First molecular characterization of *Sarcocystis* spp. in cattle in Qena Governorate, Upper Egypt

Asmaa M. El-kady¹ · Nermean M. Hussein² · Amal A. Hassan³

Received: 12 October 2017 / Accepted: 18 December 2017 / Published online: 12 January 2018
© Indian Society for Parasitology 2018

Abstract Cattle are intermediate hosts for several *Sarcocystis* species with different definitive hosts. The present study, to our knowledge, is the first to determine the prevalence of *Sarcocystis* infection and morphologically and molecularly identify *Sarcocystis* species in cattle in Qena Governorate, Upper Egypt. The samples were collected from the heart and oesophagus muscles of 84 slaughtered cattle (76 males and 8 females) aged between 11 months and 3 years from slaughterhouses in different localities in Qena Governorate, Upper Egypt. The samples were macroscopically and histologically examined, and the molecular identification of the species was performed using 18S ribosomal subunit DNA through PCR and DNA sequencing. Infection was detected in 72 out of 84 animals (85.7%) and was more prevalent in males (76.2%) than in females (9.5). Using light microscopy, the microscopic sarcocysts were observed to be thin-walled. Sequencing and genotyping revealed one isolate that had 99 and 100% identity, respectively, to *Sarcocystis cruzi*, while another isolate had 95 and 99% identity to *Sarcocystis hjorti*. The present study is the first to identify *Sarcocystis* infection in

cattle in Qena Governorate, Upper Egypt both morphologically and molecularly. *Sarcocystis cruzi* and *S. hjorti* species were isolated from cattle, which is of veterinary importance and indicates that morphologically similar *Sarcocystis* species are genetically distinct. Additionally, the results show that *Sarcocystis* species are not host-specific.

Keywords *Sarcocystis* · Upper Egypt · Molecular · *Hjorti*

Introduction

Sarcocystis spp. are obligatory intracellular protozoan parasites that require two hosts, namely, prey and predator hosts, to complete its life cycle (Fayer 2004). Narges et al. (2013) reported a large number of species of *Sarcocystis* (more than two-hundred), representing the most common protozoans infecting domestic animals. The life cycle of *Sarcocystis* has been well described (CDC 2015; Dubey and Lindsay 2006). *Sarcocystis* species have a wide range of hosts (Dubey et al. 1989), with many domestic herbivorous animals that act as prey or intermediate hosts for *Sarcocystis* spp., such as cattle, Ox, sheep, goats, buffalo, pigs and horses (Dubey 1976).

Cattle are intermediate hosts for five *Sarcocystis* species, including *S. cruzi*, *S. hirsuta*, *S. hominis*, *S. heydorni* and *S. rommeli*, with different definitive hosts, including canids, felids and humans (Dubey et al. 2015; Dubey and Lindsay 2006). In addition, Moré et al. (2014) reported the presence of *S. sinensis* in cattle. Among the several *Sarcocystis* spp. reported in cattle, only *S. hominis* may elicit symptoms in humans; infection can lead to symptomatic gastroenteritis, which presents as nausea, abdominal pain,

✉ Asmaa M. El-kady
Asmaa.elkady@med.svu.edu.eg

Nermean M. Hussein
nermeanmohu@yahoo.com

Amal A. Hassan
drasmaakady1981@gmail.com

¹ Faculty of Medicine, South Valley University, Qena 83523, Egypt

² Faculty of Science, South Valley University, Qena 83523, Egypt

³ Faculty of Science, Damanhour University, Damanhūr 21634, Egypt

and watery diarrhoea (Prakas and Butkauskas 2012) (Dubey and Lindsay 2006). Therefore, the infection is potentially zoonotic. Human hosts are infected by consuming raw or undercooked beef containing mature cysts. Human infections are distributed worldwide (Boonjaraspinyo et al. 2013; Nimri 2014).

Sarcocystis species can be differentiated on the basis of their morphological features, including the sarcocyst walls, which can be studied via histopathological examination and electron microscopy. Histopathological studies of fresh tissue samples can be used to differentiate thick ($> 3 \mu\text{m}$) and thin-walled ($< 1 \mu\text{m}$) cysts, but this method cannot be used to differentiate species of *Sarcocystis* (Fayer 2004; Nourollahi-Fard et al. 2009; Moré et al. 2011). Electron microscopy has considerable limitations, i.e., it is time consuming and costly; thus, molecular assays are the most efficient methods for epidemiological studies and for the identification of *Sarcocystis* species in intermediate hosts.

