Molecular Investigating of *Sarcocystis fusiformis* Infection in cattle (*Bos taurus*) in Basra Province/ Iraq

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https://doi.org/10.29072/basjs.20260104

ARTICLE INFO

Received: 08 September 2025

Accepted: 26 October 2025

Published: 30 April 2026

Keywords:

Molecular characterization, Sarcocystis fusiformis, 18S rRNA, ELISA

ABSTRACT

One of the most common foodborne tissue cyst-forming coccidia endangering public health as well as veterinary medicine is *Sarcocystis* species. Identifying Sarcocysts infection prevalence in slaughtered cattle (*Bos taurus*) was the goal of the presented work. Esophageal muscles underwent molecular analysis, macroscopic cyst inspection, and ELISA. *Sarcocystis* antigens were detected using ELISA test, and the results showed that 160 of 300 cattle (53.3%) were infected. The 18S rDNA gene of 160 from the sarcocyst has been amplified using PCR. Of the samples of cattle esophageal muscles that were tested, 47.5% had a positive PCR result depending on 18S rDNA *Sarcocystis*. Aligning 18S rDNA sequences as well as phylogenetic sequences of 17 PCR results indicated high identity percents with *Sarcocystis fusiformis* previously indicated in gene bank. Every Iraqi isolate was added to the gene bank as a new isolate. Age had a statistically significant impact on the molecular and ELISA results.

1. Introduction

Sarcocystosis can be defined as one of the common parasitic diseases resulting from an intra-cellular coccidian parasite in phylum Apicomplexa that has 2 obligate hosts, which are: a carnivorous definative host and an omnivorous or herbivorous intermediate hosts such as: cattle, buffaloes, camels, and sheep (1). Omnivorous animals, birds, and herbivores are examples of intermediate hosts in which the parasites reproduce asexually. (2). Its zoonotic importance is increased by down-regulating the quality regarding the meat as well as reducing the production of fiber and wool (3). Along with their economic value, livestock have become an active source of pathogens spreading throughout the environment and infecting other animals, including humans, through their products (hair, feces, crusts, hide, and meat). Since they contain and enable the pathogenic causal agents to survive, infections in nature are also known to persist. Sarcystosis can be defined as one of the zoonotic protozoan parasitic diseases that result from cyst-forming coccidia that is part of the family Scolytidae and phylum Apicomplexa (4). The parasite mainly completes its life cycle through sexual and asexual reproduction phases in herbivores (i.e., intermediate hosts) and carnivores (i.e., final hosts) (5). *Sarcocystis cruzi*, *Sarcocystis hirsuta*, *Sarcocystis bovifelis*, *Sarcocystis bovini*, *Sarcocystis heydorni*, *Sarcocystis hominis*, *Sarcocystis sigmoideus*, and *Sarcocystis rommeli* are the eight species that are presently known to infect cattle (6;7).

Although there is a considerable amount of information about sarcocystosis, it remains incomplete and requires further research. Sinc bovine sarcocystosis can lower livestock productivity, its spread poses a financial burden for food producers. Increased control in the fields of food biotechnology and veterinary parasitology is necessary to deal with the problem of malnutrition and food insecurity (8). The connection between sarcocystosis and inflammatory processes of striated muscles called bovine eosinophilic myositis. Although myositis does not have clinical manifestations, it is detected after cutting the carcass. The sale of meat from an infected animal is impossible, which causes economic losses in agriculture (6).

One of the most significant criteria for identifying *Sarcocystis* species is the lack or existence of septa, total cyst dimension, fine structural morphology regarding the wall, and cyst-wall thickness (1, 9; 10). Nonetheless, it is possible that different *Sarcocystis* species that share morphological features as observed through light and transmission electron microscopy are found in the same host. For verifying the identification of morphological species as well as ascertain whether sarcocysts in intermediate hosts that have a common morphology are of the same or distinct species, molecular indicator technique has emerged as a useful tool (9; 11).

Sarcocystosis is typically identified through looking for its macroscopic form in the muscle tissues of the tongues, diaphragm, and esophagus of infected animals during a postmortem examination (12). Sarcocystosis can be asymptomatic or look like other conditions, making antemortem diagnosis difficult. In addition to muscle weakness, anorexia, and generally low meat productivity, it can have chronic or subclinical courses and occasionally lead to death (13). Time-consuming procedures include the Dot-blot test, indirect fluorescent antibody test (IFAT), and the histological analysis of muscle biopsies. Furthermore, even with specialized equipment, it is challenging to correctly diagnose a large number of animals prior to slaughter (14; 15).

