



Serological Detection of Sarcocystiosis in Buffaloes

Ghassan J.K. Al-Abedi¹, Enas MM. Al-Eodawee², Ali A. Shatti³

¹ Lecturer, Department of Microbiology, College of Veterinary Medicine, University of Wasit, Wasit, Iraq. Email: ghjabar@uowasit.edu.iq
ORCID: <https://orcid.org/0000-0001-5040-1097>

² Lecturer, Department of Microbiology, College of Veterinary Medicine, University of Wasit, Wasit, Iraq. Email: emhihi@uowasit.edu.iq
ORCID: <https://orcid.org/0009-0002-4796-5611>

³ Assistant Lecturer, Department of Anatomy and Histology, College of Veterinary Medicine, University of Wasit, Wasit, Iraq. Email: alishati@uowasit.edu.iq
ORCID: <https://orcid.org/0000-0002-1402-6362>

*Corresponding author's E-mail: ghjabar@uowasit.edu.iq

Article History	Abstract
Received: 19 August 2022 Revised: 22 February 2023 Accepted: 28 February 2023	<p>The study aimed to serologically detect the prevalence of <i>Sarcocystis</i> infection in buffaloes using the indirect enzyme-linked immunosorbent assay (ELISA) for the first time in Iraq. An overall 184 buffaloes of different ages and sexes were selected randomly from the regions of Wasit province (Iraq) from March to June (2022), and subjected to draining of venous blood under aseptic conditions. After centrifugation, the obtained sera were examined by indirect ELISA. Totally, 33.15% of study animals were positive for IgG antibodies. According to their concentrations, the ODs level showed a significant increase ($P < 0.0186$) in mild infection (63.93%) when compared to moderate (27.87%) and strong (8.2%) infections. Subsequently, values of mild, moderate, and strong infections were 0.330 ± 0.01, 0.554 ± 0.019 and 0.912 ± 0.031 nm, respectively. Regarding age and sex factors, significantly higher positivity ($P < 0.0469$) was shown in buffaloes aged >10 years old (57.89%); while the lowest was seen in those <1 year (13.64%). Subsequently, older buffaloes appeared significantly ($P < 0.0192$) at higher risk (2.1685) when compared to other age groups. Females recorded an obvious increase ($P < 0.05$) in positivity (35.95%) and risk (1.8569) in comparison with males; 19.35% and 0.5385, respectively. The results indicate that ELISA is of great value in the diagnosis of sarcocystiosis.</p>
CC License CC-BY-NC-SA 4.0	Keywords: <i>Sarcocystis</i> , ELISA, Seropositivity, Age, Sex.

1. Introduction

Sarcocystiosis is a parasitic, chronic, and mainly asymptomatic disease caused by Apicomplexan members of *Sarcocystis* genus (Morsy et al., 2018). Normally, the disease is developed in two-host cycles; the intermediate host, in which, the parasite reproduced asexually to develop the muscle cysts; and the final host, in which, the parasite reproduced sexually in intestine to produce the mature oocysts (Dubey, 2015; Latif and Muslim, 2016). Worldwide, several *Sarcocystis* species (>250) were

distributed among different domestic (cattle, sheep, pigs, horses, dogs, cats) and wild (birds, rodents, snakes, raccoons, and opossums) animals in addition to humans. Namely, *S. cruzi*, *S. bovis*, *S. bovis*, *S. fusiformis*, *S. heydorni*, *S. S. hirsute* and *S. hominis* were demonstrated in buffaloes that act as an intermediate host for the establishment of the tissue cysts (sarcocysts); while carnivores act as the definitive host for the formation of oocysts (Connick et al., 2020; Prakas et al., 2020; Zeng et al., 2021).

