

Investigation of *Sarcocystis* spp. in slaughtered cattle and sheep by peptic digestion and histological examination in Sulaimani Province, Iraq

Shadan Hassan Abdullah 

Department of Microbiology, College of Veterinary Medicine, Sulaimani University, Sulaimaniyah, Iraq.

Corresponding author: Shadan Hassan Abdullah, e-mail: shadan.abdullah@univsul.edu.iq

Received: 01-09-2020, **Accepted:** 11-01-2021, **Published online:** 22-02-2021

doi: www.doi.org/10.14202/vetworld.2021.468-474 **How to cite this article:** Abdullah SH (2021) Investigation of *Sarcocystis* spp. in slaughtered cattle and sheep by peptic digestion and histological examination in Sulaimani Province, Iraq, *Veterinary World*, 14(2): 468-474.

Abstract

Background and Aim: Sarcocystosis is a zoonotic infection caused by various species of *Sarcocystis* organisms with a worldwide geographic distribution. This study investigated the presence of *Sarcocystis* organisms in cattle and sheep slaughtered at an abattoir in Sulaimani Province in North Iraq.

Materials and Methods: A total of 130 muscle samples were collected during May, June, and July of 2020, including 80 samples from sheep and 50 samples from cattle. Samples were examined visually for macrosarcocysts. The peptic digestion method was used to analyze fresh muscle tissue samples for detecting microsarcocysts followed by microscopic examination. Furthermore, muscle samples were fixed and stained with hematoxylin and eosin for histopathological examination.

Results: In the gross examination, macroscopic cysts were not detected in both cattle and sheep; hence, all the prevalence data were obtained through microscopic observation of muscle samples. The peptic digestion method revealed the presence of banana-shaped bradyzoites in 90% and 92.5% of slaughtered cattle and sheep muscle samples, respectively. Organ-wise prevalence revealed that 95% and 92% of esophageal samples of sheep and cattle contained *Sarcocystis* spp., respectively. Moreover, 90% and 88% of sheep and cattle diaphragms were respectively infected. Histopathological examination of tissue sections revealed two morphologically distinct types of microsarcocysts, including thin-walled and thick-walled, in both sheep and cattle.

Conclusion: The suspected *Sarcocystis* spp. were *Sarcocystis tenella* and *Sarcocystis arieticanis* in sheep and *Sarcocystis cruzi* and *Sarcocystis bovifelis* or *Sarcocystis hominis* in cattle. Infective stages of different *Sarcocystis* spp. are widespread in the study area environment.

Keywords: cattle, muscle samples, prevalence rate, *Sarcocystis*, sheep.

Introduction

Sarcocystosis is caused by unicellular coccidian protozoan parasites belonging to *Sarcocystis* species [1,2]. This genus consists of more than 189 species with global distribution [3]. The majority of the known *Sarcocystis* spp. appear to be intermediate host-specific [4].

A wide range of vertebrates, including mammals, birds, and fish, are infected by *Sarcocystis* spp. Merogony and cyst formation (asexual stage) occur in the intermediate host, whereas gametogony and sporogony (sexual stages) occur in the definitive host [5].

Definitive hosts are infected through the ingestion of *Sarcocystis* in muscle tissues, which include carnivorous predators, scavengers, as well as humans. Intermediate hosts become infected through the ingestion of oocysts/sporocysts present in feed or water

contaminated by fecal materials, which typically consist of herbivores and omnivores [4].

Most of the pathogenic *Sarcocystis* spp. cause disease only in their intermediate hosts. In general, *Sarcocystis* spp. transmitted by canids or primates are more pathogenic than those transmitted by felids [5].

Although the majority of *Sarcocystis* infections are asymptomatic for intermediate hosts, acute cases have also been reported with clinical signs of weakness, anorexia, diarrhea, weight loss, muscle twitching, and, in rare cases, death of the infected animal [6]. These symptoms occur during the development of the second generation of intravascular meronts [7]. The common pathological change associated with sarcocystosis in the intermediate host is the development of tissue cysts containing bradyzoites in the muscle tissues. Occasionally, encephalitis may occur in sheep and horses after infection with *Sarcocystis oivicans* and *Sarcocystis neurona* [8].

Humans can be infected with intestinal sarcocystosis through the consumption of raw or undercooked beef, pork, and meat products containing the bradyzoites of *Sarcocystis hominis* and *Sarcocystis suihominis* [9]. The transmission of *Sarcocystis cruzi* to humans has also been reported [10]. However,

Copyright: Abdullah. Open Access. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated.

S. cruzi has also been reported in sheep. Nevertheless, the possibility of its transmission from sheep to humans requires careful investigation [11].

Infection generally includes transient nonspecific gastrointestinal manifestations. The severity correlates with the number of ingested sarcocysts [1]. The diagnosis of intermediate hosts is commonly possible during necropsy because the disease exhibits either a subclinical or chronic course [12].

