Original Article

Molecular Prevalence of *Toxoplasma Gondii* in Butchers and Slaughtered Cattle in Middle Euphrates, Iraq

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ABSTRACT

Background: Toxoplasmosis is a common disease caused by an apicomplexian protozoa, *Toxoplasma gondii*; one of the potential sources of human zoonotic infection is meat handling and consumption.

Objectives: This study aims to determine the prevalence of *T. gondii* in the blood of butchers and slaughtered cattle by using the *B1* gene and demonstrate the role of slaughtered cattle in transmitting toxoplasmosis to humans.

Methods: The study involved 100 blood samples collected randomly from butchers (male) and 100 from cattle before slaughtering from September 2022 to September 2023 and examined by real-time PCR technique.

Results: The results revealed total infection rates of 28% and 12% in butchers and cattle, respectively, with the highest rate recorded in butchers aged 18-30 years old and the lowest at age >40 years. While in cattle, the highest rate is seen at age (2-4 years) and the lowest at age (1-2 years) without significant differences. Calves reported a higher infection rate (14.28%) compared with cows (8.10%), with a significant difference (P>0.05).

Conclusion: Cattle are an important source of zoonotic transmission of *T. gondii* infection to humans by handling meat and consuming raw or uncooked meat. We recommended a control program by awareness of butchers during meat handling, avoiding consuming raw or uncooked meat, and appropriate heat treatments for beef.

Keywords: Butcher, Cattle, Real-time PCR, *Toxoplasma gondii*, Zoonotic

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Introduction

Toxoplasma gondii is the most common zoonotic worldwide obligate intracellular parasite infecting humans via warm-blooded animals (Khalil et al., 2018). Wild and domesticated felines are the final hosts of *T. gondii* that sexually develop in the intestine, and the oocysts shed in the feces (Sakban et al., 2020; Jilo et al., 2021). Toxoplasmosis, a clinical disease caused by *T. gondii*, can probably infect all warm-blooded animals and humans and lead to several clinical symptoms, including fever, mental retardation, chorioretinitis, encephalitis, hydrocephalus in children, and abortion in livestock; however, the infection can lead to even mortality (Abdallah et al., 2019; Fazel et al., 2021; Khattab et al., 2022).

Although cattle infection is asymptomatic and leads to clinical signs or abortion, it plays a vital role in the transmission of toxoplasmosis to humans by the consumption of undercooked meat or unpasteurized milk because this parasite exits in the bloodstream and develops as tissue cysts in the muscles and glands (Opsteegh et al., 2020; Taalay et al., 2022).

There are several diagnostic methods used to identify *T. gondii*: Direct methods, such as preparation of blood smears from an infected host, histopathological examination and serological (immunological) methods, such as latex agglutination test, modified agglutination test, and enzyme-linked immunosorbent assay (Abdul Ameer Jaber & Noori, 2021; Liyanage et al., 2021). Different molecular techniques and real-time polymerase chain reaction (RT-PCR) methods have been developed to identify *T. gondii* using different samples, such as tissue biopsy and blood (Mahittikorn et al., 2005; Aghwan et al., 2021). Real-time PCR is a highly sensitive and specific method that is more accurate but costly and requires specialized detection systems (Santoro et al., 2019; Adriaanse et al., 2020; Souza et al., 2023).

Our study aimed to detect the incidence of toxoplasmosis in butchers and butchered cattle using the *B1* gene and demonstrate the role of cattle in the zoonotic transmission of infection to humans.

Materials and Methods

A total of 100 blood samples were collected (2-3 mL) from butchers (male) and cattle (both sexes) of different age groups from different abattoirs during the period from September 1st, 2022, to September 30th, 2023. The blood samples were collected in an EDTA tube with anticoagulant and stored at -20°C according to the manufacturer's instructions till used for DNA extraction.

