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Traditional, histopathological and molecular diagnosis of sarcocytosis in slaughtered sheep in Al-Diwaniyah province, Iraq

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Abstract

The presented investigation was performed to identify *Sarcocystis* species, in an intermediate-host parasite with zoonotic importance, infection in slaughtered sheep identified by traditional, histological, and molecular methods in Al-Diwaniyah Province, Iraq. Here, 70 intercostal and esophageal muscle samples 10-40 gm/each were collected from slaughtered sheep. The samples were examined for detection of the Sarcocystic bradyzoites using microscopic, histological, *18S rRNA* gene-based polymerase chain reaction (PCR) and PCR-product-dependent gene sequencing methods. The results of the microscopic method revealed that 65 (93%) of the muscle samples contained bradyzoites. The histopathological picture showed the presence of two microsarcocysts with different morphological thin or thick wall sizes. The PCR demonstrated that 68/70 (97%) of the samples were positive for the occurrence of the parasite DNA. According to the NCBI-based websites, the Phylogenetic analysis revealed that the isolates from the current study were closely similar in their nucleotide sequences with isolates from Norway, Egypt, and Iran. The present data indicate high infection levels by *Sarcocystis* spp. in slaughtered sheep, which could bring alarm for public health importance.

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Introduction

As one of the Parasites of food-borne illnesses, *Sarcocystis* are intracellular protozoa. Until recently, 196 genuine species of *Sarcocystis* have been identified, and each of them is very selective regarding the types of hosts it can live on. Cysts are produced by parasites and may be seen in the striated muscles and the brains of pigs, cattle, and sheep (1-5). Eating raw or undercooked meat carrying sarcocystosis may spread infections of *S. hominis*, *S. heydorni*, and *S. suihominis*. Both *S. hominis* and *S. suihominis* have been designated as zoonotic risks by the European Food Safety Authority (6-12). Sheep may be infected with Sarcocystosis through drinking contaminated water or eating wild feed containing the parasite's sporocysts, leading to weight loss, abortion, early delivery, and even death (13-16). Most species of *Sarcocystis* need not one but two different hosts during their life cycles, with carnivores serving as the final hosts and herbivores acting as intermediate hosts. Only four parasitic species have been confirmed to infect sheep: the non-pathogenic parasitic species (macroscopic) *S. gigantea* and *S. medusiformis* transferred by felids, and the pathogenic parasitic species (microscopic) *S. tenella* and *S. arieticanis* spread by canids (17-21). *Sarcocystis spp.* Infects various animals, especially mammals, birds, and fish. The final host is responsible for the sexual phases of gametogony and sporogony. In contrast, the intermediate host is responsible for the asexual phases of merogony and cyst development (22,23). Sarcocystis infections in intermediate hosts are often asymptomatic, but severe instances have been observed, resulting in the host's fatigue, loss of appetite, diarrhea, loss of weight, muscular twitching, and in very exceptional instances, mortality. Muscle tissue cysts carrying bradyzoites are a characteristic feature of the degenerative alteration known as sarcocystosis, which occurs in the intermediate host.

Infections by *Sarcocystis ovicanis* or *Sarcocystis neurona* has been linked to rare cases of encephalitis in sheep and horses (24,25).

The presented investigation was performed to identify *Sarcocystis* species, in an intermediate-host parasite with zoonotic importance, infection in slaughtered sheep identified by traditional, histopathological, and molecular methods in Al-Diwaniyah Province, Iraq.

Materials and methods

Samples

The presented work collected 70 intercostal and esophageal muscle samples (10-40gm/each) from slaughtered sheep from April-December, 2022. The samples were cooled-box-transported to the Laboratory of Parasitology, College of Veterinary Medicine, University of Al-Qadisiya, Al-Diwaniyah City, Iraq.

Microscopic examination

The samples were examined for Sarcocystic bradyzoites and cysts using squeezing, trichoscopy, and solution analysis methods described by Al-Bayatee (26) and Al-Dulaimi (27).

