Journal of Zoonotic Diseases
2023, 7 (4): 393-397
doi: 10.22034/jzd.2023.17025
https://jzd.tabrizu.ac.ir/article_17025.html

Original Article

Prevalence of Toxoplasmosis in Sheep Slaughtered in Malekan Slaughterhouse by Modified Agglutination Test (MAT)

Firouzivand Yaghoub*

Department of Pathobiology, Malekan Branch, Islamic Azad University, Malekan, Iran
***Corresponding author**: *Yaghuob_firoozi@yahoo.com*
(Received September 13, 2023, Accepted October 14, 2023)

Abstract
Toxoplasma gondii is one of the most common zoonotic parasites worldwide. Among livestock, sheep have a higher prevalence of *T. gondii* infection. Toxoplasmosis causes abortion in sheep and has a high economic impact on sheep breeding. Human infection mainly occurs by eating undercooked or raw meat containing tissue cysts. The present study was conducted to determine the prevalence of *T. gondii* in the sheep slaughtered at the Malekan slaughterhouse. Of 300 sheep, 63 (21%) were serologically positive for anti-Toxoplasma antibodies. Of 133 male and 167 female sheep, the infection rates were determined as 17.29% and 23.95%, respectively. The sheep were divided into three age groups: 1-2, 2-3, and over three years old, with infection rates of 23.26%, 19.02%, and 26.66%, respectively. There was no significant relationship between the age and sex of sheep with the prevalence of *T. gondii*. This study shows that sheep are an important source of *T. gondii*. Since sheep are an important animal to produce livestock production such as meat in the region, it is suggested to consume meat after it is fully cooked to prevent and reduce infection. It is recommended that more studies should be conducted to compare the results obtained with the method used in this study with other serological and molecular methods.
Keywords: Malekan, MAT, Sheep, *Toxoplasma gondii*

Introduction

Toxoplasma gondii is an intracellular protozoan parasite and the causative agent of Toxoplasmosis in humans and animals worldwide (Dubey, 2010). The parasite's final hosts are cats, while warm-blooded mammals, including humans and domestic animals such as sheep, and goats, serve as intermediate hosts (Stefanie, 2021). The final host sheds millions of oocytes through feces, contaminating the environment. When sporulated oocytes are ingested by the intermediate host, tissue cysts containing hundreds of bradyzoites can be formed in the various organs, including skeletal muscle, brain, and heart (Weiss and Kim, 2014; Armand et al., 2016). Complications of Toxoplasmosis in humans include abortion, eye problems, hydrocephalus, mental retardation, neurological problems, and, schizophrenia (Robert-Gangneux and Dard, 2012; Sugden et al., 2016). Consumption of raw or undercooked meat is a significant risk factor for *T. gondii* infection in humans (Jones et al., 2009). Toxoplasmosis usually does not show obvious clinical signs in animals, but it can

lead to abortion and economic loss in livestock production, especially in sheep (Dubey, 2010; Gutiérrez-Exposito et al., 2021). The prevalence of *Toxoplasma* infection varies based on region, weather, and diet in livestock and human communities (Tenter et al., 2000). In one study in Iran, the prevalence of *Toxoplasma* infection among pregnant women in Kashan was 42.7%, and in another study in Mashhad, 23.2% and 7.2% of the women referring to health centers were positive for IgG and IgM, respectively (Rasti et al., 2015; Sharifi et al., 2019). In the United States and the UK, it is estimated that between 16% and 40% of the population is infected with *T. gondii*, while in Canada, 20% to 40% of human cases have been reported (Tenter et al., 2000; Messier et al., 2009; Dubey, 2010). There are many reports on *T. gondii* infection in sheep and other animals based on serology. In a study in Kerman, of 108 cats, 114 goats, and 90 sheep, 2.7%, 1.7%, and 3.3% were positive by IFAT, respectively (Derakhshan and Mousavi, 2012). In Golestan province in the north of Iran, the seropositivity rate among sheep was 28.2%, according to IFA (Akhoundi and Youssefi, 2017). In a study at an abattoir in Sanandaj, serum antibodies against *T. gondii* were detected in cattle, sheep, and goats by MAT. The prevalence rates for cattle, sheep, and goats were 23.84%, 8.1%, and 12.74%, respectively (Khadem-erfan et al., 2019).