Various pathogens can be identified and distinguished primarily through molecular detection as well as phylogeny (16; 17). Due to the accuracy and reliability regarding PCR-restriction fragment length polymorphism approach, 18S and 28S (18) and ITS-1 genes (12; 19) are primarily used to define *Sarcocystis* species. However, it is still not included in other common sarcocystosis detection techniques (20; 21).

According to indigenous research, infection rates in Iraqi provinces ranged greatly, from 64% in Babylon province (22) to 64% in Baghdad province (23) to 65% in Diyala province (24) to 76% in Diwaniyah province (16).

Yet, as far as the authors are aware, nothing is known about zoonotic foodborne parasites, in particular, sarcocystosis, which is a major health concern for both humans and commercial animals in south Iraq, such as bovines. Moreover, the agricultural sector has a well-established problem with invisible sarcocysts. In order to offer information on the molecular identification, seasonal distribution, and occurrence percentages of *Sarcocystis fusiformis* from slaughtered cattle (i.e., *Bos taurus*) in Basrah, or southern Iraq, the current study was conducted.

2. Materials and methods

2.1. Sampling

Esophageal muscles Samples were collected from 300 cattle (*Bos taurus*). These animals (aged 1-7 years) slaughtered in Qurna city abattoir, Basra province, southern Iraq, through January 2024 to December 2024. After the animals were slaughtered, the esophagus muscles have been put in plastic bags with the proper labels, stored in an icebox that includes ice bags, and delivered right away to the Central Research Laboratory of the Veterinary College at Basrah

University for visual inspection. Then, Samples have been kept at -20ºC until subsequent examinations by ELISA, PCR, gene sequence and phylogenetic analysis

2.2. Macroscopic examination

Using the unaided eye, the obtained esophageal muscle samples were closely inspected. In order to view macroscopic cysts, each muscle tissue was cut into tiny pieces (3-5 mm) using a sterile blade, as shown by (25).

2.3. ELISA detection of Sarcocystis antigens

Sarcocystis in 300 bovine esophageal muscle samples was qualitatively determined using Bovine *Sarcocystis* ELISA kit (Sun Long Biotech; China). The Bovine *Sarcocystis* ELISA was used in accordance with the manufacturer's instructions.

2.4. Tissue sample preparation

Following the addition of PBS (pH 7.4), the tissue samples were homogenized. The recommended operating temperature for samples is 4°C. Following 20 minutes of centrifugation at 2,000rpm–3,000rpm, the supernatant has been collected carefully. An aliquot of the supernatant was made for the ELISA test.

2.5. ELISA procedure

To put it briefly, the appropriate micropores regarding the sample have been counted in the antibody-coated microplate. One empty well served as the blank control, two wells were used as the negative control, and two wells represented the positive controls. 50.0µl of each of the negative and positive controls was applied to the corresponding control wells. 10.0µl of sample and 40.0µl of sample dilution buffer have been added to sample wells and gently shaken to mix. For 30 minutes, the plate has been incubated at 37°C. Three hand washes with wash buffer were performed on plate. Except for the blank control well, each one of the wells received 50µl of HRP-Conjugate reagent. The wells haver then been incubated at 37ºC for 30min. As before, the wells have been washed. Each well received 50µl of Chromogen Solution A and 50µl of Chromogen Solution B, which have then been incubated for 15 mins at 37ºC. 50µL of stop solution has been used in order to stop the chromogenic process, and a plate reader (Micro ELISA auto reader, Biotech, USA) was used for reading the optical density readings at 450 nm. The next computation was used for determining the ELISA results: The average negative control value + 0.15 is the crucial value (CUT OFF). OD value ≥CUT OFF indicates a positive judgment, while if it is less than that, it indicates a negative judgment.

