Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2024.280

RESEARCH ARTICLE

Morphological Description of Fatal Sarcocystosis in Cattle with Implication of their Immunological and Histopathological Alteration

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ARTICLE HISTORY (24-539)

Received: September 1, 2024
Revised: October 31, 2024
Accepted: November 6, 2024
Published online: November 16, 2024

Key words:
Cattle
Macrocysts
Cytokines
Histopathological examination
Morphological Features
Sarcocystis fusiformis

ABSTRACT

Sarcocystis (*S.*) is a cyst-forming parasite with over 200 species in the phylum Apicomplexa. This study aimed to investigate the prevalence of *S. fusiformis* in cattle and examine its impact on the animals. A total of 200 cattle carcasses were examined for the presence of *S. fusiformis* in various tissues, including the esophagus, heart, tongue, and skeletal muscles, to assess the extent of infection. The study also focused on analyzing the associated immunological and histopathological changes in infected animals. Infected tissues, including the esophagus, hearts, and muscles, were subjected to light microscopy, transmission electron microscopy (TEM), and histopathological examination. A 10% prevalence of *S. fusiformis* was found among the examined cattle. Toluidine blue-stained sections revealed that the sarcocyst walls ranged from 1.8 to 6.5µm in thickness and displayed branching villar protrusions. These protrusions were observed to have homogeneous, papillomatous forms with a width of 100nm. TEM analysis revealed filamentous tubular structures within the sarcocyst walls, some reaching lengths up to 6µm. These tubules ran parallel to the long axis of the villar projections and lacked granules. Furthermore, the expression levels of various cytokines in the infected tissues were significantly elevated, including tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), and interferon-gamma (IFN-ɤ). Histopathological examination revealed tissue changes, with a small number of mononuclear inflammatory cells present in the affected organs, indicating an immune response to the parasite. This study provides valuable insights into the unique characteristics of *S. fusiformis* sarcocysts, helping to distinguish them from other *Sarcocystis* species found in both domestic and wild ruminants. Further research is recommended to explore potential control measures and better understand the broader implications of *S. fusiformis* infections in livestock.

To Cite This Article: Bahshwan SMA, Almayouf MA, Al-Rashidi HS, Alzahrani AM, Khan SA, Mahjoub HA, AL-Qurashi MM, Al-Hoshani N, Alqahtani MM and Attia MM, 2024. Morphological description of fatal sarcocystosis in cattle with implication of their immunological and histopathological alteration. Pak Vet J, 44(4): 1063-1072. http://dx.doi.org/10.29261/pakvetj/2024.280

INTRODUCTION

Globally, there are approximately one billion beef cattle. However, compared to pigs and chickens, they are less efficient at converting feed into meat. This means that producing beef requires more resources and potentially has a greater environmental impact (Terry *et al.*, 2020; Ţenu *et al.*, 2023). These measurements, however, do not account for the fact that beef cattle provide high-quality protein from animal foods that are unsuitable for other species of livestock (Thomas *et al.*, 2021). Principal areas of emphasis for methods to enhance the productivity of beef cattle include the structure and content of feed, host genetics, the functioning of the rumen and pulmonary

microbiomes, and breeding and operational management (Lovarelli *et al.*, 2020; Thornton *et al.*, 2022). Strategies for improving beef production must consider not only the changing environmental conditions in which cattle are raised but also the overall health and immunity of the herd (Attia *et al*., 2022a). Factors, i.e., climate change, resource availability, and disease resistance, need to be taken into account to ensure sustainable and efficient beef production (Kinley *et al.*, 2020; Palange and Dhatrak, 2021). Around 45% of the worldwide protein supply for humans is attributed to meat and milk derived from cattle and buffalo (Mottet *et al.*, 2017; Cordeiro *et al.*, 2022).

Parasitism in livestock production poses a significant threat, leading to various clinical signs, mortality, reduced production, increased condemnation rates, and economic losses (Bachaya *et al*., 2015; Swar and Shnawa, 2020; Soliman *et al.*, 2022a; Strydom *et al*., 2023; Soliman *et al.*, 2024). *Sarcocystosis* is a major parasitic disease affecting livestock, with significant economic consequences. Infected cattle can experience severe health issues, including miscarriage, reduced milk production, neurological problems, and weight loss, potentially leading to death (Ahmed *et al.*, 2016; Badr *et al.*, 2024).

According to Castro-Forero *et al.* (2022), the majority of *Sarcocystis* (*S.*) species follow a life cycle featuring two hosts: herbivores as host intermediates and carnivores as ultimate hosts. *Sarcocystis* species are transmitted to the intermediate host by ingesting sporocysts, oocysts, or both expelled from the definitive host's feces (Fig. 1). The parasite has a short duration of infective schizogony before encysting in tissues to generate sarcocysts (Mousa *et al.*, 2016; Dubey and Rosenthal, 2023). Dogs are crucial in spreading this infection since they reside close to buffalo herds (Wielgus *et al.*, 2021). Feeding dogs the raw meat of killed animals and the high frequency of interaction between animals and domestic or stray dogs provide ideal conditions for the parasite, *S. cruzi* maintenance (Mendoza Roldan and Otranto, 2023; Pal *et al.*, 2023)

Sarcocysts are embedded in the tissues of intermediate hosts, which the definitive host consumes and then gets infected. The infected body parts with macrocysts can only be identified by inspection after slaughtering the infected animals at slaughterhouses (Constable *et al.*, 2016; Valizadeh and Ebrahimi, 2022).