In Turkey, the rate of sheep Toxoplasmosis has been reported to be 10% using ELISA (Çakmak and Karatepe, 2017), while in an abattoir study in Egypt using ELISA, the infection rate was found to be 64.2% (Elfadaly et al., 2017). In Brazil, using the MAT method, 70% of sheep were positive for *T. gondii* (Consalter et al., 2019). The diagnosis of Toxoplasmosis is commonly based on serological, histological, and molecular analyses of samples from intermediate or final hosts (Verhelst et al., 2014; Dawson et al., 2020). Common serological methods for the detection of antibodies, such as ELISA, antibody immunofluorescence, and direct agglutination tests, are available (Remington et al., 2001; Dubey, 2010). In the Malekan region, sheep farming is common, but there have been no reports on *T. gondii* infection rate in sheep. Therefore, we conducted a study using the MAT method to determine the prevalence of *Toxoplasma* infection in sheep.

Materials and methods

Sample size and sampling

Based on previous studies and statistical formulas, 300 samples were selected with an accuracy of 5% and a confidence level of 95%. Referring to the twice-weekly Malekan slaughterhouse, Sheep are divided into two groups, male and female, and three age groups: 1 to 2, 2 to 3, and over three years old. Blood samples were randomly collected using vacuum blood collection tubes without anticoagulant and then immediately transferred to the Parasitology Laboratory of the Islamic Azad University, Malekan branch, left at room temperature (27 °C) for two h, and centrifuged at 2000 rpm for 10 minutes, and sera were stored at -20 °C until assayed.

Detection of anti-Toxoplasma antibodies in serum samples

A modified agglutination test was performed to determine specific antibodies against *Toxoplasma* antigens according to the method of Desmonts and Remington (1980). Briefly, serum samples were sequentially prepared using phosphate-buffered saline from 1:20 to 1:160 dilutions, and killed tachyzoites of the *Toxoplasma* RH strain were used as antigens. Twenty-five µL of diluted serum and 25 µL of antigen suspension were added to each well of the plate, and the plates were placed in a 37 °C incubator for 12 hours. Antibody titer equal to or higher than 1:20 dilution was considered a positive sample.

Statistical Analysis

The SPSS software version 24 was used. The chi-squared test was used to analyze the data. The significance level $p < 0.05$ was considered.

Results

Of 300 sheep, 63 (21%) were serologically positive for anti-*Toxoplasma* antibodies. The sample consisted of 133 males and 167 females, with infection rates of 17.29% and 23.95%, respectively (Table 1). The sheep are divided into three age groups: 1-2, 2-3, and over three years old, with infection rates of 23.26%, 19.02%, and 26.66%, respectively (Table 2).

Table 1. Comparison of *Toxoplasma gondii* infection in age groups in sheep slaughtered in Malekan slaughterhouse using Modified Agglutination Test (MAT).

Age group	No. of positive samples	No. of negative samples	Total	*p*-value
1-2 year	10(23.26%)	33(76.74%)	43	0.561
2-3 year	35(19.02%)	149(80.98%)	184	
>3 year	18(26.66%)	55(75.34%)	73	

Table 2. Comparison of *Toxoplasma gondii* infection in male and female sheep slaughtered in Malekan slaughterhouse using Modified Agglutination Test (MAT).

Gender	No. of positive samples	No. of negative samples	Total	*p*-value
Female	40(23.95%)	127(76.05%)	167	0.160
Male	23(17.29%)	110(82.70%)	133	

Discussion

Among livestock, sheep play an important role in the epidemiology of toxoplasmosis. They are potential intermediate hosts of *T. gondii*, as the parasite cysts remain in their skeletal and cardiac muscle (Schlüter et al., 2014). In the present study, anti-*T. gondii* antibodies were detected in 21% of sheep slaughtered at the Malekan slaughterhouse using the MAT method, and no significant relationship between sex and infection was observed. (p = 0.160). In most of the studies, MAT was used to evaluate the seroprevalence of *T.* gondii in different animals (Alvarado-Esquivei et al., 2012; Garcia – Bocanegra et al., 2012). There is a high agreement between MAT and other serological methods, including indirect ELISA, for detecting serum antibodies against *T.* gondii in sheep (Mainar-Jaime and Barberan, 2007). Compared to ELISA and IFA tests, MAT is preferable due to its easy application, ease of use in single samples, visual evaluation of results, no need for precise tools, and testing of sera from any specific species due to no need for species-specific conjugates (Seefeldt et al., 1989). Different results have been obtained in studies conducted in different regions of Iran. Furthermore, the prevalence of *T. gondii* infection in sheep was 12.2% in Kashan and 24.8% in Tabriz, as determined by ELISA (Rasti et al., 2015; Kavari et al., 2016). In Fars province, the prevalence of Toxoplasma infection in sheep was 29.5% using the MAT method, with no significant difference between seropositive animals and male and female animals, and no significant correlation between animal age and Toxoplasma infection (Asgari et al., 2013). Different results have been obtained in similar studies conducted in other countries. According to a slaughterhouse study, the prevalence of *T. gondii* infection in sheep was 25% in China and 30.14% in India, as determined by the MAT method (Satbige et al., 2016). Using ELISA, 8.3% of sheep were found to be infected in Algeria (Dahmani et al., 2018), and 56% in Turkey (Oncel and Vural, 2007). The differences in the prevalence of

