Open Veterinary Journal, (2023), Vol. 13(9): 1099–1105
ISSN: 2226-4485 (Print)
ISSN: 2218-6050 (Online)
Original Research
DOI: *10.5455/OVJ.2023.v13.i9.5*

Submitted: 10/06/2023 Accepted: 06/08/2023 Published: 30/09/2023

Molecular identification and phylogenetic confirmation of *Sarcocystis* species in slaughtered camels in Al-Najif province, Iraq

Ola A. Aggar* and Mohammed T. S. Al-Zubaidi

Department of Parasitology, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq

Abstract

Background: *Sarcocystis* is an intracellular parasite of particular importance as it infects many domestic animals as camels that play the role of intermediate host for the parasite.

Aim: This study aimed to identify *Sarcocystis* species in camels by molecular assay with confirmation of local isolates by phylogenetic analysis.

Methods: A total of 200 slaughtered camels (*Camelus dromedarius*) that were slaughtered in Al-Najaf province (Iraq) abattoirs from October (2021) to July (2022) were subjected to collect the fresh tissues from four organs (esophagus, diaphragm, skeletal muscle, and heart), to be tested later by the conventional polymerase chain reaction (PCR). Then, a total of 20 positive genomic DNA samples were sequenced, named, got specific access numbers (OP785703.1 to OP785722.1), and compared with the NCBI-GenBank isolates.

Results: Targeting *Cox1* gene, 80% of collected tissues were found positive by the conventional PCR assay. Phylogenetic tree analysis revealed that the local *Sarcocystis* isolates were identical to Indian *S. cameli* isolates at 99.70%–99.90%. Significantly, an increase in *Sarcocystis* infection was seen in the esophagus compared to the diaphragm, skeletal muscle, and heart; older (>4 years) than younger (≤4 years) camels, and in females more than males.

Conclusion: To the best of our knowledge, this represents the first molecular study in Iraq that identifies *Sarcocystis cameli* in camels. However, additional epidemiological and molecular studies in camel populations as well as in other domestic and wild animals appeared to be necessary.

Keywords: Sarcocystosis, *S. cameli*, *Camelus dromedarius*, PCR, Sequencing.

Introduction

Sarcocystis, first reported in 1843, is a genus of intracellular protozoan parasite which belongs to Sarcocystidae family, Eucoccidiorina order of Apicomplexa phylum. Worldwide, considerable production losses and health illness in animals have been recognized due to the presence of >200 species in the genus of *Sarcocystis* that infects a wide array of domestic and wild animals (Dubey *et al*., 2015a; Abbas *et al*., 2023). The life cycle of a parasite involves two hosts; definitive (predator) and intermediate (prey) hosts. However, *Sarcocystis* spp. having greater specificity to intermediate than definitive hosts (Prakas *et al*., 2023). In the intermediate host, the development of asexual stages of the parasite occurs usually in skeletal, cardiac, esophageal, and diaphragmatic muscles and to a lesser extent in CNS (Dubey *et al*., 2015a; Gajadhar *et al*., 2015; Prakas *et al*., 2021). The definitive host can be infected during ingestion of contaminated tissues with mature *Sarcocystis* that undergoes sexual reproduction to excrete the oocysts/sporocysts to the environment by feces (Lindsay and Dubey, 2020; Razooqi *et al*., 2022).

One-humped or dromedary camels (*Camelus dromedarius*) reared widely in arid regions of many world countries (Burger *et al*., 2019; Zhu *et al*., 2019) has been used mainly for the production of meat that is characterized by the low percentages of fats and cholesterol when comparison to meats of other animal species (Faye, 2016; Kadim *et al*., 2018). However, the existence of parasite in camel meats might potentially downgrade their quality to be consumed by humans (Hamidinejat *et al*., 2013; Mohammadpour *et al*., 2020).

Although *Sarcocystis* spp. can induce significant pathology or even mortality, most diseased camels generally exhibit subclinical illness (Metwally *et al*., 2020). Thus, the diagnosis of *Sarcocystis* spp. in camels using traditional tools represents a challenge for the researchers. Hence, molecular identification of *Sarcocystis* spp. in Iraqi camels with confirmation of local isolates by phylogeny was aimed in this study.

