



Prevalence, Risk Factors and Haemato-biochemical Alterations Associated with Naturally Occurring *Cryptosporidium* Spp. Infection in Goats

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ABSTRACT

Background: *Cryptosporidium* spp. infection is one of the most common problems that occurs in kids under first month of life. The prevalence of *Cryptosporidium* may vary from farm to farm and region to region, depending upon herd size and animal husbandry practices. In India, although cryptosporidiosis had been reported in goat kids but *Cryptosporidium* spp. infection, risk factors and haematobiochemical alterations in goats of Madhya Pradesh are least known.

Methods: A total of 290 goats <12 month of age belonging to unorganized goatry farms, situated in and around Rewa district of the Madhya Pradesh were attended and rectal contents were collected in 5 ml sterile screw capped plastic cryo vials during March 2023 to April 2024. The samples were screened for the presence of *Cryptosporidium* spp. oocysts in the faecal smear by modified Ziehl-Neelsen (mZN) method at Department of Veterinary Pathology, College of Veterinary Science and A.H., Rewa, Madhya Pradesh. To determine the haemato-biochemical parameters, 5 ml of blood samples were collected in aseptic condition from goats included in the study.

Results: Out of 290 samples, twenty six (8.96%, 26/290) cases were tested positive for the *Cryptosporidium* oocysts. The highest prevalence was observed in kids below 1 month of age (14.6%). The Prevalence of *cryptosporidium* spp. was found higher in female goats (10.20 %) than male. The prevalence was found highest in goats belonged to dam of first parity (14.28%). Haematological observations in *Cryptosporidium* infected goats showed significantly ($p < 0.05$) lowered haemoglobin, total erythrocyte count, mean corpuscular haemoglobin and lymphocyte percentage but significantly higher total leukocyte count, neutrophil and eosinophil percentage than the healthy goats. Biochemical studies revealed significant reduction in total serum protein, albumin and globulin values in the infected goats.

Key words: *Cryptosporidium* spp, Goats, Haemato-biochemical changes, Modified Ziehl-neelsen staining, Prevalence.

INTRODUCTION

The goat population in India is estimated around 148.88 million out of which 11.6 million present in Madhya Pradesh (Livestock census, 2019). The Goat rearing is an easy, less expensive, less laborious and highly profitable business. Goat rearing has emerged as an important component of a mixed farming system (Kumar, 2007). Unfortunately, the enormous number of goat population die due to digestive and respiratory tract affections at the early stage of their lives, resulting greater economic burden on animal husbandry. There are several pathogens like *E.coli*, *Salmonella* spp., *Clostridium* spp., *Rotavirus* and *Cryptosporidium* spp. are main organism responsible for causing diarrhoea in goats. Cryptosporidiosis in small and large ruminants is considered as a major cause of infection in humans, due to the dissemination of oocytes in the environment (Squire *et al.*, 2017). The *Cryptosporidium parvum* infection is one of the most common threat that occur in lamb, kids and calves in the first month of life (Delafose *et al.*, 2006; Ceballos *et al.*, 2009; Kabu *et al.*, 2023). Cryptosporidiosis causes decline in productivity, retarded growth, decreased feed efficiency, delayed maturity, loss of fertility and overall financial loss in the form of treatment of ailing animals (Praud and Chartier, 2012). Transmission is mainly by ingestion of sporulated

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oocytes through contaminated feed and water. Cross infection occurs between domestic and laboratory animals and man (Mallinath *et al.*, 2009). *Cryptosporidium* may possess a potential public health problem due to infective oocysts in the environment (Fayer and Xiao, 2008). It causes self-limited watery diarrhoea in immunocompetent animals (Caccio *et al.*, 2005) but has more severe effects on immunocompromised animals (Chen *et al.*, 2005). The insufficient colostrum feeding in kids and contamination of kidding areas has been associated with *Cryptosporidium* infection (Noordeen *et al.*, 2000; Sevinc *et al.*, 2005). However, the risk factors concerned with cryptosporidiosis in goats have not been well described. Consequently, the objective of the present study was to determine the prevalence and associated risk factors of cryptosporidiosis in goats in the region.

