Special Issue:
Emerging and Re-emerging Animal Health Challenges in Low and Middle-Income Countries

A Diagnostic and Immunological Study of the Sarcocystosis in Bovine

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Abstract | The current study was carried out to evaluate the incidence of bovine *Sarcocystosis*. Our analysis indicated that the incidence of the infection among 180 tested bovine was 26.67%, while the infection was not detected in 73.33% of the tested animals. Examining of the effect of the parasite on the liver and kidney functions showed that the infection didn't significantly affect the levels of ALP, AST, ALT, creatinine and urea. However, the infection has significantly elevated the level of serum IL-6 (44.31 Pg/ml) compared to its level in the non-infected animals (8.36 Pg/ml). Infections by *Sarcocystis* in bovine are ubiquitous worldwide, most of the cases were subclinical. It is critical to properly diagnose *Sarcocystis* species in order to evaluate their significance for the economy and public health.

Keywords | Sarcocystosis, Bovine, ELISA, IL-6

Received | August 20, 2025; **Accepted** | October 01, 2025; **Published** | October 16, 2025
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Citation | Hadi ZS, Flaih MH, Mustafa HM, Al-Snafi AE (2025). A diagnostic and immunological study of the Sarcocystosis in bovine. J. Anim. Health Prod. 13(s1): 623-627.
DOI | https://dx.doi.org/10.17582/journal.jahp/2025/13.s1.623.627
ISSN (Online) | 2308-2801

INTRODUCTION

Members of the genus *Sarcocystis*, an intracellular protozoal parasite in the phylum Apicomplexa that is extensively found in mammals, birds, and reptiles, are responsible for causing sarcocystosis (Maky and Mohammed, 2021). *Sarcocystis* has a number of stages, the oocyst stage contains two sporocysts. Depending on the parasite type, sarcocyst stages vary in size and shape; some are tiny (*S. cruzi*), while others are gigantic (*S. gigantea, S. muris*) (Ondrej and David, 2022). These parasites go through two hosts in their life cycle: The definitive host's intestines create the sexual stage, while the intermediate host's different tissues host asexual reproduction. *Sarcocystis* typically finds definite hosts in carnivores and omnivores. A few organisms can develop in more distantly related animals (e.g., cats and dogs), but most parasites are believed to use one or a group of closely related hosts. Mammals, marsupials, birds, reptiles, and perhaps fish can all serve as intermediate hosts. They are frequently omnivores or herbivores. It appears that certain *Sarcocystis* species are more host-specific than others (Chiesa *et al.*, 2013; Obijiaku *et al.*, 2013). *Sarcocystis* species occasionally cause myositis, encephalitis, and other illnesses in humans or animals, although the majority of infections appear to be asymptomatic. The dose of the parasites is believed to be one determinant, though it is unclear why some infected people get sick while others stay healthy (Dubey and Rosenthal, 2023; Howe *et al.*, 2018). Worldwide, *Sarcocystis* infections in cattle are common. The species of *Sarcocystis* that cause microscopic infection and are spread by the Canidae family are more pathogenic, the parasite toxins

lead to fever, anemia, loss of appetite, weight loss, wool loss, decreased milk production, acute myopathy, and central nervous signs such as ataxia, paralysis, muscle tremors that lead to abortion and sometimes death (Rubiola *et al.*, 2021).

The identity of *Sarcocystis* species in cattle is a topic of recent discussion. *Sarcocystis* species must be properly diagnosed in order to be evaluated for their significance to the economy and public health (Dubey and Rosenthal, 2023). Our study aimed to evaluate the incidence of bovine sarcocystosis in Nasiriyah province as an effort to provide foundational information for mitigation of the infection.

MATERIALS AND METHODS

Sample collection and processing

This study was carried out in various locations within the Thi-Qar province between 2023 and 2024. A total of 180 blood samples from bovine were chosen at random and 25 blood samples from health bovine. Using anticoagulant-free sterile tubes, jugular vein blood samples were drawn, put on ice, and then brought to the lab. Following a 15-minute centrifugation at 3,000 rpm, the serum was extracted from the coagulated blood and kept at -20 °C for further examination.

