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PREVALENCE OF *Toxoplasma* AND *Sarcocystis* SPECIES IN BUFFALOES BASED ON ABATTOIR STUDY AT MUMBAI

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Abstract: In the present study meat inspection of 920 slaughtered buffalo carcasses were performed and 90 blood samples were collected for serological analysis from Deonar abattoir Mumbai to detect the prevalence of meat borne protozoan parasites. By meat inspection 10.10 % prevalence of *Sarcocystis* species were recorded and *Sarcocyst* were mainly recovered from the skeletal muscles and oesophagus. The highest prevalence was recorded during the rainy season (16.67%) followed by summer (9.23%) and then winter (6.45%) with statistically significant differences ($P < 0.05$). By antibody ELISA test 28.8% seroprevalence was noted for *Toxoplasma gondii.* Age wise analysis revealed non-significant differences. The occurrence of these parasitic infections in buffaloes underscores the need for systematic epidemiological studies to design appropriate control strategies to safeguard animal health, ensure meat safety, and protect public health. Owing to the limitations of meat inspection advanced serological and molecular methods are recommended for detection of these meat borne parasites.
Keywords: *Toxoplasma*, *Sarcocystis*, Buffaloes, Prevalence.

INTRODUCTION

Buffaloes play a significant role in meat production and contribute substantially to the livestock economy. However, they also act as reservoirs of several meat-borne zoonotic parasites, posing risks to both public health and meat safety. Among these, Toxoplasmosis and Sarcocystosis are of major importance due to their wide distribution and zoonotic potential. Toxoplasmosis, caused by *Toxoplasma gondii*, is a globally important zoonosis, with infection in humans occurring mainly through consumption of raw or undercooked meat containing tissue cysts or ingestion of oocysts shed by cats (de Souza *et. al* 2016). Sarcocystosis, caused by *Sarcocystis* spp. is commonly detected during meat inspection and is associated with reduced meat quality and economic losses. Buffaloes usually remain asymptomatic, which facilitates the unnoticed entry of infected meat into the food chain. Transmission of these parasites is influenced by poor hygiene, inadequate meat inspection, and close contact between

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livestock and definitive hosts such as dogs and cats. Routine meat inspection methods have limited sensitivity, particularly in detecting light infections, leading to underestimation of the true prevalence. Therefore, reliable epidemiological studies are essential to assess the occurrence and impact of these infections.

MATERIALS AND METHODS

The present study was conducted from March 2025 to January 2026 at the Department of Veterinary Parasitology, Mumbai Veterinary College, MAFSU, Mumbai, India. Samples were collected from buffaloes slaughtered at Deonar abattoir, Mumbai, originating from different regions of Maharashtra. A total of 920 buffalo carcasses were examined for the presence of meat-borne sarcocystosis through routine post-mortem inspection. Detailed examination involved visual inspection, palpation, and incision of predilection sites such as masseter muscles, heart, diaphragm, and skeletal muscles. Suspected tissues were collected and examined microscopically for confirmation. The abattoir was visited twice weekly, and buffaloes above two years of age were included in the study. Information regarding age, sex, breed, and place of origin of animals was recorded. Seasons were categorized as summer (March–May), rainy (June–September), and winter (November–January).

For serological analysis, of *Toxoplasma gondii* 90 blood samples were collected from slaughtered buffaloes. Serum was separated by centrifugation and stored at −20°C until further use. Serum samples were screened for antibodies against *T. gondii* using a commercially available Bovine TOX-IgG ELISA kit (Fine Test, China).

Prevalence was calculated as the percentage of infected animals out of the total animals examined. Statistical analysis was performed using the Chi-square test to assess differences based on age, sex, and seasons.

RESULTS AND DISCUSSIONS

Prevalence of sarcocystosis

A total of 920 buffalo carcasses were examined at the Deonar abattoir, Mumbai for a period of one year, of which 93 were found positive for *Sarcocystis* sp., showing an overall prevalence of 10.10% (Table 1). Cysts were mainly recovered from skeletal muscles and the oesophagus. During carcass inspection, macroscopic *Sarcocystis* cysts appeared creamy-whitish, spindle-shaped, fusiform, globular, and resembled cooked rice grains (Fig. 1). Cyst sizes varied from a few millimetres to several centimetres. Microscopic examination revealed cyst walls with radiating septa dividing the cyst into multiple compartments. The cysts

exhibited two distinct regions a peripheral cortex containing globular metrocytes and a central region with mature, banana-shaped bradyzoites called as Rainey's corpuscles (Fig. 2).