In addition, a few firmly related intermediate hosts may have morphologically undefined sarcocysts. However, sarcocysts of the same type in different intermediate hosts are considered different species in light of the supposition that *Sarcocystis* spp. are host-specific in the intermediate host (Dubey et al. 1989; Odening 1998). Since 1990, molecular methods have been progressively used to support the identification of various *Sarcocystis* spp., particularly those infecting domestic animals, and to determine whether morphologically similar sarcocysts in a single or multiple intermediate hosts belong to the same species (Dahlgren and Gjerde 2010; Gjerde and Dahlgren 2010).

The present study, to our knowledge, is the first to determine the prevalence of *Sarcocystis* infection and morphologically and molecularly identify species in cattle in Qena Governorate, Upper Egypt.

Materials and methods

Sample collection

Samples were collected from the heart and oesophagus muscles of 84 slaughtered cattle (76 males and 8 females) aged between 11 months and 3 years from slaughterhouses in different localities in Qena Governorate, Upper Egypt in the period between May 2016 and June 2016.

Macroscopic examination

Each heart and oesophagus muscle sample was cut into smaller pieces and assessed by using a hand lens for the presence of macrocysts, which appear whitish with different shapes (described by Lam et al. (1999)). Small slices of the muscle tissues were compressed between two glass

slides and inspected for the presence of microscopic sarcocysts (Latif et al. 1999).

Histological examination

A small piece of each muscle was fixed in 10% formalin and processed for histopathology. The samples were subsequently stained with haematoxylin and eosin and microscopically examined under 10, 20, 40 and 100 $\times$ magnification.

Molecular identification using 18S ribosomal subunit DNA

Small pieces of infected heart and oesophagus tissue were incubated in ethanol (70%) for DNA analysis. After removing the ethanol, genomic DNA was extracted using the QIAamp DNA Mini and Blood Mini Kit (QIAGEN, GmbH, Germany) according to the manufacturer's instructions. For amplification of 18S ribosomal DNA genes, we used the following pair of primers: Sar-F1 Forward 5'GCACTTGATGAATTCTGGCA3', Sar-R1 Reverse 5'CACCACCCATAGAATCAAG 3' (DalimiAsl et al. 2008). PCR reactions were performed using Taq PCR Master Mix Kit (QIAGEN) according to the manufacturer's instructions for 25–35 cycles using the Applied Biosystems 9700 PCR system (Thermo-Fisher). PCR cycles were as follows: initial denaturation step at 94 °C for 3 min, followed by 25 cycles of denaturation at 94 °C for 1 min, annealing at 50–68 °C for 1 min and extension at 72 °C for 1 min, with a final extension step at 72 °C for 10 min.

The PCR products were then run on a 1.5% agarose gel. Subsequently, the gel was stained with ethidium bromide, and the appropriate bands were excised and purified with the QIAamp PCR Purification Kit (QIAGEN) according to the manufacturer's instructions. The isolates were subsequently sequenced using forward primers to construct a continuous sequence of the tested DNA using an Applied Biosystems 3500 Automatic Genetic Analyzer. The acquired sequences were compared and aligned with the *Sarcocystis* sequences deposited in GenBank using the NCBI Basic Local Alignment Search Tool (BLAST). Phylogenetic trees were created using Mega6 software (Tamura et al. 2013). We used sequences for other *Sarcocystis* spp. of cattle from GenBank with *Plasmodium vivax* as the outgroup.

Results

Light microscopy

Sarcocystis were microscopically identified, and no macrocysts were found. Infection was detected in 76 out of 84 animals (90.47%), and infection was more prevalent in males (80.95%) than females (9.52%), Table 1. Using LM, the *Sarcocystis* spp. in the heart were found to be as large as 96.7 μm wide and 326.9 μm long, while the sarcocysts in the oesophagus were as large as 48.8 μm wide and 158.1 μm long. The *Sarcocystis* wall was thin (< 1 μm thick). Some cysts were septate, and the interior compartments were packed with bradyzoites (Figs. 1, 2 and 3).

