Indian J Anim Health (2025), 64(2) : 394 - 400
DOI: https://doi.org/10.36062/ijah.2025.15724

Research Article

Study on the occurrence of cryptosporidiosis in farm animals of Jaipur

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Abstract

Jaipur, the capital city of Rajasthan, India, is experiencing rapid urbanization and industrialization, with favourable meteorological conditions for growth of *Cryptosporidium* sp. infection. *Cryptosporidium* sp., a zoonotic parasite, poses significant economic and health challenges, particularly prevalent in low-middle-income countries. Between October 2023 and March 2024, a total of 1308 faecal samples were collected from cattle, buffaloes, sheep, and goats across various locations *viz.* Jaipur city, Bassi, Chaksu, Phagi, Phulera, Renwal, Chomu, Shahpura, Kotputli, and Jamwa Ramgarh with nearly 5 L of water samples from respective places of the study area and were stained using mZN stain. Microscopic analysis of faecal samples revealed a 24.39% prevalence rate, with cattle exhibiting the highest (43.14%) infection rate, followed by buffaloes (28.91%), goats (15.34%), and sheep (07.19%). Logistic regression analysis indicated increased odds of infection in buffaloes (OR=2.245) and cattle (OR=4.189) compared to goats. Regional analysis within Jaipur revealed prevalence rates ranging from 16.30% to 34.97%, with Phulera exhibiting the highest prevalence and Jamwa Ramgarh the lowest. Positive associations with Chaksu and Phulera and negative associations with other regions were observed. Water samples also revealed traces of infection in one out of ten locations. This study sheds light on the complex dynamics of *Cryptosporidium* sp. infection within Jaipur's livestock population and underscores its implications for public health and veterinary management

Keywords: *Cryptosporidium* sp., Faecal samples, Modified Ziehl-Neelsen stain, Prevalence, Water

Highlights

- High prevalence (24.39%) of *Cryptosporidium* sp. found in livestock (cattle, buffaloes, sheep, goats)
- Highest infection found in cattle (43.14%), followed by buffaloes, goats and sheep
- Geographical risk with Phulera area showed the highest infection rate among studied locations
- Oocysts found in Jaipur city water samples, indicating environmental contamination
- Infection poses significant risk to humans, especially immunocompromised individuals.

INTRODUCTION

Cryptosporidium sp. is a zoonotic protozoan parasite that infects a variety of hosts, including humans, domestic and wild animals, worldwide (Ryan et al., 2016). Cryptosporidiosis is induced by ingestion of sporulated oocysts (round, thick-walled cyst, 5 µm in size and contain 4 sporozoites) through food and water contaminated by faecal materials. The sporozoites invade the epithelial cells of intestine and develop into schizonts. Some merozoites produced by schizogony continue repeated schizogonic cycle, while others follow gametogony, resulting in the production of oocysts. It is mainly diagnosed by microscopy, especially in low-middle-income countries (Sharma et al., 2023). Cryptosporidiosis is difficult to control because the oocysts are stable in the environment, resistant to standard methods of disinfection, and their small size (<5 µm) makes them difficult to efficiently remove from water by filtration. Over 40 species of *Cryptosporidium* described to date, of which four (*C. parvum*, *C. bovis*, *C. ryanae* and *C. andersoni*) are routinely reported in cattle (Guy et al., 2022). Cryptosporidiosis, mainly associated to calves with a wide range of clinical signs from no apparent ill effects to severe morbidity, including poor performance but has also been reported in adult animals worldwide (Fayer et al., 2010). It is known to cause huge production losses in bovines (Olson et al., 1997), and are considered potential sources of human infection as well, with pre-weaned calves and lambs recognized as important reservoirs of the zoonotic *C. parvum* in some countries (Van Kessel et al., 2011). A recent study has

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reported a €40 1 (₹3,654.68) loss per calf due to cryptosporidiosis and could save € 15.0 (₹1336.43) by applying strategic managemental practices (Roblin et al., 2023). *Cryptosporidium* sp. are the most common agents of infectious enteritis in humans and animals worldwide. From a public health perspective, it is imperative to understand the sources and routes of transmission in different geographical regions. Surface and ground water contamination with faecal pathogens is a public health concern especially in low-income settings where these sources are used untreated. *Cryptosporidium* sp., widely distributed protozoan parasites of vertebrates have recently attracted increasing interest due to several serious waterborne outbreaks, the life-threatening nature of infection in immunocompromised patients, and the realization of economic losses caused by these pathogens in livestock.

