RESEARCH ARTICLE

Prevalence, Molecular Characterization and Public Health Importance of *Cryptosporidium* spp. in Tirupati Region of Andhra Pradesh

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Abstract

Diarrheal diseases are a leading cause of illness and death in children under five years old around the world. *Cryptosporidium* is one of the most commonly found parasites that cause gastrointestinal illness in humans and animals. The present study was undertaken to determine the prevalence of *Cryptosporidium* spp. and to compare the diagnostic performance of modified Ziehl-Neelsen (mZN) staining with PCR for detection of *Cryptosporidium* spp. In addition, the study also aimed to assess the genetic diversity and evaluate the zoonotic transmission potential of *Cryptosporidium* spp. circulating in the Tirupati region. Out of 2000 samples, *viz.,* 1000 cattle faecal samples and 500 each of human stool samples and water samples screened, modified ZN staining detected 225 positive samples, whereas nested PCR confirmed 246 positives for *Cryptosporidium* spp. When compared with PCR, mZN staining demonstrated a sensitivity of 90.65 %, specificity of 99.89 % and an overall accuracy of 98.75 %. The overall prevalence of *Cryptosporidium* detected by mZN staining was 15.9 %, 5.6 % and 7.6 % in cattle faecal, human stool and water samples, respectively. In comparison, nested PCR detected higher positivity rates of 17.3 %, 6.2 % and 8.4 % in cattle faecal, human stool and water samples, respectively. Phylogenetic analysis of sequenced isolates revealed that the majority belonged to *C. parvum* (n=7), followed by *C. hominis* (n=2) and *C. ryanae* (n=1). Detection of zoonotic species, particularly *C. parvum*, indicates the potential for zoonotic transmission of *Cryptosporidium* spp. in and around Tirupati.

Keywords: Cattle, Cattle handlers, *Cryptosporidium*, Faecal samples, PCR, Phylogenetic analysis, Risk factors.

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Introduction

Cryptosporidium, a coccidian parasite, is the most common zoonotic protozoa infecting a wide range of vertebrates including humans. It causes diarrheal disease in both animals and humans, with significant morbidity and mortality among humans in developing as well as developed countries (Khan *et al*., 2010). Amongst animals, cattle are the major reservoirs for *Cryptosporidium* spp. (Zhao *et al.,* 2014) that are generally host-specific. The most common *Cryptosporidium* species found in humans are *Cryptosporidium hominis* and *Cryptosporidium parvum*, followed by *Cryptosporidium meleagridis*, *Cryptosporidium felis* and *Cryptosporidium canis*, while *Cryptosporidium parvum*, *Cryptosporidium bovis*, *Cryptosporidium ryanae* and *Cryptosporidium andersoni* are the major disease-causing species in cattle (Khan *et al*., 2010). This disease transmission occurs via the faecal-oral route, either by direct contact with contaminated faecal samples from an infected host or indirectly through contaminated water or food (Das *et al*., 2019). Hence, the risk of cryptosporidiosis is high in individuals who are in close proximity to infected animals, precisely cattle handlers, veterinarians, animal researchers, children attending camps and fairs and other persons working in low hygiene areas. Though the disease is self-limiting in immunocompetent individuals, but it is life-threatening or fatal to malnourished and immunocompromised individuals like HIV, AIDS and TB patients (Kurniawan *et al*., 2013).

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Even though, the disease has been considerable interest in recent years in transmission, but there is very little information known about the genetic diversity and zoonotic transmission of *Cryptosporidium* spp. in cattle and persons working at dairy farms in India, especially in Andhra Pradesh. Unless like other infectious enteritis causative agents, no effective vaccines and drug-based intervention strategies are available for cryptosporidiosis (Das *et al*., 2019). Hence, control measures should be focused on this disease

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prevention, by identifying infection reservoirs and mode of transmission. Genetic characterization of *Cryptosporidium* isolates obtained from cattle, humans and environmental sources may determine the mode of transmission in the respective areas (Xiao, 2010). Hence, the present study was undertaken to study the prevalence of cryptosporidiosis among animals, human beings and environmental (water) samples by staining and PCR techniques for the detection of *Cryptosporidium* and to know the risk factors and zoonotic potential of cryptosporidiosis in Tirupati region of India by comparing the prevalence in both animals and humans.