2.6. Molecular characterization

2.6.1. PCR analysis

DNA extraction

Following the manufacturer's instructions, 160 esophageal muscle samples had their genomic DNA extracted with using a DNA extraction kit (iNtRON Biotechnology, Korea). A 2% agarose gel that has been stained with ethidium bromide has been used for evaluating the extracted DNA quality. A gel documentation system (Gel Doc. Alpha-chem. imager, US) has been used to observe and take photograph of the bands after the electrophoresis gel has been evaluated in a UV transilluminator.

2.6.2. Gene amplification and purification

F: 5'GGCCCTTTTAGTGAGGGTGT3' and R: 5' TACGAATGCCCCCAACTGTC 3' were the primers utilized for amplifying the approximately 270 bp 18S rRNA sequence of *S. fusiformis* (26). For effective PCR amplification of DNA templates, PCR amplification mixture (25.0µl) contains tubes with Accu Power® PCR Master mix, containing Taq DNA polymerase, dNTPs, MgCl2, and reaction buffer at optimal condensation. These tubes have been filled with 2µl of template DNA and 1µl of every forward and reverse primer. The remaining 25 µl of the amplification mixture has been made using nuclease-free water (16 µl). After being placed in preheated thermocycler (Cleavere Scientific, USA), PCR tubes containing the amplification mix have been subjected to the following thermal cycling program: initial denaturation at 95 ºC for 5 min, followed by 35 cycles of 94ºC for 1min for DNA denaturation, annealing at 55 ºC for 1 min, extension at 72º C for 1min, and final extension at 72 ºC for 10min. The PCR product has been electrophoresed in 1× TBE buffer on a 1.50% agarose gel (AppliChem, Germany, GmbH). Approximately 20µL of the products were put into gel aperture for the gel research. The amplicons have been stained with Ethidium Bromide (0.5µg/ml), observed under UV illumination, and their sizes were clarified using a common gene indicator 100 bp ladder. A gel imaging system (Alpha Innotech, Biometra) has been utilized in order to confirm the outcome.

2.6.3. DNA Sequencing and Data Analysis.

Seventeen purified PCR products of cattle esophageal muscles samples that displayed a positive diagnostic band using 18S rRNA gene of *S. fusiformis* primer were subjected to sequencing. The Macrogen firm labrotary in Korea received the PCR amplicons. The forward

direction (5′-3′) was used to identify the clones' sequence. The same primers utilized for PCR were also utilized for sequencing 18S rRNA gene fragments. After that, the sequences have been aligned and contrasted with GenBank 18S rRNA gene sequences of *Sarcocystis* species. MEGA11 software was used to create the phylogenetic tree, which has been based on Maximum Composite Likelihood approach.

2.7. Statistical analysis

With a significance level of 5%, the chi-square test has been used for evaluating the data's correlation. SPSS software (v. 11) has been used for analyzing the data.

3. Results

3.1. Molecular Identification

As illustrated in Fig. (1) and table (1), the amplified target gene 18S rRNA was represented by the diagnostic DNA bands that appeared at approximately 270 bp for *S. fusiformis* (Fig. 1). It has been determined that 18S rRNA gene amplification was observed in 47. 5 % of cattle (76 /160). The results of Sarcocystosis prevalence in relation to age of screened cattle showed that the percentage of *Sarcocystis* infection increased with progression of age and high *Sarcocystis* infection percentage of 68.8 %(64/93) among old animals as compared to 17.9%(12/67) of young ones(Table 2). The difference between *Sarcocystis* infection percentage was found to be statistically significant (P<0.05).