18S rRNA gene-based PCR

18S rRNA gene-based PCR was performed using primers, Sar-F1: GCACTTGATGAATTCTGGCA and Sar-R1: CACCACCCATAGAATCAAG, followed by Stecher *et al.* (13). The QIAamp DNA Mini Kit (Qiagen, Germany), with steps from the kit itself was employed for the bradyzoite DNA extraction. A 1.5% agarose gel was placed under an electrophoresis process for 60mins at 100 volts and 80Amp. The gel, after that, was visualized under a UV-based imager. Stecher *et al.* (28) employed the methods in the current section.

18S rRNA-PCR product-based sequencing

The PCR-product-dependent gene sequencing method sends the PCR-purified products to Macrogen (Korea) for sequencing. The sequences were processed using MEGA X software and NCBI websites to complete the analyses and build a phylogenic tree.

Results

Microscopic test

The results of the microscopic method revealed that 65 (93%) of the muscle samples contained bradyzoites (Figure 1).

Histopathological findings

The pathohistological picture showed the presence of two microsarcocysts with different morphological thin or thick wall sizes (Figure 2).

18S rRNA gene-based PCR findings

The PCR demonstrated that 68/70 (97%) of the samples were positive for the occurrence of the parasite DNA (Figure 3).

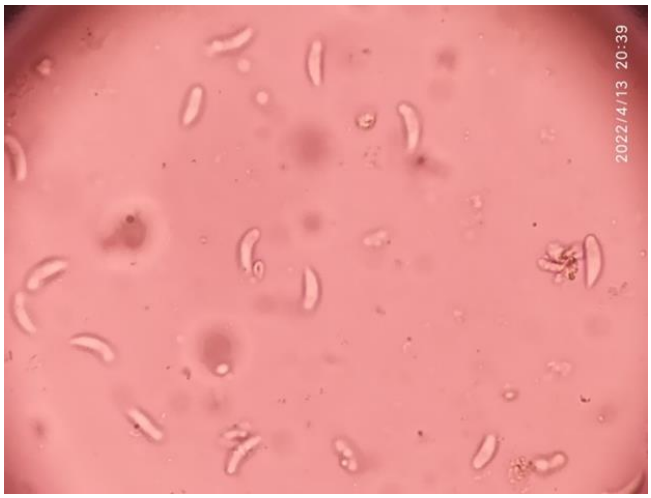

Figure 1: The bradyzoites of *Sarcocystis* spp. in sheep muscle tissue, 40X.

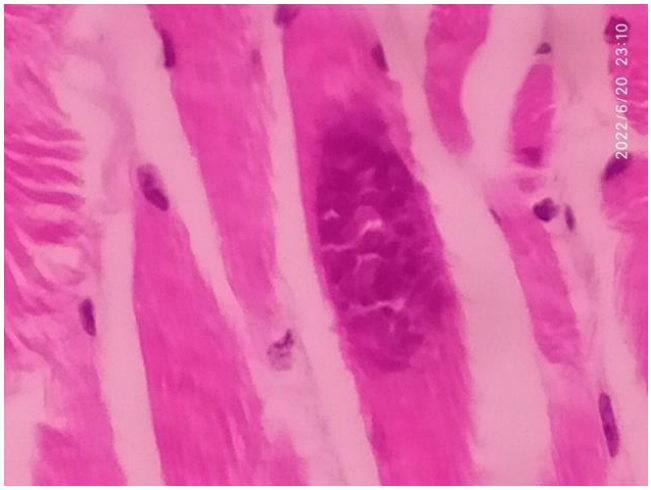

Figure 2: Encysted parasite in skeletal muscle showing internal bradyzoite nuclei separated by clear internal septations. H and E stain ×100.

Findings of *18S rRNA*-PCR product-based sequencing

According to the NCBI-based websites, the Phylogenetic analysis revealed that the isolates from the current study were closely similar in their nucleotide sequences with isolates from Norway, Egypt, and Iran (Table 1 and Figure 4).

