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Comparative analysis of *Sarcocystis* infections in cattle, sheep, and camels in Thi-Qar, Iraq: Implications for food safety

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Abstract
This study investigates the prevalence of *Sarcocystis* spp. infections in livestock in Iraq, aiming to assess the potential risk to public health. Conducted at a slaughterhouse in Thi-Qar governorate from October 2023 to March 2024, the study involved the collection of muscle samples from 150 animals (cattle, sheep, and camels). Samples underwent macroscopic and microscopic examination for parasite presence. DNA extraction and PCR analysis were employed for species identification, with sequencing and phylogenetic analysis used for further confirmation. The prevalence of suspected Sarcocyst spp. infection across different animal species. The data prevalence of suspected *Sarcocystosis* spp. infection in different animals species as (82%,78%,44%) respactly for cattle, sheep and camels of the sampled individuals testing positive for the parasites, while the prevalence of *Sarcocystis* spp. in infected animals as detected by ribosomal RNA (rRNA) analysis. Across all animal species, the overall prevalence was 72%, with 36 out of 50 samples testing positive for *Sarcocystis* infection.
Statistical analysis was performed to evaluate the association between infection rates and potential risk factors. Results revealed a high prevalence of both *Sarcocystis* spp. in the studied animals, particularly in cattle and sheep. Specific *Sarcocystis* species identified included S. tenella, *S. cruzi*, *S. hominis*, and *S. cameli*. The study concludes that these infections pose a significant public health concern in the region, emphasizing the need for effective control measures to prevent transmission.
Organ-specific analyses identified high infection rates in intercostal muscles and hearts of cattle, as well as the hearts and esophagus of sheep.

Keywords: *Sarcocystis*, livestock, prevalence, zoonotic, public health, Iraq

Introduction
Parasite-related diseases are among the major global health challenges facing humanity in the modern era, these diseases pose a serious threat to public health, with broad impacts on the economy and society, the study of diseases caused by parasites is a very important topic, as it aims to understand the nature of these diseases and their impact on humans and the environment (Sitotaw, Mekuriaw, and Damtie 2019) [17]. *Sarcocystosis* this disease are characterized by high health risks, as infection with them can lead to serious complications that threaten the lives of patients (Amairia *et al*., 2021) [1].
First, *Sarcocystosis* is a serious parasitic disease caused by the parasite *Sarcocystosis*, which usually infects mammals, including humans, this disease is known to cause severe inflammation of the muscles and internal organs, and in some cases it may lead to disruption of the functions of the body's organs and even death (Rathish and Raksha, 2022) [15].
These diseases are among the few diseases caused by parasites that result in serious complications that threaten public health, these complications cause enormous medical costs to the health system, in addition to economic losses resulting from lost productivity and costs of treatment and prevention (Fauziah *et al*., 2022) [8]. In addition, epidemiological analysis of these diseases shows that they spread widely around the world, making them a global public health threat, therefore, understanding the nature of these diseases and their transmission and spread mechanisms is vital for developing effective prevention and control strategies (Baker *et al*., 2022) [2].
The first stage: Sexual reproduction within the definitive host this multiplication usually occurs in the small intestine of the definitive host after ingestion of sarcoptic-infected meat this reproduction results in the production of aggressive forms (truncated stages) of the parasites (Dubey *et al*. 2023) [6].

The second stage: Transmission of aggressive forms to the muscle tissue of the intermediate host, Sarcocysts are stored in muscle after parasites have completed their first life cycle within the definitive host (Cawthorn and Speer 2019). The third stage: formation of cysts, this stage consists of the formation of final sarcocyst forms within the muscle tissue of the intermediate host, an outer wall is formed for the cysts to protect them and allow them to move to the next definitive host (Dubey 2022) [5]. The fourth stage: consuming cysts, cystases are consumed by the definitive host when infected meat is eaten, it occurs when the definitive host ingests the muscle tissue containing the formed cysts (Suh, Kozarsky, and Keystone 2015) [18]. The fifth stage: sexual reproduction within the new definitive host, the life cycle continues with sexual reproduction within the small intestine of the new definitive host, where the process is repeated again (Elshahawy *et al*. 2023) [7].