It has been reported that sarcocystosis in sheep (*Ovis aries*) is caused by *Sarcocystis tenella*, *Scolopendra gigantea*, *Sarcocystis medusiformis*, and *Sarcocystis arieticanis* [13]. Moreover, *Sarcocystis moulei* and *S. cruzi* have been reported in sheep [11]. At the same time, cattle (*Bos taurus*) are an intermediate host for *Sarcocystis* spp., including *S. cruzi*, *S. hominis*, *Sarcocystis hirsuta*, and *Sarcocystis bovifelis* [14]. Other species include *Sarcocystis sinensis* [15]. Recently, *Sarcocystis heydorni*, *Sarcocystis rommeli*, and *Sarcocystis bovini* have also been identified in bovines [9,14].

Sarcocystis spp. can be microscopic, whereas others are macroscopic sarcocysts [13]; macrosarcocysts in striated muscles are large enough to be distinguished by the naked eye, as they occur as elongated cylindrical bodies and milky white-colored cysts embedded in muscular tissues, with length ranging from <5 mm to >10 mm. Two forms of examined macrosarcocysts were present, including fat and thin forms [16]. In sheep, *S. gigantea* and *S. medusiformis* form macroscopic sarcocysts that are transmitted by felids, whereas other *Sarcocystis* spp. transmitted by canines are microscopic [13]. In cattle, mature *S. hirsuta* cysts are macroscopic, whereas cysts of other species are microscopic [4].

Microscopic sarcocysts are generally identified based on the morphology of the cyst wall; *S. cruzi* and *S. heydorni* have thin-walled cysts, and *S. bovini*, *S. bovifelis*, *S. hirsuta*, and *S. hominis* have thick-walled cysts [4,9]. The sarcocysts of *S. tenella* have thick cyst walls, whereas those of *S. arieticanis* have thin cyst walls [17].

The definitive identification of *Sarcocystis* species requires an electron microscope or a molecular detection method [18]. Molecular techniques have been used to differentiate *Sarcocystis* species in cattle and sheep [4,19].

Sarcocystosis, due to its severe economic, medical, and veterinary consequences, is considered as a significant public health issue in several countries [20].

Due to the lack of available data on the prevalence of *Sarcocystis* spp., this study was conducted to investigate the presence of *Sarcocystis* organisms in meat-producing animals (cattle and sheep) slaughtered at an abattoir in Sulaimani Province in the northern region of Iraq.

Materials and Methods

Ethical approval

The study is not based on *in vivo* experiments or live animals; the sampling was performed

following the slaughtering of animals in the slaughterhouse.

Study period and location

This study was conducted during May, June, and July of 2020. Muscle samples were collected from the diaphragm and esophagus of slaughtered cattle and sheep of different ages and sexes after inspection of meat at the abattoir in Sulaimani Province. The samples were examined for the presence of *Sarcocystis* spp.

Sample collection

A total of 130 muscle samples, including 80 from sheep (40 diaphragm and 40 esophagus samples) and 50 from cattle (25 diaphragm and 25 esophagus samples), were collected. Samples were placed in plastic bags, labeled, and transported in a CoolBox to the laboratory for further investigation.

Fresh tissue samples were subjected to gross examination for the presence of macrocysts, and the microsarcocysts were detected using the peptic digestion method, followed by microscopic examination and histological examination of stained muscle samples [21].

Microscopic examination using peptic digestion method

Muscle samples were minced and subjected to peptic digestion separately using the method described by Dubey *et al.* [12]. Approximately 20 g of each minced sample was processed using 50 mL of the digestion solution, comprising 1.3 g pepsin, 2.5 g NaCl, and 3.5 mL concentrated HCl in a total volume of 500 mL of distilled water.

The suspensions were set at room temperature for 30 min and then filtered using a strainer with gauze. Filtrates were collected in sterile test tubes and centrifuged at 1500 rpm for 15 min, and the sediment was suspended in 0.5 mL of distilled water [22].

The suspension was examined for the presence of *Sarcocystis* bradyzoites under a light microscope at 40×. Free bradyzoites were observed as banana-shaped bodies. In addition, smears were prepared using droplets from the same solution spread on glass slides, fixed with absolute methanol, and stained with 10% Giemsa solution for 5 min. Slides were examined under a microscope at ×100 for detecting *Sarcocystis* bradyzoites [23].

Histological examination

A total of 16 samples that were positive in the microscopic examination were subjected to histopathological investigation (eight samples from each organ) for both animal species. Slices of muscle samples were preserved, fixed in 10% neutral buffered formalin, and embedded in paraffin blocks for histological examination. The tissues were cut into 3-5 mm thick sections and stained with hematoxylin and eosin (H&E) [24]. Slides were examined for the presence of sarcocysts at 10× and further analyzed at 40× and 100×. For the morphological identification

of *Sarcocystis* spp., the cell wall structure was observed [25].