Diagnosis of *Sarcocystis*

Following the manufacturer's instructions, the Bovine *Sarcocystis* ELISA kit (SunLong Biotech Co., LTD, China) was used to qualitatively determine the presence of *Sarcocystis* in all bovine serum samples. Test efficacy for every specimen: Positive control's average value was greater than 1.00, while negative control's average value was less than 0.10. Calculating the crucial value (CUT OFF) involves multiplying the average value of negative control by 0.15. Negative samples for bovine *Sarcocystis* were concluded if the OD value was less than CUT OFF. Positive determination of the sample for bovine *Sarcocystis* when the OD value is more than or equal to CUT OFF.

Estimated of IL-6

The Bovine IL-6 (Interleukin 6) ELISA Kit (Reed Biotech Ltd, RE3186B, China) was used to estimate the levels of IL-6.

Calculation of results include following analysis. For each standard and sample, the average duplicate readings were subtracted from the average zero standard optical density. Drawing a logistic curve with four parameters on log-log graph paper, use OD values on the y-axis and standard concentration on the x-axis. Samples were retested using the proper dilution if its optical density (OD) exceeds the upper limit of the standard curve. The computed concentration times the dilution factor is the actual concentration.

Biochemical tests

Following the manufacturer's instructions, a commercially available kit (Biosystem Laboratory Products LTD, Spain) was used to measure various serum biochemical parameters (Urea, Creatinine, ALT, AST, ALP).

Statistical analysis

The Student's t test (SPSS, version 26) was used to assess the differences between the groups. The proportions were compared using the Chi-square test. The outcome was deemed significant if the p-value was 0.05 or below.

RESULTS

As shown in Table 1, the incidence of the infection among 180 tested bovine was 26.67%, which include 48 infected animals while the infection was not detected in 132 of the tested animals 73.33%.

Table 1: Infection rate in infected bovine with *Sarcocystosis* using ELISA.

Total samples	Positive sample	Negative sample	%	Sig.
180	48	132	26.67	P < 0.05

The results showed an effect of parasitic infection in infected bovine with Sarcocystosis on elevated the level of serum IL-6 (44.31 Pg/ml), and was significantly higher (P < 0.05) compared with its level in control animals (8.36 Pg/ml) Table 2.

Table 2: The serum level of IL-6 in infected bovine with *Sarcocystosis* compared to control samples.

Samples	IL-6 Pg/ml	P-value
Infected(48)	8.36±64.36	0.007
Control(25)	44.31±7.880	

Examining of the effect of the parasitic infection on the liver and kidney functions in infected bovine with Sarcocystosis, showed that the infection didn't significantly affected the levels of ALP (72.08±9.375), AST (13.88±7.343), ALT (10.40±5.615), creatinine (0.716±0.1901) and urea (32.94±13.90) compared to its level in control animals Table 3.

DISCUSSION

Animal emaciation caused by the common intracellular protozoan parasite *Sarcocystis* species has a significant financial impact on livestock. decreased weight gain, decreased quality and quantity of meat, milk, and wool, transboundary restriction, condemnation of infected

Table 3: The serum level of ALP, AST, ALT, Creatinine and Urea in the infected bovine with *Sarcocystosis* compared to control samples.

Samples	ALP (IU/l)	AST (IU/l)	ALT (IU/l)	Creatinine (mg/dl)	Urea (mg/dl)
Infected (48)	72.08±9.375	13.88±7.343	10.40±5.615	0.716±0.1901	32.94±13.90
Control (25)	78.10±14.53	16.12±4.961	12.07±3.731	0.741±0.1908	33.43±11.71
p-value	0.0432	0.1816	0.1906	0.6124	0.8787

carcasses, abortion and even death (Hussein *et al*., 2025; Fayer, 2004; Saleque *et al*., 1990). In this study, naturally infected bovine samples were used to examine the possible impacts of *Sarcocystis* infection on inflammation as well as liver and kidney function.

According to estimates, almost 90% of cows worldwide were thought to be infected with *Sarcocystis*. China and Malaysia both reported the same percentage (Tappe *et al*., 2014; Yang *et al*., 2018). Using the tissue expansion method, 94.8% of the bovines under study in Isfahan, Iran, were infected (Fayer, 2004). Infection rates in Tabriz, Iran, were almost same (Shekarforoush *et al*., 2005). However, almost 90% of the cows analyzed in Australia had *Sarcocystis* infections, In South Moravia, 75-93% of the examined cows were infected (Pena *et al*., 2001). Infection was recorded in 78% of the examined cattle in new valley governorate- Egypt (El-Mahdi *et al*., 2023).