Materials and Methods

The study was conducted following ethical clearance from SV Medical College and Institutional Animal Ethics Committee (IAEC) of College of Veterinary Science, SVVU, Tirupati (India) with proposal no. 281/go/ReBi/S/2000/CPCSEA/ CVSc/ TPTY/032/VPHE/2024 dated 07.05.2024

Collection of Samples

The study was carried in the Department of Veterinary Public Health and Epidemiology, College of Veterinary Science, Tirupati during the period from August 2024 to February 2026 under the project sanctioned by ICMR-MRHRU. A total of 1000 cattle faecal samples were collected from various farms located in Tirupati district. These samples were directly collected from the rectum of cattle with a gloved hand hygienically and transferred to laboratory in to sterile insulated containers containing ice packs. Simultaneously, a total of 500 stool samples were collected from workers of these farms who were in direct or indirect contact with animals. In addition, a total of 500 water samples were collected from different water bodies located in and around farms, the water used for disposal of farm waste and waste water. All the samples collected were transferred to 4°C and were processed within 1 to 3 days of collection. Three aliquots of each sample were frozen without preservative in 1.5 mL cryovials at -80°C for staining and PCR.

Processing of Samples for Microscopic Examination

Initially, the faecal and stool samples were smeared on a clean, grease-free glass slides and allowed to dry. The dried smears were stained with modified Ziehl-Neelsen (mZN) stain and examined under a compound microscope at 40X and 100X magnifications, and an identified single oocyst was even considered positive.

In case of water samples, each sample was initially filtered through 40 µm mesh sieve followed by filtration through a 1 µm polyester filter bag (River soft). Then, the filtered sample was mixed with 1% Tween 20 in a 50 mL tube and centrifuged at 1500 x g for 30 min, repeated twice with distilled water for washing. The samples were then concentrated using a flotation method with a saturated salt solution (Sharifuzzaman *et al*., 2015). A drop of the concentrated sample was placed on a microscope slide, stained using the modified Ziehl-Neelsen method, and examined under a microscope at 40X and 100X magnification. The positive samples were stored at 4°C for subsequent DNA extraction.

DNA Extraction and Nested PCR

Genomic DNA was isolated from all frozen faecal samples using the QIAamp DNA Stool Mini Kit (QIAGEN) as per the manufacturer's instructions. From the water samples, DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega, Madison, USA) as per manufacturer's protocol.

For molecular confirmation of *Cryptosporidium* species, 18S rRNA nested PCR was performed using specific primers as described by previous researchers (Xiao *et al*., 1999) with some modifications. The primers used with amplification size are listed in Table 1. The PCR was rum with a 25 µL PCR mixture consisting of 12.5 µL PCR master mix, 1 µL of each primer (200 nM), 2 µL of DNA sample and the remaining 8.5 µL of nuclease free water. The templates were subjected to an initial denaturation at 94°C for 3 min, followed by 35 amplification cycles (94°C for 45s, 59°C for 45s, 72°C for 60s), followed by a final extension at 72°C for 7 min. The secondary PCR protocol included an initial denaturation at 94°C for 3 min, followed by 35 amplification cycles (94°C for 30s, 58°C for 90s, 72°C for 2 min) followed by a final extension at 72°C for 7 min. All the PCR products were analysed using 1.5% agarose gel electrophoresis and visualized after ethidium bromide staining.