Statistical analysis

Data analysis was conducted using SPSS Statistic Version 19 (IBM, USA). Chi-square tests were used to determine the association between different organs (inspected esophagus and diaphragms in both cattle and sheep). $p < 0.05$ was considered to be statistically significant.

Results

The overall prevalence rates of *Sarcocystis* spp. as examined by light microscopy were 90% (45/50) and 92.5% (74/80) in slaughtered cattle and sheep, respectively. There were no significant differences ($p > 0.05$) between the overall *Sarcocystis* detection rates in the examined host species (Table-1).

Organ-wise prevalence revealed higher positive rates of *Sarcocystis* spp. in the esophagus than in the diaphragm. Approximately 95% (38/40) of esophageal samples contained *Sarcocystis*, and 90% (36/40) of diaphragm samples were positive in sheep. In cattle also, 92% (23/25) of esophageal samples were positive, and 88% (22/25) of diaphragm samples were positive for *Sarcocystis* spp. However, there were no significant differences ($p > 0.05$) between the examined organs in both cattle and sheep. The distribution of *Sarcocystis* spp. in cattle and sheep muscles is shown in Table-1.

In the gross observation, no macroscopic sarcocysts were found in any of the examined muscle samples representing cattle and sheep. This finding may be due to the variation in risk factors associated with the distribution of *Sarcocystis* infection.

In the microscopic observation, the bradyzoites appeared crescent shaped and had pointed anterior end and rounded posterior end as analyzed using the peptic digestion method (Figure-1).

Histopathological examination of H&E-stained tissue sections revealed the presence of elongated, spindle-, round-, and ovoid-shaped microscopic tissue cysts packed with banana-shaped bradyzoites (Figure-2). Microscopic observation also revealed variations in the intensity of *Sarcocystis* spp. invasion, varying from single to numerous cysts (Figure-3).

Regarding the pathological changes caused by *Sarcocystis* spp., an inflammatory response around several microcysts was detected in the infected muscle fibers accompanied by infiltration of mononuclear

cells and degeneration and necrosis of muscle fibers (Figure-4).

Observation of the microsarcocyst wall under the light microscope revealed two morphologically distinct types, including thin-walled and thick-walled *Sarcocystis* spp., in both sheep and cattle tissue samples (Figures-5-7). Based on the microsarcocyst morphology, the suspected *Sarcocystis* spp. in this study were identified as *S. tenella* and *S. arieticanis* in sheep and *S. cruzi* and *S. bovis* or *S. hominis* in cattle. This finding indicated that more significant numbers of *Sarcocystis* spp. were of the thin-walled type in both animals. Sarcocysts were found inside the striated muscle cells including the esophagus, and diaphragm (Figure-8).

Discussion

Sarcocystis is considered as one of the most prevalent livestock parasites, with potential public health importance due to the consumption of undercooked or raw meat. *Sarcocystis* spp. have been isolated from different animals throughout the world. However, to the best of our knowledge, the present study is the first to investigate *Sarcocystis* infection among slaughtered cattle and sheep in Sulaimani Province in North Iraq.

Natural infections of sheep by *Sarcocystis* spp. have been investigated in various countries, with prevalence rates varying from 9.0% to 100%, depending on the detection method [13]. Meanwhile, no macroscopic cysts were detected in the visual examination in the present study, and the overall prevalence was obtained through microscopic examination of muscle tissues, which revealed prevalence rates of 90% and 92.5% of unspecified cysts of *Sarcocystis* spp. by the digestion method in cattle and sheep, respectively.

Diversity in sarcocystosis prevalence has been reported in different geographical areas; for instance, a prevalence rate of 100% was reported by Dehaghi *et al.* [26] and Whaeeb and Faraj [27] in sheep, and

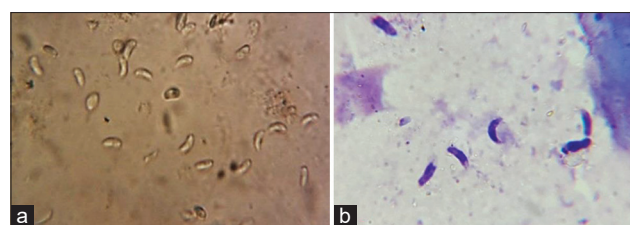


Figure-1: (a) Fresh smear preparation from microcyst, showing crescent-shaped bradyzoites (40×), (b) Giemsa stained from digested muscle samples showing *Sarcocystis* bradyzoites (100×).