Sequencing and genotyping of isolates

The partial 18S rRNA gene of the *Sarcocystis* species was amplified and yielded the expected PCR product size (600 bp) from microscopic cyst samples (Fig. 4). Genotype

identification was performed by comparing the revealed sequences with the *Sarcocystis* DNA sequences available in GenBank based on sequence analysis of the 18S rRNA region using BLAST. In the present study, one of the isolates had 99 and 100% identity to *S. cruzi* (accession number KT964008.1), while a second isolate had 95 and 99% identity to *S. hjorti* (accession number KY973332.1). Alignment was performed using BLAST software (Figs. 5, 6).

Discussion

In the present study, both morphological and molecular examinations were utilized to identify *Sarcocystis* infection in cattle in Qena Governorate, Upper Egypt. The overall *Sarcocystis* spp. infection rate among butchered animals was 85.7%, consistent with past reports in Egypt (Metwally et al. 2014; El-Dakhly et al. 2011; Fatma et al. 2008; Khalifa et al. 2008; Said 1996; El-Afi 1958; Abdel Rahman 1975, 2001; Ali 1985; Fawaz 1998). The present findings are also consistent with those of reports in different countries, such as New Zealand (Bottner et al. 1987), Brazil (Pena et al. 2001), Iran (Nourollahi-Fard et al. 2009), the United States (Fayer 2004), Hungary (Hornok et al. 2015), Northern Vietnam (Jehle et al. 2009), Nigeria (Obijiaku et al. 2013), Malaysia (Latif et al. 1999), Iran (Hamidinejat et al. 2010) and Mongolia (Fukuyo et al.

Table 1 Showing number of positive and negative cases

	Males	Females	Total
Infected	68	8	76
Not infected	8	0	8
Total	76	8	84

Fig. 1 Esophagus of a *Sarcocystis*-infected cattle showing microscopic cysts with thin cyst wall (cut section) stained with haematoxylin and eosin ($\times 63$)

Fig. 2 Esophagus of a *Sarcocystis*-infected cattle showing microscopic cysts with thin cyst wall (longitudinal section) stained with haematoxylin and eosin ($\times 40$)

Fig. 3 Heart muscles of a *Sarcocystis*-infected cattle showing microscopic cysts with thin cyst wall ($\times 40$) (longitudinal section) stained with haematoxylin and eosin ($\times 63$)

Fig. 4 The electrophoresis of the PCR product of 18S ribosomal gene of *Sarcocystis* in cattle samples presented bands at 600 bp

2002). Collier et al. (1998) reported that one reason for the high prevalence of *Sarcocystis* in cattle is the assortment of large number of hosts that shed countless resistant

sporocysts; life cycles in cattle have been established in canines (*S. cruzi*), felines (*S. hirsuta*), humans (*S. hominis*), and others (Metwally et al. 2014).

A molecular approach is widely utilized for the identification of *Sarcocystis* species variation, as transmission electron microscopy is of limited value in the detection of slight morphology variations (Gjerde 2013). In the present study, we used the 18S rRNA gene for the differentiation of *Sarcocystis* species in cattle using genus-specific primers as in previous studies (DalimiAsl et al. 2008). We found that *Sarcocystis* isolates in the cattle oesophagus belonged to *S. cruzi* (accession KT964008.1). Comparison of one of the nucleotide sequence obtained in our study with other *Sarcocystis* species in GenBank revealed 99% homology and 100% query coverage with *S. cruzi*, with only 3 unique nucleotides (Fig. 5). This finding was expected, as *S. cruzi* is highly prevalent in cattle worldwide, with prevalence ranging from 24 to 100%, as documented in several studies (only a few of these studies performed molecular identification) (Jehle et al. 2009; Moré et al. 2008; Pena et al. 2001). This finding is important for veterinary medicine, as *S. cruzi* causes serious pathogenicity in domesticated animals but does not have any pathological consequences for humans, though Agholi et al. (2016) found *S. cruzi* in the stool of an HIV-positive woman (More et al. 2014).