DNA Sequencing and Phylogenetic Analysis

Ten *Cryptosporidium* isolates obtained were selected randomly and sent to Barcode Biosciences, Bangalore for sequencing to know the species and their origin. Each DNA sequence obtained was identified and verified manually by using the software Gene tool and compared with the GenBank database. The evolutionary history of *Cryptosporidium* was inferred by aligning reference sequences from the GenBank with representative sequences from each species obtained through sequencing. All sequences were aligned using the ClustalW

Table 1: Primers used in the PCR protocol

	Primers	Primers sequence	Amplicon size (bp)
External	Forward (1F)	5'- TTC TAG AGC TAA TAC ATG CG-3'	834 bp
	Reverse (1R)	5'- CCC ATT TCC TTC GAA ACA GGA-3'	
Internal	Forward (2F)	5'- GGA AGG GTT GTA TTT ATT AGA TAA AG-3'	
	Reverse (2R)	5'- AAG GAG TAA GGA ACA ACC TCC A-3'	

technique in MEGA 7.0 software with several alignment modifications made manually to eliminate fictitious gaps. The bootstrap consensus trees that were derived from 1000 replicates were retained for analysis. Each node of the tree indicates the proportion of replicate trees where the related taxa clustered together in the bootstrap test, with a threshold of more than 50%. For the analysis, MegAlign, a program from DNA Star, was used to conduct nucleotide alignment for the pairwise identity matrix among various species of *Cryptosporidium* found in their respective host animals.

Survey through Questionnaire

To investigate the risk factors associated with the occurrence of cryptosporidiosis in the Tirupati district, a semi-structured questionnaire was constructed with the information related to individual animal characteristics, herd characteristics, housing characteristics, biosecurity measures, dairy practices, disinfection practices followed, animal handlers' behaviour, and detailed history of animal as well as owner. The respondents of this survey were randomly selected from animal handlers. They were personally interviewed in order to get their responses while collecting the samples.

Statistical Analysis

Data was analysed using SPSS for windows version 16.0. The impact of every risk factor on the probability of contracting *Cryptosporidium* infection was evaluated using binary logistic regression analysis, and the odds ratio, which is calculated as the exponent of the corresponding regression coefficient, was used to quantify the results. The significance level was set at $p < 0.05$, while the confidence level was maintained at 95%.

Results and Discussion

The modified Ziehl-Neelsen (mZN) stained *Cryptosporidium* oocysts appeared spherical to ellipsoidal, pink to bright red stained bodies with clear refractive halo against pale green background. By the mZN staining, *Cryptosporidium* was detected in 159 cattle faecal samples (15.9%), 28 human stool samples (5.6%) and in 38 water samples (7.6%) (Table 2). The prevalence observed in the present study was comparable with the findings of Sateesh *et al.* (2022), who reported a positivity rate of 14.63% in calf samples from Karnataka. In contrast, Maurya (2016) reported a lower positivity of 8.8% prevalence, whereas Bhanja *et al.* (2023) recorded a higher prevalence of 20.9%. The variations in prevalence rates across different studies may be attributed to differences in geographical location, breed susceptibility, management and husbandry practices, age groups, nursing conditions, seasonal variations, and farm hygiene. Additionally, methodological differences, including sample size and diagnostic techniques employed, may also contribute to the observed discrepancies in prevalence rates.

The nested PCR was positive in 173 cattle faecal samples (17.3%), 31 human stools (6.2%) and 42 water samples (8.4%) collected from various dairy farms in and around Tirupati (Table 2). These findings suggest that PCR is a more sensitive diagnostic tool than conventional microscopy for detecting *Cryptosporidium*, particularly in environmental samples like water, where low oocyst concentrations may hinder microscopic detection. The current overall results concurred with Das *et al.* (2026) from Tripura and Khan *et al.* (2010) from West Bengal, who reported 16% and 11.7% positivity rate, respectively. Other studies have reported comparable prevalence rates, including 22% in calves and 19% in dairy farm workers as documented by Das *et al.* (2019), and 30.2% in calves as reported by Paul *et al.* (2008), indicating a consistently high burden of infection across both animal and human populations.

