish sheep, sheep in Argentina, and sheep in Iran (30, 32-34). These findings imply that *T. gondii* contamination in sheep occurs primarily after birth and that horizontal contamination is the primary method of infection transmission in herds (19, 34). Additionally, seroprevalence rises with increasing age, which is related to an elevated cumulative risk of exposure to environmental infectious agents (32).

The findings revealed a strong positive association between the prevalence of *T. gondii* antibodies in sheep and the access of cats to water provided for livestock on farms. This suggests that interaction with feline species, specifically through exposure to water contaminated with oocysts from cats, plays a crucial role in the epidemiology of *T. gondii* infection. The presence of cats,

both domestic and stray, on farms appears to be a significant factor contributing to the high incidence of *T. gondii*-specific antibodies in sheep observed in the study. Cats can shed oocysts in their feces, contaminating the environment, including water sources. Therefore, contaminated water may be just as important as contaminated feed in transmitting *T. gondii* infection. The findings align with a recent review paper, which suggests that the transmission of *T. gondii* through water may be more widespread than previously recognized, emphasizing the potential role of waterborne transmission in the spread of toxoplasmosis (35). The "use of surface water sources for drinking" was noted as a possible risk factor for *T. gondii* seropositivity in sheep in an earlier study (36). Static water use was linked to seroprevalence, which could be explained by the fact that cats were more likely to have access to it than to rushing water. Any feces from infected cats will be dangerous because oocyst survival in soil has been observed to last for up to two years (37). Unfiltered water infected with *T. gondii* can cause illness since *T. gondii* oocysts can live in water for up to 54 months in cold water (38, 39).

Sheep sampled during wet seasons had a twofold higher likelihood of testing seropositive compared to those sampled during dry seasons. Our findings are consistent with earlier data from Iran, where it was discovered that sheep had significantly greater *T. gondii* seropositivity in humid and mild climates, which are thought to be the best environments for the growth and survival of *T. gondii* oocysts (30). In contrast, a study conducted in Egypt found that the seropositivity rate of *T. gondii* was not significantly associated with climate (40).

Environmental and climatic factors can affect the spread and survival of the oocysts, increasing the likelihood that susceptible animals will be exposed to the parasite (41, 42). The oocysts can disperse in freshwater after heavy rains due to their hydrophilic and hardly sticky surface. In addition, heavy rains may influence the flow of rivers and streams, facilitating the spread of oocysts in the environment (43). According to Afonso et al., cats that have endured rainy winters for several years have a greater seroprevalence (43). Analysis of bioclimatic variables found that places with higher levels of precipitation had a higher chance of instances, supporting this idea. A moist environment helps the oocysts survive and makes food available to the insects that serve as *T. gondii* transport hosts (42).

5.1. Conclusions

This study provides new data about the epidemiology of *T. gondii* infection in sheep. Age, the rainy season, cats' access to water sources, and surface water sources all significantly contribute to the dissemination of oocysts and the rise in the rate of oral infection in farm animals. The emphasis on the high seroprevalence and risk factors for *T. gondii* transmission in sheep may serve as a springboard for educating Iranian farmers about the significance of the issue and the necessity of implementing control measures to stop the spread of *T. gondii*.

Footnotes

Authors' Contribution: MS, AJ, and MP designed the study protocol; MP, SHK, and MS collected the data and involved in statistical analysis; MS performed the experiments; MP and MS drafted the manuscript. MS, AJ, and MF critically revised the manuscript. All authors read and approved the final version of the manuscript.

Conflict of Interests Statement: The authors declared no potential conflicts of interest with respect to the research, authorship, and or publication of this article. The second author Dr. Masoud Foroutan serves as the Director of Research and Technology of Abadan University of Medical Sciences.

Data Availability: The data used to support the findings of this study are available from the corresponding author upon reasonable request.

Ethical Approval: This study received the approval from the Behbahan Faculty of Medical Sciences Ethical Committee (IR.BHN.REC.1402.024).