In India, bovine Cryptosporidiosis was reported from several states (Swain et al., 2019; Das et al., 2020). Profuse watery diarrhoea is the primary clinical sign, contributing to high mortality rates, which may lead to economic losses (De Graaf et al., 1999; Chako et al., 2010). Infected hosts can shed huge numbers of oocysts per day, which are immediately infective to other susceptible hosts (Zambriski et al., 2013) indicating that infection can pass very quickly between animals kept in close contact to one another. Reports of infection in sheep and goats are also available from parts of India (Bhat et al., 2019) along with its risk of transmission to human population (Utaaker et al., 2017). As regards to Rajasthan, only a few studies on cryptosporidiosis on bovines (Monika et al., 2022) and goat kids (Shahnawaz et al., 2011) have been reported. However, there is severe dearth of studies on the prevalence status of the disease in this part of the country. Keeping these facts in mind the present study has been planned to assess the point prevalence of the domesticated animals along with the transmission and establishment of the infection in the region. The present study will help to understand the dynamics of *Cryptosporidium* sp. and to draw effective control strategies against the infection in the region.

MATERIALS AND METHODS

Study area: A total of 1308 faecal samples were collected from the collecting sites, *viz.* Jaipur city, Bassi, Chaksu, Phagi, Phulera, Renwal, Chomu, Shahpura, Kotputli, and Jamwa Ramgarh regions, with roughly 300–350 of each animal species, depending on the number of animals presentation the farm, between October 2023 and March 2024.

Sampling: Samples from cattle (350), buffaloes (339), sheep (306), and goat (313) populations as well as water samples (about 5 L) from different water sources at each collecting locationfor the detection of the oocysts.

Parasitological techniques: For the purpose of screening for *Cryptosporidium* sp. infection with a few minor adjustments, the faecal samples collected were subjected to modified Ziehl-Neelsen method as per protocol followed by Fayer and Xiao (2007) with the slight modifications.

Statistical analysis: Risk assessment of area wise distribution of the infection in the region was done using multivariate binary logistic regression.

RESULTS

With an overall prevalence of 24.39%, microscopic analysis using modified Ziehl-Neelsen stain showed (Fig. 1a, Fig. 1b) pinkish or red clusters of tiny ovals to circular oocysts with thick transparent outer wall and

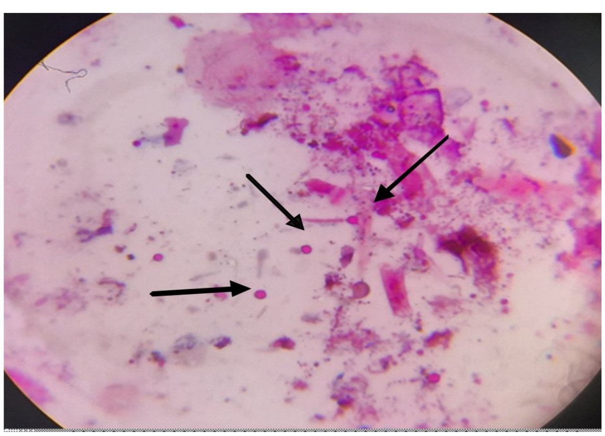

Fig. 1a

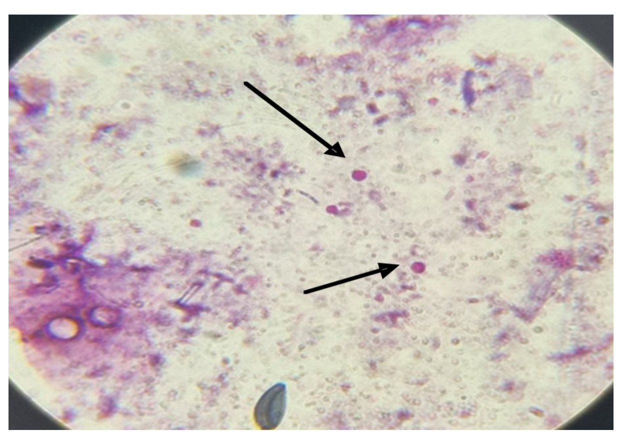

Fig. 1b

Fig. 1. mZN stained oocysts of *Cryptosporidium* sp. in the faecal samples (40X)

inner body, ranging between 3-5 μm, in 319 out of 1308 faecal samples. The oocysts of *Cryptosporidium* sp. were also found to range in size from 3.52 to 4.21 X 4.82 μm to 5.45 μm, with an average of 3.51±1.30 μm X 4.12±1.24 μm. The shape index of *Cryptosporidium* sp. oocysts was determined to be 1.25 μm.