MATERIALS AND METHODS

Collection of samples

Faecal samples and rectal contents were collected from 290 goats below 12 month of age with or without diarrhoea from unorganized goat farms located in and around Rewa district of Madhya Pradesh. Approximately, 5gm of rectal contents of goats were collected in 5 ml sterile screw capped plastic cryovials after cleaning of the anal area and beating by rectal stimulation with the index finger during March 2023 to April 2024. The collected samples were transported on ice packs to the Department of Veterinary Pathology, College of Veterinary Science and A.H., Rewa, Madhya Pradesh. Collected samples were categorized on the basis of age, sex, season (monsoon, post monsoon, summer and winter) and parity (first, second, third and fourth and above) of dam (Table 1).

Screening of faecal samples for *Cryptosporidium* spp. oocysts

The smears were prepared directly from the faecal samples on clean glass slides and air dried. Then smears were fixed in methanol for 3 minutes and were stained with carbol fuchsin for 15-20 minutes, followed by rinsing in tap water, then decolorized in acid alcohol (1% HCl in methanol) for 20 seconds and rinsing in tap water thereafter. Finally, the smears were counter stained with methylene blue for 1 minute, followed by rinsing and air drying (Henriksen and Pohlenz, 1981). Smears were examined using 100 X objectives under the light microscope.

Collection of blood sample

Blood sample (2 ml with anticoagulant) was collected aseptically from jugular vein of goats of different age groups which were positive for *cryptosporidium* infection but free from other gastro-intestinal parasites on faecal sample examination. For analysis of the biochemical parameters, 5 ml of blood samples were collected without anticoagulant in vacutainer tubes. The vacutainer tubes were kept in slanting position at room temperature for clotting and then centrifuged at 3000 rpm for 5 min. The separated serum

samples were collected in screw capped plastic vials and stored at -20°C for further investigation. For comparison of haematobiochemical values ten healthy goats, which were free from *Cryptosporidium* oocysts and other gastro-intestinal parasites kept as control group. The samples were analyzed on the same day for haematological parameters like Total Erythrocyte Count (TEC, million/cmm), Total Leucocyte Count (TLC, cells/cmm), Differential Leukocyte Count (DLC, %), Hemoglobin (Hb, g/dl), Packed Cell Volume (PCV, %) and erythrocyte indices using haemoglobinometer, hemocytometer and blood smear examination (staining with Field stain A and B) for differential leukocyte count. Biochemical parameter like albumin, globulin, total protein and albumin globulin ratio were measured using semiautomatic biochemical analyzer.

Statistical analyses

Data generated was analyzed by using SPSS 15.00 for Windows (SPSS, 2012). The generated data was subjected to independent t test for analysis and statistical significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Prevalence and risk factors

A total of twenty six (8.96%, 26/290) cases out of 290 faecal samples were tested positive for the *Cryptosporidium* oocysts on mZN screening (Table 1). Dark red, small, round or oval shaped oocysts were seen in faecal smear examinations of goats (Fig 1). Prevalence was higher in diarrhoeic goats (10.50%, 23/219) than non-diarrhoeic goats (4.22%, 3/71). As compared with present study Maurya *et al.* (2013) reported lower prevalence of *cryptosporidium*

Table: 1: Prevalence of *Cryptosporidium* spp. in goats.

Type of distribution	Number of sample	Number of positive sample	Prevalence %
Age of animals			
Up to 1 month	89	13	14.6
1-3 month	68	6	8.82
3-6 month	55	4	7.27
6-12 month	78	3	3.84
Sex of animals			
Female	147	15	10.20
Male	143	11	7.69
Parity of dam			
First	119	17	14.28
Second	88	6	6.81
Third	52	2	3.84
Fourth and above	31	1	3.22
Seasons			
Winter	85	06	7.0
Summer	60	02	3.3
Monsoon	101	17	16.83
Post-monsoon	44	01	2.2