Funding/Support: This study was financially supported by Grant No: 401046 from the Behbahan Faculty of Medical Sciences, Behbahan, Iran.

References

1. Dubey JP. The history of Toxoplasma gondii–the first 100 years. *J Eukaryot Microbiol*. 2008;**55**(6):467-75. [PubMed ID: 19120791]. https://doi.org/10.1111/j.1550-7408.2008.00345.x.
2. Weiss LM, Dubey JP. Toxoplasmosis: A history of clinical observations. *Int J Parasitol*. 2009;**39**(8):895-901. [PubMed ID: 19217908]. [PubMed Central ID: PMC2704023]. https://doi.org/10.1016/j.ijpara.2009.02.004.
3. Rostami A, Riahi SM, Gamble HR, Fakhri Y, Nourollahpour Shiadeh M, Danesh M, et al. Global prevalence of latent toxoplasmosis in pregnant women: a systematic review and meta-analysis. *Clin Microbiol Infect*. 2020;**26**(6):673-83. [PubMed ID: 31972316]. https://doi.org/10.1016/j.cmi.2020.01.008.
4. Wang ZD, Liu HH, Ma ZX, Ma HY, Li ZY, Yang ZB, et al. Toxoplasma gondii Infection in Immunocompromised Patients: A Systematic Review and Meta-Analysis. *Front Microbiol*. 2017;**8**:389. [PubMed ID:

28337191]. [PubMed Central ID: PMC5343064]. https://doi.org/10.3389/fmicb.2017.00389.