Table 2: Prevalence of *Cryptosporidium* in various types of samples

Type of sample	Samples screened	Diagnostic test employed	
		mZN staining	PCR
Cattle faecal samples	1000	159 (15.9%)	173 (17.3%)
Human stool samples	500	28 (5.6%)	31 (6.2%)
Water samples	500	38 (7.6%)	42 (8.4%)

Considering PCR as the gold standard, the modified Ziehl-Neelsen (mZN) staining technique detected 223 of the 246 PCR-confirmed positive samples and correctly identified 1752 of the 1754 PCR-negative samples. The diagnostic performance of mZN staining revealed a sensitivity of 90.65%, specificity of 99.89%, positive predictive value of 99.11%, negative predictive value of 98.70%, and an overall accuracy of 98.75%. These findings demonstrate a high level of agreement between mZN staining and PCR, indicating that mZN is a reliable and effective screening tool for the detection of *Cryptosporidium* infection, particularly in routine diagnostic laboratories where molecular techniques may not be readily available.

The modified Ziehl-Neelsen (mZN) staining technique demonstrated excellent diagnostic performance when compared with PCR for the detection of *Cryptosporidium* spp. The sensitivity of 90.65% indicates that mZN successfully identified the majority of PCR-confirmed positive samples, although 23 infections were missed. The very high specificity (99.89%) shows that the test rarely produced false-positive results, with only two samples incorrectly classified as positive.

The positive predictive value (99.11%) indicates that most samples identified as positive by mZN staining were truly positive, while the negative predictive value (98.70%) shows that most negative samples were truly negative. The overall accuracy of 98.75% demonstrates strong agreement between mZN staining and PCR. These results indicate that mZN staining is a simple, rapid, and cost-effective method for routine screening of *Cryptosporidium*, especially in resource-limited laboratories. However, PCR is more sensitive and can detect low-level infections that may be missed by microscopy.

The present findings were in agreement with earlier reports that recorded high specificity and good sensitivity of modified Ziehl-Neelsen (mZN) staining for the detection of *Cryptosporidium* oocyst when compared with PCR. Previous studies also reported that mZN staining is a rapid, economical and reliable diagnostic method suitable for routine laboratory screening, particularly in resource-limited settings, although PCR remains more sensitive for detecting low–intensity infections and confirming species identity (Chalmers *et al*., 2011, Aghamolaie *et al*., 2016).

Ten SSU rRNA gene amplicons of *Cryptosporidium* spp. were sequenced. The results showed that *C. parvum* was the most common (7 samples), followed by *C. hominis* (2 samples) and *C. ryanae* (1 sample). The sequences are available in GenBank under accession numbers PQ650926 to PQ650967. Using MEGA software for phylogenetic analysis, the study isolates were found to cluster with global reference isolates, indicating low genetic differences among samples from various regions (Table 3). Specifically, *C. parvum* was closely related to strains from the USA, Egypt, Spain, China, Brazil, Iran, Turkey, and India (Fig. 1). The *C. ryanae* isolate also showed similarity to sequences from China, Brazil, and Bangladesh. Human-derived isolates of *C. hominis* clustered with reference sequences as well, confirming their identity and showing similarities with isolates from Punjab, Sweden, Iran, Turkey, Zambia, Slovenia, Tenerife, and Kenya (Fig. 2). Overall, the analysis indicates that *Cryptosporidium* spp. exhibit a high degree of genetic conservation and close relationships across different geographical areas. This genetic relatedness supports the zoonotic importance of *Cryptosporidium*, highlighting the role of livestock and contaminated environmental sources as potential reservoirs for human infection. These findings also suggest that both zoonotic and anthroponotic transmission of *Cryptosporidium* may occur in and around Tirupati, emphasizing the public health significance of proper animal management, sanitation, and safe water practices to reduce the risk of transmission.