The second isolate from heart muscle had a similarity of 95 and 96% coverage to *S. hjorti* (accession number

Range 1: 259 to 787 [GenBank](#) [Graphics](#) ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
957 bits(518)	0.0	528/532(99%)	3/532(0%)	Plus/Plus
Query 1	ACCGATTACTGTTGAGAAAATTAGAGTGT	TTTGAAGCAGGCTAATTGCCTTGAATACTGCA	60	
Sbjct 259	ACCG- TTACT- TTGAGAAAATTAGAGTGT	TTTGAAGCAGGCTAATTGCCTTGAATACTGCA	316	
Query 61	GCATGGAATAACAATATAGGATTTTCGGTTC	ATTTTGTGGTTTCTAGGACTGAAATAAT	120	
Sbjct 317	GCATGGAATAACAATATAGGATTTTCGGTTC	ATTTTGTGGTTTCTAGGACTGAAATAAT	376	
Query 121	GATTAATAGGGACAGTTGGGGGCATTCGTAT	TTAACTGTCAGAGGTGAAATTCCTTAGATT	180	
Sbjct 377	GATTAATAGGGACAGTTGGGGGCATTCGTAT	TTAACTGTCAGAGGTGAAATTCCTTAGATT	436	
Query 181	TGTTAAAGACGAACACTACTGCGAAAGCAT	TTGCCAAAGATGTTTTCTTAATCAAGAACGA	240	
Sbjct 437	TGTTAAAGACGAACACTACTGCGAAAGCAT	TTGCCAAAGATGTTTTCTTAATCAAGAACGA	496	
Query 241	AAGTTAGGGGCTCGAAGACGATCAGATAC	CGTCGTAGTCTTAACCATAAACTATGCCGAC	300	
Sbjct 497	AAGTTAGGGGCTCGAAGACGATCAGATAC	CGTCGTAGTCTTAACCATAAACTATGCCGAC	556	
Query 301	TAGAGATAGGAAAATGTCATTTTTCTGACT	TCTCCTGCCCTTATGAGAAATCAAAGTCT	360	
Sbjct 557	TAGAGATAGGAAAATGTCATTTTTCTGACT	TCTCCTGCCCTTATGAGAAATCAAAGTCT	616	
Query 361	TTGGGTTCTGGGGGGAGTATGGTCGCAAG	GCTGAAACTTAAAGGAATTGACGGAAGGGCA	420	
Sbjct 617	TTGGGTTCTGGGGGGAGTATGGTCGCAAG	GCTGAAACTTAAAGGAATTGACGGAAGGGCA	676	
Query 421	CCACCAGGCGTGGAGCCTGCGGCTTAAT	TGACTCAACACGGGGAACTCACCAGGTCCA	480	
Sbjct 677	CCACCAGGCGTGGAGCCTGCGGCTTAAT	TGACTCAACACGGGGAACTCACCAGGTCCA	736	
Query 481	GACATGGGAAGGATTGACAGATTGATAGCT	CCTTCTTGATTCTATGGTGTGG	532	
Sbjct 737	GACATGGGAAGGATTGACAGATTGATAGCT	CCTTCTTGATTCTATGG- GTGG	787	

Fig. 5 DNA sequence alignment of 18S ribosomal RNA gene in isolated heart samples compared with the published sequences of *S. cruzi* on genebank (accession KT964008.1) showing sequence matches and gaps using BLAST software

KY973332.1) (Fig. 6). To our knowledge, this study is the first to report *S. hjorti* in cattle, although Dahlgren and Gjerde (2010) reported that this species is not host-specific and can infect different intermediate hosts such as deer and moose. A maximum likelihood phylogenetic tree was created using partial 18S ribosomal RNA sequences, and the results showed that the Egyptian *Sarcocystis* species

isolated from the heart muscle are closely related to *S. hjorti* (accession number KY973332.1). This is consistent with the findings of Gjerde (2013) who generated a phylogenetic tree for different *Sarcocystis* spp. infecting different intermediate hosts, such as cattle, sheep and cervids. The results showed that *S. hjorti* is closely related to cattle-infecting *S. cruzi* (Fig. 7).