Table-1: Prevalence of microsarcocyst of *Sarcocysts* spp. in slaughtered cattle and sheep at Sulaimani abattoir, north Iraq.

| Inspected organs | Examined animal | | | | Total infected |
|------------------|-----------------|------------------|---------------|------------------|----------------|
| | Cattle | | Sheep | | |
| | Inspected no. | Infected no. (%) | Inspected no. | Infected no. (%) | |
| Esophagus | 25 | 23 (92) | 40 | 38 (95) | 65 |
| Diaphragm | 25 | 22 (88) | 40 | 36 (90) | 65 |
| Total | 50 | 45 (90) | 80 | 74 (92.5) | 130 |

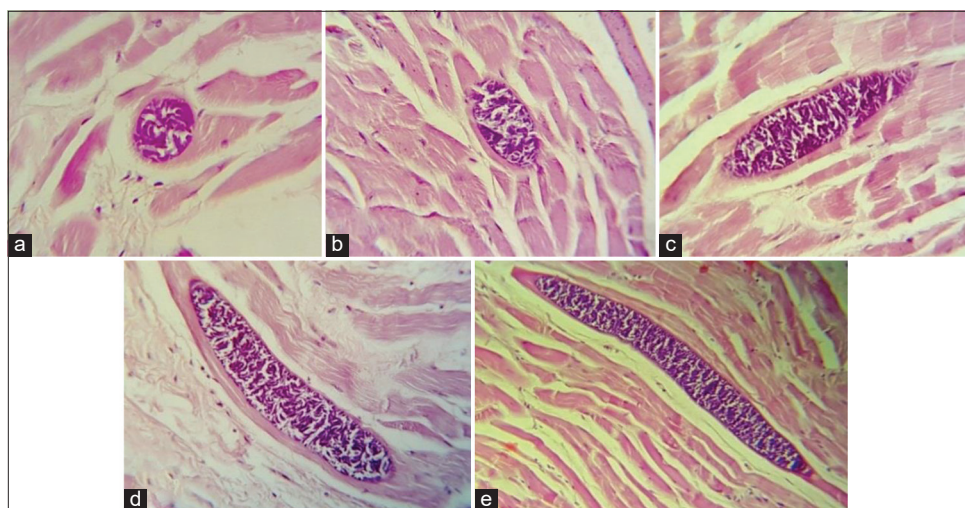


Figure-2: (a-e) Longitudinal section of *Sarcocystis* spp. revealing different shapes of microcyst with hematoxylin and eosin stain of diaphragm muscles (10×).

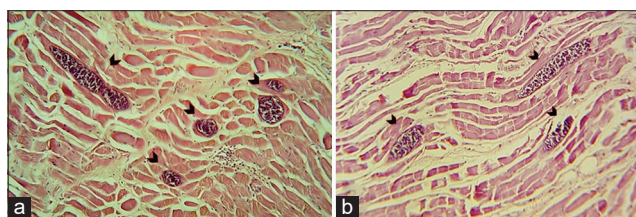


Figure-3: Longitudinal section of unidentified *Sarcocystis* spp. (a and b) Revealing different microcysts with hematoxylin and eosin stain of diaphragm muscles (10×).

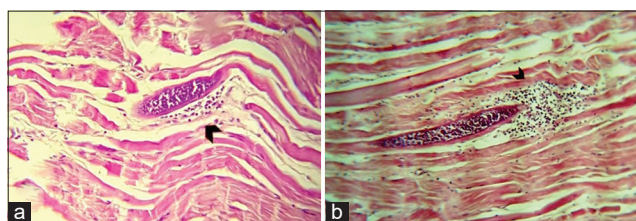


Figure-4: Histological sections from muscle tissue with an unidentified *Sarcocystis* spp. hematoxylin and eosin stain (a and b) showing inflammatory reaction (10×).

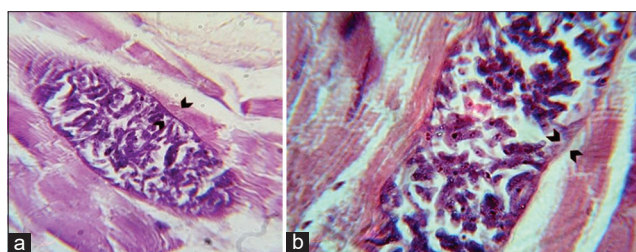


Figure-5: Light microscopic appearance of thin wall microcysts from sheep diaphragm with hematoxylin and eosin stain, (a) 40× and (b) 100×.

prevalence rates of 95.37% and 96.9% were reported by Fukuyo *et al.* [5] and Abuelwafa [28], respectively.

Similarly, the prevalence in bovine muscle was almost 100% in most of the world regions [2]. A prevalence of 100% was reported by Shekarforoush *et al.* [29]. The prevalence rates of 95.5%, 91.33%, and 88.2% were reported by Mavi *et al.* [30], Mounika *et al.* [31], and Shahraki *et al.* [32], respectively.

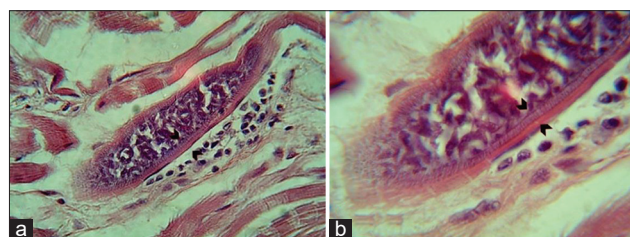


Figure-6: Light microscopic appearance of thick wall microcysts from sheep diaphragm with hematoxylin and eosin stain, (a) 40× and (b) 100×.