Table 3: Speciation of *Cryptosporidium* in dairy cattle and humans through DNA sequence analysis of various isolates, along with their accession numbers

Isolate number	Location	Host	*Cryptosporidium* spp.	Accession Number
A11	Tirupati	Humans	*C. hominis*	PQ650926
C16	Tirupati	Cattle	*C. parvum*	PQ650930
D18	Vadamalapeta	Cattle	*C. parvum*	PQ650936
E23	Gajulamandyam	Cattle	*C. parvum*	PQ650938
F26	Srikalahasti	Cattle	*C. parvum*	PQ650942
G30	Kota	Humans	*C. hominis*	PQ650963
H41	Chinnagottigallu	Cattle	*C. parvum*	PQ650964
I42	Rayalacheruvu	Cattle	*C. ryanae*	PQ650965
J44	Peruru	Cattle	*C. parvum*	PQ650966
L422	Reningunta	Cattle	*C. parvum*	PQ650967

A risk factor analysis was conducted using questionnaires from animal owners to examine the factors associated with *Cryptosporidium* infection. The risk factors were divided into four categories: animal-level, housing, biosecurity, and owner-related variables. The results showed that calves under one month had the highest infection rate at 21.96%. Female cattle had a slightly higher infection rate (20.49%) compared to males (18.34%). Descript breeds were also more affected (21.46%) than non-descript breeds (16.66%). Cryptosporidium was found more often in diarrhoeic faecal samples (20.77%) than in non-diarrhoeic samples (18.05%). In terms of farming practices, cattle in intensive systems had a higher infection rate (22.06%) than those in free-range systems (15.94%), though this was not statistically significant. Farms using groundwater for drinking had a higher prevalence (23.06%) compared to those using surface water (14.94%). Poor waste management practices, such as inadequate faeces disposal (28.72%) and farm wastewater management (29.31%), were significantly linked to higher infection rates. At the owner level, illiterate individuals and those involved in agriculture had a higher prevalence of infection, as did those living close to household animals. A Chi-square test identified factors with a value over 2.483 as having a significant association with disease.

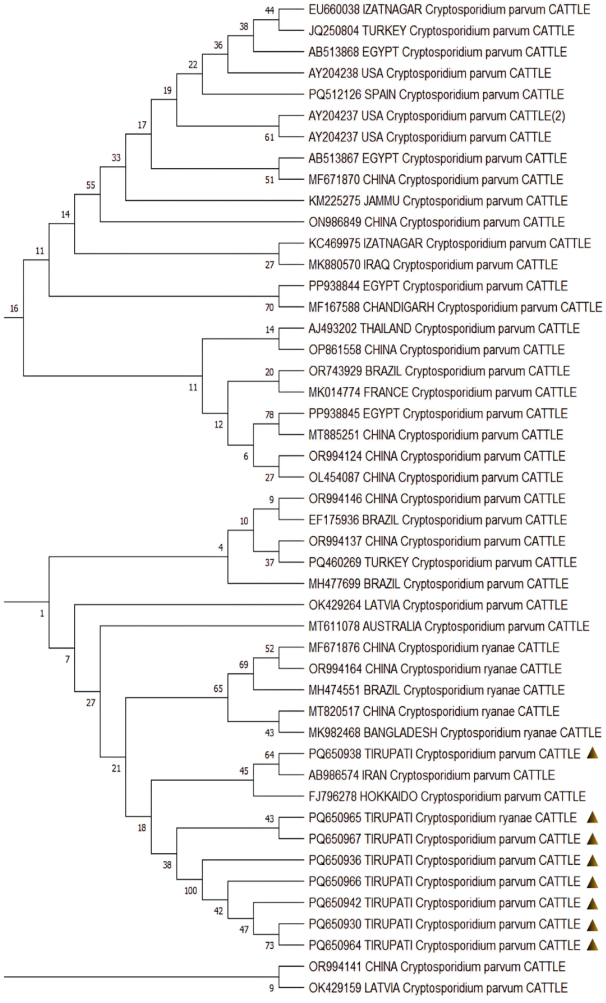


Fig. 1: The phylogram of *Cryptosporidium* Spp. inferred based on the nucleotide sequences of SSU rRNA gene. Bootstrap values are indicated on the branches. The evolutionary analyses are conducted in MEGA X. The *Cryptosporidium* isolates identified in this study are pointed with arrow marks.