The discovery of the *Sarcocystis* life cycle in 1972 initially led scientists to believe that only one species, *S. fusiformis*, infected both cattle and water buffalo. However, further research revealed that cattle harbor three distinct species: *S. hirsuta*, *S. hominis*, and *S. cruzi*. The *S. fusiformis* was then used solely to identify the species responsible for forming larger cysts (Mousa *et al.*, 2016; Dubey *et al.*, 2023).

Compared to cattle, the life cycles of *Sarcocystis* species that infect buffalo are poorly understood, especially the stages involving schizont and sarcocyst formation. Four species, i.e., *S. fusiformis*, *S. buffalonis*, *S. levinei*, and *S. dubeyi*, have been definitively identified in water buffalo (Dubey *et al.*, 2014; Ras, 2021). The largest sarcocysts in the genus are those of *S. fusiformis* (Barakat *et al.*, 2023; Menshawy *et al.*, 2023). Additionally, the study of El-Mahdi *et al.* (2023) investigated the life cycle and disease-causing potential of *S. cruzi* by examining infected cattle in El-Kharga, Egypt, and then studying how the parasite develops and affects dogs, its final host. This study identified diverse forms of *S. cruzi* oocysts in cattle from Egypt's New Valley Governorate. Genetic analysis confirmed these parasites were indeed *S. cruzi*, highlighting this species as the most common in the region's cattle population. A high prevalence (76%) of *Sarcocystis* infection was found in mustelids, with six species typically associated with canids identified for the first time in these animals. *S. arieticanis*, *S. bertrami*, *S. capracanis*, and *S. morae* were the most common, each infecting around 40% of the examined mustelids (Attia *et al.*, 2022b; Šneideris *et al.*, 2024; Al-Nabati *et al*., 2024).

Researchers have extensively studied the structure of the *S. fusiformis* sporocyst wall, which is branching and contains microtubules, as observed in transmission electron microscopy (TEM) studies (Gjerde *et al.*, 2020; El-Morsey and Abdo, 2024). While different studies have reported varying details about *S. fusiformis* metrocytes, both light microscopy and TEM have been used to describe these structures. The current study aims to provide a detailed description of *Sarcocystis* cysts, their contents, and the histopathological changes observed in affected organs, alongside genetic analysis of the implicated tissues, such as muscles and hearts.

MATERIALS AND METHODS

Sampling: A post-mortem inspection was conducted on 200 female cattle (6 years old) to detect *S. fusiformis* in the 60 esophagi, 30 hearts, and 60 tongues. The parasite was found in 10% of the animals, and the affected tissues were then sent to a parasitology laboratory, where the white cyst was separated from the skeletal muscles and preserved in two tubes: one with 10% formalin and the other with 2.5% glutaraldehyde. The diseased tissues were subjected to histopathological analysis (using the formalin-preserved tissues), while the remaining tissues were stored in a refrigerator for gene expression studies.

Parasitological examination: The sarcocyst was carefully removed from the surrounding muscle tissue and placed on a clean slide. After air drying, the sarcocyst was fixed with methanol, dehydrated, and stained with Giemsa for 30min. The slide was then rinsed with water, allowed to dry, and examined under an Olympus CX40 optical microscope (Motamedi *et al.*, 2011).

Histopathological examination: Skeletal muscle samples showing visible white cysts were embedded in paraffin wax to create a solid block for cutting. Thin sections (3-4µm thick) of the muscle tissue were then cut from the paraffin block using a microtome. The paraffin was removed from these sections, and they were stained with hematoxylin and eosin (H&E) to highlight the cellular structures for examination under a microscope (Bancroft and Gamble, 2008). Olympus optical microscope supplied with a digital camera was used to inspect the tissue sections (Tokyo, Japan).

Ultrathin preparation: The isolated *Sarcocystis* cysts from skeletal muscles were prepared for transmission electron microscope (TEM). Briefly, the fixed samples were dehydrated using a series of graded ethanol, fixed in

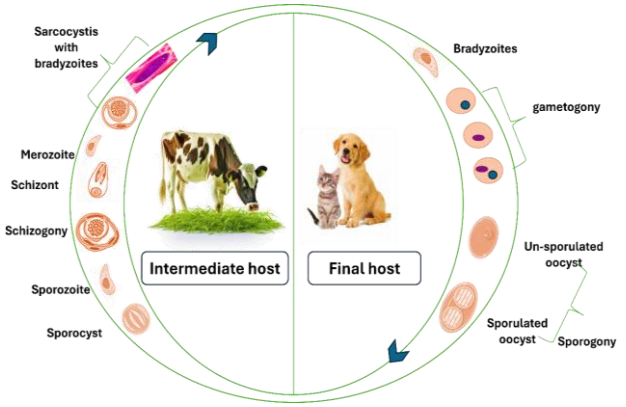

Fig. 1: Life cycle of *Sarcocystis fusiformis* between final host and intermediate host.

a 1% osmium tetroxide solution in Millonig's buffer at a pH of 7.4, penetrated with a mixture of epoxy resin and propylene oxide, encased in absolute resin, and polymerized overnight at 60°C. Ultrathin resin slices of macrocysts were contrasted using lead citrate and uranyl acetate agents; they were then investigated in an 80 kV-operated TEM (JEOL, JEM-1400 TEM) according to (Dubey *et al.*, 2015).

Inflammatory markers: Muscles (masseter muscles), esophagus, and heart parts from *S. fusiformis*-infected cattle were collected in the same manner as those from five young, uninfected cattle calves (negative controls), and stored aseptically in a refrigerator.