Table 1: Homology sequence identity (%) in local *Sarcocystis* sp in sheep

Obtained Accession number	Identical to	GenBank Accession number	Country	Identity (%)	Host
OP835896	*Sarcocystis tenella*	MT560372	Iran	99.38	sheep
OP835897	*Sarcocystis tenella*	MT569891	Iran	100	sheep
OP835898	*Sarcocystis tenella*	MK420019	Norway	99.80	sheep
OP835899	*Sarcocystis tenella*	MG515221	Egypt	99.59	sheep
OP835900	*Sarcocystis tenella*	MT569891	Iran	100	sheep
OP835901	*Sarcocystis tenella*	MT569891	Iran	100	sheep
OP835902	*Sarcocystis tenella*	MT569891	Iran	100	sheep
OP835903	*Sarcocystis tenella*	MT569891	Iran	100	sheep
OP835904	*Sarcocystis tenella*	MT569891	Iran	99.59	sheep
OP835905	*Sarcocystis tenella*	MT569891	Iran	100	sheep

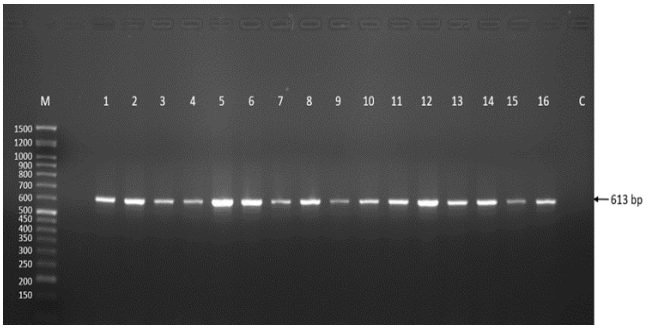

Figure 3: Image of agarose gel electrophoresis. It shows the *18S rRNA* gene-based piece amplification of *Sarcocystis* spp. of sheep. 1-16: Positive samples. C: Negative control (No DNA). M: Molecular marker.

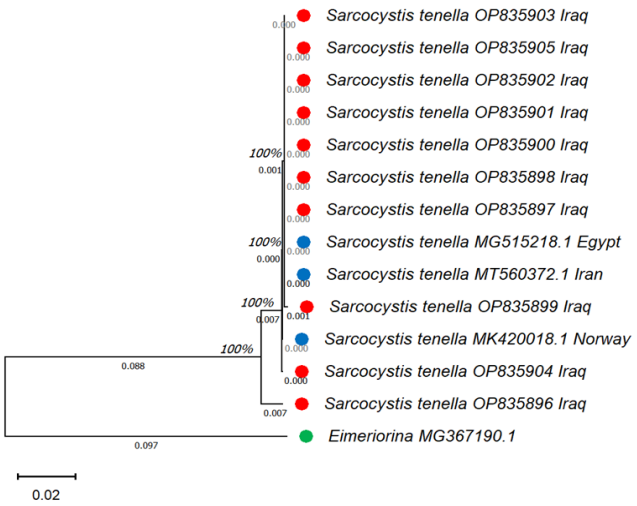

Figure 4: Phylogenetic tree of *Sarcocystis* spp in sheep based on the *18S rRNA* gene.

Discussion

Similar to the earlier findings from Baghdad 91.93% (26). this research found a prevalence of Sarcocystis infection in sheep of 93%. And in Mongolia, the sheep have an infection rate of 97%, a 100% in Iran's sheep population, and 84 % in American sheep (29). Molecular methods, such as PCR amplification of species-specific 18s rRNA, are necessary for identifying species in this genus, which cannot be done only by microscopy. These results, at 97%, show that molecular diagnosis for sarcocystosis has a greater infection rate than traditional methods. Likewise, 90.78 % of the sheep samples in Karbala Province (30), Iraq, were infected with the parasite (31-33).