Real-time PCR amplification

DNA extraction from blood samples using a Genomic DNA Mini Kit (Geneaid USA). Real-time PCR was done to detect *T. gondii* in blood samples using the primers and TaqMan probe for the *B1* gene. This method was done based on the technique designated by Lin et al. (2000), quantitative PCR master mix existed organized using NEXpro™ qPCR Master Mix (Probe). The master mix was prepared following instructions of the company as follows: DNA template 5 µL, Forward B1 primer (5′-TCCCCTCTGCTGGCGAAAAGT-3′) (10 pmol) 1 µL, Reverse B1 primer (5′-AGCGTTCGTGGTCAACTATCGATTG-3′) (10 pmol) 1 µL (product size 94 bp), B1 probe (5′- FAM-TCTGTGCAACTTTGGTGTATTCGCAG-TAMRA- 3′) (20 pmol) 1 µL, quantitative PCR master PCR mix 10 µL and PCR water 2 µL. Subsequently, PCR master mix components were transported into an ExiSpin vortex centrifuge at 3000 rpm for 3 min. RT-PCR thermocycler situations were fixed according to the annealing temperature of primer and RT-PCR TaqMan kit instructions by BioRad thermocycler system of real-time PCR as in the follows: One cycle pre-denaturation 5 min in 95°C, denaturation 20 s in 95°C in 45 cycles, annealing extension 30 s in 60°C in 45 cycles and revealing for 30 s in 60°C in 45 cycles. The data analysis was performed using the threshold series number (CT value) calculation, which obtained the positive amplification of the *T. gondii B1* gene in real-time PCR cycle numbers (Lin et al., 2000).

Statistical analysis

Computerized statistical analyses were performed using SPSS software, version 31. The chi-square test was employed to evaluate the variables (Joda, 2008).

Results

Total infection rates in butchers and cattle were 28% and 12%, respectively, out of 100 blood samples from each one, examined using real-time PCR (Table 1; Figures 1and 2).

The infection rate of toxoplasmosis in butchers and cattle concerning age groups

Butchers 18-30 years old had the highest percentage (34.21%), followed by the age group 30-40 years

Table 1. Total rate of infection of toxoplasmosis in butchers and cattle by real-time PCR

Host	No. Samples		Rate of Infection (%)
	Examined	Positive	
Butchers	100	28	28
Cattle	100	12	12

Table 2. Total infection rates of toxoplasmosis in butchers according to age groups by real-time PCR

Age (y)	No. Samples		Rate of Infection (%)
	Examined	Positive	
18-30	38	13	34.21
30-40	40	11	27.5
<40	22	4	18.18
Total	100	28	28

Table 3. Total infection rates of toxoplasmosis in cattle according to the age groups by real-time PCR

Age (y)	No. Samples		Rate of Infection (%)
	Examined	Positive	
<1-2	30	3	10
2-4	42	6	14.28
<4	28	3	10.71
Total	100	12	12

Table 4. Total infection rates of toxoplasmosis in cattle according to sex by real-time PCR

Sex	Samples (No.)		Rate of Infection (%)
	Examined	Positive	
Male (calve)	63	9	14.28
Female (cow)	37	3	8.10
Total	100	12	12

(27.50%). In contrast, the lowest (18.18%) belonged to the age group <40 years without significant (P>0.05) difference (Table 2).

The age group 2-4 years of cattle recorded the highest infection rate (14.28%), and the lowest rate was recorded at the age group <1-2 years (10%) without significant (P>0.05) difference (Table 3).

The infection rates of toxoplasmosis in cattle regarding the sex

Males had the highest infection rate (14.24%) compared to females (8.10%), with a significant difference (P>0.05) (Table 4).