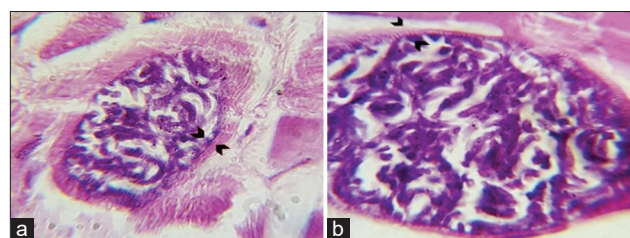


Figure-7: Light microscopic appearance of microcysts from cattle diaphragm with hematoxylin and eosin stain, showing two types of cyst (a) thin wall and (b) thick wall (100×).

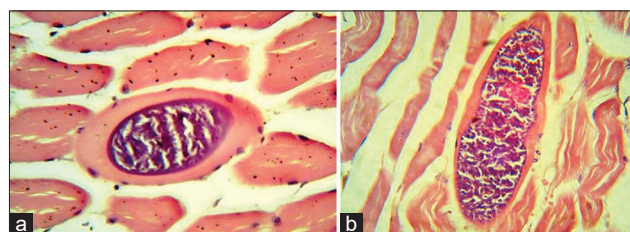


Figure-8: Histological sections of an unidentified *Sarcocystis* spp. showing the microcysts inside the muscle cells from infected cattle (a) esophagus, (b) diaphragm with hematoxylin and eosin stain (40×).

In the present study, examination of the esophagus and diaphragm muscles revealed higher prevalence rates of 92% and 95% in the esophagus than those of 88% and 90% in the diaphragm for cattle and sheep, respectively. A similar finding was also reported by Dehaghi *et al.* [26] and Ahmed *et al.* [33].

Positive rates of 91% and 58% in cattle esophagus and diaphragm were also reported by Nourani *et al.* [34].

In contrast to our findings, high prevalence rates of *Sarcocystis* spp. of 100% and 93.2% in the diaphragm were reported by Fukuyo *et al.* [5] and Bahari *et al.* [35], respectively, and also by Dubey and Livingston [20] who reported the prevalence rates of 22.4% in the diaphragm and 0.23% in the esophagus of sheep.

Sarcocystis was also detected in cattle heart at prevalence rates of 100% and 17.9% from Brazil and Romania by Ferreira *et al.* [36] and Imre *et al.* [37], respectively.

Variations in the prevalence rates of *Sarcocystis* spp. depend on several factors such as management conditions, presence of dogs and cats in the surrounding area, and the number of sporocysts disseminated by them. Survivability of sarcocysts in the environment is another factor affecting the prevalence of *Sarcocystis* controlled by climatic conditions such as temperature, rainfall, and humidity [38].

The present study results showed that the pepsin digestion technique is a sensitive method for investigating the presence of *Sarcocystis* in slaughtered animals [32,39], which produced the highest positive rate. This technique is also simpler and faster than other conventional procedures [16]. However, *Sarcocystis* spp. cannot be appropriately identified using this method.

Similar to the present study result, no macroscopic sarcocysts were observed by Shekarforoush *et al.* [29], Nourani *et al.* [34], and Shahraki *et al.* [32], although low prevalence rates of 17.08% and 7.5% were reported by Farhang-Pajuh *et al.* [16] and Ahmed *et al.* [33] in sheep and cattle, respectively.

Macrosarcocyst species are considered as symptomatic. However, they can affect the quality of meat and lead to economic losses [19] in the form of subsequent downgrading, partial rejection, or even condemnation of the animal carcasses at slaughterhouses [28].

Primarily, felids comprise the definitive hosts for macrosarcocyst forming species. Due to lesser contact with felids, lesser numbers of oocytes are shed, which thus explain the low prevalence of macroscopic cysts [30].

Dogs play a more significant role than cats in the transmission of sarcocystosis in farm animals, which might be attributed to the close association with dogs that are retained for herd protection. Moreover, dogs can easily access the offal of infected sheep, thus making it easier for them to transmit *Sarcocystis* [28].

In the present study, histopathological examination of H&E-stained tissue sections revealed the presence of two morphologically distinct microscopic types of sarcocysts. Consistent with El-Morsei *et al.* [17], the observed microsarcocysts in sheep muscle tissues were of thin-walled and thick-walled types, which belong to *S. arieticanis* and *S. tenella*, respectively, and those of *S. tenella* had striated thick-walled

sarcocysts (Figure-7). Moreover, the bovine species *S. cruzi* has been reported to have thin-walled microsarcocysts detected in sheep [11]. Similarly, both *S. tenella* and *S. arieticanis* were reported through light microscopic examination by Hu *et al.* [40].

Sarcocystis spp. in cattle were also of two types. *S. cruzi* and *S. heydorni* have thin-walled cysts, and *S. bovini*, *S. bovifelis*, and *S. hominis* have thick-walled cysts [25].