These are spherical cysts with a diameter of 10-12 micrometers, surrounded by a thick double-layered membrane. They are formed in the epithelial cells of the intestines in the final host, the cats, through the formation of gametes by a process called gametogony and sexual reproduction (Dubey, 2017). After the epithelial cells rupture, the oocysts are released into the intestinal cavity and then excreted with the cat's feces, these oocysts are unripe and non-infectious when excreted and undergo a maturation process for the spores within about 1-5 days, depending on suitable environmental conditions such as ventilation and temperature (Lindsay and Weiss, 2004).

The unsporulated oocysts are surrounded by a colorless double-layered membrane, and the sporont usually fills the oocyst, under suitable conditions, the sporont transforms into two structures called sporoblasts, which later elongate to form two sporocysts, each containing four sporozoites (Mahmud *et al*., 2017) [2]. These oocysts are resistant to environmental conditions; they can withstand cold and freezing conditions (Sherwood, 2011). They have the ability to survive for several months to a year if provided with suitable conditions, but they do not resist high temperatures; they are killed at 70 °C within 10 minutes. The optimal temperature for the growth of these oocysts is 20-30 °C, and the best type of soil for the growth of oocysts in large numbers is sandy loam soil compared to other types of soil (Al-Abdely, 2011).

Materials and Methods

Study Design and Sample Collection

This cross-sectional study was conducted at the main slaughterhouse in Thi-Qar governorate, Iraq, from October 2023 to March 2024. A total of 150 animals were included: 50 cattle, 50 sheep, and 50 camels. Muscle samples (heart, diaphragm, intercostal muscles, esophagus) were collected from each animal. Samples were labeled, transported in a cool box, and processed in the laboratory.

Macroscopic and Microscopic Examination

- **Macroscopic Examination**: Muscle samples were visually inspected for the presence of macroscopic sarcocysts.
- **Microscopic Examination**: Muscle dgestion 2 grams of Muscle were digested using a pepsin solution, filtered, and centrifuged. The sediment was stained with Giemsa and examined microscopically (40x magnification) for the presence of cystozoites (bradyzoites) of *Sarcocystis* spp.

DNA Extraction and PCR Analysis

DNA Extraction: Genomic DNA was extracted from Muscle samples using a commercial DNA extraction kit. Primers targeting the small subunit ribosomal RNA (ssrRNA) gene of *Sarcocystis* spp. were designed. PCR was performed using a standard PCR protocol.

DNA Sequencing and Phylogenetic Analysis:

DNA Sequencing: PCR products were sequenced to confirm the identity of the parasites. Sequences were compared to reference sequences from GenBank using BLAST. A phylogenetic tree was constructed to analyze the genetic relationships between the isolated parasites and reference sequences.

Statistical Analysis

The prevalence of *Sarcocystis* spp. was calculated for each animal species, organ, age group, and gender.

Results

Prevalence of Suspected diagnosis *Sarcocyst* spp. in Infected Animals, by Species

The prevalence of suspected Sarcocyst spp. infection across different animal species. The data reveals for Cattle demonstrated the highest prevalence of infection, with 82% of the sampled individuals testing positive for the parasites. This suggests that cattle are particularly susceptible to Sarcocyst spp. in the studied region. Sheep showed a slightly lower prevalence of 78%, indicating that they are also significantly affected by these parasites, though potentially to a lesser extent than cattle. Camels exhibited the lowest prevalence among the three species, with 44% of the samples testing positive as Table (1) presents it.

Table 1: Prevalence of Suspected diagnosis *Sarcocyst* spp. in Infected Animals, by Species.

Species	No. of samples	Positive sample	Percentage %
Cattel	50	41	82%
Sheep	50	39	78%
Camels	50	22	44%
total	150	102	75%

Prevalence of *Sarcocysts* spp. in infected animals according to the ribosomal RNA by PCR

The prevalence of *Sarcocystis* spp. in infected animals as detected by ribosomal RNA (rRNA) analysis. Across all animal species, the overall prevalence was 72%, with 36 out of 50 samples testing positive for *Sarcocystis* infection. This indicates a significant presence of the parasite in the studied animal population.