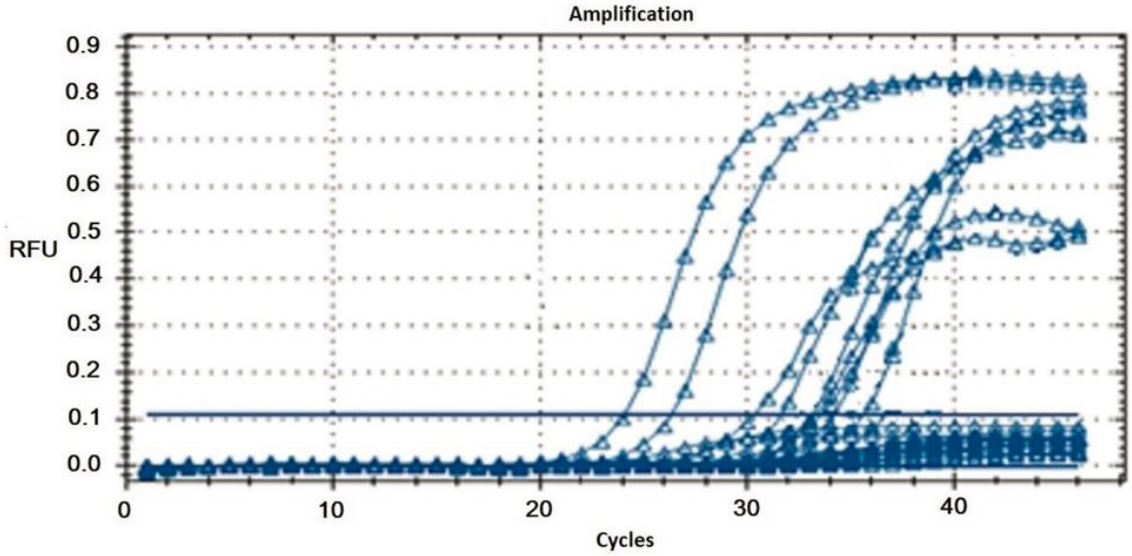

Figure 1. Positive results of Butchers toxoplasmosis by real time-PCR baseB1 gene.

Discussion

Toxoplasmosis is a common zoonotic disease worldwide caused by intracellular protozoan *T. gondii*, infecting a wide range of warm-blooded animals. The parasite can infect humans horizontally when consuming the infective stage (sporulated oocyst) in contaminated food and water or unpasteurized milk, undercooked, or meat containing bradyzoites (tissue cysts) of *T. gondii*. So cattle meat is an essential source of human infection, especially meat handlers (butchers).

Real-time PCR is a highly accurate and suitable technique for diagnosis, providing a rapid, high sensitivity, specificity, and quantitative pathway for the detection of *T. gondii* infection in clinical specimens without false-positive results in comparison with serological tests (Awad & AL-Muffti, 2020; Adriaanse et al., 2020; Souza et al., 2023).

The overall prevalence found in butchers agreed with the results of Al-Khafaji (2014), who found 29.43% in meat cookers and sellers, as they frequently possess high-risk factors with parasites. However, it disagrees with Ibrahim et al. (2017), who mentioned 57.37% by re-