***Corresponding Author:** Ola A. Aggar. Department of Parasitology, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq. Email: *Ula.Abd1105a@covm.uobaghdad.edu.iq*

Materials and Methods

Study animals and sampling

Totally, 200 slaughtered camels (*C. dromedarius*) of different ages (<2 to ≤5 years) and sexes (107 males and 93 females) were selected to the present study from the abattoir of Al-Najaf province (Iraq) during October (2021)–July (2022). Approximately, 100 g of fresh meat were obtained from the esophagus, diaphragm, skeletal muscle, and heart of each slaughtered camel, kept in individual plastic labeled containers, and saved frozen at −20C for later molecular examination.

Molecular examination

Conventional polymerase chain reaction (PCR)

In accordance with the manufacturer instruction of the gSYAN DNA Extraction Kit (iNtRON Biotechnology, South Korea), the DNAs were extracted from collected samples and examined directly by the Nanodrop System (Thermo Scientific, UK) to estimate the purity and concentration of these DNAs samples (Gharban *et al*., 2023). According to the manufacturer instructions of GoTaqTM Green PCR MasterMix Kit (Bioneer, Korea), the Mastermix tubes were prepared at a modified final volume of 25 µl (5 µl of DNA template, 2 µl of forward primer, 2 µl of reverse primer, 4 µl of Green PCR Mastermix, and 12 l of nuclease-free water) using one set of primers [(F: 5′-ATG GCG TAC AAC AAT CAT AAA GAA-3′) and (R: 5-ATA TCC ATA CCG CCA TTG CCC AT-3′)] targeting *Cox1* gene at a product size of 1,058 bp (Metwally *et al*., 2020). The PCR thermocycler was carried as follows: one cycle for initial denaturation (5 minutes/95°C); 35 cycles for denaturation (30 seconds/95°C), annealing (30 seconds/54°C) and extension (1 minute/72°C); and one cycle for final extension (5 minutes/72C). Agarose gel stained with 3 µl of Ethidium Bromide and Ladder Marker (100–2,000 bp) were analyzed by electrophoresis at 80 Volt, 80 Am for 90 minutes. PCR products of previously diagnosed and confirmed *Sarcocystis* spp. isolate was used as a positive control; while nuclease-free water was used as a negative control.

Finally, the DNA fragments were visualized under the UV-Transilluminator (ATTA/Korea).

Phylogenetic analysis

Sequencing of some positive DNAs was performed to confirm the species of *Sarcocystis*, and study the genetic relationship between the local and NCBI-BLAST *Sarcocystis* isolates. Targeting the *Cox1* gene, a total of 20 positive DNA samples in addition to forward primer were sent for the Macrogen Company (South Korea) to be sequenced by the AB DNA Sequencing System. First, the local isolates of *Sarcocystis* species were recorded in the National Center For Biotechnology Information (NCBI)-GenBank to obtain their access numbers; and then, the Phylogenetic tree analysis was performed by the MEGA 6.0 Software.

Statistical analysis

GraphPad Prism (*GraphPad Software Inc., USA*) Software was served to evaluate significant variation between results of this study using the one way-analysis of variance. Values were represented as percentages (%), and significant differences were $p < 0.05$ (Gharban, 2023).

Ethical approval

The current study was licensed by the Scientific Committee of the Department of Parasitology in the College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq.

Results

Targeting *Cox1* gene, 80% (160/200) of meat samples were positive by the conventional PCR assay at a product size of 1,058 bp (Fig. 1).

The genomic DNAs of 20 positive samples were named respectively as *Sarcocystis* spp. IQN.1 to IQN.20. Comparative analysis of local isolates with the NCBI-GenBank *Sarcocystis* isolates showed that there were nucleotide alignment similarities (*****) and substitution mutations in *Cox1* gene.

Comparative homology sequence identity (%) to detect genetic variations between the local *Sarcocystis* spp. IQN-isolates (total no. 20) and the NCBI-BLAST *Sarcocystis cameli* Indian (MW651858.1) isolate of *cox1* gene. This identity showed that there was 99% of similarity and 0.150%–0.50% of total genetic mutations/changes (Table 1, Fig. 2).

According to organ, significantly higher increases ($p < 0.05$) in the prevalence of sarcocysts were detected in the esophagus (91.43%), while the lower one ($p < 0.05$) was seen in the heart (62.5%). However, insignificant variation ($p > 0.05$) in the prevalence of *Sarcocystis* spp. between diaphragm (85.42%) and skeletal muscle (85.42%) was reported (Table 2).