In this regard, based on the morphological appearance, the thin-walled microsarcocysts observed in the present study might be *S. cruzi* and *S. heydorni*. Moreover, the thick-walled type might belong to *S. hominis*, *S. bovini*, *S. sinensis*, and *S. bovifelis*, as these species are very similar morphologically, and their cyst walls are provided with finger-like protrusions [41]. The cysts cannot be distinguished by light microscopy, whereas a few differences can be identified by transmission electron microscopy [4,6].

Based on the histological examination of the microsarcocyst wall, the suspected *Sarcocystis* spp. were identified as *S. tenella* and *S. arieticanis* in sheep and *S. cruzi* and *S. bovifelis* or *S. hominis* in cattle, as the availability of other species was inadequate.

Based on the data reported by Nourani *et al.* [34], the thin-walled sarcocysts in cattle were consistent with *S. cruzi* with higher prevalence, and the thick-walled sarcocysts could represent *S. hominis* with lower prevalence. Both *S. cruzi* and *S. tenella* were found at higher prevalence rates of 100% and 80% by Daryani *et al.* [20] and Rahdar and Kardooni [42], respectively.

Both intermediate and final hosts can harbor one or more *Sarcocystis* spp. [28], which is consistent with the present study finding in which both thin- and thick-walled microsarcocysts were observed in the same muscle tissue section.

Sarcocystis spp. cyst invasions in the muscle tissue can produce inflammation around the muscle fibers and between the cysts, showing mononuclear cells infiltration (neutrophils, lymphocytes, eosinophils, and plasma cells) and degeneration and necrosis of muscle fibers [25]. Consistently, inflammatory reactions were also detected around some of the observed microsarcocysts in the present study (Figure-4).

Identification of *Sarcocystis* spp. is not possible using a microscopic analysis [25]. Therefore, the exact identification and differentiation of *Sarcocystis* spp. in slaughtered animals necessitate other investigations using accurate detection methodologies, including molecular procedures.

Identification of *Sarcocystis* spp. through molecular characterization is a fundamental approach to evaluate the pathogenic species involved in cattle and sheep infections that consequently cause economic losses. In addition, identifying the final host responsible for shedding each of *Sarcocystis* spp. and evaluating the risk of zoonotic infections are essential steps in managing the disease and designing control programs.

Conclusion

This study reports the presence of *Sarcocystis* spp. in both sheep and cattle from Sulaimani Province. The prevalence was primarily related to the presence of microsarcocysts. Therefore, meat should be cooked sufficiently or frozen before use to prevent health hazards to the consumer. The pepsin digestion procedure was found to be a sensitive method for investigating unidentified *Sarcocystis* spp. Based on the morphology of the microsarcocyst wall, the suspected *Sarcocystis* spp. recovered in this study were identified as *S. tenella* and *S. arieticanis* in sheep and *S. cruzi* and *S. bovis* or *S. hominis* in cattle.

Author's Contributions

SHA designed the study and contributed to the collection of muscle samples, the practical parts, analysis of data, and writing and organization of the paper. SHA has read and approved the final manuscript.

Acknowledgments

The author would like to thank Dr. Dyary Heiwa from the College of Veterinary Medicine, University of Sulaimani, for his kindness in commenting and improving the manuscript. The author did not receive any funds for this study.

Competing Interests

The author declares that he has no competing interests.

Publisher's Note

Veterinary World remains neutral with regard to jurisdictional claims in published institutional affiliation.