Toxoplasma infection can be attributed to differences in weather conditions, feeding habits, and how the animals are raised and kept (Mostavafvi., 2012; Torgerson and Mastroiacovo, 2013). The present study was conducted in the spring, and the sheep grazed in the pasture, which may have contributed to the results. A study in two mountainous regions in China found that the incidence of infection was highest in spring (22.5%) and summer (23.6%), while it was lower in autumn (18.4%) and winter (16.5%) (Yin et al., 2015). Other reasons for differences in rates of toxoplasmosis include the sensitivity of diagnostic methods, the frequency of definite hosts, breeding methods and how they are fed, as well as age differences of animals (Tenter et al, 2000). Age is also an important risk factor for Toxoplasmosis in sheep, with the prevalence of infection increasing as the age of animals increases, indicating the transmission of the disease in the postnatal period (Andrade et al., 2013; Tegegne et al., 2016; Sah et al., 2018; Tagel et al., 2019). The prevalence of *T. gondii* infection in sheep over three years of age was higher than in sheep under three years of age in the present study. However, there was no statistically significant relationship between age groups and the level of infection level (p = 0.561). Katzer et al. (2011) reported that the seropositivity rate in sheep increased from 37.7% in 1-year-old sheep to 73.8% in sheep older than six years. Studies conducted in Iran have also confirmed this observation on age.

Nematollahi et al. (2014), reported that the prevalence of Toxoplasma infection in 101 sheep under two years of age was 9.9% and 3.5% over two years of age, respectively. In the study of Raeghi et al. (2011), the prevalence in sheep over six months old was 26%, and sheep under six months old was 2.7%, respectively. In another study in Razavi Khorasan, the prevalence of *T. gondii* infection in young and adult sheep was 97.4% and 29.6%, respectively (Movassaghi et al., 2016).

Conclusion

This study shows that sheep are an important source of *T. gondii*. It is recommended to fully cook sheep meat to prevent and reduce infection since it is an important animal for livestock production, including meat in the region and Iran. It is suggested that more studies should be conducted to compare the results obtained with the method used in this study with other serological and molecular methods.

Acknowledgments

Not applicable.

Ethical approval

Not applicable.

Conflict of Interests

The authors declare no conflict of interest.

References

Akhoundi S. & Youssefi M.R. Seroprevalence of sheep toxoplasmosis in north of Iran. *Trakia Journal of Sciences*, 2017, 15(1), 79-82. doi:10.15547/tjs.2017.01.013.

Alvarado-Esquivel C., Estrada-Malacón M.A., Reyes-Hernández S.O., Pérez-Ramírez J.A., Trujillo-López J.I., Villena I. & Dubey J.P. Seroprevalence of *Toxoplasma gondii* in domestic sheep in Oaxaca State, Mexico. *The Journal of Parasitology*, 2013, 99(1), 151-152. doi: 10.1645/GE-3220.1.

Andrade M.M.C., Carneiro M., Medeiros A.D., Neto V.A. & Vitor R.W. Seroprevalence and risk factors associated with ovine toxoplasmosis in Northeast Brazil. *Parasite*, 2013, 20. doi: 10.1051/parasite/2013019.

Armand B., Solhjoo K., Shabani-Kordshooli M., Davami M.H. & Sadeghi M. *Toxoplasma* infection in sheep from south of Iran monitored by serological and molecular methods; risk assessment to meat consumers. *Veterinary World*, 2016, 9: 850-855. doi: 10.14202/vetworld.2016.850-855.