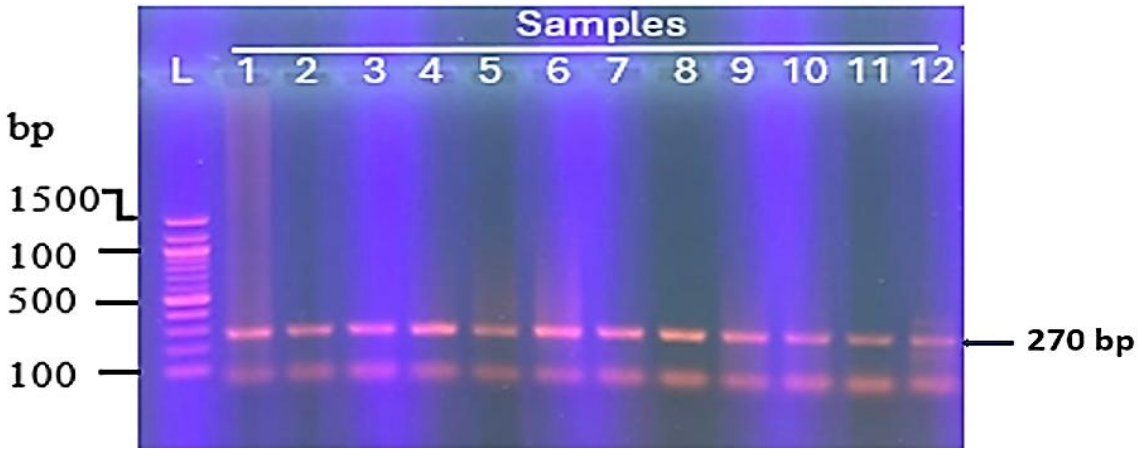

Figure (1): PCR product (approx.270 bp) of 18Sr RNA gene for *Sarcocystis Species* in DNA extracted from esophagus muscles samples. Lane L: 100bp DNA ladder;. Lane 1-12 positive samples

Table (1): PCR results of *Sarcocystis fusiformis* in cattle esophageal muscles

Examined animals	*S. fusiformis*			
160	**+veNo.**	**%**	**-veNo.**	**%**
	76	47. 5	84	52.5

Table (2): PCR results of *Sarcocystis fusiformis* in cattle according to age groups

Age	Examined N. (%)	*Sarcocystis* infection n.(%)	Chi-squared	95% CI	Significance level
< 2 years	**67 (41. 8)**	**12(17.9)**	**40.208**	**36.153% to 62.149%**	**<0.0001**
≥2 years	**93(58.2)**	**64(68.8)**			

3.2. The Gene sequencing analysis

The BLAST investigation of *Sarcocystis DNA* was performed on 17 PCR positive samples. The results of this investigation showed that all current Iraqi isolates were deposited in gene bank) (Table 3). The results of this analysis also confirmed that *Sarcocystis* DNA genes sequence displayed a 100% identity with sequences of *S. fusiformis* previously reported in gene bank(Table 4).

3.3. The evolutionary network analysis

An evolutionary network of *18S rRNA* gene fragment of *Sarcocystis fusiformis* sequences showed the relationship among current *fusiformis* sequences and counterparts from Iraq and different countries (figure 2). S1 to S17 represent *Sarcocystis fusiformis* sequences. All current local *Sarcocystis fusiformis* subgroups were found to be positioned within *S. fusiformis* phylogenetic area. Subgroup 1(S1) included eight of the present study subgroups sequences (PQ062106.1, PQ062107.1, PQ062108.1, PQ062113.1, PQ062114.1, PP968434.1, PP968437.1 and PP968438.1), and one Iranian sequence (MN986970.1), two Indian sequences (MF595831.1 and Chinese sequence (AF176927.1). While S2 contained one of the current study sequences (PQ062110.1) and four Egyptian sequences (KR186119.1, KR186121.1 and KR186131.1, KR186132.1). One Egyptian sequence (KR186122.1) present in S3.Nine Indian

sequences (MF595832.1, MF595834.1 - MF595841.1) were positioned in S4. One Indian sequence (MF595836.1) was localized in S5. While each subgroups of S6,S8, S10, S11,S12,S13 and S14 contained one of the current study sequences PQ062109.1;PQ062112.1; PQ062116.1; PQ062115.1;PQ062111.1; PP968436.1. and PQ062117.1 respectively. Each one of the two subgroups S7 and S9 contained one of Iraqi sequences from a previous study including PP994756.1.. and PP994757.1 respectively. Other subgroups S15; S16 and S17;contained one of *Sarcocystis fusiformis* sequences from China, Iran and swede including AF176926.1;MN986971.1 and U03071.1 respectively.