References

- Fayer, R. (2004) *Sarcocystis* spp. in human infections. *Clin. Microbiol. Rev.*, 17(4): 894-902.
- Vangeel, L., Houf, K., Chiers, K., Vercruyse, J., D'Herde, K. and Ducatelle, R. (2007) Molecular-based identification of *Sarcocystis hominis* in Belgian minced beef. *J. Food Prot.*, 70(6): 1523-1526.
- Poulsen, C.S. and Stensvold, C.R. (2014) Current status of epidemiology and diagnosis of human sarcocystosis. *J. Clin. Microbiol.*, 52(10): 3524-3530.
- Gjerde, B. (2016a) Molecular characterization of *Sarcocystis bovis*, *Sarcocystis bovini* n. sp., *Sarcocystis hirsuta* and *Sarcocystis cruzi* from cattle (*Bos taurus*) and *Sarcocystis sinensis* from water buffaloes (*Bubalus bubalis*). *Parasitol. Res.*, 115(4): 1473-1492.
- Fukuyo, M., Battsetseg, G. and Byambaa, B. (2002) Prevalence of *Sarcocystis* infection in meat-producing animals in Mongolia. *Southeast Asian J. Trop. Med. Public Health*, 33(3): 490-495.
- Moré, G., Pantchev, A., Skuballa, J., Langenmayer, M., Maksimov, P., Conraths, F., Venturini, M.C. and Schares, G. (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.
- Morsy, K., Saleh, A., Al-Ghamdi, A., Abdel-Ghaffara, F., Al-Rasheid, K., Bashtar, A.R., Al Quraisy, S. and Mehlhorn, H. (2011) Prevalence pattern and biology of *Sarcocystis capracanis* infection in the Egyptian goats: A light and ultrastructural study. *Vet. Parasitol.*, 181(2-4): 75-82.
- Formisano, P., Aldridge, B., Alony, Y., Beekhuis, L., Davies, E., Del Pozo, J., Dunn, K., English, K., Morrison, L. and Sargison, N. (2013) Identification of *Sarcocystis capracanis* in cerebrospinal fluid from sheep with neurological disease. *Vet. Parasitol.*, 193(1-3): 252-255.
- Dubey, J., van Wilpe, E., Calero-Bernal, R., Verma, S. and Fayer, R. (2015b) *Sarcocystis heydorni*, n. sp. (*Apicomplexa: Sarcocystidae*) with cattle (*Bos taurus*) and human (*Homo sapiens*) cycle. *Parasitol. Res.*, 114(11): 4143-4147.
- Agholi, M., Shahabadi, S.N., Motazedian, M.H., and Hatam, G.R. (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.
- Anvari, D., Narouei, E., Hosseini, M., Narouei, M.R., Daryani, A., Shariatzadeh, S.A., Pagheh, A.S., Gholami, S., Sarvi, S. and Sargazi, D. (2020) Sarcocystosis in ruminants of Iran, as neglected food-borne disease: A systematic review and meta-analysis. *Acta Parasitol.*, 65(3): 555-568.
- Dubey, J., Speer, C. and Fayer, R. (1989) Sarcocystosis of Animals and Man. CRC Press, Boca Raton.
- Dubey, J., Calero-Bernal, R., Rosenthal, B., Speer, C. and Fayer, R. (2015a). Sarcocystosis of Animals and Humans. CRC Press, Boca Raton.
- Gjerde, B. (2016b) The resurrection of a species: *Sarcocystis bovis* Heydorn *et al.*, 1975 is distinct from the current *Sarcocystis hirsuta* in cattle and morphologically indistinguishable from *Sarcocystis sinensis* in water buffaloes. *Parasitol. Res.*, 115(1): 1-21.
- 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.
- Farhang-Pajuh, F., Yakhchali, M. and Mardani, K. (2014) Molecular determination of abundance of infection with *Sarcocystis* species in slaughtered sheep of Urmia, Iran. *Vet. Res. Forum*, 5(3): 181-186.
- El-Morsey, A., Abdo, W., Sultan, K., Elhawary, N.M. and AbouZaid, A.A. (2019) Ultrastructural and molecular identification of the sarcocysts of *Sarcocystis tenella* and *Sarcocystis arieticanis* infecting domestic sheep (*Ovis aries*) from Egypt. *Acta Parasitol.*, 64(3): 501-513.
- Rosenthal, B.M., Dunams, D.B. and Pritt, B. (2008) Restricted genetic diversity in the ubiquitous cattle parasite, *Sarcocystis cruzi*. *Infect. Genet. Evol.*, 8(5): 588-592.
- Kalantari, N., Khaksar, M., Ghaffari, S. and Hamidekish, S.M. (2016) Molecular analysis of *Sarcocystis* spp. isolated from sheep (*Ovis aries*) in Babol area, Mazandaran Province, Northern Iran. *Iran. J. Parasitol.*, 11(1): 73-80.
- Daryani, A., Alaei, R., Dehghan, M., Arab, R., Sharif, M. and Ziaei, H. (2006) Survey of *Sarcocystis* infection in slaughtered sheep and buffaloes in Ardabil, Iran. *J. Anim. Vet. Adv.*, 5(1): 60-62.
- JyothiSree, C.H., Venu, R., Samatha, V., Malakondaiah, P. and Rayulu, V.C. (2017) Prevalence and microscopic studies of *Sarcocystis* infection in naturally infected water buffaloes (*Bubalus bubalis*) of Andhra Pradesh. *J. Parasit. Dis.*, 41(2): 476-482.
- Dubey, J. and Livingston, J.C. (1986) *Sarcocystis capracanis* and *Toxoplasma gondii* infections in range goats from Texas. *Am. J. Vet. Res.*, 47(3): 523-524.
- Prayson, B., McMahon, J.T. and Prayson, R.A. (2008) Fast food hamburgers: What are we really eating? *Ann. Diagn. Pathol.*, 12(6): 406-409.
- Avapal, R.S., Sharma, J. and Juyal, P. (2004) Pathological changes in *Sarcocystis* infection in domestic pigs (*Sus scrofa*). *Vet. J.*, 168(3): 358-361.
- Januškevičius, V., Januškevičienė, G., Banys, A., Dailidavičienė, J. and Anilienė, A. (2018) Biochemical profile of blood in naturally occurring *Sarcocystis* infection