When examining the prevalence by species, both cattle and sheep exhibited a high infection rate of 83%. In cattle, 10 out of 12 samples were positive, while in sheep, 20 out of 24 samples tested positive. This suggests that cattle and sheep are highly susceptible to *Sarcocystis* infection in this region. In contrast, camels showed a considerably lower prevalence of 42%, with 6 out of 14 samples testing positive as Table (2) presents it.

Table 2: Prevalence of *Sarcocysts* spp. in infected animals according to the ribosomal RNA by PCR.

Animals	No. of samples	Positive sample	Percentage%
Cattle	12	10	83%
Sheep	24	20	83%
Camel	14	6	42%
Total	50	36	72%

Prevalence of Suspected diagnosis *Sarcocyst* spp. in Cattle, by Organ

The provided data reveals a concerning prevalence of suspected *Sarcocyst spp.* in various organs of slaughtered animals in the Thi-Qar governorate. Specifically, heart, diaphragm, intercostal muscles, and esophagus samples were examined, with intercostal muscles showing the highest infection rate at 100%. This suggests that these muscles are particularly vulnerable to parasitic invasion. The heart also exhibited a high prevalence of 86%, raising significant concerns about the safety of meat products and potential public health risks. Overall, a substantial proportion (82%) of the sampled animals tested positive for these parasites, as shown in Table (3).

Table 3: Prevalence of Suspected diagnosis *Sarcocyst* spp. in Cattle, by Organ.

Organs	No. of samples	Positive sample	percentage
Heart	23	20	86
Diaphragm	16	11	68
Intercostal	9	9	100
Esophagus	2	1	50
Total	50	41	82

Prevalence of Suspected diagnosis *Sarcocyst* spp. in sheep according to organs

The prevalence of suspected *Sarcocyst* spp. infection in sheep, with a focus on different organ tissues. The data indicates a high overall prevalence of infection, with 78% of the total sheep samples (39 out of 50) testing positive for these parasites.

When examining the prevalence by organ, the heart exhibited the highest susceptibility to infection, with a striking 93% of heart samples (15 out of 16) testing positive. The esophagus also showed a relatively high prevalence of 80% (8 out of 10). In contrast, the diaphragm and intercostal muscles displayed lower infection rates, with 61% (8 out of 13) and 72% (8 out of 11) positive samples, respectively as Table 4 presents it.

Prevalence of Suspected diagnosis *Sarcocyst* spp. in camel according to organs

The prevalence of suspected *Sarcocyst* spp. infection in camels, focusing on different organ tissues. The overall infection rate across all sampled organs was 44%, with 22 out of 50 samples testing positive. However, the infection prevalence varied considerably among the different organs. The esophagus exhibited the highest susceptibility, with a striking 81% of esophageal samples (9 out of 11) testing positive for the parasites. The heart also showed a moderately high prevalence of 54% (6 out of 11). In contrast, the intercostal muscles and diaphragm displayed significantly lower infection rates, with 20% (3 out of 15) and 30% (4 out of 13) positive samples, respectively as Table 5 provides a breakdown of it.

Table 4: Prevalence of Suspected diagnosis *Sarcocyst* spp. in sheep according to organs.

Organs	No. of sample	Positive sample	percentage
Heart	16	15	93
Diaphragm	13	8	61
Intercostal	11	8	72
Esophagus	10	8	80
Total	50	39	78

Table 5: Prevalence of Suspected diagnosis *Sarcocyst* spp. in camel according to organs.