Although, many *Sarcocystis* infection in bovine animals is usually asymptomatic; the acute phase of parasitism characterizes by fever, discomfort, muscle tremor, anorexia, shivering, and lethargy (Chimelli and Keohane, 2020). Economic loss is further marked by increased body metabolism, and decreased milk yield with growth retardation which is possibly caused by the parasite that is capable of tipping the balance between nutrition-endocrine interactions and growth (Aráoz et al., 2019; Falkenberg et al., 2013; Januskevicius et al., 2018). However, the dose of sporocysts and the immune status of a host could of great importance in developing clinical symptoms of sarcocystosis (Decker Franco et al., 2018; Dubey et al., 2013).

Antemortem diagnosis is challenging due to the signs of the disease are usually similar to that observed in other diseases; in addition, a biopsy is commonly time-consuming and is not possible for screening large numbers before slaughtering animals (Dasmabai and Kumar, 2022; Verma et al., 2018). For these reasons, serological tests are usually the method of choice for diagnosing and differentiation of infected animals based on the detection of specific antibodies; whereas, negative results always rule out the existence of disease (García-Lunar et al., 2015; Banothu et al., 2017). Enzyme-linked immunosorbent assay (ELISA) is an advanced serological technique that is considered a safe and eco-friendly, highly specific and sensitive, and simple diagnostic assay (Aydin, 2015; Sakamoto et al., 2018). In Iraq, no serological studies were reported in buffalo. Therefore, this study aimed at conducting a serological screening of *Sarcocystis* spp. in buffaloes of Wasit province for the first time in Iraq.

2. Materials And Methods

Ethical Approval

The present study was approved by the Scientific Ethical Committee of the College of Veterinary Medicine (University of Wasit, Wasit, Iraq).

Samples

An overall 184 buffaloes of different ages and sexes were selected randomly from the regions of Wasit province (Iraq) from March to June (2022). Under aseptic conditions, all study animals were subjected to sampling 5 ml of venous blood into a free-anticoagulant glass gel tube for obtaining the sera by centrifugation at 5000 rpm / 5 min. Finally, sera were kept frozen at -20°C until tested.

Serological Assay

According to the manufacturers' instructions for the Bovine *Sarcocystis* ELISA Kit (SunLong Biotech, China), the sera and kit components were prepared and processed. To determine the results, optical densities (ODs) of the control and samples were read at 450 nm by the ELISA Microplate Reader (BioTek, USA), and critical value (CUT OFF) was calculated. The CUT OFF of positive samples was identified at an OD of ≥ 0.2275 . The positive ODs were divided according to their concentrations into 3 levels of infection; mild, moderate, and severe.

Statistical Analysis

One-Way ANOVA in GraphPad Prism (version 6.0.1) was applied to detect significant differences in the distribution of positive results among different levels of infection; while, Odds Ratio test was used to estimate the association of positive results to the targeted risk

factors (age and sex). Values were recorded as $M \pm SE$ (Mean \pm Standard Error), and variation is significant at $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***) and $P < 0.0001$ (****), (Ajaj et al., 2021; Gharban, 2022).

3. Result And Discussion

Among 184 tested samples by indirect ELISA, 61 (33.15%) revealed positive ODs for IgG antibodies (Figure 1). According to their concentrations, the level of ODs showed a significant increase ($P < 0.0186$) in mild infection (63.93%) when compared to moderate (27.87%) and strong (8.2%) infections (Figure 2).

Subsequently, values of mild, moderate, and strong infections were 0.330 ± 0.01 , 0.554 ± 0.019 and 0.912 ± 0.031 nm, respectively (Table 1).