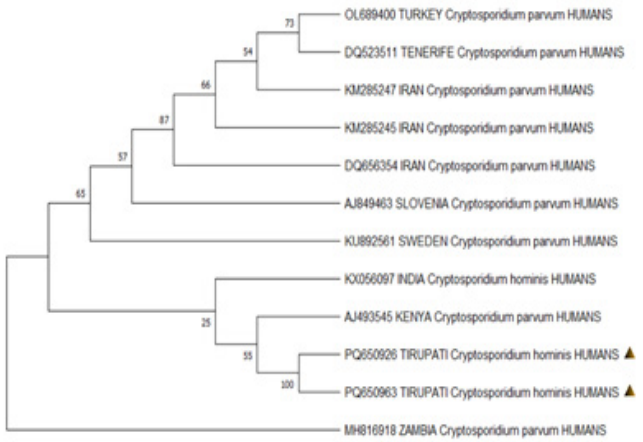


Fig. 2: Phylogenetic relationship between *Cryptosporidium parvum* and *C. hominis* was inferred based on the nucleotide sequences of SSU rRNA gene. Bootstrap values are indicated at each node. The *Cryptosporidium* isolates identified in this study are pointed with arrow marks.

From the previous research, contaminated drinking water increases *Cryptosporidium* prevalence due to the resilience of its oocyst, which can survive for long periods. Dairy cattle also contribute to the spread, as their faeces can pollute water sources, especially in areas with poor waste management (Thomson *et al.,* 2017). Additionally, many cattle owners lack awareness that cryptosporidiosis is a zoonotic disease, and the absence of vaccination programs for dairy calves exacerbates the situation. Overall, the moderate prevalence rate, combined with low public awareness and risky behaviour, makes *Cryptosporidium* infection a significant public health threat. Therefore, improving awareness, sanitation, animal management, and safe water practices is essential for effective control and prevention of the disease.

Conclusions

This study examined the prevalence of *Cryptosporidium* infections in cattle, farm workers, and water sources in Tirupati, Andhra Pradesh. It found moderate infection levels in all groups, indicating that the parasite is widespread and can spread between animals, humans, and water sources. The analysis identified strains of *Cryptosporidium parvum, C. hominis*, and *C. ryanae*, suggesting both animal-to-human and human-to-human transmission. In cattle, key risk factors included young age (especially calves), diarrhoea, intensive farming, and poor hygiene. In humans, higher infection rates were linked to close contact with animals, low awareness, and inadequate sanitation. Contaminated water and improper waste disposal also played significant roles in spreading the infection. In conclusion, the study emphasizes the need for better farm hygiene, clean drinking water, proper waste management, and increased public awareness to reduce infections. Regular monitoring is also essential for effective control.

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References

Aghamolaie, S., Rostami, A., Fallahi, S., Tahvildar Biderouni, F., Haghighi, A., & Salehi, N. (2016). Evaluation of modified Ziehl-Neelsen, direct fluorescent-antibody and PCR assay for detection of *Cryptosporidium* spp. in children faecal specimens. *Journal of Parasitic Diseases*, *40*(3), 958-963.

Bhanja, R., Biswas, J., Dule, S., Pandit, S., Jas, R., Nandi, A., Barua, R., Chaudhuri, S., Dutta, T., Datta, S., & Batabyal, K. (2023). Prevalence of *Cryptosporidium* species isolated from calves from North 24 Parganas, South 24 Parganas and Nadia Districts of West Bengal. *Indian Journal of Veterinary Public Health, 9*(3), 70.