Organs	No. of samples	+	Percentage
Heart	11	6	54
Esophagus	11	9	81
Intercostal	15	3	20
Diaghram	13	4	30
Total	50	22	44

DNA Sequence results

The DNA sequencing method was carried out to genetic species typing analysis in small subunit ribosomal RNA gene in local *Sarcocystis* species Sheep, Cattle, and Camel isolates that aligned with NCBI-Genbank related *Sarcocystis* species isolates. The phylogenetic tree was constructed using Unweighted Pair Group method with Arithmetic Mean (UPGMA tree) in (MEGA 6.0 version). The local *Sarcocystis* spp. Cattle isolate No.1, No.2, and No.4 were showed closed related to NCBI-BLAST LC171830.1 *Sarcocystis* cruzi (LC171830.1) and local *Sarcocystis* spp. Cattle isolate No.3, No.2, and No.4 were showed closed related to NCBI-BLAST LC171830.1 *Sarcocystis* hominis (KF954731.1). The local *Sarcocystis* spp. Sheep isolate (No.1-No4) were showed closed related to NCBI-BLAST *Sarcocystis tenella* (MK420019.1). The local *Sarcocystis* sp. Camel isolate (No.1-No2) were showed closed related to NCBI-BLAST *Sarcocystis* cameli (OP959654.1) at total genetic changes (0.02-0.01%) as showed in figure (1). The homology sequence identity between local *Sarcocystis* species Sheep, Cattle, and Camel isolates and NCBI-Genbank related *Sarcocystis* species isolates were showed genetic homology sequence identity ranged from (98.43-98.90%).

Figure 1 shown phylogenetic tree analysis based small subunit ribosomal RNA gene partial sequence in local *Sarcocystis* species Sheep, Cattle, and Camel isolates that used for genetic species typing analysis. The phylogenetic tree was constructed using Unweighted Pair Group method with Arithmetic Mean (UPGMA tree) in (MEGA 6.0 version).