- in cattle. *Acta Vet. Brno*, 87(3): 205-211.
26. Dehaghi, M.M., Fallahi, M., Sami, M. and Radfar, M.H. (2013) Survey of *Sarcocystis* infection in slaughtered sheep in Kerman Abattoir, Kerman, Iran. *Comp. Clin. Pathol.*, 22(3): 343-346.
 27. Whaeeb, S.T. and Faraj, A.A. (2016) Molecular identification and phylogeny of microscopic *Sarcocystis* sheep in Baghdad province. *Int. J. Adv. Res. Biol. Sci.*, 3(12): 50-56.
 28. Abuelwafa, S.A., Alaraby, M.A., Abbas, I.E. and Elmishmishy, B.M. (2016) Prevalence of *Sarcocystis* species infecting sheep from Egypt. *Egypt. Vet. Med. Soc. Parasitol. J.*, 12(7): 74-90.
 29. Shekarforoush, S., Razavi, S., Dehghan, S. and Sarihi, K. (2005) Prevalence of *Sarcocystis* species in slaughtered goats in Shiraz, Iran. *Vet. Rec.*, 156(13): 418-20.
 30. Mavi, S.A., Teimouri, A., Mohebbali, M., Yazdi, M.K.S., Shojae, S., Rezaian, M., Salimi, M. and Keshavarz, H. (2020) *Sarcocystis* infection in beef and industrial raw beef burgers from butchereries and retail stores: A molecular microscopic study. *Heliyon*, 6(6): e04171.
 31. Mounika, K., Chennuru, S., Ravipati, V., Tumati, S.R. and Krovvidi, S. (2018) Studies on prevalence and histomorphology of *Sarcocystis* species infecting cattle in Andhra Pradesh, India. *J. Parasit. Dis.*, 42(1): 77-80
 32. Shahraki, M.K., Ghanbarzehi, A. and Dabirzadeh, M. (2018) Prevalence and histopathology of *Sarcocystosis* in slaughtered carcasses in southeast Iran. *J. Adv. Vet. Anim. Res.*, 5(4): 381-387.
 33. 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): 1461-1465.
 34. Nourani, H., Matin, S., Nouri, A. and Azizi, H. (2010) Prevalence of thin-walled *Sarcocystis cruzi* and thick-walled *Sarcocystis hirsuta* or *Sarcocystis hominis* from cattle in Iran. *Trop. Anim. Health Prod.*, 42(6): 1225-1227.
 35. Bahari, P., Salehi, M., Seyedabadi, M. and Mohammadi, A. (2014) Molecular identification of macroscopic and microscopic cysts of *Sarcocystis* in sheep in North Khorasan Province, Iran. *Int. J. Mol. Cell Med.*, 3(1): 51-56.
 36. Ferreira, M.S.T., Vogel, F.S.F., Sangioni, L.A., Cezar, A.S., Patrícia Braunig, P., Botton, S.A., Camillo, G. and Portella, L.P. (2018) *Sarcocystis* species identification in cattle hearts destined to human consumption in southern Brazil. *Vet. Parasitol. Reg. Stud. Rep.*, 14: 94-98.
 37. Imre, K., Dărăbuș, G., Tîrziu, E., Morariu, S., Imre, M., Plutzer, J., Boldea, M.V. and Morar, A. (2019) *Sarcocystis* spp. in Romanian slaughtered cattle: Molecular characterization and epidemiological significance of the findings. *Biomed Res. Int.*, 2019: 4123154.
 38. El-Dakhly, K.M., El-Nesr, K.A., El-Nahass, E.S., Hirata, A., Sakai, H. and Yanai, T. (2011) Prevalence and distribution patterns of *Sarcocystis* spp. in buffaloes in Beni-Suef, Egypt. *Trop. Anim. Health Prod.*, 43(8): 1549-1554.
 39. Davoudi, J., Shabestari, A.B., Mozahheb, P., Norouzi, R., Maadinia, M. and Mahmoudi, R. (2017) A study of *Sarcocystis* infection in mincemeat using digestion method in Ghazvin, Iran. *Arch. Hyg. Sci.*, 6(2): 178-181.
 40. Hu, J.J., Huang, S., Wen, T., Esch, G.W., Liang, Y. and Li, H.L. (2017) *Sarcocystis* spp. in domestic sheep in Kunming City, China: Prevalence, morphology, and molecular characteristics. *Parasite*, 24: 30.
 41. Chiesa, F., Muratore, E., Dalmaso, A. and Civera, T. (2013) A new molecular approach to assess the occurrence of *Sarcocystis* spp. in cattle and products thereof: Preliminary data. *Trop. Anim. Health Prod.*, 2(3): e41.
 42. Rahdar, M. and Kardooni, T. (2017) Molecular identification of *Sarcocystis* spp. in sheep and cattle by PCR-RFLP from Southwest of Iran. *Jundishapur J. Microbiol.*, 10(8): e12798.