DNA extraction: Infected muscle tissue was digested, and the resulting sediment was washed three times with phosphate-buffered saline (PBS) to collect the zoites. These were stored in conical tubes at -20°C (Motamedi *et al.*, 2011). Genomic DNA was then extracted from the positive samples using a phenol-chloroform method (Köchl *et al.*, 2005; Salem *et al*., 2022a) and stored at -20°C.

RNA isolation: The RNA obtained from 100mg of tissue under examination was isolated using the total RNA kit (Ambion, Applied Biosystems) following the manufacturer's instructions. The Nano-Drop (Thermo Scientific, USA) was used to quantify and assess the purity of the RNA. Specifically, 500ng mRNA was produced using DNase I amplification grade (Invitrogen) according to the directions provided by the manufacturer. A reverse transcription reaction was conducted on the treated RNA using the High-Capacity cDNA Archive Kit from Applied Biosystems (Saad *et al*., 2017; Yu *et al.*, 2021).

Quantitative real-time PCR protocol: The PCR program consisted of an initial denaturation step at 94°C for 5 minutes, followed by 30 cycles of denaturation at 94°C for 2 minutes, annealing at 57°C for 30 seconds, Extension at 72°C for 2 minutes, and A final extension step at 72°C for 5 minutes completed the amplification (Hamidinejat *et al.*, 2014; Attia *et al*., 2022c; Soliman e*t al.*, 2022b).

Specific PCR primers were designed to amplify genes for important immune molecules in cattle to study the immune response to the parasite, tumor necrosis factor-alpha (TNF-α), interleukin-1β-8, and interferon-gamma (IFN-δ). These primer sequences were based on information from the Gene Bank (Table 1).

Table 1: List of primers used for amplification of pro-inflammatory genes

Gene	Primers Sequence (F/R)
TNF-α	F- CAAGTAACAAGCCGGTAGCC/ R-TGGAAGACCCCTCCCTGGTA
IL-1β	F-CTAGCCCATGTGTGCTGAAG/ R-CCTTTACTTGGCTCTTCACC
IFN-ϒ	F- ATAACCAGGTCATTCAAAGG/ -R- AT TCTGACTTCTCTTCCGCT
IL-8	F-ATGACTTCCAAGCTGGCTGTT/R-CATGGATCTTGCTTCTCAGC
Beta-actin	F- CGCACCACTGGCATTGTCAT/ R-TCCAAGGCGACGTAGCAGAG

The gene for beta-actin, a protein involved in cell structure, was used as a reference gene to normalize the expression levels of the immune genes. These genes were analyzed in a separate set of cDNA samples obtained from five parasite-free animals (Yu *et al.*, 2021).

Statistical analysis: The triplicate data are presented as mean ± SE. The means of the groups (positive and negative PCR-testing cattle) were then statistically compared using the t-test on independent samples. Statistical significance was determined at a p-value of less than 0.05 using SPSS v 26 (IBM, Chicago, IL, USA).

RESULTS

Parasites incidence during macroscopic and microscopic examination: Twenty out of 200 (20/200) examined female cattle (5 years old) were positive for *S. fusiformis* with a prevalence of 10%. *S. fusiformis* cysts were white, ranging from 4–12mm long and 3–6mm wide, and they were simple to separate from the host tissue. The cyst contains bradyzoites, which are usually fusiform (broad in the center and tapered ends); some appear to have blunt ends (Fig. 2); the nucleus either centrally or toward one end, and the other end contains granules.

Histopathological examination of the cysts reveals that the macroscopic cysts were detected mostly in skeletal muscles of cattle which indicated the presence of thick-walled sarcocyst containing bradyzoites embedded in muscle with mild tissue reaction as interstitial edema and few eosinophilic inflammatory cells (Fig. 3a&b), other cases showed the presence of large one with thick-walled sarcocyst with severe tissue reaction in the form of eosinophilic inflammatory cells (Fig. 3c &d; Fig. 4), hyaline degeneration of muscle fibers (Fig. 3e), The interstitial tissue was infiltrated mostly by eosinophilic inflammatory cells (Fig. 3f). In some cases, there was also significant infiltration of mononuclear inflammatory cells and muscle degeneration with a large cyst wall (Fig. 3g).

Semi thin ultrastructure of *S. fusiformis:* *Sarcocystis* cyst was composed of a wall with different layers, several cells of metrocytes, and a different compartment. This compartment is separated by septa, filled with bradyzoites in the periphery, leaving the center free from bradyzoites. The bradyzoites appear as a banana shape with an anterior point and a broad posterior (Fig. 5).