Animal sp. wise prevalence: Among the various animal species involved in the study the prevalence of *Cryptosporidium* sp. infection was reported to be the highest in cattle population (43.14%) of the region with statistically highly significant difference ($p<0.01$, $\chi^2=133.792^{**}$) followed by buffaloes (28.91%), goat (15.34%) and lowest in sheep (07.19%) as shown in Table 1. Risk assessment among various livestock components using binary logistic regression analysis for *Cryptosporidium* sp. infection revealed a negative association with sheep (B=-0.849) as compared to goat population i.e. odds for infection were decreased by 57% (OR=0.428). Whereas, a positive association was revealed in buffaloes (B=0.809) and cattle (B=1.433) i.e. the risk of *Cryptosporidium* infection was 2.2 times (2.245) and 4.2 times (OR=4.189) respectively, higher when compared to goat sp. (Table 2).

Area wise prevalence and risk assessment: The *Cryptosporidium* infection among different farm animals was observed throughout the study area i.e. in 10 different locations of Jaipur city with a range of 16.30% to 34.97% (Table 3). Area wise *Cryptosporidium* infection was detected highest in Phulera area (57/163) with prevalence of 34.97% followed by Kotputli (30.23%), Phagi (27.27), Shahpura (22.68), City (23.22), Chomu (20.62), Renwal (17.70), Bassi 17.34) and lowest in Jamwa Ramgarh (22/135) with prevalence of 16.30% with a highly significant difference of $p<0.01$, $\chi^2= 32.37^{**}$ as shown in Table 3. Risk assessment of area wise distribution of the infection in the region using multivariate binary logistic regression revealed positive association with area Chaksu and Phulera, as compared to Kotputli (Odds ratio<1) i.e. odds for infection were increased by 1.2 times (OR=1.206) and 1.24 times (OR=1.241) respectively. Whereas, a negative association was revealed in Jamwa Ramgarh, Bassi, Chomu, Renwal, Shahpura, Jaipur city and Phagi, i.e. the risk of *Cryptosporidium* infection was 55% (OR=0.449), 52% (OR=0.484), 50% (OR=0.496), 40% (OR= 0.599), 32% (OR= 0.677), 30% (OR= 0.698) and 13% (OR=0.865) respectively decreased as compared to Kotputli with complete details in Table 4.

Table 1. Animal sp. wise prevalence of *Cryptosporidium* sp.

Animal sp.	Examined	Infected	Prevalence (%)
Cattle	350	151	43.14
Buffalo	339	98	28.91
Sheep	306	22	07.19
Goat	313	48	15.34
χ^2		133.792**	
Total	**1308**	**319**	**24.39%**

Table 3. Area wise prevalence of *Cryptosporidium* sp. infection

Area	Examined	Infected	Prevalence (%)
City	211	49	23.22
Chaksu	134	46	34.33
Bassi	173	30	17.34
Jamwa Ramgarh	135	22	**16.30**
Phagi	99	27	27.27
Phulera	163	57	**34.97**
Renwal	113	20	17.70
Chomu	97	20	20.62
Shapura	97	22	22.68
Kotputli	86	26	30.23
Chi-Square Statistic		32.367**	
Total	**1308**	**319**	**24.39%**

Table 2. Binary logistic regression analysis among sp.

Animal sp.	Logistic regression coefficient (B)	Std. Error	Wald	df	p value	odd ratio
	-1.709	0.157	118.628	1	0.000	
Cattle	1.433	0.190	56.602	1	0.000	4.189
Buffalo	0.809	0.197	16.786	1	0.000	2.245
Sheep	-0.849	0.271	9.805	1	0.002	0.428
Goat (constant)	0^b			0		

*Significant (p=0.05) **Highly Significant (p=0.01)

Analysis of water samples: The study reported morphologically similar oocysts only from the water samples of Jaipur city area among the ten collection sites in the study area (Fig. 2a, Fig. 2b).