Seasonal variation was observed in *Sarcocystis* prevalence. The highest prevalence was recorded during the rainy season, where 50 out of 300 animal carcasses examined were found positive, showing a prevalence rate of 16.67%. This was followed by the summer season, during which 12 out of 130 animals were positive, accounting for a prevalence of 9.23%. The lowest prevalence was observed during the winter season, with 31 positive cases out of 480 animals examined, resulting in a prevalence of 6.45%. When these values are subjected to the Chi square test it was found to be statistically significant (χ^2 =21.129, P < 0.05) (Table 1).

Table 1- Season-wise prevalence of *Sarcocystis* spp. in buffaloes

Sr. No	Season	No. of animals examined	No. positive	Prevalence (%)	χ^2 value	P value	Significance
1	Rainy (June–Sept)	300	50	16.66[a]	21.129	0.000298	Significant
2	Summer (March–May)	130	12	9.23[b]			
3	Winter (Oct–Jan)	480	31	6.45[b]			
4	**Overall Prevalence**	920	93	10.10			

*Percent prevalence which showing different superscripts are statistically significant.

The higher prevalence in the rainy season may be attributed to increased environmental humidity, which favors oocyst survival and transmission. Climatic conditions especially temperature and humidity play an important role in determining the survival and longevity of sporocysts in the environment (Dubey *et al.,* 1989; Mounika *et al*., 2017). These findings highlight the epidemiological significance of *Sarcocystis* infection in buffaloes and its potential impact on meat quality and public health. Age wise infection was observed to be more common in old age animals. This finding correlate with the finding of Abu-Elwafa *et al*. (2015) & Ahmed *et al*. (2016). Furthermore, in present study female buffaloes showed a higher rate of infection compared to male animals. Similar finding was also reported by (Elsharkawy *et al.* 2025).

The findings of the present study align with the finding of Ahmed *et al.* (2016) and Abu-Elwafa *et al.* (2015) who reported a prevalence of 8.33% and 8.72% respectively in

buffaloes in Egypt where comparatively low prevalence of sarcocystosis was recorded. In comparison to present study lower prevalence was reported by Mounika *et al*. (2018) as 6% prevalence in cattle from Chittoor district, Andhra Pradesh and Oryan *et al.* (2010) as 3% prevalence in buffaloes slaughtered in Khuzestan Province, Iran. Whereas, Metwally *et al.* (2014) reported a comparatively moderate prevalence of 25.5 %, and Ras (2021) reported a higher prevalence of 41.5% in water buffaloes from Egypt.

In contrast to present study several authors from different parts of India have reported a high prevalence of *Sarcocystis* infection in cattle and buffaloes. In a previous study conducted by Dhotare (2023) documented a high prevalence of 42.85% in buffaloes slaughtered at the Deonar Abattoir, Mumbai which is surprisingly contrasting to the finding of the present study. Deonar abattoir is a very large abattoir in India where large number of the buffaloes are coming from different parts of west India for slaughter. That may be the reason for the differences in the prevalence rate found in the previous and present study. Dafedar *et al.* (2011) also recorded a very high prevalence of 84% in cattle carcasses slaughtered in Bangalore, Karnataka, indicating widespread infection. Dar *et al.* (2017) reported an extremely high prevalence of 95.5% in buffaloes slaughtered in Punjab. Jyothi Sree *et al*. (2017) observed a prevalence of 66.42% in water buffaloes slaughtered at local abattoirs in Andhra Pradesh.

Differences in the reported prevalence of Sarcocystis infection across various regions of India may be influenced by several epidemiological and environmental factors. Among these, climatic conditions especially temperature and humidity play an important role in determining the survival and longevity of sporocysts in the environment (Dubey *et al*., 1989; Mounika *et al.,* 2017). Regions with climatic conditions that are unfavorable for sporocysts survival are therefore likely to show lower infection rates in livestock.

The comparatively low prevalence recorded in the present investigation may also be related to the use of routine macroscopic meat inspection, which is known to underestimate infection levels as microscopic sarcocyst are not detectable by visual examination alone. Metwally *et al.* (2014) reported that visual inspection is considerably less sensitive than microscopic, histological and molecular diagnostic techniques for detecting Sarcocystis infections. this could be the reason behind low prevalence observed in the present study where slaughter house macroscopic meat inspection was the sole technique employed for the detection of *Sarcocystis* which is the limitation of this study.

Furthermore, positive changes in animal husbandry practices in India may have contributed to the reduced prevalence. The increasing adoption of intensive and semi-intensive

rearing systems limits direct exposure of buffaloes to contaminated soil, water, and feed, thereby reducing the likelihood of ingestion of sporocysts shed by definitive hosts. Additionally, proper disposal of infected organs or part of the carcasses and awareness of not feeding raw meat to dogs and cats have effectively reduced infection to definitive hosts, thereby interrupting the parasite's transmission cycle. Collectively, these factors may contribute towards the low prevalence of Sarcocystis infection observed in this study.