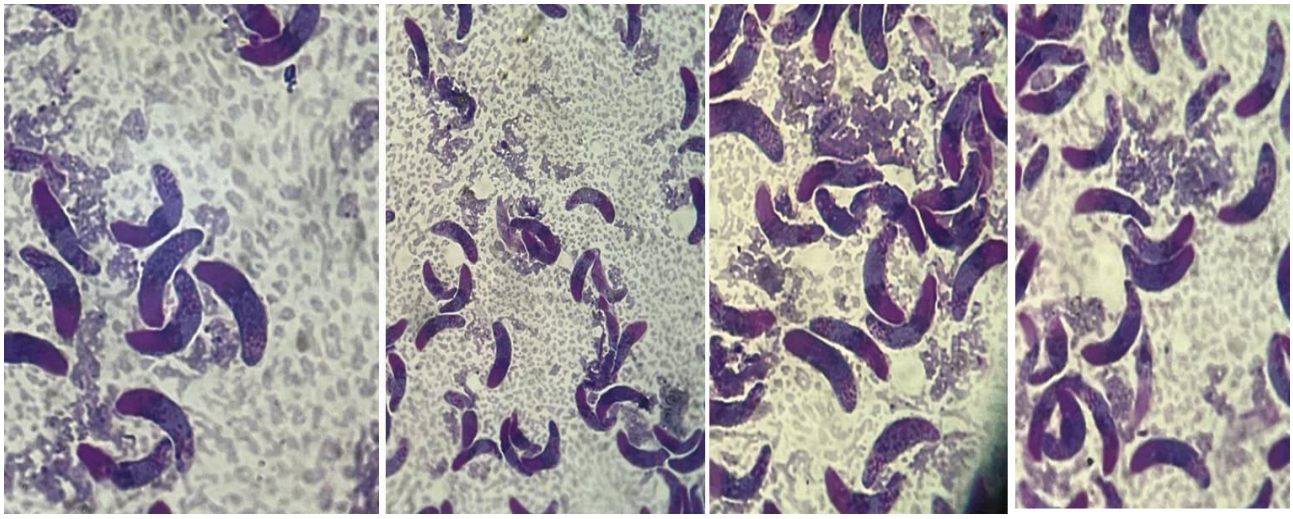

Fig. 2: Photomicrograph of cattle *S. fusiformis* showing the banana shape bradyzoites with tapering ends (one end pointed and the other is broad). H&E ×40.

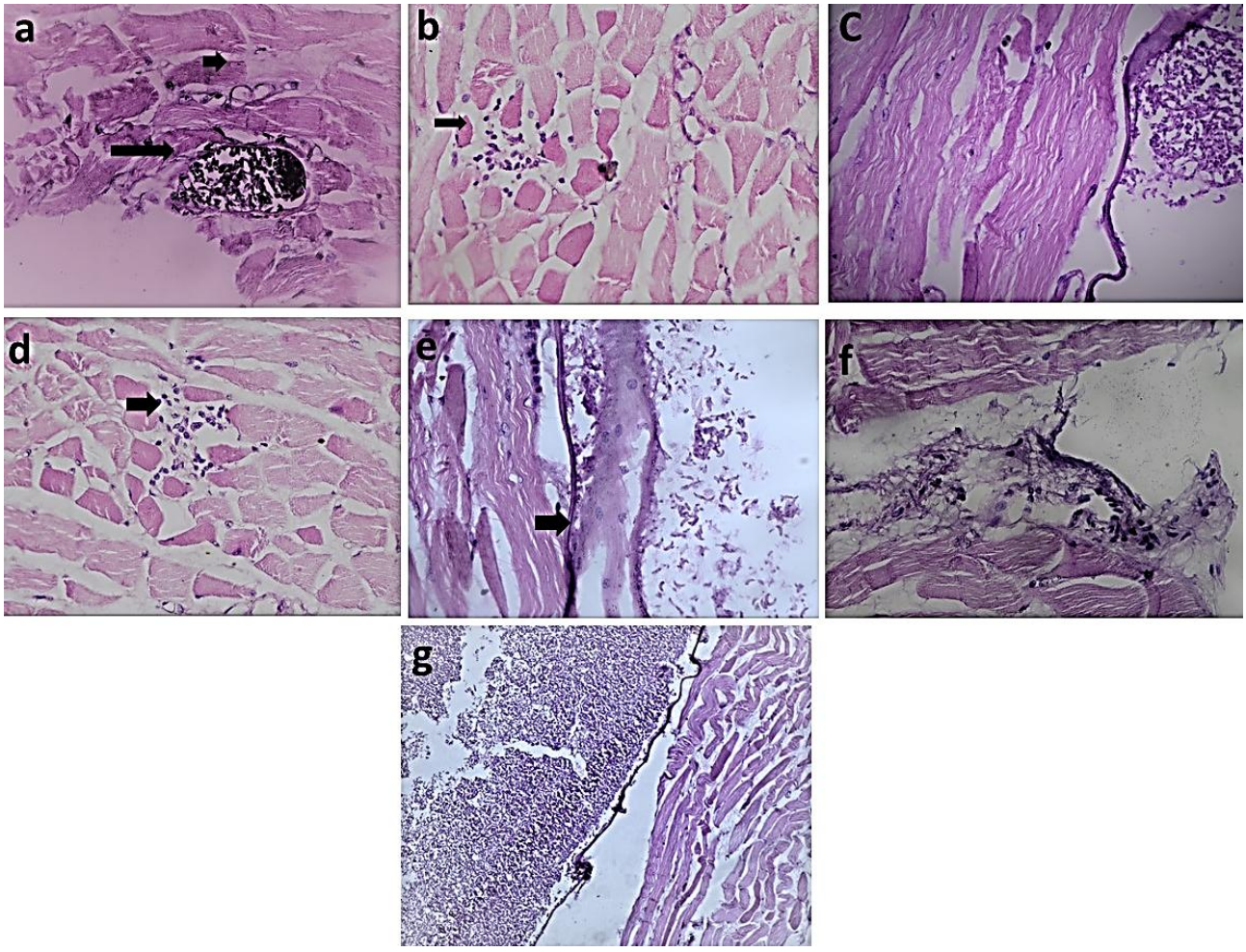

Fig. 3: Photomicrograph of cattle skeletal muscles showing (a) thick-walled sarcocyst containing bradyzoites (long arrow) and interstitial edema (short arrow) (H&EX400), (b) showing interstitial edema and few mononuclear inflammatory cells infiltration (arrow) (H&EX400). (c) large cyst with thick-walled sarcocyst which embedded in muscles (arrows) (H&EX400). (d) showing infiltration with mononuclear inflammatory cells (short arrow) (H&EX400). (e) Hyaline degeneration of skeletal muscle fibers (long arrows) and interstitial mononuclear inflammatory cells infiltration with thick large cyst wall (H&EX400). (f) Infiltration of the interstitial tissue of muscle fibers with esinophils (H&EX400). (g) thick-walled sarcocyst with muscle degeneration (H&EX400).