spp. in goats. Higher prevalence of *cryptosporidium* spp. reported by several other workers (Ahamed *et al.*, 2015; Khurshed *et al.*, 2018; Dixit *et al.*, 2019). The lower prevalence of *Cryptosporidium* spp. in the present study could be due to the variation in the sample size screened and the type of diagnostic tests used. In the present study, only staining of faecal smear with mZN stain was used. Use of more sensitive tests like polymerase chain reaction may further increase the proportion of positive samples (Al-Robaiee and Al-Farwachi, 2013). The age-wise prevalence of *cryptosporidium* was higher in 0-1 month of age (14.60%, 13/89) thereafter prevalence decreased with increasing age (Table 1). The same has been described by previous workers (Bejan *et al.*, 2009; Ceballos *et al.*, 2009; Ahamed *et al.*, 2015; Khurshed *et al.*, 2018; Shanmathi *et al.*, 2019). The age has been found to be the greatest influencing factor in causing *Cryptosporidium* infections. Higher prevalence of *cryptosporidium* infection under one month of age may be due to underdeveloped immune system and lack of maternal antibody in kids (Paraud *et al.*, 2014). Prevalence of *cryptosporidium* was higher in female goats (10.20%, 15/147) than that of male (7.69%, 11/143). Higher prevalence in female goats in the present study were in agreement with the findings of Ahamed *et al.* (2015), Maurya *et al.* (2013), Akinkuotu *et al.* (2016), El-Shahawy (2016), Khan *et al.* (2018) and Shanmathi *et al.* (2019) in goats. Contrary to present study higher prevalence of *cryptosporidium* reported in male goats than female goats (Abare *et al.*, 2019). Higher susceptibility of infections to female goats may be due to stress associated with pregnancy, parturition and lactation (Akinkuotu *et al.*, 2014). This issue has been contradicted by others and stated that the prevalence of *Cryptosporidium* did not significantly relate to sex of animals (Rahman *et al.*, 1985; Shobhamani, 2007; Venu *et al.*, 2012). Season wise prevalence of *cryptosporidium* spp. was higher in monsoon season (16.83) as compared to other season. Same finding were reported by Singh *et al.* (2018) in calves. In addition, Khan *et al.* (2018) reported higher prevalence of *Cryptosporidium* in summer season. High temperature and humidity with frequent rains in the monsoon season support faster transmission of the oocysts from the infected to healthy animals (Khan *et al.*, 2018; Jafari *et al.*, 2012). Parity-wise prevalence of *cryptosporidium* was higher in first parity (14.28%, 17/119) than it decreased with parity of dam. Similar observations were also recorded by Sharma and Joshi (2020) and Singh *et al.* (2018) in calves. First and second parity dam have significantly lower levels of immunoglobulins in their colostrum than higher parity dam and this is the reason for higher incidence of diarrhoea in new born animals during first parity (Logan *et al.*, 1981; Svensson *et al.*, 2003; Afshari *et al.*, 2012). Since, *cryptosporidium* with other polymicrobial agents are the main agents of diarrhoea so their prevalence may be influenced by parity of dams (Millemann, 2009; Cho and Yoon, 2014; Meganck *et al.*, 2015).

Hemato-biochemical changes

Hemato-biochemical parameters were recorded in 26 goats, which were found positive for *Cryptosporidium*

infection (Table 2 and 3). The average hemoglobin level were significantly ($P<0.05$) decreased in *cryptosporidium* infected group (10.51 ± 0.10) as compared the control group

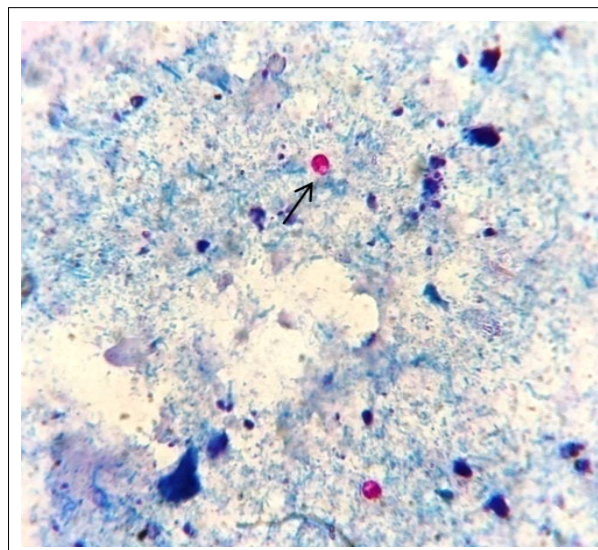


Fig 1: Dark red, small, round or oval smooth wall oocysts of *Cryptosporidium* spp. (arrow) in direct faecal smear of goats stained by modified Zeil-Nelson staining method(100X).