Table 3: New *S. fusiformis* isolates from Iraqi cattle that were deposited in the gene bank

Sarcocystis	**Query Accession**	**Subject**	**% Identity**
Sarcocystis fusiformis isolate EBF1	PP968434.1	AF176927.1	100%
Sarcocystis fusiformis isolate EBF2	PP968435.1	AF176927.1	100%
Sarcocystis fusiformis isolate EBF3	PP968436.1	AF176927.1	97.84%
Sarcocystis fusiformis isolate EBF4	PP968437.1	AF176927.1	99.62%
Sarcocystis fusiformis isolate EBF5	PP968438.1	AF176927.1	99.24%
Sarcocystis fusiformis isolate EBF6	PQ062106.1	AF176927.1	99.13%
Sarcocystis fusiformis isolate EBF7	PQ062107.1	AF176927.1	99.11%
Sarcocystis fusiformis isolate EBF8	PQ062108.1	AF176927.1	98.87%
Sarcocystis fusiformis isolate EBF9	PQ062109.1	AF176927.1	98.07%
Sarcocystis fusiformis isolate EBF10	PQ062110.1	MF327256.1	100%
Sarcocystis fusiformis isolate EBF11	PQ062111.1	AF176927.1	99.59%
Sarcocystis fusiformis isolate EBF12	PQ062112.1	AF176927.1	96%
Sarcocystis fusiformis isolate EBF13	PQ062113.1	AF176927.1	100%
Sarcocystis fusiformis isolate EBF14	PQ062114.1	AF176927.1	99.52%
Sarcocystis fusiformis isolate EBF15	PQ062115.1	AF176927.1	98.76%
Sarcocystis fusiformis isolate EBF16	PQ062116.1	AF176927.1	98.49%
Sarcocystis fusiformis isolate EBF17	PQ062117.1	AF176927.1	98.46%

Table 4: The divergence of the obtained subgroups sequences with the sequence of their counterparts from Iraq and different countries.

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17
S1	100	98.87	98.49	96.56	96.18	95.07	93.3	94.19	88.76	98.11	98.76	99.59	98.27	97.95	99.25	98.87	95.85
S2		100	99.62	96.18	95.8	94.58	92.78	93.6	88.2	97.74	97.93	98.77	97.4	97.44	98.87	98.49	96.23
S3			100	95.8	95.42	94.09	92.27	93.02	87.64	97.36	97.51	98.36	96.97	96.92	98.49	98.11	95.85
S4				100	98.85	91.5	90.58	90.53	85.14	95.42	95.38	96.27	94.74	95.31	96.56	96.18	93.89
S5					100	92	90.05	89.94	84.57	95.04	94.96	95.85	94.3	94.79	96.18	95.8	93.51
S6						100	90.58	94.77	86.93	93.1	93.6	94.58	93.07	94.03	94.58	94.58	90.2
S7							100	90.12	88.76	91.24	92.27	92.78	92.23	93.98	92.78	92.78	88.72
S8								100	86.71	91.86	92.44	93.6	91.81	91.34	93.6	93.6	88.44
S9									100	86.52	87.08	88.2	88.14	84.96	88.2	88.76	83.8
S10										100	97.1	97.95	96.54	97.95	98.11	97.74	94.72
S11											100	98.34	96.97	96.49	98.34	97.93	96.27
S12												100	97.84	97.7	99.18	98.77	95.49
S13													100	96.3	97.84	97.4	93.94
S14														100	97.95	97.44	93.33
S15															100	98.87	96.6
S16																100	96.6
S17																	100

3.4. Detection of Sarcocystis antigens

ELISA results based on parcocystis antigens showed that 160 out of 300 cattle (53.3%) tested positive for *sarcocystis* infection. According to the cattle's age, the distribution of ELISA results showed that the percentage of *sarcocystis* infection rose as animals aged, with a higher percentage of *sarcocystis* infection (61.3%) in older animals than in younger ones (29.3%). Older animals had a higher mean for the OD values of *sarcocystis* antigens (1.204±0.296) than younger ones (0.576±0.207). A statistically significant ($P<0.05$) correlation is found between age groups and the mean of OD values (Table 5).

According to PCR and ELISA positive results in the current study, *S. fusiformis* infection prevalence in cattle varies throughout the year, peaking in February (64 %), April (62.5%) and November (53.3 %), The lowest percentage value was found in March (21.4%). The seasonal distribution of *Sarcocystis* infection was displayed in table (6). Spring had the highest prevalence rate of Sarcocystis infection (51.2%), followed by Autumn 50.9%), and summer had the lowest rate (35.3%). However the seasonal difference,, has not been statistically significant ($P>0.05$).