5. Rostami A, Riahi SM, Contopoulos-Ioannidis DG, Gamble HR, Fakhri Y, Shiadeh MN, et al. Acute Toxoplasma infection in pregnant women worldwide: A systematic review and meta-analysis. *PLoS Negl Trop Dis*. 2019;**13**(10). e0007807. [PubMed ID: 31609966]. [PubMed Central ID: PMC6822777]. https://doi.org/10.1371/journal.pntd.0007807.
6. Robert-Gangneux F, Darde ML. Epidemiology of and diagnostic strategies for toxoplasmosis. *Clin Microbiol Rev*. 2012;**25**(2):264-96. [PubMed ID: 22491772]. [PubMed Central ID: PMC3346298]. https://doi.org/10.1128/CMR.05013-11.
7. Gos ML, Manazza JA, Spath EJA, Pardini L, Fiorentino MA, Unzaga JM, et al. Seroprevalence of Toxoplasma gondii and Neospora caninum infections in goats from two Argentinean provinces. *Open Vet J*. 2017;7(4):319-22. [PubMed ID: 29138747]. [PubMed Central ID: PMC5681730]. https://doi.org/10.4314/ovj.v7i4.5.
8. Pablos-Tanarro A, Ortega-Mora LM, Palomo A, Casasola F, Ferre I. Seroprevalence of Toxoplasma gondii in Iberian pig sows. *Parasitol Res*. 2018;**117**(5):1419-24. [PubMed ID: 29536166]. https://doi.org/10.1007/s00436-018-5837-3.
9. Schluter D, Daubener W, Schares G, Gross U, Pleyer U, Luder C. Animals are key to human toxoplasmosis. *Int J Med Microbiol*. 2014;**304**(7):917-29. [PubMed ID: 25240467]. https://doi.org/10.1016/j.ijmm.2014.09.002.
10. Abdallah MC, Kamel M, Karima B, Samir A, Djamel K, Rachid K, et al. Cross-Sectional Survey on Toxoplasma gondii Infection in Cattle, Sheep, and Goats in Algeria: Seroprevalence and Risk Factors. *Vet Sci*. 2019;**6**(3). [PubMed ID: 31295942]. [PubMed Central ID: PMC6789635]. https://doi.org/10.3390/vetsci6030063.
11. Sharif M, Sarvi S, Shokri A, Hosseini Teshnizi S, Rahimi MT, Mizani A, et al. Toxoplasma gondii infection among sheep and goats in Iran: a systematic review and meta-analysis. *Parasitol Res*. 2015;**114**(1):1-16. [PubMed ID: 25378258]. https://doi.org/10.1007/s00436-014-4176-2.
12. Sharif M, Gholami S, Ziaei H, Daryani A, Laktarashi B, Ziapour SP, et al. 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. [PubMed ID: 16919980]. https://doi.org/10.1016/j.tvjl.2006.07.004.
13. Raeghi S, Akaberi A, Sedeghi S. Seroprevalence of Toxoplasma gondii in sheep, cattle and horses in Urmia North-West of Iran. *J Iran Parasitol*. 2011;**6**(4):90. [PubMed ID: 22347318].
14. Desmonts G, Remington JS. Direct agglutination test for diagnosis of Toxoplasma infection: method for increasing sensitivity and specificity. *J Clin Microbiol*. 1980;**11**(6):562-8. [PubMed ID: 7000807]. [PubMed Central ID: PMC273461]. https://doi.org/10.1128/jcm.11.6.562-568.1980.
15. Raeghi S, Latifpour M, ZIA AN, SADIGHI SIMIN. Using human umbilical cord matrix cells (HUCM) for culturing Toxoplasma gondii for serological and molecular assays. *J Armaghan*. 2007.
16. Stelzer S, Basso W, Benavides Silvan J, Ortega-Mora LM, Maksimov P, Gethmann J, et al. Toxoplasma gondii infection and toxoplasmosis in farm animals: Risk factors and economic impact. *Food Waterborne Parasitol*. 2019;**15**. e00037. [PubMed ID: 32095611]. [PubMed Central ID: PMC7033994]. https://doi.org/10.1016/j.fawpar.2019.e00037.
17. Edwards JF, Dubey JP. 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*. 2013;**192**(1-3):129-36. [PubMed ID: 23099088]. https://doi.org/10.1016/j.vetpar.2012.09.037.
18. Foroutan M, Dalvand S, Daryani A, Ahmadpour E, Majidiani H, Khademvatan S, et al. Rolling up the pieces of a puzzle: a systematic review and meta-analysis of the prevalence of toxoplasmosis in Iran. *J Alexandria Medicine*. 2018;**54**(3):189-96.
19. Dubey JP. Toxoplasmosis in sheep--the last 20 years. *Vet Parasitol*. 2009;**163**(1-2):1-14. [PubMed ID: 19395175]. https://doi.org/10.1016/j.vetpar.2009.02.026.