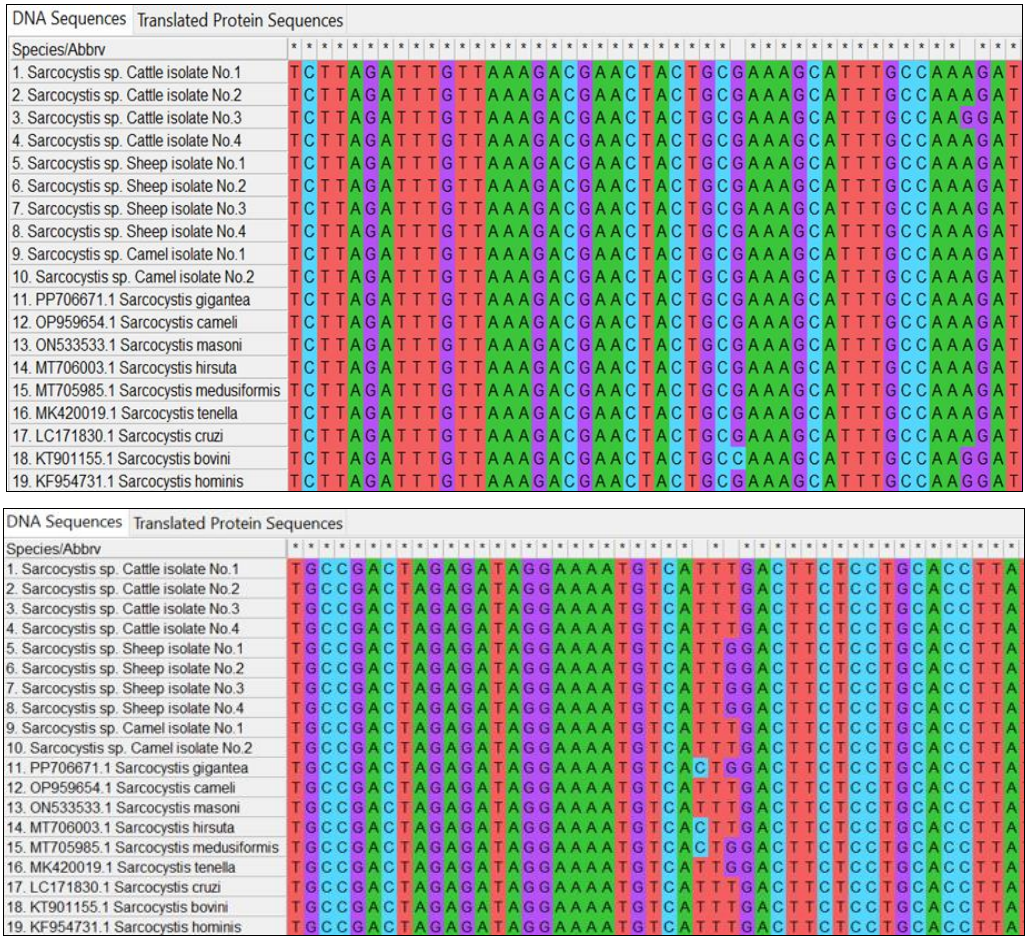

Fig 1: Multiple sequence alignment analysis of small subunit ribosomal RNA gene in (local *Sarcocystis* species Sheep, Cattle, and Camel isolates) and NCBI-Genbank related *Sarcocystis* species isolates. That dine using (ClustalW alignment tool. Online). The analysis was showed the nucleotide alignment similarity as (*) and substitution mutations.

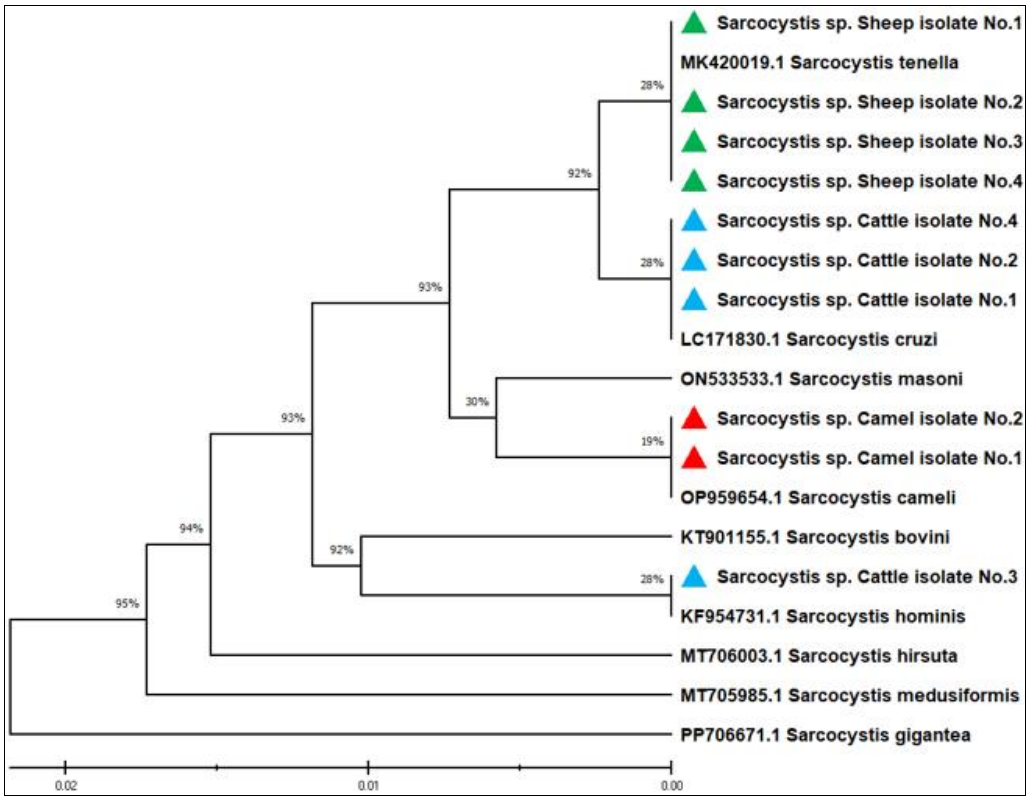

Fig 2: Phylogenetic tree analysis based small subunit ribosomal RNA gene partial sequence in local *Sarcocystis* species Sheep, Cattle, and Camel isolates that used for genetic species typing analysis.