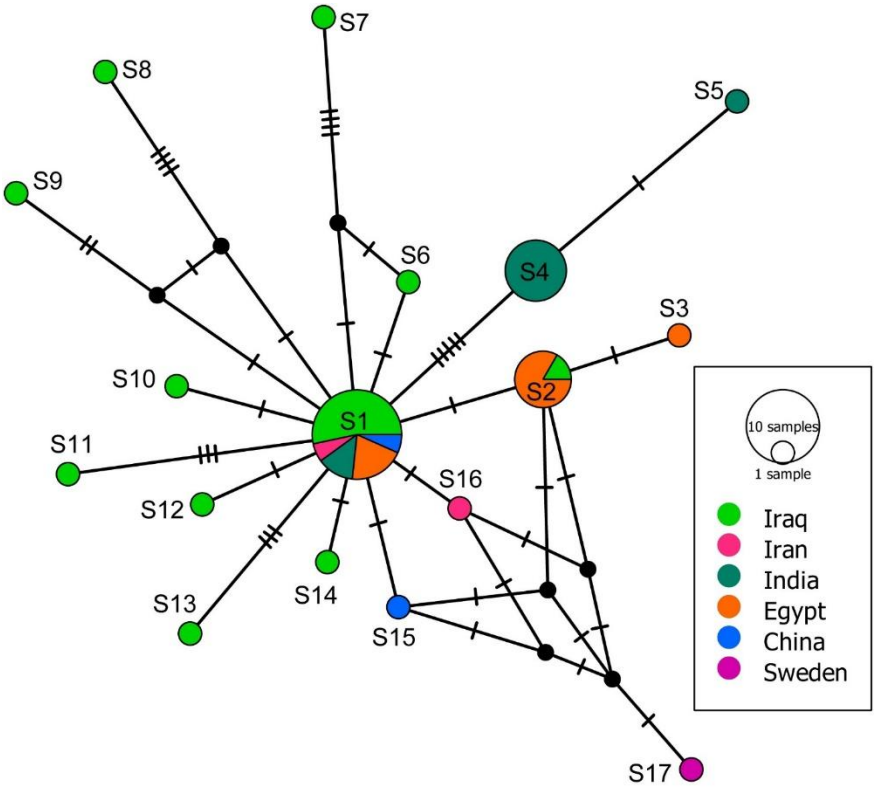

Figure2. An evolutionary network of *18S rRNA* gene fragment of *Sarcocystis* local isolate shows the relation of current study *Sarcocystis fusiformis* sequences with their counterparts from Iraq and different countries. S1 to S17 represent subgroups. A line between subgroups represents mutation. Th green color refers to sequenced *Iraqi* variants, while the other colors indicate related referring NCBI *Sarcocystis fusiformis* deposited species.

Table (5): Optical Density (OD) values of *Sarcocystis* antigens- ELISA according to cattle age

Age	Sarcocystis infected N.(%)	Mean ±SD	95% Confidence Intervals	t-statistic	P-value
<2year	22 (29.3)	0.576 ± 0.207	0.503 to 0.757	9.81	< 0.0001
≥2 year	138 (61.3)	1.204± 0.296			

Table (6) Monthly and seasonal distribution of *Sarcocystis* infection among cattle according to *Sarcocystis* antigens based ELISA and PCR

Months	ELISA positive N.	S. fusiformis-PCR positive	
		Infected n.	%
January	10	3	30
February	25	16	64
March	14	3	21.4
Winter	49	22	44.9
April	16	10	62.5
May	13	5	38.5
June	14	7	50
Spring	43	22	51.2
July	5	2	40
August	5	2	40
September	7	2	28.6
Summer	17	6	35.3
October	11	5	45.5
November	30	16	53.3
December	10	5	50
Autumn	51	26	50.9
Total	160	76	47.5
Statistics	Chi-square:1.628; freedom degrees:3; P value:0.653		