According to the age of study camels, this study has seen that the parasite is more prevalent in camels aged >4 years (96%) than those aged ≤4 years (53.33%), (Table 3).

Regarding the sex of study camels, the infection rate of sarcocystosis was increased significantly ($p < 0.05$) in females (87.78%) in comparison with males (73.64%), (Table 4).

Discussion

Sarcocystis infections have been demonstrated in different animal species such as cattle, buffalo, goats, and sheep (Dakhil *et al*., 2017; Al-Saadi *et al*., 2020; Swar and Shnawa, 2020; Abdullah, 2021). The molecular technique is one of the most useful diagnostic methods for the identification of many pathogens, but there is low molecular data about *Sarcocystis* infection in camels. In this study, the prevalence of *Sarcocystis* spp. in slaughtered camels was 80%. This agrees with the finding reported in camels (85.5%) in Egypt (Rabie *et al*., 2021).

In this study, the *Cox1* gene revealed a high efficacy in the detection of *Sarcocystis* spp. in tissue samples of slaughtered camels. Different studies reported that DNA barcoding system using the *Cox*1 gene

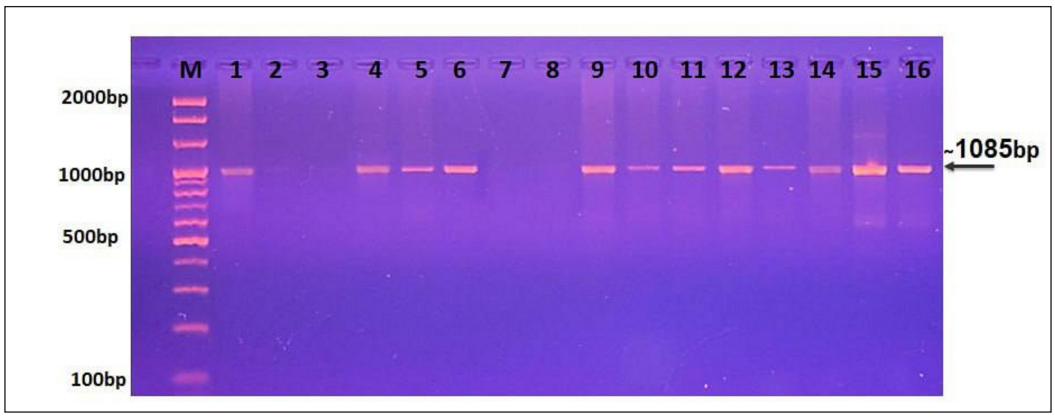

Fig. 1. Representative image shows the agarose-gel electrophoresis of PCR products targeting the *Cox1* gene to detect the genus of *Sarcocystis*. Lane (M): Ladder marker (100–2,000 bp); Lane (1): positive control; Lane (2): negative control; Lanes (3, 7, 8): negative samples; Lanes (4–6 and 9–16): positive samples for *Sarcocystis* spp. at 1,058.

Table 1. NCBI-BLAST Homology sequence identity between local and GenBank isolates.

Local isolates			NCBI-GenBank isolate		
No.	**Access No.**	**Species**	**Country**	**Access No.**	**Identity (%)**
IQN.1	OP785703.1	*S. cameli*	India	MW651858.1	99.70
IQN.2	OP785704.1	*S. cameli*	India	MW651858.1	99.70
IQN.3	OP785705.1	*S. cameli*	India	MW651858.1	99.70
IQN.4	OP785706.1	*S. cameli*	India	MW651858.1	99.80
IQN.5	OP785707.1	*S. cameli*	India	MW651858.1	99.70
IQN.6	OP785708.1	*S. cameli*	India	MW651858.1	99.80
IQN.7	OP785709.1	*S. cameli*	India	MW651858.1	99.70
IQN.8	OP785710.1	*S. cameli*	India	MW651858.1	99.60
IQN.9	OP785711.1	*S. cameli*	India	MW651858.1	99.70
IQN.10	OP785712.1	*S. cameli*	India	MW651858.1	99.90
IQN.11	OP785713.1	*S. cameli*	India	MW651858.1	99.70
IQN.12	OP785714.1	*S. cameli*	India	MW651858.1	99.80
IQN.13	OP785715.1	*S. cameli*	India	MW651858.1	99.70
IQN.14	OP785716.1	*S. cameli*	India	MW651858.1	99.70
IQN.15	OP785717.1	*S. cameli*	India	MW651858.1	99.70
IQN.16	OP785718.1	*S. cameli*	India	MW651858.1	99.70
IQN.17	OP785719.1	*S. cameli*	India	MW651858.1	99.80
IQN.18	OP785720.1	*S. cameli*	India	MW651858.1	99.80
IQN.19	OP785721.1	*S. cameli*	India	MW651858.1	99.70
IQN.20	OP785722.1	*S. cameli*	India	MW651858.1	99.70