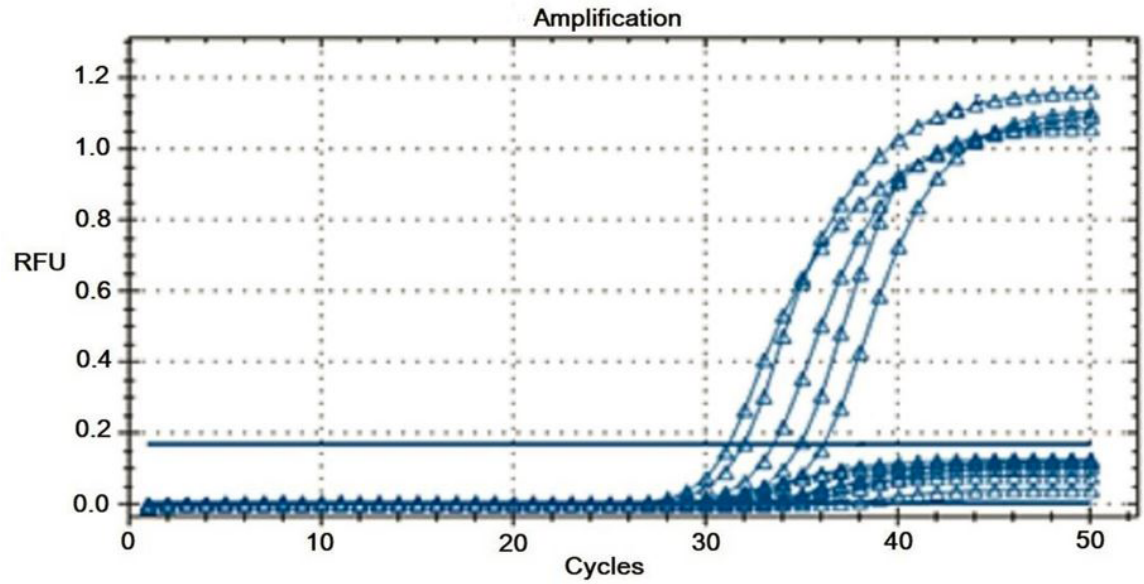

Figure 2. Positive results of cattle toxoplasmosis by real time-PCR baseB1 gene.

al-time PCR for women consumed under-cooked meat, and Ebrahimzadeh et al. (2018), who recorded 68.47% in humans by nested PCR using the *B1* gene. Ali et al. (2017) reported a lower infection rate of 0.62% in Saudi Arabia using the PCR *B1* gene, while Al-Hadraawy & Hadi (2017) recorded 20.28% using the PCR *B1* gene in Al-Najaf governorate, Iraq. Also, Khan et al. (2020) reported a lower infection rate of 20.64% in Pakistani males. These variations should be due to the differences in eating habits, method of cooking, kind of meat, social, economic, cultural habits, and sanitation coverage.

The highest percentage belonged to the age range 18-30 years, while the lowest belonged to age <40. This finding was in line with Khater et al. (2013), who detected the highest rate (33.3%) among those aged 21-30 years, (the highest percentage in those aged 20-29 years (Ghasemi et al., 2015), and disagreed with Khan et al. (2020) reported higher infection rate at age group 41-60 years (37.36%). In contrast, Nassef et al. (2018) and Mohamed (2020) noticed a non-significant relation to the age groups. The variation in young and adult human beings could be due to the difference in the immune status of persons, hygienic status, environmental conditions, different nutrition, the presence of sociocultural differences, and variations in terrain contribute to the exposure of varying age groups within the population to the infective form of the parasite.

The overall prevalence in cattle was in agreement with Tonouhewa et al. (2017), who found 12% (8%-17%) in Africa, Taalay et al. (2022), who recorded 12.2% in Pakistani cattle and Khattab et al. (2022), who reported 13.46% in Egyptian cattle. Majeed & Abbas (2018) found 14.91% in cattle in Basra City, Iraq, but disagree with Hassanain et al. (2013), who mentioned that the infection rate in Egyptian cattle was 29.4% using PCR. Shariatzadeh et al. (2021) recorded 16.94% in cattle and Fazel et al. (2021) recorded 56% in Iranian cattle. While Azizi et al. (2014) reported that the infection rate in Iranian cattle was 8.57%, Ji et al. (2023) detected that the infection rate in beef cattle was 2.9% in Korea. These differences could be due to the area of sample collection, number of collected samples, grassing type and distribution of stray cats in pastures, as well as the age of cattle (old age is more resistant to infection due to acquired immunity).

The highest percentage of *T. gondii* infection is seen in age group 2-4 years, while the lowest is at age <1-2 years. These results were in agreement with Blaga et al. (2019) and showed a strong age effect that adult cattle were more highly infected than calves in the age group (<8 months). Azizi et al. (2014) showed no significant correlation between age and infection rate in cattle. Kim & Seo (2023) showed the highest infection rate in the age group >6 years and the lowest prevalence among the age group <1 year. Sroka et al. (2020) reported that the infection rate of *T. gondii* increases with age of animals, indicating that age is a risk factor for *T. gondii* infection because older individuals are exposed to the parasite longer than younger individuals.

The infection rate was higher in male cattle (14.28%) than in females (8.10%). These results were in agreement with Fazel et al. (2021) and Kim & Seo (2023). They recorded that the prevalence of *T. gondii* was higher in male cattle than females. They disagreed with Sroka et al. (2020), who recorded the infection rate in female cattle higher than males.