Range 1: 800 to 1326 GenBank Graphics					▼ Next Match ▲ Previous Match	
Score	Expect	Identities	Gaps	Strand		
813 bits(440)	0.0	499/528(95%)	1/528(0%)	Plus/Plus		
Query 1	TTACTTTGACAAAATTATAGTGT	TTTGAAGCAGGCTCATTGCCTTGA	ACTGCACCATGG	60		
Sbjct 800	TTACTTTGAGAAAATTAGAGTGT	TTTGAAGCAGGCTAATTGCCTTGA	ACTGCAGCATGG	859		
Query 61	AATAACAATATAGGATTTTCGGT	TCTATTTTGTGGTTTCTAGGACT	GAAATAATGATTAA	120		
Sbjct 860	AATAACAATATAGGATTTTCGGT	TCTATTTTGTGGTTTCTAGGACT	GAAATAATGATTAA	919		
Query 121	TAGGGACAGTTGGGGGCAATTCG	TATTTAACTGTCTTAGGTGGAAT	TCTTAGATTTGTTAA	180		
Sbjct 920	TAGGGACAGTTGGGGGCAATTCG	TATTTAACTGTCTAGGAGTGAAAT	TCTTAGATTTGTTAA	979		
Query 181	AAACGAACTACTGCGAAAGCAT	TTGCCAAAGATGTTTTCATTAAT	CAAGAACGAAAGTTA	240		
Sbjct 980	AGACGAACTACTGCGAAAGCAT	TTGCCAAAGATGTTTTCATTAAT	CAAGAACGAAAGTTA	1039		
Query 241	GGGGCTCGAAGACGATCAAATAC	CGTCGTAATCTTAACCATAAACT	TATGCCGACTAAAGA	300		
Sbjct 1040	GGGGCTCGAAGACGATCAGATAC	CGTCGTAATCTTAACCATAAACT	TATGCCGACTAGAGA	1099		
Query 301	TAGGAAAATGTCATTTTTCTGAC	TCTCCTGCCCTTATGACAAATCA	AAAGTCATTGGGT	360		
Sbjct 1100	TAGGAAAATGTCATTTTTCTGAC	TCTCCTGCACCTATGAGAAATCA	AAAGTCATTGGGT	1159		
Query 361	TCTGGGGGAGTAGGGTCGCAAG	GGTGAAACTTAAAGGAATTGAC	GGAAGGGCACCACCG	420		
Sbjct 1160	TCTGGGGGAGTAGGGTCGCAAG	GGTGAAACTTAAAGGAATTGAC	GGAAGGGCACCACCA	1219		
Query 421	GGCGTGGAGCCTGCGGCTTAAT	TGACTCAACTCGGGGAACTCAC	CAAGTCCAGACATG	480		
Sbjct 1220	GGCGTGGAGCCTGCGGCTTAAT	TGACTCAACACGGGGAACTCAC	CAGGTCCAGACATG	1279		
Query 481	GCAAGGATTGACAGATTGATAG	CTCTTTCTTGATCTTATTTGGT	GGTG	528		
Sbjct 1280	GGAAGGATTGACAGATTGATAG	CTCTTTCTTGATCTATG-GGT	GGTG	1326		

Fig. 6 DNA sequence alignment of 18S ribosomal RNA gene in isolated heart samples compared with the published sequences of *S. hjorti* isolate *CeSl.48* on genebank (accession No.KY973332.1) showing sequence matches and gaps using BLAST software

Fig. 7 Phylogeny of *S. hjorti* isolated in the present study with different *Sarcocystis* spp. recorded in cattles by the program Mega v.6. using maximum likelihood method based on 18S rRNA gene. The reference sequences accession numbers are included. *Plasmodium vivax* was used as outgroup

Conclusion

This study is the first to molecularly identify the *Sarcocystis* species in cattle in Qena Governorate, Upper Egypt. In the present study, we isolated *Sarcocystis* species from cattle and demonstrated that these parasites were *S. cruzi*, a

species that causes major veterinary health problems. We also provide the first report of *S. hjorti* in cattle.

Author's contributions NH, AEK and AH conceived the study. NH, AEK and AH performed the laboratory experiments, literature

searches, and data analysis. AEK drafted the manuscript. NH and AEK revised the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