Asgari Q., Sarnevesht J., Kalantari M., Sadat S.J.A., Motazedian M.H. & Sarkari, B. Molecular survey of *Toxoplasma* infection in sheep and goat from Fars province, Southern Iran. *Tropical Animal Health*

and Production, 2011, 43, 389-392. doi: 10.1007/s11250-010-9704-1.

Asgari Q., Sarkari B., Amerinia M., Panahi S., Mohammadpour I. & Sarvestani A.S. *Toxoplasma* infection in farm animals: a seroepidemiological survey in Fars Province, south of Iran. *Jundishapur Journal of Microbiology*, 2013, 6(3), 269-272. doi: 10.5812/jjm.5195.

Çakmak D.Ö. & Karatepe B. Seroprevalence of *Toxoplasma gondii* in sheep from Nevşehir province in Turkey. *Türkiye Parazitolojii Dergisi*, 2017, 41(3), 148-151. doi: 10.5152/tpd.2017.5245.

Consalter A., Frazão-Teixeira E., Dubey J.P., Zanella E.L., da Silva A.F., de Souza G.N. & Ferreira A.M. Epidemiological investigation of *Toxoplasma gondii* infections in commercial sheep flock in an endemic area for ocular toxoplasmosis in Southern Brazil. *Acta Parasitologica*, 2019, 64, 514-519. doi: 10.2478/s11686-019-00081-5.

Dahmani A., Harhoura K., Aissi M., Zenia S., Hamriouri B., Guechi N., Athmane M.A., & Kadour R. The zoonotic protozoan of sheep carcasses in the north of Algeria: A case of ovine toxoplasmosis. *Journal of the Hellenic Veterinary Medical Society*, 2018, 69(2), 1004-1012. doi: 10.12681/jhvms.18385.

Dawson A.C., Ashander L.M., Appukuttan B., Woodman R.J., Dubey J.P., Whiley H. & Smith J.R. Lamb as a potential source of *Toxoplasma gondii* infection for Australians. *Australian and New Zealand Journal of Public Health*, 2020, 44, 49-52. doi: 10.1111/1753-6405.12955.

Derakhshan M. & Mousavi M. Serological survey of antibodies to *Toxoplasma gondii* in cats, goats, and sheep in Kerman, Iran. *Comparative Clinical Pathology*, 2014, 23, 267-268. doi: 10.1007/s00580-012-1605-4.

Desmonts G. & Remington J.S. Direct agglutination test for diagnosis of *Toxoplasma* infection: method for increasing sensitivity and specificity. *Journal of Clinical Microbiology*, 1980, 11(6), 562-568. doi: 10.1128/jcm.11.6.562-568.1980.

Dubey J.P.2010, Toxoplasmosis of animals and humans. 2nd ed. Boca Raton, CRC Press. Florida.

Dubey J.P., Murata F.H.A., Cerqueira-Cézar C.K., Kwok O.C.H.& Su, C. Economic and public health importance of *Toxoplasma gondii* infections in sheep: 2009–2020. *Veterinary Parasitology*, 2020, 286, 109195. doi: 10.1016/j.vetpar.2020.109195.

Elfadaly H.A., Hassanain N.A., Shaapan R.M., Hassanain M.A., Barakat A.M., & Abdelrahman K.A. Molecular detection and genotyping of *Toxoplasma gondii* from Egyptian isolates. *Asian Journal of Epidemiology*, 2017, 10(1), 37-44. doi: 10.3923/aje.2017.37.44.

Firouzivand Y., Ahmadi B.K., & Afsari, M. Evaluation of the Effects of Toxoplasmosis in Small Ruminants on the Epidemiology of Human Toxoplasmosis by Indirect ELISA in Kaleybar Region, North West of Iran. *International Journal of Medical Parasitology and Epidemiology Sciences*, 2020, 1(3), 65-69. doi: 10.34172/ijmpes.2020.19.

García-Bocanegra I., Cabezón O., Arenas-Montes A., Carbonero A., Dubey J.P., Perea A. & Almería S. Seroprevalence of Toxoplasma gondii in equids from Southern Spain. *Parasitology International*, 2012, 61(3), 421-424. doi: 10.1016/j.parint.2012.02.003.

Gutiérrez-Expósito,D., Tejerina bF., Gutiérrez J., Fernández-Escobar M., Ortega-Mora L.M., Mantecón A.R., Dagleish M.P., Pérez V. & Benavides, J. Direct economic losses of *Toxoplasma gondii* abortion outbreaks in two Spanish sheep flocks. *Veterinary Parasitology: Regional Studies and Reports*, 2021, 26, 100623. doi: 10.1016/j.vprsr.2021.100623.