has a high efficacy in discrimination between the invertebrate and vertebrate species (Rodrigues *et al*., 2017; Mioduchowska *et al*., 2018; Ahmed *et al*., 2022). Gjerde *et al*. (2015) concluded that the *Cox1* gene can work better with DNA samples derived from *Sarcocystis*, and sequences of the *Cox1* gene can vigorously distinguish between different species of *Sarcocystis*. Rodrigues *et al*. (2017) demonstrated the suitability of *Cox*1 as a gene of parasitic reorganization from those of highly related. Other studies reported that *Cox1* has a well phylogenetic character than other genes (Stöger *et al*., 2016; Wang *et al*., 2017). Furthermore,

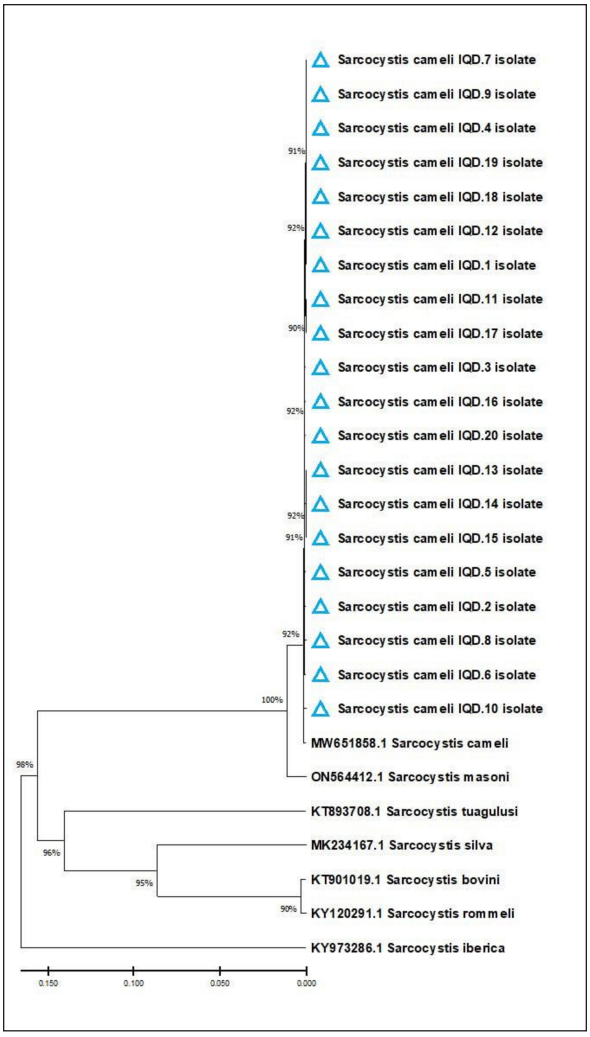

Fig. 2. Phylogenetic tree analysis based on *Cox1* gene partial sequence in local IQN *S. cameli* isolates and NCBI-GenBank *S. cameli* isolates. Comparative identity for the genetic variations between the local isolates and NCBI-BLAST isolates showed that similarity was 98%; whereas, the total genetic mutations/changes were 0.2%–1.5%.

some authors claimed that the evolution of the *Cox1* gene enables researchers to distinguish between closely related species and investigate intraspecific variation (Changbunjong *et al*., 2016; Viricel and Rosel, 2022). The *Cox1* has been demonstrated as the potential barcode marker for joint analysis of nuclear and mitochondrial markers of various species (Rodrigues *et al*., 2017; Ahmed *et al*., 2022).

Despite the discovery of five different *Sarcocystis* species in camels, multiple studies have shown that only *S. cameli* and *S. ippeni* are considered to be the real species infecting dromedary camels, namely *S. cameli*, *S. ippeni*, *S. camelicanis*, *S. camelocanis*, and *S. miescheri* (Dubey *et al*., 2015b; Omer *et al*., 2017; Rabie *et al*., 2021).