Prevalence of toxoplasmosis

Clinical diagnosis of toxoplasmosis is often challenging because the disease usually does not produce specific or distinctive clinical signs. Also, the microcyst in the tissue of infected buffaloes cannot be detected by the routine meat inspection method. Hence in the present study antibody ELISA technique was used to determine the seroprevalence to *Toxoplasma gondii* in buffalo serum sample.

A total of 90 buffalo serum samples were screened for *Toxoplasma gondii* antibodies using a commercial TOXO-IgG ELISA kit. The calculated cut-off value was 0.19, and samples with OD values above this threshold were considered positive (Fig. 3). Out of 90 samples, 26 were positive, giving an overall seroprevalence of 28.8% (Table 2). The findings of the present study are consistent with the reports of Chhabra and Mahajan (1978) from Chhattisgarh and Deshpande (2012) from Mumbai who reported a seroprevalence of 23.7% and 30% in buffaloes respectively. Comparatively lower seroprevalence rates have been reported by Katam (2018) as 9.91%, Balamurugan *et al*. (2022) as 11.4%, Hamidinejat *et al*. (2010) as 14.33%, Fengcai *et al.* (2015) as 7.5%, Cuica *et al.* (2020) as 13.7%, and Selim *et al*. (2023) as 7.4%, at Punjab, Karnataka, Iran, China, Italy and Egypt respectively. Whereas a distinctly high prevalence of toxoplasmosis was also observed from different parts of the world by different authors such as Kuraa *et al*. (2016) reported 74.5% in buffaloes in Egypt, Santos *et al*. (2009) reported 71% in cattle in Brazil.

The observed variation in seroprevalence across different regions may be attributed to differences in environmental conditions. In addition, variations in grazing practices, density and access of cats to livestock premises, and differences in livestock management systems may significantly influence the level of environmental contamination. Cat is the principle definitive host for *Toxoplasma gondii* and it is a common practice that domestic cats are always intermix with the large ruminants and their farms which act as a source of infection for cattle and buffaloes.

T. gondii antibodies are less prevalent in buffaloes comparing with the other ruminants, suggesting buffaloes are more resistance to *T. gondii.* The seroprevalence of toxoplasmosis in buffaloes in India ranges between 10-24% (Dubey and Beattie 1988). In the present study the higher seroprevalence observed in the buffaloes than previous published reports may be due to fact that cats are widely distributed throughout the study area. The climatic condition in the study area is hot and humid because of the coastal region. A high prevalence of toxoplasmosis reported in the hot and humid environments in tropical areas is due to longer viability of *T. gondii* oocyst under the humid condition. (Fayer 1981). Rainfall is very important in transporting water borne pathogens in the terrestrial area and also transfer of the oocyst from land to water bodies leading to high level of water contamination and spread of water borne toxoplasmosis. Moist environment creates prolong survival of sporulated oocyst in the soil and grass for several months, thus increases the opportunity for buffaloes to become expose to the oocyst while grazing on pasture (Inpankaew *et al.* 2021).

Age-wise analysis revealed that buffaloes aged 2–5 years had a seroprevalence of 23.3% (7/30), while those above 5 years showed 31.7% (19/60) (Table 2). Although higher prevalence was observed in older buffaloes, the difference was statistically non-significant ($\chi^2 = 0.676$, $P = 0.713$). Similar observations have been reported by Inpankaew *et al.* (2021), who recorded higher seroprevalence in advance age groups. The high prevalence of *T. gondii* detected in the older animal may be explained by cumulative exposure, indicating high probability of exposure of oocyst throughout their life than the younger animals. In integrated farming system buffaloes are freely allowed to graze on contaminated grass land or water bodies as advancing age increases the likelihood of repeated contact with infective oocysts is a risk factor for *T. gondii* infection. Fengcai (2015) reported non-significant differences in the age wise prevalence of toxoplasmosis in buffaloes.

Table 2 - Age wise seroprevalence of Toxoplasma *gondii*

Age group	**No. of samples tested**	**No. positive**	**Prevalence (%)**	**χ^2 value**	**P value**	**Significance**
2–5 years	30	7	23.3	0.676	0.713	Non-significant
>5 years	60	19	31.7			
Overall prevalence	90	26	28.8			

A moderate seroprevalence of *T. gondii* in buffaloes was found in the study region compare to the other investigation from different parts of India reveling a potential risk for the humans from buffaloes. Oocyst contaminated water, soil and pasture are the source of the infection for the buffaloes. The high prevalence of toxoplasmosis in humans has been associated with the consumption of the raw or undercooked contaminated meat which highlight the epidemiological importance of present study in buffaloes in tropical countries like India that might be used as basis for disease management practices. There are reports stated that infected buffaloes milk showed presence of toxoplasma gondii DNA given that unpasteurized buffalo milk and meat when consumed inadequately cooked from infected animals is a potential source of human toxoplasmosis. Further research is needed to assess the risk for infection in humans associated with the ingestion of raw milk or undercooked meat from ruminants infected with *T. gondii.*

One of the limitation of present study could be due to the indirect diagnosis by ELISA tests that may show some level of cross-reactivity. The present study having the limitation such as discrepancy in sample size, lack of crucial information during sampling because of the abattoir study and host factors were not available for multifactorial analysis. Further research is necessary to elucidate the crucial risk factor, for *T. gondii* transmission in the buffaloes and their indirect effect on human health through beef consumption.