Havakhah Y., Esmaeili Rastaghi A.R., Amiri S., Babaie J., Aghighi Z., & Golkar, M. Prevalence of *Toxoplasma gondii* in sheep and goats in three counties of Gilan Province, North of Iran the more humid climate the higher prevalence. *Journal of Medical Microbiology and Infectious Diseases*, 2014, 2(2), 80-83. http://jommid.pasteur.ac.ir/article-1-62-en.html.

Johnson S.K. & Johnson P.T. Toxoplasmosis: recent advances in understanding the link between infection and host behavior. *Annual Review of Animal Biosciences*, 2021, 9, 249-264. doi: 10.1146/annurev-animal-081720-111125.

Jones J.L., Dargelas V., Roberts J., Press C., Remington J.S., & Montoya, J.G. Risk factors for *Toxoplasma gondii* infection in the United States. *Clinical Infectious Diseases*, 2009, 49(6),.878-884. doi: 10.1086/605433.

Katzer F., Brülisauer F., Collantes-Fernández E., Bartley P.M., Burrells A., Gunn G., Maley S.W., Cousens C., & Innes E.A. Increased *Toxoplasma gondii* positivity relative to age in 125 Scottish sheep flocks; evidence of frequent acquired infection. *Veterinary Research*, 2011, 42(1), 1-9. doi: 10.1186/1297-9716-42-121.

Kavari A., Nowzari N., Moazeni Jula G., Moazeni Jula F., & Hashemzadeh, H. A serological and molecular study on *Toxoplasma gondii* infection in sheep and goat in Tabriz. *Archives of Razi Institute*, 2016, 68(1), 29–35. doi: 10.7508/ari.2013.01.005.

Khadem-erfan M.B., Shariati S., Faridi A., Ghaderi E., Javan K. & Zamini G. Prevalence of *Toxoplasma gondii* Antibody in Livestock Slaughtered in Sanandaj Slaughterhouse with Agglutination Method in 2015. *Journal of Veterinary Research*, 2019, 74(1), 19-26. doi: 10.22059/jvr.2018.222972.2555.

Khamesipour F., Doosti A., Mobarakeh H.I., & Komba, E.V. *Toxoplasma gondii* in cattle, camels and sheep in Isfahan and Chaharmahal va Bakhtiary Provinces, Iran. *Jundishapur Journal of Microbiology*, 2014, 7(6), e17460. doi: 10.5812/jjm.17460.

Mainar-Jaime RC., Barberán M. Evaluation of the diagnostic accuracy of the modified agglutination test and an indirect ELISA for the detection of serum antibodies against *Toxoplasma gondii* in sheep through Bayesian approaches. *Veterinary Parasitology*, 2007, 148(2), 122-129. oi:10.1016/j.vetpar.2007.05.018.

Messier V., Levesque B., Proulx J.F., Rochette L., Libman M.D., Ward B.J., Serhi, B., Couillard M., Ogden N.H., Dewailly E. & Hubert, B. Seroprevalence *of toxoplasma gondii* among Nunavik Inuit (Canada). *Zoonoses and Public Health*, 2009, 56(4), 188-197. doi: 10.1111/j.1863-2378.2008.01177.x.

Mostavafvi J.J.L. A systematic review of published studies on the epidemiology of toxoplasmosis in Iran. *Medical Journal of Isfahan Uni*versity, 2012, 30, 76.

Movassaghi A.R., Rassouli M., Fazaeli A. & Salimi-Bejestani M.R. Outbreak of ovine congenital toxoplasmosis in Iran, confirmed by different diagnostic methods. *Journal of Parasitic Diseases*, 2016, 40, 152-156. doi: 10.1007/s12639-014-0467-x.

Nematollahi A., Shahbazi P., & Nosrati S. Serological survey of antibodies to *Toxoplasma gondii* in sheep in north-west of Iran. *International Journal of Advanced Biological and Biomedical Research*, 2014, 2(9), 2605-8. https://www.sid.ir/FileServer/JE/5700002 0140917.pdf.

Oncel T. & Vural G. Occurrence of *Toxoplasma gondii* antibodies in sheep in Istanbul, Turkey. *Veterinarski Arhiv*, 2006, 76(6), 547-553. https://hrcak.srce.hr/22260.