Chalmers, R.M., Campbell, B.M., Crouch, N., Charlett, A., & Davies, A.P. (2011). Comparison of diagnostic sensitivity and specificity of seven Cryptosporidium assays used in the UK. *Journal of Medical Microbiology*, *60*(11), 1598-1604.

Das, K., Nair, L.V., Ghosal, A., Sardar, S.K., Dutta, S., & Ganguly, S. (2019). Genetic characterization reveals evidence for an association between water contamination and zoonotic transmission of a *Cryptosporidium* spp. from dairy cattle in West Bengal, India. *Food and Waterborne Parasitology, 17,* e00064.

Das, P., Deka, D., & Lalrinkima, H. (2026). Molecular characterisation of *Cryptosporidium* parvum among cattle and cattle handlers from Tripura (India) and the associated risk factors. *Indian Journal of Animal Research, 60*(2), 300-306.

Khan, S.M., Debnath, C., Pramanik, A.K., Xiao, L., Nozaki, T., & Ganguly, T. (2010). Molecular characterization and assessment of zoonotic transmission of *Cryptosporidium* from dairy cattle in West Bengal, India. *Veterinary Parasitology, 171,* 41-47.

Kurniawan, A., Dwintasari, S.W., Connelly, L., Nichols, R.A., Yunihastuti, E., Karyadi, T., & Djauzi, S. (2013). *Cryptosporidium* species from human immunodeficiency-infected patients with chronic diarrhea in Jakarta, Indonesia. *Annals of Epidemiology, 23*(11), 720-723.

Maurya, P.S. (2016). Molecular diagnosis of *Cryptosporidium parvum* infection in calves. *Doctoral Dissertation*. Indian Veterinary Research Institute, Izatnagar, Bareilly, India.

Paul, S., Chandra, D., Ray, D.D., Tewari, A.K., Rao, J.R., Banerjee, P.S., Baidya, S., & Raina, O.K. (2008). Prevalence and molecular characterization of bovine *Cryptosporidium* isolates in India. *Veterinary Parasitology, 153,* 143-146.

Sateesh, A.G., Patil, N.A., Vivek, R.K., Dilipkumar, D., & Basavaraj Awati, R.B. (2022). Study on prevalence and risk factors of

bovine cryptosporidiosis. *The Pharma Innovation Journal, SP 11*(7), 2038-2042.

Sharifuzzaman, S., Shahiduzzaman, M., Anisuzzaman, A., Parvez, M.S., & Barragan, A. (2015). Isolation and identification of *Cryptosporidium* from water samples in Bangladesh. *International Journal of Natural and Social Sciences, 2,* 118-124.

Thomson, S., Hamilton, C.A., Hope, J.C., Katzer, F., Mabbott, N.A., Morrison, L.J., & Innes, E.A. (2017). Bovine cryptosporidiosis: impact, host-parasite interaction and control strategies. *Veterinary Research, 4,* 1-16.

Xiao, L. (2010). Molecular epidemiology of cryptosporidiosis: An update. *Experimental Parasitology, 124*(1), 80-89.

Xiao, L., Escalante, L., Yang, C., Sulaiman, I., Escalante, A.A., Montali, R.J., Fayer, R., & Lal, A.A. (1999). Phylogenetic analysis of *Cryptosporidium* parasites based on the small-subunit rRNA gene locus. *Applied and Environmental Microbiology, 65*(4), 1578-1583.

Zhao, W., Wang, R., Zhang, W., Liu, A., Cao, J., Shen, Y., Yang, F., & Zhang, L. (2014). MLST subtypes and population genetic structure of *Cryptosporidium andersoni* from dairy cattle and beef cattle in northeastern China's Heilongjiang Province. *PLoS One, 9*(7), e102006.