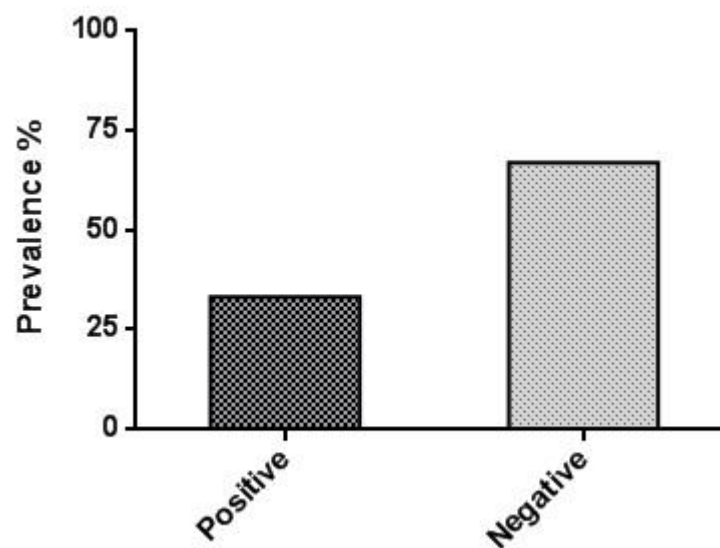


Figure (1): Total results for examination 184 sera by indirect ELISA

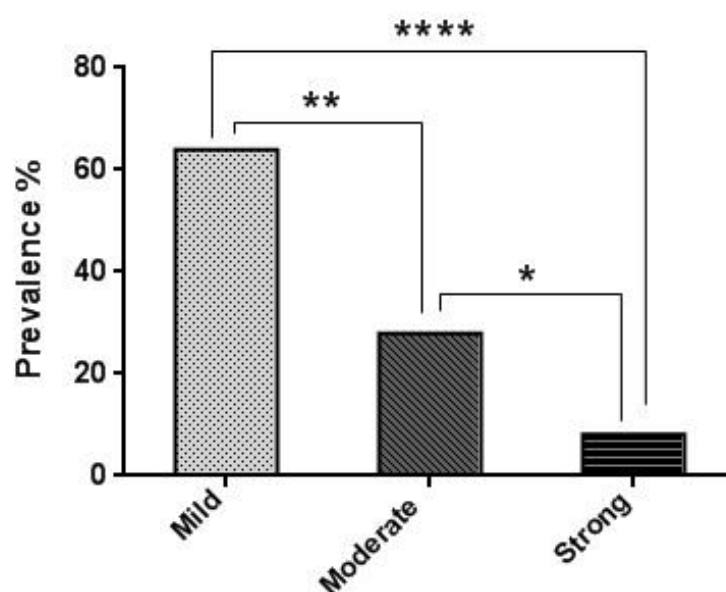


Figure (2): Classification of infection according to positive ODs

Table (1): Concentration of positive ODs in mild, moderate and strong infections

Category	Titer	
	M ± SD	Range
Mild	0.330 ± 0.01	0.23 – 0.471
Moderate	0.554 ± 0.019	0.479 – 0.716
Severe	0.912 ± 0.031	0.805 – 0.989
<i>p-value</i>	P≤0.0001	-

Source: Authors

Regarding age and sex of study animals, significantly higher positivity ($P < 0.0469$) was shown in buffaloes > 10 years old (57.89%); while the lowest was seen in those < 1 year (13.64%). Subsequently, older buffaloes appeared significantly ($P < 0.0192$) at higher risk (2.1685) when compared to other age groups. Concerning sex factors, females revealed a significant elevation ($P < 0.05$) in positivity (35.95%) and risk (1.8569) in comparison with males; 19.35% and 0.5385, respectively (Table 2).

Table (2): Association of age and sex to positive infection (Total No: 61 buffaloes)

Factor (group)	Total No.	Positive	Odd ratio	Risk
<i>Age (Year)</i>				
< 1	22	3 (13.64%)	0.2832	0.381
1-4	51	12 (23.53%)	0.5283	0.6394
> 4 - 10	73	24 (32.88%)	0.98	0.9874
> 10	38	22 (57.89%) *	3.767 *	2.1685 *
<i>p-value</i>	-	0.0469	0.0173	0.0192
<i>Sex</i>				
Female	153	55 (35.95%) *	2.338 ***	1.8569 **
Male	31	6 (19.35%)	0.4277	0.5385
<i>p-value</i>	-	0.0196	0.0007	0.0029

Significance * ($P < 0.05$)