Ultrathin structure of the *S. fusiformis:* The wall consisted of a layer with a high electron density, measuring up to 50 nm in thickness, which surrounded the outermost parasitophorous vacuolar membrane (ppm) of the sporocyst wall. Metrocytes had a comparatively lower electron density than bradyzoites. The particles were spherical to elongated in form and were 7–15µm in length (Fig. 6). The structures comprised a nucleus,

endoplasmic reticulum, lipid droplets, fissures, and a few numbers to many amylopectin granules. Fig. 5 shows that bradyzoites obtained from 5 *Sarcocysts* were 15.5µm (11.5–16.5) in length and 2.5-4.0µm in width when sliced longitudinally.

Transcript levels of the pro-inflammatory cytokines: The analysis of IL-1β levels in *S. fusiformis*-infected cattle revealed (8.5±1) levels in muscles, while 9.7±1 in esophagus and 10.5±1 in hearts, which were greater than those of healthy cattle (3.5±0.50; Fig. 7). However, IFN-ɤ levels in *S. fusiformis* -infected cattle were 7±1 in muscles, while in esophagus, 7.5±1.5 and 9.6±1.49 in hearts, which were higher than those in healthy cattle (3± 0.55; Fig. 8). The analysis of TNF-α levels in *S. fusiformis* -infected cattle revealed (9±1) levels in muscles, while 10.5±1 in esophagus, and 10.0±1 in hearts, which were greater than those of the healthy cattle (3±0.50; Fig. 9). The analysis of IL-8 levels in *S. fusiformis* -infected cattle revealed 10±1 in muscles, 12±1 in esophagus, and 95±1 in hearts, which were higher than in healthy cattle (3.5±0.59; Fig. 10).

DISCUSSION

In this study, the prevalence of *Sarcocystis* infection in cattle was 10% (Sarafraz *et al.*, 2020). Dubey *et al.* (2023) detected sarcocysts of *S. dubeyi* in 11 (30%) of 35 cattle. Mohamed *et al.* (2020) investigated the prevalence of *Sarcocystis* infection in imported cattle at the Duhok abattoir and evaluated the effectiveness of traditional detection methods. Muscle samples (esophagus, heart, and diaphragm) were collected from 150 cattle from three different countries. A high percentage of the samples were infected with *Sarcocystis*: 94% of the esophagus, 92% of the diaphragm, and 41.3% of heart samples. This study highlights the common occurrence of *Sarcocystis* in imported cattle and emphasizes the need for further epidemiological investigations to assess the extent of the infection within the country.

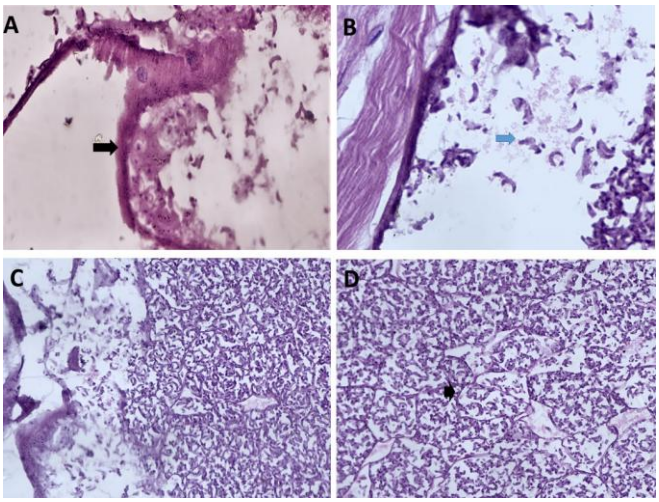

Fig. 4: Photomicrograph of cattle skeletal muscles showing (a) thick-walled sarcocyst containing bradyzoites (long arrow) (H&EX400), (b) showing degenerated muscle fibers with the wall of the cyst and banana-shaped bradyzoites (H&EX400). (c) large cyst showing the compartment of the cyst (H&EX400). (d) Higher magnification of the cyst in the center shows the trabeculae, which differentiate the cyst into compartments; (short arrow) (H&EX400).

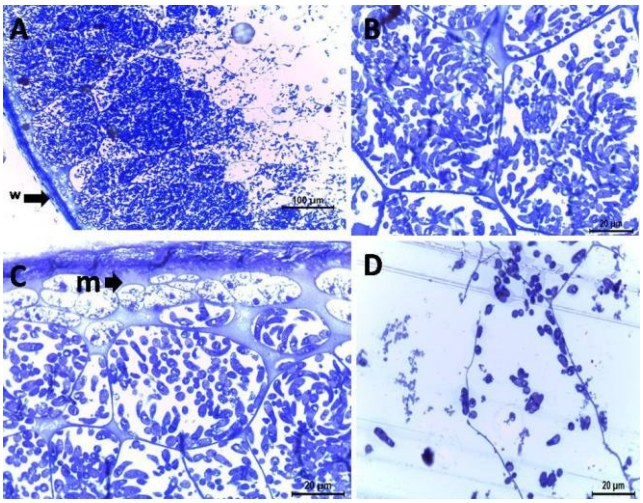

Fig. 5: Semi-thin section of *Sarcocystis fusiformis* showing the cyst wall; metrocyte and merozoites. **A**: *Sarcocystis* cyst was composed of a wall (w) with different layers, several cells of metrocytes, and a different compartment. **B**: compartment in the cyst separated by septa, which filled with merozoites. **C**: The mertocyte and compartment of the cyst are closely packed with merozoites. **D**: the center of the cyst appears nearly free from merozoites.