- Abdel Rahman AM (1975) Studies on protozoa of ruminants in Assiut Governorate. Ph. D. Thesis, Faculty of Veterinary Medicine, Assiut University
- Abdel Rahman SM (2001) Serodiagnosis of two zoonotic parasites (*Toxoplasma* & *Sarcocystis*) in cattle. In: 1st Congress of food hygiene & human health, Faculty of Veterinary Medicine, Assiut, Egypt
- Agholi M, Shahabadi SN, Motazedian MH, Hatam GR (2016) Prevalence of enteric protozoan oocysts with special reference to *Sarcocystis cruzi* among fecal samples of diarrheic immunodeficient patients in Iran. *Korean J Parasitol* 54(3):339–344. <https://doi.org/10.3347/kjp.2016.54.3.339> (PubMed: 27417091)
- Ali GAT (1985) Studies on *Sarcocystis* of animals in Assiut province. M. Sc. Thesis, Faculty of Medicine, Assiut University
- Boonjaraspinyo S, Boonmars T, Kaewsamut B, Ekobol N, Laummaunwai P, Aukkanimart R, Sriraj P (2013) A cross-sectional study on intestinal parasitic infections in rural communities, Northeast Thailand. *Korean J Parasitol* 51(6):727–734
- Bottner A, Charleston WA, Promroy WE, Rommel M (1987) The prevalence and identity of *Sarcocystis* in beef cattle in New Zealand. *Vet Parasitol* 24(3–4):157–168
- CDC (Centers for Disease Control and Prevention) (2015) Sarcocystosis. <http://www.cdc.gov/dpdx/sarcocystosis>. Date accessed: 15 October 2015
- Collier L, Balows A, Sussman M (1998) *Sarcocystis*, *Isospora* and *Cyclospora*. In: Gransden WR (ed) *Topley and Welson's: microbiology and microbial infections*, vol 5.9. Oxford University Press Inc., New York, pp 319–326
- Dahlgren SS, Gjerde B (2010) The red fox (*Vulpes vulpes*) and the arctic fox (*Vulpes lagopus*) are definitive hosts of *Sarcocystis alces* and *Sarcocystis hjorti* from moose (*Alces alces*). *Parasitology* 137:1547–1557
- DalimiAsl AH, Mutamedi G, Paykari H et al (2008) Detection of *Sarcocystis* spp. of slaughtered sheep in GazvinZiaran slaughter house by molecular assay. *J Modarres Med Sci* 11:65–72 (In Persian)
- Dubey JP (1976) A review of *Sarcocystis* of domestic animals and of other coccidia of cats and dogs. *J Am Vet Med Assoc* 169(10):1061–1078
- Dubey JP, Lindsay DS (2006) Neosporosis, toxoplasmosis, and sarcocystosis in ruminants. *Vet Clin North Am Food Anim Pract* 22:645–671
- Dubey JP, Speer CA, Fayer R (1989) *Sarcocystosis* of animals and man. CRC Press Inc., Boca Raton, FL, pp 105–145
- Dubey JP, van Wilpe E, Calero-Bernal R, Verma SK, Fayer R (2015) *Sarcocystis heydorni*, n. sp. (Apicomplexa: Sarcocystidae) with cattle (*Bos taurus*) and human (*Homo sapiens*) cycle. *Parasitol Res* 114(11):4143–4147
- El-Afifi A (1958) If there is a relationship between sarcosporidia and the eosinophilic myositis of cattle. *Vet Med J* 5:107–112
- El-Dakhly KM, El-Nesr KA, El-Nahass E-S, Hirata A, Sakai H, Yanai T (2011) Prevalence and distribution patterns of *Sarcocystis* spp. in buffaloes in Beni-Suef, Egypt. *Trop Anim Health Prod* 43:1549–1554
- Fatma GS, Maha SIS, Mohsen IA, Hoda MK (2008) *Sarcocystis* infection in cattle at Assiut abattoir: microscopical and serological studies. *Ass Univ Bull Environ Res* 11:47–58
- Fawaz AA (1998) Incidence of *Toxoplasma* and *Sarcosporidia* in slaughtered animals in Qena Governorate. PhD thesis, Faculty of Veterinary Medicine, Assiut University
- Fayer R (2004) *Sarcocystis* spp. in human infections. *Clin Microbiol Rev* 17:894–902
- Fukuyo M, Battsetseg G, Byambaa B (2002) Prevalence of *Sarcocystis* infection in meat-producing animals in Mongolia. *South-east Asian J Trop Med Public Health* 33:490–495
- Gjerde B (2013) Phylogenetic relationships among *Sarcocystis* species in cervids, cattle and sheep inferred from the mitochondrial cytochrome c oxidase subunit I gene. *Int J Parasitol* 43(7):579–591. <https://doi.org/10.1016/j.ijpara.2013.02.004> (PubMed: 23542092)