DISCUSSION

Cryptosporidium sp. is a significant intestinal parasite (Yadav, 2017). The Modified Ziehl-Neelsen (mZN) staining method (Henriksen & Pohlenz, 1981) is widely employed as the gold standard for detecting *Cryptosporidium* sp. oocysts in faecal samples (Kali, 2014), oocysts appear pinkish, oval to round in shape (Nabi et al., 2023), with a thick outer wall. Primary diagnostic method remains Microscopy for *Cryptosporidium* sp. identification, especially in low-middle-income countries (Sharma et al., 2023). The microscopic appearance of the *Cryptosporidium* sp. using modified Zeihl-Neelsen staining is similar to the standard keys (Taylor et al., 2007; Paniker & Ghosh, 2017) and is in agreement to the findings of Mphephu et al. (2021), Monika et al. (2022). Further, the micrometric dimensions were in range as per the standard dimensions (Taylor et al., 2007) and are in congruent of the findings of Fayer (2004), Sahu et al. (2010), and Bhanja et al. (2023). In various livestock animals worldwide, the prevalence rate of Cryptosporidiosis infections varies greatly, ranging from 0.5% (Utaaker et al., 2017) to 65% (Ahamed et al., 2015). The current study's total prevalence rate of 24.39% was consistent with the findings of Indian studies by Brar et al. (2017), and Agrawal et al. (2018). However, a number of researchers have documented varying incidence rates worldwide, including in India and Rajasthan (Monika et al., 2022; Kuraa & Malek, 2023).

The variation in the prevalence rates may be attributed to the different topographic locations of study area, sample size, managemental and animal husbandry practices, nutritional management, immune status of animals, Veterinary care as well as awareness of the livestock owners (Hatam- Nahavandi et al., 2019; Golomazou et al., 2024). The present study has presented the prevalence of infection in all the components of livestock of the region that is cattle, buffalo, sheep and goat. Various researchers have reported the infection in different livestock animals

Table 4. Area wise risk assessment using binary logistic regression analysis

Area	Logistic regression coefficient (B)	Std. Error	Wald	df	p value	odd ratio
	-0.836	0.235	12.685	1	0.000	
City	-0.360	0.286	1.582	1	0.208	0.698
Chaksu	**0.188**	0.297	0.399	1	0.528	**1.206**
Bassi	-0.725	0.309	5.513	1	0.019	0.484
Jamwa Ramgarh	-0.800	0.331	5.850	1	0.016	0.449
Phagi	-0.145	0.326	0.197	1	0.657	0.865
Phulera	**0.216**	0.287	0.568	1	0.451	**1.241**
Renwal	-0.701	0.340	4.236	1	0.040	0.496
Chomu	-0.512	0.344	2.218	1	0.136	0.599
Shapura	-0.390	0.338	1.337	1	0.248	0.677
Kotputli (constant)	0^b			0		

*Significant (p=0.05) **Highly Significant (p=0.01)