20. Ibrahim HM, Mohamed AH, El-Sharaawy AA, El-Shqanqery HE. Molecular and serological prevalence of Toxoplasma gondii in pregnant women and sheep in Egypt. *Asian Pac J Trop Med*. 2017;**10**(10):996-1001. [PubMed ID: 29111196]. https://doi.org/10.1016/j.apjtm.2017.09.012.
21. Ahmed H, Malik A, Arshad M, Mustafa I, Khan MR, Afzal MS, et al. Seroprevalence and Spatial Distribution of Toxoplasmosis in Sheep and Goats in North-Eastern Region of Pakistan. *Korean J Parasitol*. 2016;**54**(4):439-46. [PubMed ID: 27658595]. [PubMed Central ID: PMC5040087]. https://doi.org/10.3347/kjp.2016.54.4.439.
22. Subedi S, Sharma B, Singh S, Bindari YR. Sero-prevalence of Toxoplasma gondii in sheep in different geographical regions of Nepal. *Vet Anim Sci*. 2018;**5**:7-9. [PubMed ID: 32734039]. [PubMed Central ID: PMC7386727]. https://doi.org/10.1016/j.vas.2018.01.001.
23. Magalhaes FJ, Ribeiro-Andrade M, Alcantara AM, Pinheiro J, Sena MJ, Porto WJ, et al. Risk factors for Toxoplasma gondii infection in sheep and cattle from Fernando de Noronha Island, Brazil. *Rev Bras Parasitol Vet*. 2016;**25**(4):511-5. [PubMed ID: 27580399]. https://doi.org/10.1590/S1984-29612016051.
24. Dahourou LD, Gbati OB, Savadogo M, Yougbare B, Dicko A, Combari AHB, et al. Prevalence of Toxoplasma gondii and Neospora caninum infections in households sheep "Elevage en case" in Dakar, Senegal. *Vet World*. 2019;**12**(7):1028-32. [PubMed ID: 31528028]. [PubMed Central ID: PMC6702564]. https://doi.org/10.14202/vetworld.2019.1028-1032.
25. Pastiu AI, Mircean V, Mercier A, Passebosc-Faure K, Plault N, Darde ML, et al. Toxoplasma gondii infection in sheep from Romania. *Parasit Vectors*. 2023;**16**(1):24. [PubMed ID: 36691063]. [PubMed Central ID: PMC9872358]. https://doi.org/10.1186/s13071-022-05634-8.
26. Rasti S, Marandi N, Abdoli A, Delavari M, Mousavi SGA. Serological and molecular detection of Toxoplasma gondii in sheep and goats in Kashan, Central Iran. *J Food Safety*. 2018;**38**(2). e12425. https://doi.org/10.1111/jfs.12425.
27. Gharekhani J, Yakhchali M, Esmaeilnejad B, Mardani K, Majidi G, Sohrabei A, et al. Seroprevalence and risk factors of Neospora caninum and Toxoplasma gondii in small ruminants in Southwest of Iran. *J Archives of Razi Institute*. 2018;**73**(4):305-10. [PubMed ID: 31077120].
28. Mahami-Oskouei M, Moradi M, Fallah E, Hamidi F, Akbari NAR. Molecular detection and genotyping of Toxoplasma gondii in chicken, beef, and lamb meat consumed in Northwestern Iran. *J Iranian Journal of Parasitology*. 2017;**12**(1):38. [PubMed ID: 28761459].
29. Yousefvand A, Mirhosseini SA, Ghorbani M, Mohammadzadeh T, Moghaddam MM, Mohammadyari S. Molecular and serological detection and of Toxoplasma gondii in small ruminants of southwest Iran and the potential risks for consumers. *J Verbrauch Lebensm*. 2021;**16**(2):117-27. [PubMed ID: 33424528]. [PubMed Central ID: PMC7776314]. https://doi.org/10.1007/s00003-020-01306-w.
30. Izadyar N, Abd Nikfarjam B, Esmaeili Rastaghi AR, Alizadeh SA, Heydarian P, Saraei M. A serologic study on Toxoplasma gondii infection in slaughtered sheep and goats in Qazvin Province, Iran. *Trop Anim Health Prod*. 2019;**51**(5):1289-93. [PubMed ID: 30689155]. https://doi.org/10.1007/s11250-019-01832-2.
31. de Barros RAM, Torrecilhas AC, Marciano MAM, Mazuz ML, Pereira-Chioccola VL, Fux B. Toxoplasmosis in Human and Animals Around the World. Diagnosis and Perspectives in the One Health Approach. *Acta Trop*. 2022;**231**:106432. [PubMed ID: 35390311]. https://doi.org/10.1016/j.actatropica.2022.106432.
32. Katzer F, Brulisauer F, Collantes-Fernandez E, Bartley PM, Burrells A, Gunn G, et al. Increased Toxoplasma gondii positivity relative to age in 125 Scottish sheep flocks; evidence of frequent acquired infection.