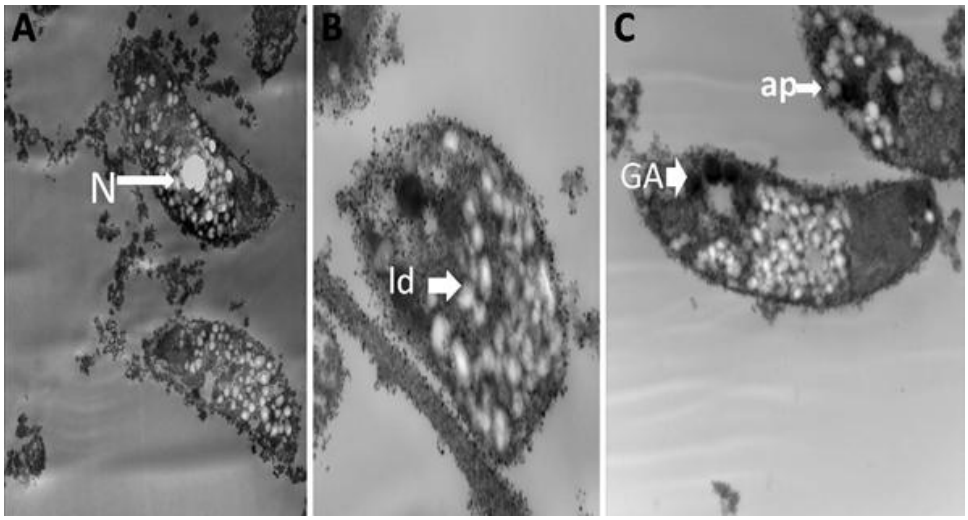

Fig. 6: TEM study of Sarcocytis cyst showing several bradyzoites which were globular to oblong in shape; A: They contained a nucleus (N), endoplasmic reticulum, lipid droplets (ld), a few to several amylopectin granules (ap) and golgi apparatus (GA).

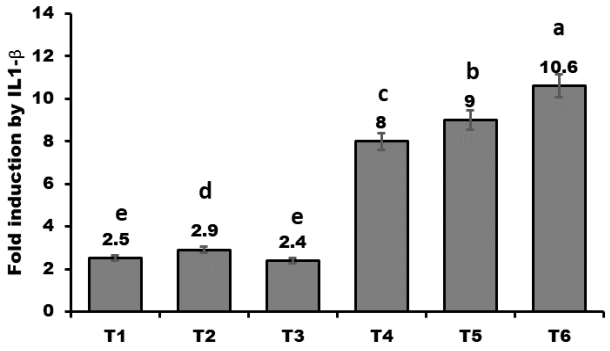

Fig. 7: Transcript levels of IL1-β in different organs infected with *S. fusiformis* in cattle. T1-T3 were control samples of muscle, tongue, and heart, while T4-T6 were *S. fusiformis*- infected muscle, tongue, and heart samples, respectively.

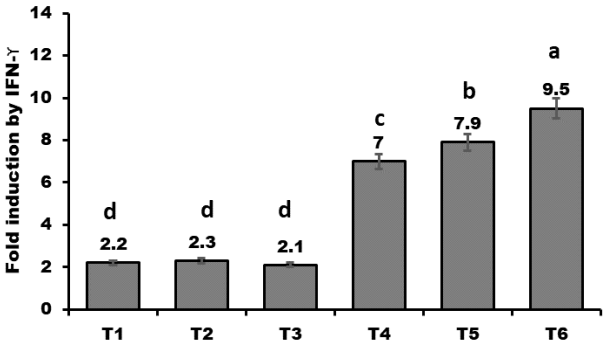

Fig. 8: Transcript levels of IFN-γ in different organs infected with *S. fusiformis* in cattle**.** T1-T3 were control samples of muscle, tongue, and heart, while T4-T6 were *S. fusiformis*- infected muscle, tongue, and heart samples, respectively.

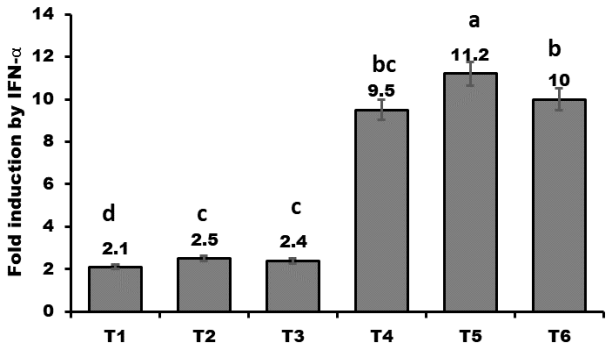

Fig. 9: Transcript levels of TNF-α in different organs infected with *S. fusiformis* in cattle. T1-T3 were control samples of muscle, tongue, and heart, while T4-T6 were *S. fusiformis*- infected muscle, tongue, and heart samples, respectively.