Source: Authors

Discussion

Sarcocystis is one of the most prevalent parasites in livestock, worldwide (Castro-Forero et al., 2022; Sarafraz et al., 2020; Shaapan, 2016). In some hosts such as buffalo, the occurrence rate of infection is required to improve due to global insufficient data. Our findings revealed that 33.15% of study buffaloes were positive suggesting that exposure of buffaloes to infection is repeated as a result of their close contact with domestic and wild animals that play an intermediate role and final hosts to different *Sarcocystis* species. In Iraq, *Sarcocystis* infection was reported in buffaloes in only one previous study by the macroscopical and microscopical examinations (Latif et al., 1999), in which, there were 15.9% and 82.9% positive animals. In other countries, buffalo's sarcocystosis has been reported in South Africa (Quandt et al., 1997), the Philippines (Claveria and Cruz, 2000), Vietnam (Jehle et al., 2009; Huong, 1999), China (Dubey et al., 2014; Li et al., 2002), Egypt (El-Dakhly et al., 2011; Metwally et al., 2014), Iran (Daryani et al., 2006; Gjerde, 2016), Hungary (Hornok et al., 2015), Thailand and Cambodia (Hongchuta et al., 2021), and India (Daptardar et al., 2016; Sudan et al., 2023). However, the comparison between the findings of these studies had many challenges as different techniques served to define infection status and species identification and different types of samples were collected from different locations. For example, Metwally et al. (2014) demonstrated the prevalence of *Sarcocystis* infection was 25.5%, 27.7%, and 94% by the macroscopic, microscopic and serological (ELISA) assays, respectively. Accordingly, the researchers strongly recommended the application of ELISA in the diagnosis of *Sarcocystis* infection because of the low sensitivity of macroscopic, microscopic and histological testing of samples.

Variations in the severity of infections with the prevalence of mild positivity seen in this study might be associated with the number of infected organs, parasitic overload, age and strength of the immune response, *Sarcocystis* species, and single or mixed infection (Burezq, 2021; Singh, 2020). Fayer

(2004) reported that the severity of infection is dependent on a dose of ingested sporocysts. Also, the source of antigen used in the coating of an ELISA plate might play a role in the diagnosis of positivity and detection of the severity of infections.

The increasing rate of prevalence of infection observed in the current study with advancing age might be attributed to the chronic nature of the disease, the low immune response of the elderly towards the parasite, the high resistance of young animals to infection, and more commonly due to repeated exposure of elderly buffaloes to sarcocystosis that result in a gradual accumulation of the parasite within the muscles. However, our findings were similar to that recorded by many studies (El-Dakhly et al., 2011; Gerab et al., 2022; Oryan et al., 2010; Said, 1996) and compatible with others (JyothiSree et al., 2017; Metwally et al., 2014) who detected no significant differences between age groups.

Females showed more positivity than males in this study. This is in agreement with several studies (Daryani et al., 2006; Gerab et al., 2022; Mousa et al., 2021), and disagreed with others (El-Dakhly et al., 2011; Huong, 1999; Oryan et al., 2010) who found no significant differences between males and females in particular in elderly animals. Our results might be caused by many reasons such as the exposure of females to additional physiological stress due to gestation and milk production which attenuate the immune response of females rather than males. The low number of males subjected to the current study might have contributed to these results. Also, the low percentage of infected males may be attributed to the animal management system as most of the males are kept only for the fattening system and slaughtering at approximately 1-2 years old; while females are kept for long times for milk production (El Shanawany et al., 2019; Gerab et al., 2022).

4. Conclusion

The study aimed to serological detection of the prevalence of Sarcocystis infection in buffaloes using the indirect enzyme-linked immunosorbent assay (ELISA). This is the first serological study for Sarcocystis infection in buffaloes in Iraq. These results revealed that the prevalence rate of infection in study animals is relatively high with a significant increase in disease in elderly buffaloes and females. More importantly, the use of ELISA in the diagnosis of Sarcocystis infection in animals is recommended to control the disease in animals and to avoid human infection due to the presence of hidden or subclinical infections of Sarcocystis, especially in large animals.

Conflict of Interest

The authors declare no conflict of interest.

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