Conclusion

The detection of *T. gondii* in cattle blood explains that cattle meat is an important source of zoonotic transmission of toxoplasmosis to humans. We recommended establishing a strategy to control infection by health education on the zoonotic significance of toxoplasmosis, maintaining a high standard of personal hygiene, avoiding consuming raw or uncooked meat, and appropriate heat treatments for beef and milk.

Ethical Considerations

Compliance with ethical guidelines

All animal procedures were performed following the standards outlined in the guidelines of the Animal Welfare, Ethics and Experimentation Committee (No.: BMS/0231/016) of the Faculty of Veterinary Science, University of Babylon.

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References

Abdallah, M. C., Kamel, M., Karima, B., Samir, A., Djamel, K., & Rachid, K., et al. (2019). Cross-sectional survey on Toxoplasma gondii infection in cattle, sheep, and goats in Algeria: seroprevalence and risk factors. *Veterinary Sciences, 6*(3), 63. [DOI:10.3390/vetsci6030063]

Abdul Ameer Jaber, K., & Aamer Noori, R. (2021). Comparisons of toxoplasma gondii prevalence in rural and urban areas of Al-Najaf Province of Iraq using serological methods. *Archives of Razi Institute, 76*(6), 1695–1701. [PMID]

Adriaanse, K., Firestone, S. M., Lynch, M., Rendall, A. R., Sutherland, D. R., & Hufschmid, J., et al. (2020). Comparison of the modified agglutination test and real-time PCR for detection of Toxoplasma gondii exposure in feral cats from Phillip Island, Australia, and risk factors associated with infection. *International Journal for Parasitology: Parasites and Wildlife, 12*(8), 126-133. [Link]

Aghwan, S. S., Al-Bakri, H. S., & Albaqqal, S. M. (2021). Comparison the efficiency of different techniques for the diagnosis of Toxoplasma gondii infection in slaughtered ewes. *Iraqi Journal of Veterinary Sciences, 35*(2), 19-23. [DOI:10.33899/ijvs.2021.127058.1452]

Al-Hadraawy, S. K., & Hadi, F. A. (2017). Immunological and Molecular Study of Toxoplasma gondii in Al-Najaf Governorate-Iraq. *International Journal of Pharmacognosy and Phytochemical Research, 9*(4), 482-492. [Link]

Ali, A., Mohamed, K., & Toulah, F. (2017). Prevalence of Toxoplasma gondii in women population of Rafha city, Saudi Arabia. P*akistan Journal of Zoology, 49*(3), 1039-1047. [Link]

Al-Khafaji, A. M. (2014). Serological diagnosis and histopathological lesion of Toxoplasma gondii infection in human and sheep at Kut city [MA thesis]. Baghdad: University of Baghdad.

Awad, R. N., & Al-Muffti, S. A. (2020). Detection of toxoplasma gondii among women in Duhok City-Kurdistan Region of Iraq. *The Journal of Duhok University, 23*(2), 20-33. [DOI:10.26682/ajuod.2020.23.2.5]

Azizi, H., Shiran, B., Boroujeni, A. B., & Jafari, M. (2014). Molecular survey of toxoplasma gondii in sheep, cattle and meat products in Chaharmahal va Bakhtiari Province, Southwest of Iran. *Iranian Journal of Parasitology, 9*(3) 429-434. [PMID]

Blaga, R., Aubert, D., Thébault, A., Perret, C., Geers, R., & Thomas, M., et al. (2019). Toxoplasma gondii in beef consumed in France: Regional variation in seroprevalence and parasite isolation. *Parasite, 26*(77), 1-14. [DOI:10.1051/parasite/2019076]

Ebrahimzadeh, A., Shahraki, M. K., & Mohammadi, A. (2018). Serological and molecular diagnosis of Toxoplasma gondii in patients with schizophrenia. *Journal of Parasitic Diseases, 42*(2), 177-181. [DOI:10.1007/s12639-018-0979-x]

Fazel, R., Rezanezhad, H., Solhjoo, K., Kalantari, M., Erfanian, S., & Armand, B., et al. (2021). PCR-based detection of Toxoplasma gondii from cattle in southern Iran. *Comparative Immunology, Microbiology and Infectious Diseases, 77*, 101677. [DOI:10.1016/j.cimid.2021.101677]