Table 2. Total infection rate of sarcocystosis in camels according to organ.

Organ	Total no.	Positives	
		No.	%
Esophagus	70	64	91.43*
Diaphragm	48	41	85.42*
Skeletal muscle	50	35	85.42*
Heart	32	20	62.5
Total	200	160	80
Chi-square (χ^2)	-	-	5.417

*$p < 0.043$.

Table 3. Total infection rate of sarcocystosis in camels according to age.

Age (year)	Total no.	Positives	
		No.	%
≤4	75	40	53.33
>4	125	120	96*
Total	200	160	80
Chi-square (χ^2)	-	-	4.425

*Vertical comparison between values (%) refers to significant differences at ($p < 0.05$).

Table 4. Total infection rate of sarcocystosis in camels according to sex.

Sex	Total no.	Positives	
		No.	%
Male	110	81	73.64
Female	90	79	87.78*
Total	200	160	80
Chi-square (χ^2)	-	-	4.009

*Vertical comparison between values (%) refers to significant differences at ($p < 0.05$).

In this study, the phylogenetic analysis discovered that the local study isolates were identical to the global NCBI-GenBank Indian *S. cameli* isolate. This is in agreement with the results of other studies as *S. cameli* is the more prevalent species of *Sarcocystis* in camels to yet (Valinezhad *et al*., 2008; Dubey *et al*., 2015b; Omer *et al*., 2017; Asopa *et al*., 2023). For the first molecular proof of *S. cameli* in camels in Iran (Motamedi *et al*., 2011), the bradyzoite DNA was amplified from the *18S rRNA* gene fragment using traditional PCR.

Sarcocystis species can frequently infect the muscular tissue of the heart, tongue, esophagus, and diaphragm (Wahba *et al*., 2014; Ahmed *et al*., 2016). Our findings revealed that *Sarcocystis* spp. has existed in all examined organs but more commonly in the

esophagus. Some research suggested that sarcocystosis is more frequent in the cremaster muscle (Bucca *et al*., 2011; Sağlam and Keleş, 2016). Shekarforoush *et al*. (2006) demonstrated that the heart is the most infected organ; while Al-Ani and Amr (2017) noted that camel's diaphragm was the most frequently affected organ. According to Oryan *et al*. (2010), *Sarcocystis* appears to prefer the esophagus, tongue, and heart. In contrast, Gareh *et al*. (2020) reported that *Sarcocystis* can spread in various organs, mostly in the esophagus with a prevalence rate of 49%. Rabie *et al*. (2021) reported *Sarcocystis* in the esophageal, heart, and ocular muscles in addition to other organs, with a higher infection rate in the esophagus (85.5%). Asopa *et al*. (2023) showed that *Sarcocystis* spp. was prevalent in the tongue and esophagus of dromedary camels from Rajasthan's Bikaner area. This variance in the distribution of *S. cameli* strains may be accounted for differences in the prevalence of *Sarcocystis* among camel organs (Hamidinejat *et al*., 2013).

Another substantial risk factor for infection was age. In this study, the infection rate was considerably higher in older camels (>4 years) than in younger camels (<4 years). Other reports in Iran (Hamidinejat *et al*., 2013), Saudi Arabia (Omer *et al*., 2017), and Egypt (El-Bahy *et al*., 2019) have revealed similar results. The slower development of detectable cysts may account for the low prevalence of *Sarcocystis* in young camels. Another explanation for increasing the rate of infection in older camels is that the older animals are slaughtered at a higher rate than younger ones. In addition, keeping young camels indoors may reduce their exposure to disease (Valinezhad, *et al*., 2008, Hamidinejat *et al*., 2013, Omer *et al*., 2017).

Sex was discovered as a crucial factor linked to infection. Our findings were consistent with numerous studies conducted in southern Ethiopia (Woldemeskel and Gumi, 2001), Iran (Valinezhad *et al*., 2008), and Egypt (Rabie *et al*., 2021) which found that male camels were more susceptible to infection than females. Omer *et al*. (2017) showed that the slaughtered female camels were usually over 4 years old, whereas male camels were under 2 years old. This disparity might be explained by the fact that most males are grazed outside for hard work, while most females are kept indoors for breeding under good and hygienic management. This might make males more susceptible to infection than females (Romero *et al*., 2017). An insignificant difference in the frequency of sarcocystosis between male and female camels was identified by Hamidinejat *et al*. (2013). A lack of relationship between sex and infection rates has been shown in similar studies on camels (Woldemeskel and Gumi, 2001; Shekarforoush *et al*., 2006; Valinezhad *et al*., 2008).