The local *Sarcocystis* spp. Cattle isolate No.1, No.2, and No.4 were showed closed related to NCBI-BLAST LC171830.1 *Sarcocystis* cruzi (LC171830.1) and local *Sarcocystis* spp. Cattle isolate No.3, No.2, and No.4 were showed closed related to NCBI-BLAST LC171830.1 *Sarcocystis* hominis (KF954731.1). The local *Sarcocystis* spp. Sheep isolate (No.1-No4) were showed closed related to NCBI-BLAST *Sarcocystis tenella* (MK420019.1). The local *Sarcocystis* spp. Camel isolate (No.1-No2) were showed closed related to NCBI-BLAST *Sarcocystis* cameli (OP959654.1) at total genetic changes (0.02-0.01%).

Discussion

The results in Table 1 reveal varying prevalence of suspected Sarcocyst spp. infection across different animal species, with cattle exhibiting the highest prevalence (82%), followed by sheep (78%) and camels (44%). This suggests that cattle and sheep in the studied region are at a higher risk of infection compared to camels. These findings are consistent with previous studies reporting a higher prevalence of *Sarcocystis* spp. in cattle and sheep compared to camels (Gareh *et al*. 2020) [9]. The variation in prevalence could be attributed to differences in feeding habits, grazing patterns, and exposure to contaminated environments among these species.

The results presented in Table 2, based on ribosomal RNA (rRNA) analysis, indicate a high overall prevalence of *Sarcocystis* spp. infection in the studied animal population, with 72% of samples testing positive. Notably, both cattle and sheep exhibited a high infection rate of 83%, suggesting their susceptibility to *Sarcocystis* infection in the region. In contrast, camels showed a considerably lower prevalence of 42%. These findings are consistent with previous studies reporting a high prevalence of *Sarcocystis* spp. in cattle and sheep (Mirzaei and Rezaei 2016) [13]. However, the lower prevalence observed in camels in this study contradicts some previous reports, which have suggested a higher prevalence of *Sarcocystis* spp. in camels (Valinezhad, Oryan, and Ahmadi 2008) [19]. This discrepancy could be attributed to differences in sample size, geographical location, or the specific *Sarcocystis* species involved.

The DNA sequencing results, as depicted in Figure 1 and Table 1, reveal that the local *Sarcocystis* isolates from sheep, cattle, and camels exhibited high homology (98.43-98.90%) with *Sarcocystis tenella*, *S. cruzi*/*S. hominis*, and *S. cameli*, respectively, suggesting the presence of these species in the studied region. These findings align with previous studies reporting the prevalence of S. tenella in sheep (Minuzzi *et al*. 2019) [12], *S. cruzi* and *S. hominis* in cattle (Nourani *et al*. 2010) [14], and *S. cameli* in camels (Sazmand, Joachim, and Otranto 2019) [16]. The high genetic similarity between the local isolates and the reference sequences from GenBank confirms the accuracy of species identification and supports the validity of using rRNA gene sequencing for *Sarcocystis* species differentiation.

The results in Table 4 demonstrate a high overall prevalence of suspected *Sarcocyst* spp. in sheep, with the heart being the most frequently infected organ (93%), followed by the esophagus (80%).The diaphragm and intercostal muscles showed lower, but still substantial, infection rates. These findings suggest that these parasites have a predilection for certain tissues in sheep, particularly the heart.

The results in Table 5. Reveal a varying prevalence of suspected *Sarcocyst* spp. infection in camel organs, with the esophagus showing the highest susceptibility (81%), followed by the heart (54%). In contrast, the intercostal muscles and diaphragm exhibited significantly lower infection rates (20% and 30%, respectively).

Conclusions

This study highlights the significant prevalence of *Sarcocystis* spp. infections in cattle, sheep, and camels in Thi-Qar, Iraq. The high prevalence rates observed, particularly in cattle and sheep, emphasize the potential public health risk associated with the consumption of undercooked meat from these animals. The presence of *S. cruzi*, *S. hominis*, and S. tenella in the studied region raises concerns due to their zoonotic potential.

Declarations

Acknowledgments

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Conflict of interest

The authors declare that they have no conflicts of interest.

Authors' contributions

Eman Hadi Zaib: Conceptualization, methodology, investigation, data curation, writing - original draft.

Prof. Dr. Ghaidaa Abbas Jasim: Supervision, validation, resources, writing - review & editing.

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Data availability

The datasets generated and analyzed during the current study are available from the corresponding author

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