Ghasemi, F. S., Rasti, S., Bandehpour, M., Kazemi, B., Piroozmand, A., & Mousavi, G. A. (2015). Molecular diagnosis of Toxoplasma gondii in aborted women. J*undishapur Journal Of Microbiology, 8*(1), e15925. [DOI:10.5812/jjm.15925]

Hassanain, M. A., El-Fadaly, H. A., Hassanain, N. A., Shaapan, R. M., Barakat, A. M., & Abd El-Razik, K. A. (2013). Serological and molecular diagnosis of toxoplasmosis in human and animals. *World Journal of Medical Sciences, 9*(4), 243-247. [Link]

Ibrahim, H. M., Mohamed, A. H., El-Sharaawy, A. A., & El-Shqanqery, H. E. (2017). Molecular and serological prevalence of Toxoplasma gondii in pregnant women and sheep in Egypt. *Asian Pacific Journal of Tropical Medicine, 10*(10), 996-1001. [DOI:10.1016/j.apjtm.2017.09.012]

Ji, M. J., Cho, H. C., Park, Y. J., Jang, D. H., Park, J., & Choi, K. S. (2023). Molecular detection of toxoplasma gondii in blood samples of domestic livestock in the Republic of Korea. *Pathogens, 12*(4), 547. [DOI:10.3390/pathogens12040547]

Jilo, K., Tegegne, D., Kasim, S., Dabasa, G., & Zewdei, W. (2021). Seroprevalence and public health significance of toxoplasmosis in small ruminants of pastoral community in Yabello district, Borana zone, southern Ethiopia. *Veterinary Medicine International, 2021*, 1-11. [Link]

Joda, M. (2008). *The progressive statistical analysis by using SPSS.* Amman: Wales house editions. [Link]

Khalil, M., Baothman, M., Alserhan, F., Almunyif, A., Alsharbe, G., & Samaren, H., et al. (2018). Prevalence of Toxoplasma gondii infection in diabetic patients in Makkah AL Mukarramah, Saudi Arabia. *Tropical Biomedicine 35*(2), 464-471. [PMID]

Khan, M. B., Khan, S., Rafiq, K., Khan, S. N., Attaullah, S., & Ali, I. (2020). Molecular identification of Toxoplasma gondii in domesticated and broiler chickens (Gallus domesticus) that possibly augment the pool of human toxoplasmosis. *PloS One, 15*(4), e0232026. [PMID]

Khater, H. F., Khalifab, N. O., & Barakatc, A. M. A. (2013). Serological and molecular studies of ovine and human toxoplasmosis with a trial of treatment of infected ewes. *Scientific Journal of Veterinary Advances, 2*(11), 157-168. [Link]

Khattab, R. A., Barghash, S. M., Mostafa, O. M. S., Allam, S. A., Taha, H. A., & Ashour, A. A. E. (2022). Seroprevalence and molecular characterization of Toxoplasma gondii infecting ruminants in the North-West of Egypt. *Acta Tropica, 225*, 106139. [PMID]

Kim, K. T., & Seo, M.G. (2023). Molecular epidemiology of Toxoplasma gondii in cattle in Korea. *Parasites, Hosts and Diseases, 61*(2), 210-215. [DOI:10.3347/PHD.23016]

Lin, M. H., Chen, T. C., Kuo, T.T., Tseng, C. C., & Tseng, C. P. (2000). Real-time PCR for quantitative detection of Toxoplasma gondii. *Journal of Clinical Microbiology, 38*(11), 4121-4125. [DOI:10.1128/JCM.38.11.4121-4125.2000]

Liyanage, K. L. D. T. D., Wiethoelter, A., Hufschmid, J., & Jabbar, A. (2021). Descriptive comparison of ELISAs for the detection of Toxoplasma gondii antibodies in animals: A systematic review. *Pathogens, 10*(5), 605. [PMID]