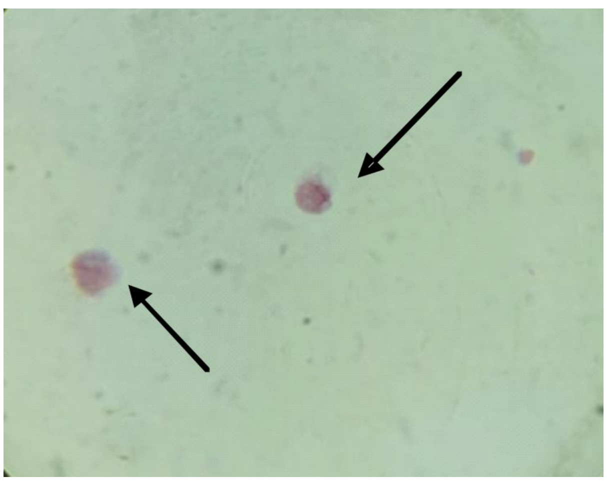

Fig. 2a

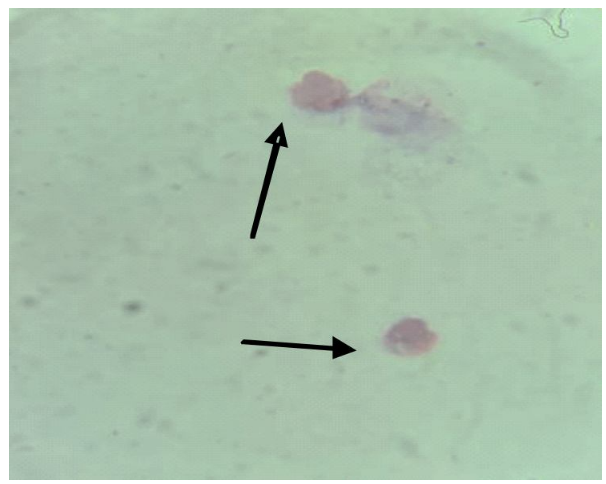

Fig. 2b

Fig. 2. mZN stained oocysts of *Cryptosporidium* sp. (100X) in the water samples

throughout the world *viz*. Monika et al. (2022), and Kuraa and Malek (2023). Variations in the socioeconomic position of owners, animal rearing methods, and the nutritional health of the animals may be the cause of the highly significant differences in infection prevalence rates among the various animal species included in the study (Maharana et al., 2016; Utaaker et al., 2017). According to risk analysis, the region's cattle population is most at risk of infection, which can be linked to inadequate pastures for nutritional input, relatively poor management practices, and a lack of veterinary care for the species (Maharana et al., 2016; Sharma et al., 2023). Prior reports of the infection from goat kids and dairy of the state came have been recorded by Shahnawaz et al. (2011) and Monika et al. (2022), respectively. The current investigation, however, has shown that all of the livestock components from various study region locations had a significant viral prevalence.

Area wise risk analysis revealed Phulera at the highest risk of infection when compared to Kotputli with a highly significant difference in the prevalence rates of Cryptosporidiosis infections in different locations of the study area may be due to the difference in the urban and rural animal raising of the animals as well as veterinary care (Fletcher et al., 2012; Utaaker et al., 2017; Golomazou et al., 2024). Various workers have reported significant difference within the zone wise analysis of gastrointestinal helminthic infections from the state (Faran et al., 2024; Kumari et al., 2024).

Cryptosporidium sp. is a parasitic protozoan that can cause gastrointestinal illness in humans and animals, commonly transmitted through contaminated water or food (Ahmed & Karanis, 2020; Golomazou et al., 2024). *Cryptosporidium* sp. oocysts, which are the dormant form of the parasite, can be present in various water sources such as lakes, rivers, streams, ponds, and even swimming pools and water parks. These oocysts are resistant to chlorine (Paudyal et al., 2013), the common disinfectant used in water treatment plants, making them particularly challenging to eradicate. The primary source of *Cryptosporidium* sp. in water is through the feces of infected humans or animals. People can become infected by ingesting contaminated water or food, through drinking untreated or inadequately treated water, consuming raw or undercooked food washed with contaminated water, or swallowing water while swimming in contaminated recreational water sources. Traces of morphologically similar oocysts to the findings of Vesey and Slade (1991) and Utaaker et al. (2017), have been reported in Jaipur city water samples, indicating the possibilities of contamination Zahedi et al. (2020), however, the molecular confirmation is highly recommended. The results of this investigation showed that the environment of the study area is favourable for the spread of the infection (Singh et al., 2018; Mphephu et al., 2021).

The study shows a considerable overall prevalence of *Cryptosporidium* sp. infection (24.39%) in the livestock of the Jaipur area. The risk is statistically significant ($p<0.01$) higher in cattle (OR=4.189) and buffaloes (OR=2.245) than in goats, and lower in sheep (OR=0.428). Multivariate binary logistic regression was used to estimate spatial risk and found that Jamwa Ramgarh had the lowest prevalence (16.30%, OR=0.449), while Phulera (34.97%, OR=1.241) and Chaksu (34.33%, OR=1.206) were epidemiologically critical zones. The finding of morphologically similar oocysts in Jaipur city's municipal water raises the possibility of environmental contamination and the pathogen's ability to spread through water. These findings highlight the necessity of molecular confirmation, ongoing monitoring, focused herd-level interventions, and strict water treatment procedures in order to reduce zoonotic risk and the resulting financial losses in the area.

Conflict of interest: Authors declare that they do not have any conflict of interest in the study.

Author's contribution: NK: Conducted, carried out the experiments and statistical analysisof the research for the manuscript; AG: Design and supervised for manuscript drafting; KK: Sample collection and data compilation for the research. All authors have considerably contributed for the conduction of research and preparation of the manuscript.

Data availability statement: The data mentioned in the article will be available with the corresponding author.

Use of artificial intelligence tools: During the preparation of this work, the author used QuillBot (free version) to paraphrase the written content. After using this tool, the author reviewed and edited the content as needed and take full responsibility for the content of the publication.

ACKNOWLEDGEMENT

The office of Dean, College of PGIVER, Jaipur for the providing the necessary facility for the project and research.

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Received - 27.11.2024, Accepted - 18.08.2025, Published - 14.09.2025 (Online), 01.12.2025 (Print)
Section Editor: Dr. Suman Biswas, Editorial Board Member