The Sartcocystis includes Sarcosporidia, known for their versatility in where they invade and grow inside the intermediate host. In addition, investigational transfer of the muscle cysts from sheep to dog puppies revealed the coccidian character of Sarcocystis, where the parasite was found at a higher incidence (34). Sarcocysts, only seen in the muscular parts of intermediate hosts, were characterized as evidence of a wide variety of host species and global dissemination. The current research demonstrated that natural infection rates in the tested hosts' skeletal muscles and organs varied. These findings are consistent with those found previously in Riyadh (35-37).

Alternatively, other nations reported lower levels. This research demonstrated a very varied rate of Sarcoystis distribution throughout several sheep organs. Oocyst exposure, changes in host immunity owing to abnormalities in the environmental and nutritional state, and parasite speciation are all possible causes for the observed variances in intensity among the tested hosts, as revealed by different studies (38). Other reports of detecting *Sarcocystis* spp. in different countries, such as Saudi Arabia, Tunisia, and China (39-41).

Conclusion

The present data indicate high infection levels by *Sarcocystis* spp. in slaughtered sheep, which could bring alarm for public health importance.

Acknowledgments

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Conflict of interests

The authors have not received any funding or benefits from industry, financing agency, or elsewhere to conduct this study.

References

1. Dubey JP, van Wilpe E, Calero-Bernal R, Verma SK, Fayer R. *Sarcocystis heydorni*, n. sp. (Apicomplexa: Sarcocystidae) with cattle (*Bos taurus*) and human (*Homo sapiens*) cycle. Parasitol Res. 2015;114(11):4143–7. DOI: 10.1007/s00436-015-4645-2
2. Enas SM, Alhayali NS, Suleiman EG. Pathological and molecular study of ovine diaphragms naturally infected by *Sarcosystis spp*. Iraqi J of Vet Sci. 2021;35(4):749-755. DOI: 10.33899/ijvs.2021.128327.1570
3. Alfatlawi MA, Jasim AA, Jarad NE, Khlaif SF. Clinical and molecular identification of ruling *Theileria annulata* strains in cattle calves in Al-Diwaniyah province, Iraq. Iraqi J Vet of Sci. 2021;35(1):115-119. DOI: 10.33899/ijvs.2020.126429.1319
4. Klaif SF, Abid AJ, Al-Fatlawi MA, Ali MJ. Major-surface-protein-4-gene-based detection of *Anaplasma marginale* isolated from sheep in Al-Diwaniyah province, Iraq. Iraqi J Vet Sci. 2022;36(1):85-88. DOI: 10.33899/ijvs.2021.129230.1635
5. Alfatlawi MA, Ismail YK, Ali MJ, Karawan AC, Ibadi IN. Molecular differentiation of *Thysaniezia* (Helictometra) *giardi* and Moniezia species based on 18s rRNA gene in small ruminants. Iraqi J Vet Sci. 2021;35(1):105-108. DOI: 10.33899/ijvs.2020.126407.1313
6. Ayazian MS, Teimouri A, Mohebali M, Sharifi Yazdi MK, Shojaee S, Rezaian M, Salimi M, Keshavarez H. Sarcocystis infection in beef and industrial raw beef burgers from butcheries and retail stores: A molecular microscopic study. Heliyon. 2020;6(6):e04171. DOI: 10.1016/j.heliyon.2020.e04171
7. Fayer R, Esposito DH, Dubey JP. Human Infections with Sarcocystis Species. Clin Microbiol Rev. 2015;28(2):295–311. DOI: 10.1128/CMR.00113-14
8. de Freitas BR, Roman IJ, Reis BR, Rodrigues F, Braünig P, Cargnelutti J, Cunha RC, Vogel FF. Sarcocyst quantification and viability: Freezing treatment as an alternative to carcass condemnation. Acta Parasitol. 2023;68(11):277-281. DOI: 10.1007/s11686-022-00656-9
9. Gorman TR, Alcaíno HA, Muñuz H, Cunazza C. *Sarcocystis sp*. in guanaco (*Lama guanicoe*) and effect of temperature on its viability. Vet Parasitol. 1984;15(2):95-101. DOI: 10.1016/0304-4017(84)90025-6
10. Panziera W, Vielmo A, De Lorenzo C, Heck LC, Pavarini SP, Sonne L, Soares JF, Driemeier D. Characterization of parasitic lesions of sheep observed at slaughter line. Pesqui Vet Bras. 2018;38(8):1491-1504. DOI: 10.1590/1678-5150-PVB-5549
11. Minuzzi CE, Cezar AS, Bräunig P, Portella LP, Rodrigues FS, Sangioni LA, Vogel FF. Occurrence of *Sarcocystis gigantea* macrocysts and high frequency of *S. tenella* microcysts in sheep from southern Brazil. Vet Parasitol Reg Stud Rep. 2019;15(1):100256. DOI: 10.1016/j.vprsr.2018.12.002