In our study, 48 bovines were infected out of 180 (26.67%) tested, the reason of the low percent of the infection cold be attributed to using of *Sarcocystis* ELISA which is more accurate diagnostic test. Furthermore, the majority of these studies were carried out on the slaughtered bovine with high contamination rate reaching to 75-93% by the digestion method in bovine (Pena *et al*., 2001).

The serum level of IL-6 was significantly elevated in bovine infected with *Sarcocystis*. In response to infections and tissue damage, the soluble cytokine mediator interleukin 6 (IL-6) is quickly and temporarily produced. In the early stages of inflammation, IL-6 is generated in a local lesion and then circulated to the liver, where it rapidly initiates the production of a wide range of acute phase proteins, such as fibrinogen, haptoglobin, serum amyloid A (SAA), C-reactive protein (CRP), and α1-antichymotrypsin. It supports host defense by encouraging hematopoiesis, immunological responses, and acute phase responses (Tanaka *et al*., 2014).

Many studies showed elevated IL-6 in *Trypanosoma, Trichomonas* and *Toxoplasma* infections and suggested that IL-6 mediates anti-parasite protective responses (Al-Hadraawy *et al*., 2014; Gao and Pereira, 2002). An infection with *Sarcocystis* is a major contributing factor to a number of serious inflammatory conditions, including intestinal infections, hepatitis, encephalitis, and encephalomyelitis (Fayer, 2004; AbuBakar *et al*., 2013). Infection with *Sarcocystis camelicanis* increased the expression of IL-6 in many organs of the infected animals (Metwally *et al*., 2021).

Fresh *Sarcocystis* extract significantly raised the levels of several cytokines, including IL-1β, IL-18, IL-15, IL-12, IL-10, and IL-13, in rats used in experimental infection (Badr *et al*., 2020).

During protozoan infections, the immune system generates a number of defense mechanisms, such as innate immune responses to extracellular protozoan parasites by immune cells like natural killer (NK) cells, neutrophils, and macrophages. Both innate and adaptive immune responses to intracellular parasites depend on the cytokines generated by natural killer cells and activated macrophages (Melby *et al*., 2019).

Our result showed that *Sarcocystis* didn't significantly change hepatic and renal functions. However, the effect of the parasite on the values of these tests depend on the severity and distribution of the parasite, When the parasite invaded liver, it cased hepatocellular injury represented by hepatocytic and sinusoidal necrosis and alterations. The hepatocyte nuclei showed a pleomorphic appearance with dilatation of the core veins and pale-violet staining. The border architecture of the central vein and sinusoids was uneven and often looked torn. When it entered the kidney, a part of the renal cortex showed highly damaged renal corpuscles with a destroyed glomerulus, dilated lumen, proximal and distal convoluted tubules that were twisted and dilated, and leukocyte infiltration. These histological changes associated with alterations of liver and kidney function tests (El-Mahdi *et al*., 2023).

CONCLUSIONS AND RECOMMENDATIONS

This study evaluated the incidence of bovine sarcocystosis in Thi-Qar province. The incidence of the infection reached 26.67%, most of the cases were subclinical. Sarcocystosis species must be properly diagnosed in order to be evaluated for their significance to the economy and public health. Researchers recommend conducting more molecular studies and studying the histological effects on animals infected with the *Sarcocystis* parasite.

ACKNOWLEDGEMENT

The authors sincerely thank the Research Laboratories, Department of Biology, College of Education for Women, University of Al-Shatrah, Iraq, for their infrastructure and technical support. We also thank the staff of the Veterinary Hospital in Shatrah and Nasiriyah, Iraq, for their assistance in sample collection.

NOVELTY STATEMENT

The novelty of our research is that it includes studying the effect of parasitic infection in bovine, which are considered an important source of human food, with few studies in Thi-Qar Governorate that include the effect of this infection on Immunological and biochemical parameters in infected bovine.

AUTHOR'S CONTRIBUTION

ZSH: Preparing the research idea, collecting samples, parasites diagnosed and writing the research. Immunological and biochemical parameters work.
MHF: Preparing and providing the necessary materials to complete the immunological parameters work.
HMM: Preparing and providing the necessary materials to complete the biochemical parameters work.
AEA-S: Preparing the research idea, writing the research, supervising it and analyze the results.

Generative AI and AI-assisted technology statement

In this manuscript, AI was not used.

Conflict of interest

The authors have declared no conflict of interest.

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