CONCLUSION

In the present study it has been determined that the occurrence of these zoonotic protozoan infections is still continued at a considerable level. In addition, variations in grazing practices, density and access of cats and dogs to livestock premises, may significantly influence the level of environmental contamination of oocyst of *Toxoplasma* and sporocyst of *Sarcocystis*. Domestic cats and dogs are always intermixing with the large ruminants and their farms which act as a source of infection for buffaloes.

The occurrence of these parasitic infections in buffaloes underscores the need for systematic epidemiological studies to design appropriate control strategies to safeguard animal health, ensure meat safety, and protect public health. Owing to the limitations of meat inspection advanced serological and molecular methods are recommended for detection of these meat borne parasite.

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Fig 1. Macroscopic sarcocyst in muscle

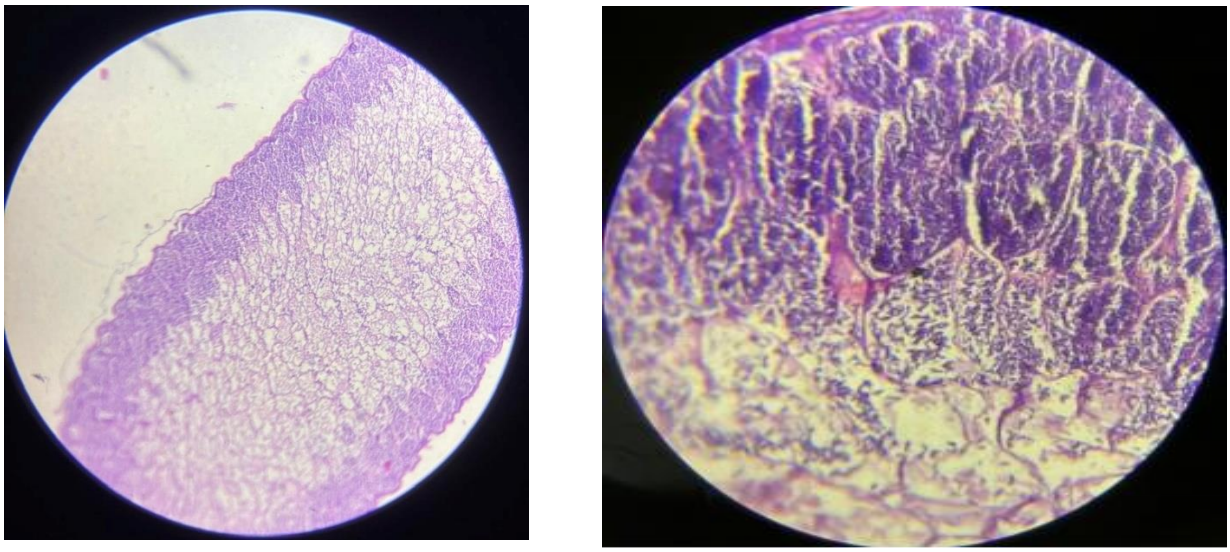

Fig 2. Microscopic sarcocyst in H/P section (L.S. and T.S.)

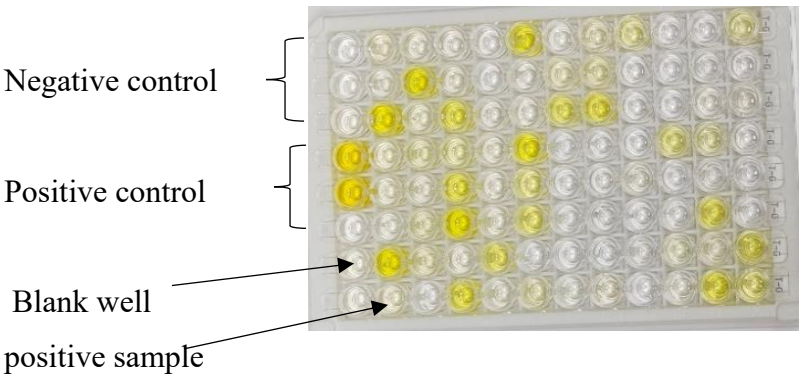

Fig. 3. Positive serum sample for *T. gondii* in ELISA plate