12. Marciano MM, Silva RA, Barbosa ML, Ferreira AS, Pereira-Chioccola VL. Determination of the viability of *Toxoplasma gondii* oocysts by PCR real-time after treatment with *Propidium monoazide*. Rev Inst Med Trop São Paulo. 2020;62(3):1-7. DOI: 10.1590/S1678-9946202062084
13. Dong H, Lu Y, Yang Y. Epidemiology and classification of Sarcocystis in sheep and goat. Chinese J Zoonoses. 2017;33(9):828-36. [available at]
14. Duarte AP, Carvalho GM, Franzen AJ, Junior AH, Fernandes C, Fortes FA, Seabra SH. Anti-*Toxoplasma gondii* effect of metalocomplex compounds N0414 and N5814. Braz J Dev. 2021;7(2):16541-16555. DOI: 10.34117/bjdv7n2-330
15. Alves MM, Cadore GC, Oliveira CS, Portella LP, Sangioni LA, Vogel FF. Molecular characterization of *Sarcocystis spp*. in samples of meat. Pesqui Vet Bras. 2018;38(3):425-429. DOI: 10.1590/1678-5150-PVB-4606
16. Moré G, Abrahamovich P, Jurado S, Bacigalupe D, Marin JC, Rambeaud M, Venturini L, Venturini MC. Prevalence of *Sarcocystis spp*. in Argentinean cattle. Vet Parasitol. 2011;65(8):256-258. DOI: 10.2478/s11686-019-00114-z
17. Dalimi A, Jalosian F, Biderouni FT, Ghaffarifar F. Identification of *Sarcocystis gigantea* by PCR-RFLP. J Vet Res. 2010;65(1):43–6. DOI: 10.1016/j.cimid.2020.1015662
18. Salehi M, Spotin A, Rostamian M, Adami M. Prevalence and molecular assessment of Sarcocystis infection in livestock in northeast Iran. Comp Immunol Microbiol Infect Dis. 2022;80(1):101738. DOI: 10.1016/j.cimid.2021.101738
19. Martínez-Navalón B, Anastasio-Giner B, Cano-Fructuoso M, Sanchez-Maertínez P, Llopis-Morant A, Perez-Castarlenas B, Goyena E, Berriatua E. Short communication. Sarcocystis infection: A major cause of carcass condemnation in adult sheep in Spain. Span J Agric Res. 2012;10(2):388-392. DOI: 10.5424/sjar/2012102-523-11
20. Bidone NB, Gonçalves AP, Bremm C, Girotto AF, Girotto-Soares A, Santos P, Pinto AT, Soares JF. Slaughter condemnation in bovine due to parasitic lesions and their economic impact in federal inspection system establishments in Brazil and in state inspection system in Rio Grande do Sul State. Braz J Vet Parasitol. 2021;30(1):1-10. DOI: 10.1590/S1984-296120201079
21. Harada S, Furukawa M, Tokuoka E, Matsumoto K, Yahiro S, Miyasaka J, Saito M, Kamata Y, Watanabe M, Irikura D, Matsumoto H, Sugita-Konishi Y. Control of toxicity of *Sarcocystis fayeri* in horsemeat by freezing treatment and prevention of food poisoning caused by raw consumption of horsemeat. J Food Hyg Soc Japan. 2013;54(3):34-44. DOI: 10.3358/shokueishi.54.198
22. Poulsen CS, Stensvold CR. Current status of epidemiology and diagnosis of human Sarcocystosis. J Clin Microbiol. 2014;52(10):3524–30. DOI: 10.1128/JCM.00955-14
23. Verma SK, Lindsay DS, Grigg ME, Dubey JP. Isolation, culture and cryopreservation of Sarcocystis species. Curr Protoc Microbiol. 2017;45(1). DOI: 10.1002/cpmc.32
24. Lau YL, Chang PY, Tan CT, Fong MY, Mahmud R, Wong KT. *Sarcocystis nesbitti* Infection in human skeletal muscle: Possible transmission from snakes. Am J Trop Med Hyg. 2014;90(2):361–4. DOI: 10.4269/ajtmh.12-0678
25. Rogers KH, Arranz-Solís D, Saeij JPJ, Lewis S, Mete A. *Sarcocystis calchasi* and other Sarcocystidae detected in predatory birds in California, USA. Int J Parasitol Parasites Wildl. 2022;17(4):91–9. DOI: 10.1016/j.ijppaw.2021.12.008
26. AL-Bayatee SM. Study in epidemiology of bovine sarcocystosis in Baghdad area. University of Baghdad; 1993. DOI: 10.1016/s0304-4017(99)00046-12
27. Al-Dulaimi SS. Parasitic etiology of diarrhea in Al-Anbar province. Al Mustansiriya J Sci. 1992;7(2):64–8. DOI: 10.37652/juaps.2014.1239101
28. Stecher G, Tamura K, Kumar S. Molecular evolutionary genetics analysis (MEGA) for macOS. Mol Biol Evol. 2020;37(4):1237–9. DOI: 10.1093/molbev/msz312
29. Fard SN, Asghari M, Nouri F. Survey of Sarcocystis infection in slaughtered cattle in Kerman, Iran. Trop Anim Health Prod. 2009;41(8):1633–6. DOI: 10.1007/s11250-009-9358-z
30. Al-Saadi SM, Al-Mussawi KM, Muhammed HA. Molecular identification of Sarcocystis species infection in sheep in Karbala governorate – Iraq. Med Leg Updat. 2020;20(1):889–95. DOI: 10.37506/v20/i1/2020/mlu/194708
31. Baranauskaitė A, Strazdaitė-Žielienė Ž, Servienė E, Butkauskas D, Prakas P. Molecular identification of protozoan Sarcocystis in different