4. Discussion

Sarcocystis, an intra-cellular protozoan infecting a wide range of livestock, vertebrates, and humans, is one parasite that plays a role in the development regarding food-borne diseases. Precautions for food safety are therefore crucial. In addition to potentially endangering the health of consumers, *Sarcocystis* is believed to be one of the primary sources of financial losses in livestock industry (27). The results regarding almost all previous revisions (28, 29, and 30) confirmed the results that were reported, which demonstrated that visual inspection regarding the muscle tissues indicated no obvious cysts in the screened muscle tissue samples. Nonetheless, numerous investigations discovered macro cysts in cattle (31, 32, 24; 33). The animal age, breeding system, and anatomical site of diagnosis have been among the variables that affected the variations (34). Using microscopical examination through ELISA test for *Sarcocystis* diagnosis has been strongly advised because of the existence of microscopic or hidden cysts in order to prevent human infection of such a zoonotic parasite and to control the resulting disease. Furthermore, because they frequently reproduce in areas where stray cats and canines are present or where infectious species pollute the environment, bovines are

frequently vulnerable to infection (35). Therefore, ELISA kits that are specific, sensitive, and inexpensive are useful for prevalence research and early diagnosis. (36, 37). Prior research has demonstrated that purified antigen is a more effective way to identify sarcocystosis, which explains why whole cyst antigen has a lower diagnostic efficiency (38). As a result, the *sarcocystis* ELISA kit is used in this research to estimate the disease's prevalence in cattle. 160 out of 300 cattle, or 53.3%, tested positive for *sarcocystis* infection, according to the most recent results. In Iraq, there is relatively little research on using the *sarcocystis* antigen to diagnose sarcocystosis. Yet, only a small number of investigations carried out in Basrah, Iraq, utilized the crude antigen from S. fusiformis whole cysts to diagnose sarcocystosis in humans and buffaloes (39; 40). Prior research has examined the significance of Apicomplexa antigens; in Basrah province, Iraq, three surface antigens have been employed in the molecular diagnosis regarding human toxoplasmosis (41). Because they're branched fibrilles that are visible on the surface of *sarcocystis* wall, ground substances of *sarcocystis* close to the cyst wall, and merozoite septa and plasmalemma, the glycoprotein of mature *sarcocystis* plays a functional role. It was anticipated that such structural characteristic would mediate the interaction between the host immune system and the parasite in order to evoke the cellular and humoral response (42). In addition, tests that identify antigens rather than antibodies facilitate the separation of active cases from past parasite infections or may aid in tracking the effectiveness of treatment (43).

The distribution regarding ELISA data by cattle age revealed that the proportion of *sarcocystis* infection increased with age, with older animals having a greater percentage of *sarcocystis* infection (61.3%) compared to younger animals (29.3%). Previous studies conducted in Iraq (44, 45, 46) and other nations (47, 35) have likewise revealed a consistent rise in infection with advancing age. As most bulls in Egypt are kept in the fattening system and slaughtered at about 2 years old, whereas cows are kept for long periods of time for milk production, the animal management organization might be to blame for low percentage of infected bulls. The findings of (49;50;47), who concluded that the infection incidence regarding bovine sarcocystosis has been statistically insignificant across age groups, did not align with the current findings. The environmental circumstances, breeding system, and variations in management conditions may be the cause of the notable variation in infection rates (45; 51). The months with the highest monthly percentage were February, April, and November. The spring season has the highest seasonal share, however. Results of this study demonstrated a strong relationship between the prevalence of sarcocystosis and seasonality. Conversely, (52; 47) discovered no discernible seasonal variation in parasite frequency. April

had the lowest parasite outbreak (4%), while June had the greatest (16.13%) (53). (47) reported a higher occurrence frequency in the fall (97.20%). The discrepancies between the current results and those of other people could be due to the lengthier grazing durations. The viability and survival of sporocysts in the environment is an additional factor that influences seasonal changes in infection distribution, which are arranged according to climatic parameters as humidity, temperature, and rainfall (47). Longer grazing periods in the spring could be the source of the highest frequency of *Sarcocystis* infection (91.7%) during this season (54).