Table 2: Haematological parameters (Mean $\pm$ SE) in *Cryptosporidium* infected goats in Rewa district of Madhya Pradesh.

Hematological parameters	Control group	<i>Cryptosporidium</i> Infected group
Hb(g/dl)	11.81 ^A $\pm$ 0.13	10.51 ^B $\pm$ 0.10
PCV (%)	33.10 ^A $\pm$ 0.45	31.80 ^A $\pm$ 0.25
TEC(10 ⁶ X)	13.89 ^A $\pm$ 0.56	11.68 ^B $\pm$ 0.52
TLC (10 ³ X)	7.86 ^A $\pm$ 0.12	11.65 ^B $\pm$ 0.13
Neutrophil (%)	37.80 ^A $\pm$ 0.29	46.50 ^B $\pm$ 0.49
Lymphocyte (%)	57.20 ^A $\pm$ 0.27	47.23 ^B $\pm$ 0.43
Monocyte (%)	3.40 ^A $\pm$ 0.22	3.88 ^A $\pm$ 0.90
Eosinophil (%)	1.40 ^A $\pm$ 0.16	2.46 ^B $\pm$ 0.99
Basophil (%)	0.00 ^A $\pm$ 0.00	0.00 ^A $\pm$ 0.00
MCV (fl)	24.58 ^A $\pm$ 0.46	26.29 ^A $\pm$ 1.02
MCH (pg)	8.51 ^A $\pm$ 0.12	9.00 ^B $\pm$ 0.17
MCHC (g/dl)	34.67 ^A $\pm$ 0.54	43.41 ^A $\pm$ 1.30

Superscript differ in letter significant difference, should be read row wise ($p<0.05$).

Table 3: Biochemical parameter (Mean $\pm$ SE) in *Cryptosporidium* infected goats.

Parameters	Control group	Infected group
Albumin	3.23 ^A $\pm$ 0.13	2.75 ^B $\pm$ 0.11
Globulin	3.29 ^A $\pm$ 0.01	2.79 ^B $\pm$ 0.16
Total protein	6.53 ^A $\pm$ 0.08	5.54 ^B $\pm$ 0.04
A:G ratio	0.9848 ^A $\pm$ 0.001	0.9839 ^A $\pm$ 0.013

Superscript differ in letter significant difference, should be read row wise ($p<0.05$).

(11.81±0.13). These findings were in accordance with Thakre *et al.* (2016) in calves and Aziz *et al.* (2021) in sheep. Decrease in hemoglobin concentration might be attributed to hemoconcentration and the loss of body fluid along with electrolytes in extreme diarrheic and dehydrated condition in goats. The mean packed cell volume (PCV) values were decreased (31.80±0.25) in case of infected goats but it was not significantly related to the control group (33.10±0.45). Same findings were reported by Rekha *et al.* (2011) in calves and Aziz *et al.* (2021). The total erythrocyte count were significantly ($P<0.05$) decreased in infected group (11.68±0.52) as compared to the corresponding value of the control group (13.89±0.56). Similar findings were observed by Thakre *et al.* (2016) in calves and Aziz *et al.* (2021) in sheep. The total erythrocyte count in *cryptosporidium* infected goats decreased due to dehydration and hemoconcentration caused by diarrhoea (Molina *et al.*, 1994). The findings of present study that is decrease in hemoglobin, packed cell volume and total erythrocyte count were disagree with the findings of Al-Doori (2018), Abdel-Saeed and Salem (2019) in *cryptosporidium* infection in rabbit and lambs respectively. They reported a significant increase in red blood cells, hemoglobin and packed cell volume. This difference may be due to the loss of epithelial cells of intestine and reduce absorptions from the villi, resulting diarrhoea, dehydration and anemia (Al-dujaily *et al.*, 2017). Total leukocyte counts were significantly higher in (11.65±0.13) goats with cryptosporidiosis as comparison with healthy goats (7.86±0.12). These findings were in accordance with earlier workers (Thakre *et al.*, 2016; Aziz *et al.*, 2021). Contrary to the present findings Shobhamani *et al.* (2007) reported lower values of TLC in *cryptosporidium* infected calves. The infection with *cryptosporidium* or any other parasite may predispose to the animals for secondary bacterial invasions which lead to the increased total leukocyte counts. There was a significant ($P<0.05$) increase in neutrophil counts in infected goats (46.50±0.45) as compared to control group (37.80±0.29) (Table 2). These findings were in agreement with Thakre *et al.* (2016) in calves. Intestinal inflammation and acute enteritis may be the cause of neutrophilia in the present study. There was a significant decrease in lymphocyte count in goats with cryptosporidiosis. These findings were accordance with the findings of Thakre *et al.* (2016) in calves. Contrary to the present findings Khan *et al.* (2020) reported increased lymphocyte count in *cryptosporidium* infected goats. Decreased lymphocyte count may be due to stress and depletion of lymphoid follicle. The values of eosinophil counts were significantly ($P<0.05$) higher (2.46±0.99) in infected goats than that of control group (1.40±0.16). The present finding was in agreement with Khan *et al.* (2020) in goats. The elevation of eosinophils in circulating blood is common in parasitic infections of intestines (Howard and Smith, 1999).