Conclusion

To the best of our knowledge, this represents the first molecular study in Iraq that identifies *Sarcocystis cameli* in camels. However, additional epidemiological and molecular studies in camel populations as well as in other domestic and wild animals appeared to be necessary.

Acknowledgments

The authors are thankful to the Colleges of Veterinary Medicine, University of Baghdad for providing the necessary facilities for the study. However, all materials used in this study were purchased by the authors and no external funds were received for this study.

Conflict of interest

The authors declare that there is no conflict of interest.

Author's contribution

OAA: Collection of tissue samples, extraction of DNAs, and preparation of Mastermix tubes; MTSA: PCR analysis, sequencing, and statistical analysis of obtained results. All authors have written the manuscript and approved the final copy of it.

Funding

There is no external fund (private funding).

Data availability

All data supporting the findings of this study are available within the manuscript.

References

Abbas, I., Baghdadi, H.B., Rizk, M.A., El-Alfy, E.S., Elmishmishy, B. and Gwida, M. 2023. Gastrointestinal parasites of dogs in Egypt: an update on the prevalence in Dakahlia Governorate and a meta-analysis for the published data from the country. Animals (Basel) 13(3), 1–21.

Abdullah, S.H. 2021. Investigation of *Sarcocystis* spp. in slaughtered cattle and sheep by peptic digestion and histological examination in Sulaimani Province, Iraq. Vet. World 14(2), 468–477.

Ahmed, A.M., Elshraway, N.T. and Youssef, A.I. 2016. Survey on *Sarcocystis* in bovine carcasses slaughtered at the municipal abattoir of El-Kharga, Egypt. Vet. World 9(12), 1461–1469.

Ahmed, S., Ibrahim, M., Nantasenamat, C., Nisar, M.F., Malik, A.A., Waheed, R. and Alam, M.K. 2022. Pragmatic applications and universality of DNA barcoding for substantial organisms at species level: a review to explore a way forward. BioMed. Res. Int. 2022, 1–15.

Al-Ani, F.K. and Amr, Z. 2017. *Sarcocystis* spp. prevalence in camel meat in Jordan. J. Dairy. Vet. Sci. 4, 1–3.

Al-Saadi, S.A., Al-Mussawi, K.A. and Muhammed, H.A. 2020. Molecular identification of *Sarcocystis* species infection in sheep in Karbala Governorate–Iraq. Med. Legal. Update 20, 889–895.

Asopa, S., Joshi, A., Dadhich, H. and Vyas, I. 2023. Prevalence of sarcocysts in tongue and oesophagus of dromedary camels from Bikaner district of Rajasthan. Ind. J. Vet. Sci. Biotechnol. 19(1), 96–98.

Bucca, M., Brianti, E., Giuffrida, A., Ziino, G., Cicciari, S. and Panebianco, A. 2011. Prevalence and distribution of *Sarcocystis* spp. cysts in several muscles of cattle slaughtered in Sicily, Southern Italy. Food Control 22(1), 105–108.

Burger, P.A., Ciani, E. and Faye, B. 2019. Old world camels in a modern world—a balancing act between conservation and genetic improvement. Anim. Gen. 50(6), 598–612.

Changbunjong, T., Weluwanarak, T., Samung, Y. and Ruangsittichai, J. 2016. Molecular identification and genetic variation of hematophagous flies, (Diptera: Muscidae: Stomoxyinae) in Thailand based on *cox1* barcodes. J. Asia Pac. Entomol. 19 (4), 1117–1123.

Dakhil, H.G., Abdallah, B.H. and Abdallah, F.A. 2017. Molecular identification of *Sarcocystis fusiformis* and *S. moulei* infecting water buffaloes (*Bubalus bubalis*) in southern Iraq. World J. Pharm. Res. 6(3), 215–229.

Dubey, J.P., Calero-Bernal, R., Rosenthal, B.M., Speer, C.A. and Fayer, R. 2015a. Sarcocystosis of animals and humans, 2nd ed. Boca Raton, FL: CRC Press, pp: 235–238.