Approaches for diagnosing livestock must be improved (55). Microscopic cysts within the tissues of several organs, such as the esophagus, are investigated using a range of diagnostic procedures beyond visual examination. Minute cysts have been discovered in a variety of bovine tissues in a small number of investigations on different hosts (48; 35; 56). In the presented investigation, PCR diagnostic testing for the 18S rRNA gene was performed on esophageal muscles collected from slaughtered carcasses. *Sarcocystis* species-specific distinction is achieved by the application of genetic markers in molecular approaches that depend on the recognition regarding the 18S rRNA gene. According to earlier reports, these methods do not amplify DNA from other hosts or the environment (50). Therefore, in this study, PCR amplification of 18S rRNA gene verified *Sarcocystis spp*. infection in 47. 5 examined samples of cattle esophageal muscles. This emphasizes 18S rRNA importance in the differentiation of *Sarcocystis* species from other cystic diseases. Globally, 18S rRNA fragment is thought to be an effective method for differentiating *Sarcocystis* species (47). The present work's identification of *Sarcocystis* species utilizing phylogenetic analysis and gene sequencing of 18S rRNA gene showed that *S. fusiformis* was found in the bovine carcasses under investigation. This conclusion was confirmed by earlier studies (57; 58; 23). Additionally, (4) noted that molecular genetic study identified three distinct sarcocyst pathogen types in the muscles regarding cattle from Kazakhstani farms: *S. bovifelis*, *S. cruzi*, and *S. dehongensis*. Infesting Asian buffalo, the latter is found mostly in China and the Netherlands (57; 58). One possible explanation for the high rate of infection in the present study is that seasonal variations in infection, impacted by climate factors such high temperatures and humidity that are characteristic of examined area, have an impact on the viability and survival of sporocysts in the environment (47). Furthermore, (59) discovered that close contact between the intermediate and final hosts is linked to the distribution regarding sarcocystosis. *Sarcocystis* sporocysts are believed to be released into the environment in extremely large amounts by infected final hosts. These sporocysts could survive for long periods of time for a variety of

reasons, such as high temperatures. These contaminated feces are easily consumed by pastoral cattle, which after that contributes to additional environmental contamination (60). The ELISA and PCR techniques shown in this research might be helpful for detecting *sarcocystis* infection, particularly when combined with a particular primer for *S. fusiformis* and traditional PCR, gene sequencing, and phylogenetic analysis.

Ethics approval

Every animal experiment was carried out in compliance with the guidelines set forth by Animal Welfare. The Ethics Committee of the Veterinary College of Basra University granted ethical approval for the ongoing research.

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دراسة جزيئية لعدوى *Sarcocystis fusiformis* في الابقار (*Bos taurus*) في محافظة البصرة ، العراق

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المستخلص

واحدة من أكثر أنواع الكوكسيديا المتسببة في تكوين الأكياس النسيجية المنقولة بالغذاء شيوعًا والتي تشكل خطرًا على الصحة العامة وكذلك الطب البيطري هي أنواع المتكيسات العضلية. كان هدف البحث المقدم هو تحديد انتشار عدوى المتكيسات العضلية في الأبقار المذبوحة (*Bos taurus*). خضعت عضلات المريء لتحليل جزيئي، وفحص عياني للاكياس، واختبار الفحص المناعي الممتز المرتبط بالانزيم (ELISA). تم الكشف عن مستضدات المتكيسات العضلية باستخدام اختبار الفحص المناعي الممتز المرتبط بالأنزيم (ELISA)، وأظهرت النتائج إصابة 160 رأسًا من أصل 300 رأس ابقار (53.3%). تم تضخيم جين 18S rDNA في 160 رأسًا من المتكيسات العضلية باستخدام تفاعل البلمرة المتسلسل (PCR). من بين عينات عضلات مريء الأبقار التي فُحصت، أظهرت 47.5 %منها نتيجة إيجابية لتفاعل البلمرة المتسلسل (PCR)، وذلك اعتمادًا على جين 18S rDNA للمتكيسات العضلية. أشارت اصطفافات تسلسلات 18S rDNA ، بالإضافة إلى التسلسلات التطورية لـ 17 نتيجة تفاعل البلمرة المتسلسل (PCR)، إلى نسب تطابق عالية مع *Sarcocystis fusiformis* المُشار إليها سابقًا في بنك الجينات. أُضيفت كل عزلة عراقية إلى بنك الجينات كعزلة جديدة. كان للعمر تأثير ذو دلالة إحصائية على النتائج الجزيئية ونتائج اختبار الفحص المناعي الممتز المرتبط بالأنزيم (ELISA).

الكلمات المفتاحية: التوصيف الجزيئي ، *Sarcocystis fusiformis*، 18S rRNA ، الفحص المناعي الممتز المرتبط بالأنزيم (ELISA).