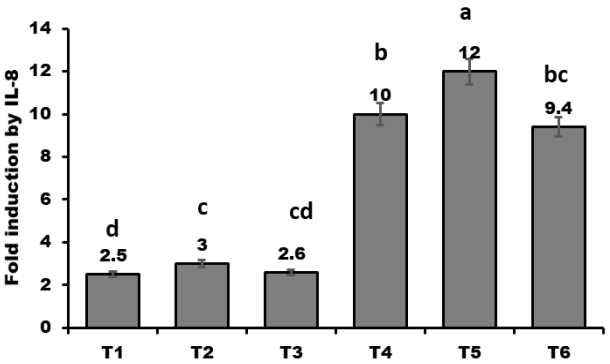

Fig. 10: Transcript levels of IL-8 in different organs infected with *S. fusiformis* in cattle. T1-T3 were control samples of muscle, tongue, and heart, while T4-T6 were *S. fusiformis*- infected muscle, tongue, and heart samples, respectively.

Based on the assessed incidence of sarcocystosis in this study, Egypt has several appropriate conclusive and intermediate hosts for various parasite species (El-Morsey *et al.*, 2021). The variation in the prevalence percentage between the other studies may be attributed to the differences in sampling time, localities, hygienic measures applied on the various farms, and preventive medication and control strategies applied on each farm (Belina *et al.*, 2021; Alsulami *et al.*, 2023; Alsulami *et al.*, 2024). It is noteworthy that the animals afflicted with the macroscopic sarcocyst forms also contracted the microscopic ones suggests that they had previously come into contact with both canines and felines that were infected (Ashmawy *et al.*, 2014; Idland *et al.*, 2022).

Previous studies can distinguish between different types of *Sarcocystis* by examining the microscopic structure of the parasite's cyst wall. This detailed analysis involves using powerful imaging techniques like TEM and histological staining (El-Kady *et al.*, 2018; Swar and Shnawa, 2020). Microscopic examination of infected muscle tissue showed the presence of *Sarcocystis* cysts with thick walls containing numerous merozoites (Morsy *et al*., 2018; Salem *et al*., 2022b). The surrounding muscle tissue exhibited signs of damage and inflammation, including interstitial edema as fluid buildup between muscle fibers and hyaline degeneration as the change in muscle fibers where they appear glassy or translucent. Inflammatory cell infiltration is the presence of immune cells, predominantly eosinophils, in the spaces between muscle fibers. Some cases also displayed a significant presence of mononuclear inflammatory cells, indicating that the parasite triggers a significant inflammatory response in the affected muscle tissue (Faghiri *et al.*, 2019). Our results agreed with those recorded by Farhangpazhouh *et al.* (2020) and Shams *et al.* (2022) reported similar histopathological changes in infected cattle tissues with *Sarcocystis* infection in Iran.

The immune response to sarcocystosis is species-specific, and the host protection against one species of the parasite cannot provide immunity against heterologous challenges (Hettiarachchi and Rajapakse, 2008; Florin-Christensen *et al.*, 2021). Cell-mediated and humoral immunity can be developed after immunizing the intermediate hosts against crude antigens isolated from cystizoites and merazoites of *Sarcocystis* species (Salem *et al*., 2022c; Mousa *et al*., 2024). In cell-mediated immunity, immune cells such as macrophages and lymphocytes migrate and disseminate through visceral and muscular tissues (El Shanawany *et al.*, 2019; Delgado and Lennon-Duménil, 2022).

The humoral immune response usually appears after 30 days of infection. Cell-mediated immunity in Sarcocystosis is more significant than humoral immunity (Hussein *et al.*, 2025). Skin or delayed-type hypersensitivity tests enable crude antigen stimulation of cell-mediated immunity response to the specific antigen injected intradermally (Hussein *et al.*, 2025).

This study explores how *Sarcocystis* infection affects different tissues in livestock, by analyzing microscopic

changes in infected tissues and examining the roles of various immune system molecules (cytokines and chemokines) to develop effective disease prevention strategies. Consisting with our study Teufel *et al.* (2022) focused on understanding the activity of IL-1 cytokines, which are important in regulating the body's inflammatory response and natural defenses against infection. This knowledge could lead to new treatments using recombinant cytokines to combat *Sarcocystis* infection. While IL-1Ra binds to IL-1RI to have a potent anti-inflammatory impact, IL-1α and IL-1β are potent inducers of inflammation (Mingala *et al.*, 2007; Makaremi *et al.*, 2022). The IL-1 family members that have been investigated the most are IL-1α and IL-1β (Bhadaniya *et al.*, 2019). IL-1α is typically connected to the plasma membrane of several cell types (Ezzat Alnakip *et al.*, 2014; Pyrillou *et al.*, 2020). Only in its fully developed state does IL-1β function, and this protein is released, circulates throughout the body, and functions as a paracrine factor (Cavalli *et al.*, 2021).

The current study agreed with El Shanawany *et al.* (2019), who stated that older female cattle were much more likely to be infected (90.7%) with *Sarcocystis* than young males (20%). Analysis of the immune response showed a shift towards a Th-2 type response in infected animals, with suppression of pro-inflammatory signals: Levels of the Th-1 cytokine IFN-γ, which normally helps activate a strong immune response, were reduced. Increase in anti-inflammatory signals: Levels of the Th-2 cytokine IL-5, which is associated with suppressing inflammation, were elevated. Antibody production: Higher levels of IgG and IgE antibodies were also found in infected animals. At the infection site, the immune response involved a mix of inflammatory cells, granuloma formation, eosinophil infiltration, fluid buildup (edema), and tissue death (necrosis). This indicates a complex immune reaction to the parasite.