Dubey, J.P., Hilali, M., Van Wilpe, E., Calero-Bernal, R., Verma, S.K. and Abbas, I.E. 2015b. A review of sarcocystosis in camels and redescription of *Sarcocystis cameli* and *Sarcocystis ippeni* sarcocysts from the one-humped camel (*Camelus dromedarius*). Parasitology 142(12), 1481–1492.

El-Bahy, N., El-Bagory, A.E.R., AbouLaila, M., Elkhatam, A. and Mady, H.M. 2019. Prevalence of *Sarcocystis fusiformis* and hydatid cyst among different ruminants at Menofia Governorate, Egypt. J. Curr. Vet. Res. 1(1), 1–10.

Faye, B. 2016. The camel, new challenges for a sustainable development. Trop. Anim. Health. Prod. 48(4), 689–692.

Gajadhar, A.A., Lalonde, L.F., Al-Adhami, B., Singh, B.B. and Lobanov, V. 2015. Foodborne apicomplexan protozoa: Coccidia. In Foodborne parasites in the food supply web. Sawston, UK: Woodhead Publishing, pp: 101–147.

Gareh, A., Soliman, M., Saleh, A.A., El-Gohary, F.A., El-Sherbiny, H.M., Mohamed, R.H. and Elmahallawy, E.K. 2020. Epidemiological and histopathological investigation of *Sarcocystis* spp. in slaughtered dromedary camels (*Camelus dromedarius*) in Egypt. Vet. Sci. 7(4), 1–10.

Gharban, H.A. (2023). Molecular prevalence and phylogenetic confirmation of bovine trichomoniasis in aborted cows in Iraq. Vet. World 16(3), 580–587.

Gharban, H.A., Al-Shaeli, S.J. and Hussen, T.J. 2023. Molecular genotyping, histopathological and immunohistochemical studies of bovine papillomatosis. Open Vet. J. 13(1), 26–41.

Gjerde, B., Hilali, M. and Mawgood, S.A. 2015. Molecular characterisation of three regions of the nuclear ribosomal DNA unit and the mitochondrial *cox1* gene of *Sarcocystis* fusiformis from water buffaloes (*Bubalus bubalis*) in Egypt. Parasitol. Res. 114, 3401–3413.

Hamidinejat, H., Hekmatimoghaddam, S., Jafari, H., Sazmand, A., Haddad Molayan, P., Derakhshan, L. and Mirabdollahi, S. 2013. Prevalence and distribution patterns of *Sarcocystis* in camels (*Camelus dromedarius*) in Yazd province, Iran. J. Parasit. Dis. 37, 163–165.

Kadim, I.T., Al-Amri, I.S., Al Kindi, A.Y. and Mbaga, M. 2018. Camel meat production and quality: a review. J. Camel Pract. Res. 25(1), 9–23.

Lindsay, D.S. and Dubey, J.P. 2020. Neosporosis, toxoplasmosis, and sarcocystosis in ruminants: an update. Vet. Clin. Food. Anim. Pract. 36(1), 205–222.

Metwally, D.M., Al-Otaibi, T.T., Al-Turaiki, I.M., El-Khadragy, M. F. and Alajmi, R.A. 2020. Identification of *Sarcocystis* spp. in one-humped camels (*Camelus dromedarius*) from Riyadh and Dammam, Saudi Arabia, via histological and phylogenetic approaches. Animals (Basels) 10(7), 1108.

Mioduchowska, M., Czyż, M.J., Gołdyn, B., Kur, J. and Sell, J. 2018. Instances of erroneous DNA barcoding of metazoan invertebrates: Are universal *cox1* gene primers too "universal"? PLoS One 13(6), 1–13.

Mohammadpour, R., Champour, M., Tuteja, F. and Mostafavi, E. 2020. Zoonotic implications of camel diseases in Iran. Vet. Med. Sci. 6(3), 359–381.

Motamedi, G.R., Dalimi, A., Nouri, A. and Aghaeipour, K. 2011. Ultrastructural and molecular characterization of *Sarcocystis* isolated from camel (*Camelus dromedarius*) in Iran. Parasitol. Res. 108, 949–954.

Omer, S.A., Alzuraiq, A.A. and Mohammed, O.B. 2017. Prevalence and molecular detection of *Sarcocystis* spp. infection in the dromedary camel (*Camelus dromedarius*) in Riyadh city, Saudi Arabia. Biomed. Res. 28(11), 1–10.