- Gjerde B, Dahlgren SS (2010) Corvid birds (Corvidae) act as definitive hosts for *Sarcocystis ovalis* in moose (*Alces alces*). *Parasitol Res* 107:1445–1453
- Hamidinejat H, Jalali M, Nabavi L (2010) Survey on *Sarcocystis* infection in slaughtered cattle in south-west of Iran, emphasized on evaluation of muscle squash in comparison with digestion method. *J Anim Vet Adv* 9:1724–1726
- Hornok S, Mester A, Takács N, Baska F, Majoros G, Fok É, Biksi I, Németh Z, Hornyák Á, Jánosi S, Farkas R (2015) *Sarcocystis* infection of cattle in Hungary. *Parasites Vectors* 8:69
- Jehle C, Dinkel A, Sander A, Morent M, Romig T, Luc PV, De TV, Thai VV, Mackenstedt U (2009) Diagnosis of *Sarcocystis* spp. in cattle (*Bos taurus*) and water buffalo (*Bubalus bubalis*) in Northern Vietnam. *Vet Parasitol* 23:314–320
- Khalifa RM, El-Nadi NA, Sayed FG, Omran EK (2008) Comparative morphological studies on three *Sarcocystis* species in Sohag, Egypt. *J Egypt Soc Parasitol* 38(2):599–608
- Lam KKH, Watkins KL, Chan CW (1999) First report of equine protozoal myeloencephalitis in Hong Kong. *Equine Vet Educ* 11:54–56
- Latif B, Al-Delemi JK, Mohammed BS, Al-Bayati SM, Al-Amiry AM (1999) Prevalence of *Sarcocystis* spp. in meat producing animals in Iraq. *Vet Parasitol* 84:85–90
- Metwally A, Abd Ellah M, Al-Hosary A, Omar M (2014) Microscopical and serological studies on *Sarcocystis* infection with first report of *S. cruzi* in buffaloes (*Bubalus bubalis*) in Assiut, Egypt. *J Parasit Dis* 38(4):378–382 ([PMC free article] [PubMed])
- More G, Pantchev A, Skuballa J, Langenmayer MC, Maksimov P, Conraths FJ et al (2014) *Sarcocystis sinensis* is the most prevalent thick-walled *Sarcocystis* species in beef on sale for consumers in Germany. *Parasitol Res* 113(6):2223–2230. <https://doi.org/10.1007/s00436-014-3877-x> (PubMed:24700022)
- More G, Basso W, Bacigalupe D, Venturini MC, Venturini L (2008) Diagnosis of *Sarcocystis cruzi*, *Neospora caninum*, and *Toxoplasma gondii* infections in cattle. *Parasitol Res* 102(4):671–675
- More G, Abrahamovich P, Jurado S, Bacigalupe D, Marin JC, Rambeaud M, Venturini MC (2011) Prevalence of *Sarcocystis* spp. in Argentinean cattle. *Vet Parasitol* 177(1–2):162–165
- Narges K, Masomeh B, Salman G (2013) *Sarcocystis cruzi*: first molecular identification from cattle in Iran. *Int J Mol Cell Med* 2:125–130
- Nimri L (2014) Unusual case presentation of intestinal *Sarcocystis hominis* infection in a healthy adult. *JMM Case Rep* 1(4):e004069. <https://doi.org/10.1099/jmmcr.0.T00019>
- Nourollahi-Fard SR, Asghari M, Nouri F (2009) Survey of *Sarcocystis* infection in slaughtered cattle in Kerman, Iran. *Trop Anim Health Prod* 41:1633–1636

- Obijiaku IN, Ajogi I, Umoh JU, Lawal IA, Atu BO (2013) *Sarcocystis* infection in slaughtered cattle in Zango abattoir, Zaria, Nigeria. Vet World 6:346–349
- Odening K (1998) The present state of species-systematics in *Sarcocystis* Lankester, 1882 (Protista, Sporozoa, Coccidia). Syst Parasitol 41:209–233
- Pena HF, Ogassawara S, Sinhorini IL (2001) Occurrence of cattle *Sarcocystis* species in raw kibbe from Arabian food establishments in the city of São Paulo, Brazil, and experimental transmission to humans. J Parasitol 87(6):1459–1465
- Prakas P, Butkauskas D (2012) Protozoan parasites from genus *Sarcocystis* and their investigations in Lithuania. Ekologija 58:45–58. <https://doi.org/10.6001/ekologija.v58i1.2349>
- Said MS (1996) Muscular parasites in slaughtered animals in Assiut Governorate. Unpublished PhD thesis, Faculty of Veterinary Medicine Assiut University
- Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30:2725–2729