الطرق التقليدية، النسيجية المرضية والجزيئية لتشخيص الحويصلات الصنوبرية في الأغنام المذبوحة في محافظة الديوانية، العراق

هديل هادي جواد و غيداء عباس جاسم

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الخلاصة

تم إجراء البحث المقدم هنا للتعرف على وجود خمج الحويصلات الصنوبرية، وهو طفيلي المضيف الوسيط وذو أهمية للأمراض حيوانية المصدر، في الأغنام المذبوحة التي تم تحديد الخمج بالطرق التقليدية والنسيجية والجزيئية في محافظة الديوانية، العراق. تم جمع ٧٠ عينة من العضلات ١٠-٤٠ جم / لكل منهما من الأغنام. تم فحص العينات لوجود الحويصلات بطيئة التكاثر باستخدام تفاعل البلمرة المتسلسل للجين الرايبوسومي وطرق دراسة تعاقب القواعد النيتروجينية لمنتجات تفاعل البلمرة المتسلسل. أظهرت نتائج الطريقة المجهرية أن ٦٥ (٩٣٪) من عينات العضلات احتوت على الحويصلات بطيئة التكاثر. أظهرت الصورة المرضية النسجية وجود اثنين من الأكياس المجهرية ذات حجم جدار رقيق أو سميك. أظهر تفاعل البلمرة المتسلسل أن ٧٠/٦٨ (٩٧ ٪) من العينات كانت إيجابية لوجود الحمض النووي للطفيلي في العينات المفحوصة. كشف تحليل تطور النسب أن العزلات من الدراسة الحالية كانت متشابهة إلى حد كبير في متواليات القواعد النيتروجينية مع عزلات من النرويج ومصر وإيران. تشير البيانات الحالية إلى مستويات عالية من الإصابة بطفيلي الحويصلات الصنوبرية. في الأغنام المذبوحة، الأمر الذي يمكن أن ينذر بأهمية الصحة العامة.