Oryan, A., Ahmadi, N. and Mousavi, S.M.M. 2010. Prevalence, biology, and distribution pattern of *Sarcocystis* infection in water buffalo (*Bubalus bubalis*) in Iran. Trop. Anim. Health Prod. 42, 1513–1518.

Prakas, P., Bea, A., Juozaitytė-Ngugu, E., Olano, I., Villanúa, D., Švažas, S. and Butkauskas, D. 2021. Molecular identification of *Sarcocystis* halieti in the muscles of two species of birds of prey from Spain. Parasites Vect. 14, 1–7.

Prakas, P., Moskaliova, D., Šneideris, D., Juozaitytė-Ngugu, E., Maziliauskaitė, E., Švažas, S. and Butkauskas, D. 2023. Molecular identification of *Sarcocystis rileyi* and *Sarcocystis* sp. (closely related to *Sarcocystis wenzeli*) in intestines of

mustelids from Lithuania. Animals (Basels)13(3), 467.

Rabie, S., Hassanine, R.M.E.S., A Hassan, A., Abo Elhussien, O. and BM EL-Mahdi, M. 2021. Morphological and molecular characterization of *Sarcocystis cameli* and *Sarcocystis ippeni* from the muscles of one-humped camel (*Camelus dromedarius*) in New valley Governorate, Egypt. J. Vet. Sci. 4(3), 103–118.

Razooqi, M.A., Gharban, H.A. and Al-Kaabi, M.A. 2022. Molecular and seroprevalence of toxoplasmosis in goats' blood and milk in Iraq. Arch. Razi. Instit. 77(5), 1749–1755.

Rodrigues, M.S., Morelli, K.A. and Jansen, A.M. 2017. Cytochrome c oxidase subunit 1 gene as a DNA barcode for discriminating *Trypanosoma cruzi* DTUs and closely related species. Parasites Vect. 10(1), 1–18.

Romero, S., Carletti, T., Franco, C.D., Moré, G., Schnittger, L. and Florin-Christensen, M. 2017. Seropositivity to Sarcocystis infection of llamas correlates with breeding practices. Vet. Parasitol. Reg. Stud. Reports 10, 65–70.

Sağlam, K. and Keleş, H. 2016. Sarcocystosis in the cremaster muscle of an infertile bull, spermiostasis and orchitis. Kocatepe Vet. J. 9(3), 252–254.

Shekarforoush, S.S., Shakerian, A. and Hasanpoor, M.M. 2006. Prevalence of *Sarcocystis* in slaughtered one-humped camels (*Camelus dromedarius*) in Iran. Trop. Anim. Health Prod. 38(4), 301–303.

Stöger, I., Kocot, K.M., Poustka, A.J., Wilson, N.G., Ivanov, D., Halanych, K.M. and Schrödl, M. 2016. Monoplacophoran mitochondrial genomes: convergent gene arrangements and little phylogenetic signal. BMC Evolut. Biol. 16(1), 1–18.

Swar, S.O. and Shnawa, B.H. 2020. Ultrastructural and molecular characterization of *Sarcocystis* species derived from macroscopic sarcocysts of domestic sheep and goats in Soran City, Erbil, Iraq. World Vet. J. 10(4), 540–550.

Valinezhad, A., Oryan, A. and Ahmadi, N. 2008. *Sarcocystis* and its complications in camels (*Camelus dromedarius*) of eastern provinces of Iran. Korean J. Parasitol. 46(4), 229–234.

Viricel, A. and Rosel, P.E. 2022. Evaluating the utility of *cox1* for cetacean species identification. Marine Mam. Sci. 28(1), 37–62.

Wahba, A., Ayoub, M. and Soliman, K. 2014. Light and ultrastructure of *Sarcocystis* spp. of camels and associated pathological changes. Anim. Health Res. J. 2, 143–158.

Wang, J., Zhang, L., Zhang, Q.L., Zhou, M.Q., Wang, X.T., Yang, X.Z. and Yuan, M.L. 2017. Comparative mitogenomic analysis of mirid bugs (Hemiptera: Miridae) and evaluation of potential DNA barcoding markers. Peer J. 5, e3661.

Woldemeskel, M. and Gumi, B. 2001. Prevalence of sarcocysts in one-humped camel (*Camelus dromedarius*) from Southern Ethiopia. J. Vet. Med. 48(3), 223–226.

Zhu, S., Zimmerman, D. and Deem, S.L. 2019. A review of zoonotic pathogens of dromedary camels. Ecohealth 16, 356–377.