In the current study, the analysis of IL-1β levels in *S. fusiformis* -infected cattle revealed (8.5±1) levels in muscles, while 9.7 ± 1 in the esophagus and 10.5±1 in hearts, which were greater than those of the healthy cattle (3.5±0.50) however, IFN-ɤ levels in *S. fusiformis* -infected cattle were 7±1 in muscles, while in esophagus 7.5±1.5 and 9.6±1.49 in hearts, The reported values were greater than those of healthy cattle (3±0.55), also, the analysis of TNF-α levels in *S. fusiformis* -infected cattle revealed (9 ± 1) levels in muscles, while 10.5±1 in the esophagus, and 10.0±1 in hearts compared to control (3±0.50), and the analysis of IL-8 levels in *S. fusiformis* -infected cattle revealed 10±1 in muscles and 12±1 in the esophagus, and 95±1 in hearts, compared to control animals (3.5±0.59). There were different studies in concur with our observation, such as the parallel research by Aboelenin *et al.* (2017), who noticed that TNFα is a cytokine signaling protein crucial for regulating the immune system and influencing female reproductive function and its pathogen response. Also, Abou Mossallam *et al.* (2015) found that the inflammatory cytokines released during infection are endometriosis, which is prompted to release cytokines and chemokines, such as IL-8. This attracts and activates neutrophils and monocytes to clear germs and prevent illness. Dini *et al.* (2023) reported the first case of bovine eosinophilic myositis (BEM) in a beef cow where *Toxoplasma gondii* DNA was detected alongside *S. hominis*. This finding suggests that co-infection with these two parasites may contribute to the development of BEM, a muscle disease that causes significant economic losses and potential public health concerns. IL-8 was recognized as the first member of the chemokine superfamily. Comparative analysis of IL-8 expression was conducted in Egyptian buffalo with and without endometritis (Abou Mossallam *et al.*, 2015; Farghali *et al.*, 2022).

The expression of IL8 was examined in the previous study in Egyptian buffalo's ovaries, uteruses, and mammary glands, both with and without endometritis (Raheel *et al.*, 2020). Only the uteruses of buffalo with endometritis exhibited a considerable increase (26.6-fold) in IL8 expression compared to buffalo without endometritis (Elsayed *et al.*, 2020; Taha *et al.*, 2021).

Prior research on the key cytokine genes in the buffalo immune system, the conservation of this high-level sequence (Abdallah *et al.*, 2021; Yehia *et al.*, 2023). For the host to defend itself against pathogenic invasion, IL-12 must be expressed early. The transcription of the p40 chain regulates the secretion of the physiologically active heterodimer, or p70. Non-mammalian p40, such as avian (Zhang and Su, 2023) and Fugu p40 (Yoshiura *et al.*, 2003), has been shown to exhibit constitutive expression in tissues of non-lymphoid origin, such as the kidney, brain, heart, and intestine. The constitutive expression of buffalo p35 was seen in both lymphoid tissues, such as the spleen, and non-lymphoid tissues, such as the brain. Exposure to polyclonal mitogens in lymphoid cells leads to immunological activation, which in turn promotes the expression of IL-12 p40 (Lima-Junior *et al.*, 2022; McDaniel *et al.*, 2022).

One powerful inducer of the acute phase response is IL-6. Quick IL6 synthesis aids in tissue damage and infection response on the part of the host, but overproduction of IL6 leads to disease pathology (Sreekumar *et al.*, 2002). Upon recognition of pathogens by toll-like receptors (TLRs) at the infection site or tissue damage, myeloid cells, including macrophages and dendritic cells, initiate a portion of the innate immune response (Ni and Chen, 2023). The transcript levels of different genes were upgraded in different tissues according to inflammatory reactions.

Conclusions: This study found a 10% prevalence of *S. fusiformis* in cattle. The parasite's ultrastructure was characterized, and its impact on host tissues, including upregulation of inflammatory cytokines and histopathological changes, was documented. Advanced molecular studies are necessary to determine the relationship between *Sarcocystis* species infecting buffalo and cattle in this region. Considering the economic importance of cattle, more research is crucial to fully grasp the effects of *Sarcocystis* infection. This includes deeper investigation into the parasite's biology, life cycle, and variations. Furthermore, controlling the movement of canine and feline hosts on farms should be explored as a potential method for mitigating the impact of *Sarcocystis*.

Funding: The authors gratefully acknowledge Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2024R437), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia. The authors extend their appreciation to the Deanship of Research and Graduate Studies at King Khalid University for funding this work through Large Research Project under grant number (R.G.P2/478/45).

Competing interests: The authors declare that they have no competing interests.

Acknowledgements: The authors gratefully acknowledge Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2024R437), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia. The authors extend their appreciation to the Deanship of Research and Graduate Studies at King Khalid University for funding this work through Large Research Project under grant number (R.G.P2/478/45).

Authors contribution: Conceptualization, SMAB, MAA, HSAR, AMA, and SAK, formal analysis, HAM, MMAQ, NAH, MMA, and MMA, investigation, SMAB, MAA, HSAR, AMA, and SAK, data curation, HAM, MMAQ, NAH, MMA, and MMA, writing original draft preparation, SMAB, MAA, HSAR, AMA, and SAK, writing final manuscript and editing, HAM, MMAQ, NAH, MMA, and MMA, visualization and methodology, SMAB, MAA, HSAR, AMA, SAK, HAM, MMAQ, NAH, MMA, and MMA. All authors have read and agreed to the published version of the manuscript.

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