Mahittikorn, A., Wickert, H., & Sukthana, Y. (2005). Comparison of five DNA extraction methods and optimization of a B1 nested PCR (nPCR) for detection of Toxoplasma gondii tissue cysts in mouse brain. *The Southeast Asian Journal of tropical Medicine and Public Health, 36*(6), 1377–1382. [PMID]

Majeed, B. A., & Abbas, W. H. (2018). Serological and molecular detection of Toxoplasma gondii in meat and minced meat in Basra City. *Basrah Journal of Veterinary Research, 17*(3), 491-505. [Link]

Mohamed, K. (2020). Toxoplasmosis in humans and animals in Saudi Arabia: A systematic review. *The Journal of Infection in Developing Countries, 14*(8), 800-811. [DOI:10.3855/jidc.12648]

Nassef, N. E., Abd El-Ghaffara, M. M., El-Nahasa, N. S., Hassanain, M. E. A., El-Dina, S.A.S., & Ammara, A. I. M. (2018). Seroprevalence and genotyping of Toxoplasma gondii in Menoufia governorate. *Menoufia Medical Journal, 185*(128), 37-146. [DOI:10.4103/1110-2098.165828]

Opsteegh, M., Dam-Deisz, C., de Boer, P., DeCraeye, S., Faré, A., & Hengeveld, P., et al. (2020). Methods to assess the effect of meat processing on viability of Toxoplasma gondii: Towards replacement of mouse bioassay by in vitro testing. *International Journal for Parasitology, 50*(5), 357–369. [DOI:10.1016/j.ijpara.2020.04.001]

Sakban, F. M., & A'aiz, N. N. (2020). Investigate the Toxoplasma gondii infection in the consumed beef in Al-Diwaniyah province. *Iraqi Journal of Veterinary Sciences, 34*(1), 95-99. [DOI:10.33899/ijvs.2020.164336]

Santoro, M., Viscardi, M., Sgroi, G., D□Alessio, N., Veneziano, V., & Pellicano, R., et al. (2019). Real-time PCR detection of Toxoplasma gondii in tissue samples of wild boars (Sus scrofa) from southern Italy reveals high prevalence and parasite load. *Parasites & Vectors, 12*(1), 335. [DOI:10.1186/s13071-019-3586-5] [PMID]

Shariatzadeh, S. A., Sarvi, S., Hosseini, S. A., Sharif, M., Gholami, S., & Pagheh, A. S., et al. (2021). The global seroprevalence of Toxoplasma gondii infection in bovines: A systematic review and meta-analysis. *Parasitology, 148*(12), 1417-1433. [DOI:10.1017/S0031182021001116]

Souza, I. M. F. N. B., Siqueira, V. D. S., Ribeiro, I. D. C., Moraes, L. S. P., Prado, D. P. G. D., & Rezende, S. R., et al. (2023). Molecular and serological diagnosis of toxoplasmosis: A systematic review and meta-analysis. *Revista do Instituto de Medicina Tropical de São Paulo, 65*, 19. [PMID]

Sroka, J., Karamon, J., Wójcik-Fatla, A., Piotrowska, W., Dutkiewicz, J., & Bilska-Zając, E., et al. (2020). Toxoplasma gondii infection in slaughtered pigs and cattle in Poland: Seroprevalence, molecular detection and characterization of parasites in meat. *Parasites & Vectors, 13*(1), 223. [DOI:10.1186/s13071-020-04106-1]

Taalay, I., Iqbal, R. K., Asif, M., Ahmad, A., Amjad, M., & Anwar, F. N., et al. (2022). Molecular survey of Toxoplasma gondii in cattle and buffaloes and phylogenetic position of Pakistani isolates based on ITS-1 gene. C*omparative Immunology, Microbiology and Infectious Diseases, 84*, 101782. [DOI:10.1016/j.cimid.2022.101782]

Tonouhewa, A. B. N., Akpo, Y., Sessou, P., Adoligbe, C., Yessinou, E., & Hounmanou, Y.G., et al. (2017). Toxoplasma gondii infection in meat animals from Africa: Systematic review and meta-analysis of sero-epidemiological studies. *Veterinary World, 10*(2), 194-208. [Link]

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