Vet Res. 2011;**42**(1):121. [PubMed ID: 22189159]. [PubMed Central ID: PMC3284422]. https://doi.org/10.1186/1297-9716-42-121.

33. Gazzonis AL, Veronesi F, Di Cerbo AR, Zanzani SA, Molineri G, Moretta I, et al. Toxoplasma gondii in small ruminants in Northern Italy - prevalence and risk factors. *Ann Agric Environ Med*. 2015;**22**(1):62-8. [PubMed ID: 25780830]. https://doi.org/10.5604/12321966.1141370.
34. Hecker YP, Masson FM, Armendano JI, Cora J, Olivares CF, Gual I, et al. Evaluation of frequency of antibodies against Toxoplasma gondii,Neospora caninum and Sarcocystis spp. and transmission routes in sheep from Humid Pampa, Argentina. *Acta Parasitol*. 2018;**63**(2):416-21. [PubMed ID: 29654669]. https://doi.org/10.1515/ap-2018-0048.
35. Otte MJ, Gumm ID. Intra-cluster correlation coefficients of 20 infections calculated from the results of cluster-sample surveys. *Prev Vet Med*. 1997;**31**(1-2):147-50. [PubMed ID: 9234433]. https://doi.org/10.1016/s0167-5877(96)01108-7.
36. Vesco G, Buffolano W, La Chiusa S, Mancuso G, Caracappa S, Chianca A, et al. Toxoplasma gondii infections in sheep in Sicily, southern Italy. *Vet Parasitol*. 2007;**146**(1-2):3-8. [PubMed ID: 17383099]. https://doi.org/10.1016/j.vetpar.2007.02.019.
37. Cenci-Goga BT, Rossitto PV, Sechi P, McCrindle CM, Cullor JS. Toxoplasma in animals, food, and humans: an old parasite of new concern. *Foodborne Pathog Dis*. 2011;**8**(7):751-62. [PubMed ID: 21486145]. https://doi.org/10.1089/fpd.2010.0795.
38. Lindsay DS, Dubey JP. Long-term survival of Toxoplasma gondii sporulated oocysts in seawater. *J Parasitol*. 2009;**95**(4):1019-20. [PubMed ID: 20050010]. https://doi.org/10.1645/GE-1919.1.
39. VanWormer E, Fritz H, Shapiro K, Mazet JA, Conrad PA. Molecules to modeling: Toxoplasma gondii oocysts at the human-animal-environment interface. *Comp Immunol Microbiol Infect Dis*. 2013;**36**(3):217-31. [PubMed ID: 23218130]. [PubMed Central ID: PMC3779781]. https://doi.org/10.1016/j.cimid.2012.10.006.
40. Fereig RM, Mahmoud H, Mohamed SGA, AbouLaila MR, Abdel-Wahab A, Osman SA, et al. Seroprevalence and epidemiology of Toxoplasma gondii in farm animals in different regions of Egypt. *Vet Parasitol Reg Stud Reports*. 2016;**3-4**:1-6. [PubMed ID: 31014492]. https://doi.org/10.1016/j.vprsr.2016.05.002.
41. Meerburg BG, Kijlstra A. Changing climate-changing pathogens: Toxoplasma gondii in North-Western Europe. *Parasitol Res*. 2009;**105**(1):17-24. [PubMed ID: 19418068]. [PubMed Central ID: PMC2695550]. https://doi.org/10.1007/s00436-009-1447-4.
42. Yan C, Liang LJ, Zheng KY, Zhu XQ. Impact of environmental factors on the emergence, transmission and distribution of Toxoplasma gondii. *Parasit Vectors*. 2016;**9**:137. [PubMed ID: 26965989]. [PubMed Central ID: PMC4785633]. https://doi.org/10.1186/s13071-016-1432-6.
43. Afonso E, Germain E, Poulle ML, Ruette S, Devillard S, Say L, et al. Environmental determinants of spatial and temporal variations in the transmission of Toxoplasma gondii in its definitive hosts. *Int J Parasitol Parasites Wildl*. 2013;**2**:278-85. [PubMed ID: 24533347]. [PubMed Central ID: PMC3862504]. https://doi.org/10.1016/j.ijppaw.2013.09.006.