The significant increase in eosinophil count may be due to body defense mechanism as eosinophils are potent inflammatory cells that play an integral role in defense mechanism against parasites. The mean value of MCH was

significantly higher in infected group. These finding were in agreement with the findings of Thakre *et al.* (2016), who observed a significantly increased value of MCH in *Cryptosporidium* infection. Contrary to the present findings Darabus *et al.* (2009) reported reduction of MCH in cryptosporidium infected calves. Higher MCH value may be due to the dehydration and hemoconcentration arising from diarrhoea in *Cryptosporidium* infected goats. The mean value MCV and MCHC increased but not significantly related between *Cryptosporidium* infected group and control group.

There was a significant decrease value of albumin was observed in *cryptosporidium* infected group (2.75±0.0.11) as compared to that of healthy animals (3.23±0.13g/dl) (Table 3). Same findings were reported by Khan *et al.* (2020) and Aziz *et al.* (2021). Albumin is a negative acute phase protein and its concentration decreases during inflammation of intestine (Hashemnia *et al.*, 2014). The globulin value significantly decreased in *cryptosporidium* infected goat. The present finding was in agreement with Rekha *et al.* (2011) and Mokhbatly and Selim (2003). The values of total proteins significantly decrease in infected group (5.54±0.04 g/dl) than that of healthy animals (6.53±0.08 g/dl). The findings of present study were in accordance with earlier workers (Molina *et al.*, 1994; Shobhamani, 2005; Rekha *et al.*, 2011; Khan *et al.*, 2020; Aziz *et al.*, 2021). The Albumin/globulin ratio were decrease in *cryptosporidium* infected goats but it was not significantly related to the healthy animals. Similar findings reported by Shobhamani (2005), Rekha *et al.* (2011) and Asati *et al.*, (2008). The decrease in the albumin, globulin and total proteins may be due to the loss of above proteins in enteritis associated with *cryptosporidium* infection. Here, our findings revealed significant decrease in total erythrocyte count, PCV, hemoglobin, lymphocyte count, total protein, albumin and globulin levels whereas increase in neutrophil, eosinophil and total leukocyte count in *Cryptosporidium* infection.

CONCLUSION

From the present study it is evident that several predisposing factors like age of the animals, sex, season and parity of the dams might be contributing factors for the *Cryptosporidium* infection. It occurs more commonly in the neonatal kids, first parity dams and in monsoon season. Therefore, predisposing factors is one of the most important aspects, which should be kept in mind to minimize the *Cryptosporidium* infection. The infection with *Cryptosporidium* in goats had an effect on the hemato-biochemical parameters. There was a significant decrease in hemoglobin, total erythrocyte count, total protein, albumin, globulin and lymphocyte count and an increase in neutrophil, eosinophil and total leukocyte count in *Cryptosporidium* infected goats.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Conflict of interest

The authors declare no competing interest.

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