types of water bodies in Lithuania. Life. 2023;13(1):51–60. DOI: 10.3390/life13010051

32. Agholi M, Shahabadi SN, Motazedian MH, Hatam GR. Prevalence of enteric protozoan oocysts with special reference to *Sarcocystis cruzi* among fecal samples of diarrheic immunodeficient patients in Iran. Korean J Parasitol. 2016;54(3):339–44. DOI: 10.3347/kjp.2016.54.3.339
33. Hamidinejat H, Moetamedi H, Alborzi A, Hatami A. Molecular detection of Sarcocystis species in slaughtered sheep by PCR–RFLP from south-western of Iran. J Parasit Dis. 2014;38(2):233–7. DOI: 10.1007/s12639-012-0231-z
34. Dehaghi MM, Fathi S, Asl EN. Survey of Sarcocystis infection in slaughtered goats in Kerman abattoir, southeast of Iran. J Anim Vet Adv. 2011;10(9):1205–8. DOI: 10.3923/javaa.2011.1205.1208
35. Omer SA, Alzuraiq AA, Mohammed OB. Prevalence and molecular detection of *Sarcocystis spp*. infection in the dromedary camel (*Camelus dromedarius*) in Riyadh city, Saudi Arabia. Biomed Res. 2017;28(11):4962–5. DOI: 10.21608/svu.2021.80987.11301
36. Al-Hoot AS, Al-Qureishy SA, Al-Rashid K, Bashtar AR. Microscopic study on *Sarcocystis moulei* from sheep and goats in Saudi Arabia. J Egypt Soc Parasitol. 2005;35(1):295–312. DOI: 10.1007/s00436-014-4050-2.
37. Morsy K, Saleh A, Al-Ghamdi A, Abdel-Ghaffara F, Al-Rasheid K, Bashtar AR, Al-Quraishy S, Mehlhorn H. Prevalence pattern and biology of *Sarcocystis capracanis* infection in the Egyptian goats: A light and ultrastructural study. Vet Parasitol. 2011;181(2–4):75–82. DOI: 10.1016/j.vetpar.2011.05.010
38. Al Quraishy S, Morsy K, Bashtar AR, Ghaffar FA, Mehlhorn H. *Sarcocystis arieticanis* (Apicomplexa: Sarcocystidae) infecting the heart muscles of the domestic sheep, *Ovis aries* (Artiodactyla: Bovidae), from K. S. A. on the basis of light and electron microscopic data. Parasitol Res. 2014;113(10):3823–31. DOI: 10.1007/s00436-014-4050-2
39. Metwally DM, Al-Damigh MA, Al-Turaiki IM, El-Khadragy MF. Molecular Characterization of Sarcocystis species isolated from sheep and goats in Riyadh, Saudi Arabia. Anim. 2019;9(5):256. DOI: 10.3390/ani9050256
40. Amairia S, Amdouni Y, Rouatbi M, Rjeibi MR, Awadi S, Gharbi M. First detection and molecular identification of *Sarcocystis spp*. in small ruminants in north-west Tunisia. Transbound Emerg Dis. 2018;65(2):441–6. DOI: 10.1111/tbed.12722
41. Dong H, Ruijing S, Wang Y, Tong Z, Zhang L, Yang YR, Hu J. Sarcocystis species in wild and domestic sheep (*Ovis ammon* and *Ovis aries*) from China. BMC Vet Res. 2018;14(1):377. DOI: 10.1186/s12917-018-1712-9