Raeghi S., Akaberi A., & Sedeghi, S. Seroprevalence of *Toxoplasma gondii* in sheep, cattle and horses in Urmia North-West of Iran. *Iranian Journal of Parasitology*, 2011, 6(4), 90-94. https://www.sid.ir/FileServer/JE/1149201 10412.

Rasti S., Marandi N., Abdoli A., Delavari M., & Mousavi S.G.A. Serological and molecular detection of *Toxoplasma gondii* in sheep and goats in Kashan, Central Iran. *Journal of Food Safety*, 2018, 38(2), 12425. doi: 10.1111/jfs.12425.

Robert-Gangneux F. & Dardé M.L. Epidemiology of and diagnostic strategies for toxoplasmosis. *Clinical Microbiology Reviews*, 2012, 25(2), 264-266. doi: 10.1128/cmr.05013-11.

Sah R.P., Talukder M.H., Rahman A.A., Alam M.Z. & Ward, M.P. Seroprevalence of *Toxoplasma gondii* infection in ruminants in selected districts in Bangladesh. *Veterinary Parasitology: Regional Studies*

and Reports, 2018, 11, 1-5. doi: 10.1016/j.vprsr.2017.10.008.

Satbige A.S., Vijaya Bharathi M., Ganesan P.I., Sreekumar C. & Rajendran, C. Detection of *Toxoplasma gondii* in small ruminants in Chennai using PCR and modified direct agglutination test. *Journal of Parasitic Diseases*, 2016, 40, 1466-1469. doi: 10.1007/s12639-015-0713-x.

Schlüter D., Däubener W., Schares G., Groß U., Pleyer U., & Lüder, C. Animals are key to human toxoplasmosis. *International Journal of Medical Microbiology*, 2014, 304(7), 917-919. doi: 10.1016/j.ijmm.2014.09.002.

Seefeldt S.L., Kirkbride C.A. & Dubey J.P. Comparison of enzyme-linked immunosorbent assay, indirect fluorescent antibody test, and direct agglutination test for detecting *Toxoplasma gondii* antibodies in naturally aborted ovine fetuses. *Journal of Veterinary Diagnostic Investigation*, 1989, 1(2), 124-127. doi: 10.1177/104063878900100206.

Sugden K., Moffitt T.E., Pinto L., Poulton R., Williams B.S. & Caspi A. Is *Toxoplasma gondii* infection related to brain and behavior impairments in humans? Evidence from a population-representative birth cohort. *PLoS One*, 2016, 11(2), e0148435. doi: 10.1371/journal.pone.0148435.

Tagel M., Lassen B., Viltrop A., & Jokelainen, P. Large-scale epidemiological study on *Toxoplasma gondii* seroprevalence and risk factors in sheep in Estonia: age, farm location, and breed associated with seropositivity. *Vector-Borne and Zoonotic Diseases*, 2019, 19(6), 421-429. doi: 10.1089/vbz.2018.2343.

Tegegne D., Abdurahaman M. & Yohannes, M. Seroepidemiology and associated risk factors of *Toxoplasma gondii* in sheep and goats in Southwestern Ethiopia. *BMC Veterinary Research*, 2016, 12(1), 1-6. doi: 10.1186/s12917-016-0906-2.

Tenter A.M., Heckeroth A.R. & Weiss, L.M. *Toxoplasma gondii*: from animals to humans. *International Journal for Parasitology*, 2000, 30(12-13), 1217-1258. doi: 10.1016/S0020-7519(00)00124-7.

Torgerson P.R. & Mastroiacovo P. The global burden of congenital toxoplasmosis: a systematic review. *Bulletin of the World Health Organization*, 2013, 91, 501-508. doi: 10.2471/BLT.12.111732.

Verhelst D., DeCraeye S., Entrican G., Dorny P. & Cox, E. Parasite distribution and associated immune response during the acute phase of *Toxoplasma gondii* infection in sheep. *BMC Veterinary Research*, 2014, 10. 1-12. doi: 10.1186/s12917-014-0293-5.

Weiss L.M. and Kim K. 2014, *Toxoplasma gondii* the model of Apicomplexan-prospectives and methods. 2nd ed., Elsevier, UK.

Yin M.Y., Wang J.L., Huang S.Y., Qin S.Y., Zhou D.H., Liu G.X., Tan Q.D. & Zhu, X.Q. Seroprevalence and risk factors of *Toxoplasma gondii* in Tibetan Sheep in Gansu province, Northwestern China. *BMC Veterinary Research*, 2015, 11, 1-5. doi: 10.1186/s12917-015-0358-0.