RESEARCH ARTICLE

Prevalence, Haemato-Biochemical Changes and Pathology of Colibacillosis in Slaughtered Goats

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Abstract

The study aimed to investigate the prevalence, risk factors and pathological changes in intestine of slaughtered goats affected with colibacillosis in Rewa districts of Madhya Pradesh. In this study, out of a total of 305 goats examined from different slaughter houses of Rewa district 108 goats showing intestinal lesions were included for further study. Of the 108 intestinal contents collected, 33 (30.55%) cases were found positive for the *E. coli* infection. The age-wise prevalence of colibacillosis was higher in 6-12 month of age (33.87%) and decreased with advancement of age. Sex wise prevalence was found higher in female goats (36%) than males (28.91%). Goats with colibacillosis had significantly lower level of haemoglobin and lymphocyte count, and significantly higher levels of PCV, TLC, TEC, neutrophils and eosinophils. Biochemical analysis showed significantly lower level of sodium and higher level of potassium and alkaline phosphatase in colibacillosis affected goats. Thirty three slaughtered goats that were positive for colibacillosis based on microscopic examination included, catarrhal enteritis (9), haemorrhagic enteritis (6), necrotizing enteritis (8), necro-haemorragic enteritis (6), and fibrinous enteritis (4).

Key words: Colibacillosis, Goats, Haemato-biochemical profile, Pathomorphology, Prevalence.

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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). Goats are used worldwide for milk, meat, hair and skin. Regretfully, a large portion of the goat population dies due to enteritis at a young age. Major enteric pathogens responsible for enteritis in animals are *Escherichia coli, Salmonella* spp., *Clostridium perfringens*, *Cryptosporidium parvum,* Rotavirus, Coronavirus and *Eimeria spp.* (Cho and Yoon, 2014). The prevalence of each pathogen may vary from farm to farm and region to region, depending upon herd size and animal husbandry practices. *Escherichia coli,* a member of enterobacteriaceae family, is a part of normal intestinal microflora of mammals and plays an important role in host metabolism, immunology and nutrition (Tenaillon *et al*., 2010). However infection by one or several *E. coli* pathotypes may cause diarrhea in animals and humans (Ngeleka *et al.,* 2019). The pathotypes have different virulence factors by which they colonize in the small intestine of host, avoiding the immune response and stimulating the inflammatory diarrhoea (Croxen and Finlay, 2010). *E. coli* infections produce mild and scattered lesions throughout the small and large intestine. The lesions are most often found in caecum and colon, but the distal jejunum and ileum might also be involved. The uniform thick layers of adherent bacteria on the brush border epithelium of the distal jejunum, ileum and large intestine is observed on histopathology (Uzal *et al*., 2023).

Grossly, the small and large intestines may be filled with fluid content and reveal erosive fibrinohaemorrhagic enterocolitis and/or fibrinous enteritis. The large intestine particularly caecum and colon, may reveal marked degree of hyperplasia of goblet cells with degeneration of epithelium lining (Awad *et al*., 2020). Conventional cultural technique is used to isolate *E. coli* with commonly used media like McConkey agar, EMB agar and tryptic soya broth. In addition to these conventional techniques, several immunoassays (ELISA, LAT and RIA) and multiplex PCR have been applied for the detection of different *E. coli* pathotypes (Turkyilmaz *et al*., 2014). In India, although colibacillosis, a disease caused by *E. coli,* has been reported in goats, but the pathomorphology of colibacillosis, associated risk factors and haemato-biochemical changes in slaughtered goats in Rewa district of Madhya Pradesh are least studied, hence were evaluated in this study.

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Materials and Methods

Collection of Tissue Samples

A total 305 goats from different slaughter houses in and around the Rewa district of Madhya Pradesh (India), ranging from 6 month to 2 years of age, were observed for the presence of any type of gross pathological lesions in the intestine and mesenteric lymph nodes. Out of 305 slaughtered goats that were studied, only 108 goats revealed gross pathological lesions that were included for further study. Intestinal swab samples were collected before the ligation of both ends of intestine from slaughtered goats. The collected samples were transported on ice to the laboratory for bacterial isolation and stored at 4°C for further study. Tissues for histoplathological examination were collected and preserved in 10% neutral buffered formalin solution. The collected samples were divided into age and sex-wise distribution.

Isolation and Identification of *E. coli*

For the identification and characterization of *E. coli* in pure culture the method described by Markey *et al*. (2013) was adopted. For the isolation of *E. coli*, TBX agar was streaked and incubated at 42°C for 18-24 h. The typical growth of *E. coli* colonies observed as dark blue green round colonies, were indicative of β-glucuronidase activity. After streaking an isolated typical TBX colony on MacConkey agar, it was incubated for 24 h at 37°C. This produced pink lactose fermenting colonies, which were indicative of the presence of *E. coli*. Microscopical confirmation of *E. coli* by Gram's staining revealed a small, rod-shaped, pink, Gram-negative organism found in single or paired short colonies. IMViC reactions were carried out on pure cultures of *E. coli*. As a result, it provides + + - - IMViC test pattern for every isolate. The isolates produced a yellow butt color and a slant with gas production on Triple Sugar Iron (TSI) agar indicating *E. coli*.

Haemato-Biochemical Study

To determine the haematological parameters, 5 mL of blood samples were collected in aseptic condition from jugular vein of all slaughtered goats in a sterile vials containing anticoagulant (EDTA @ 2 mg/mL of blood). For biochemical parameters, 5 mL of blood samples were collected in vacutainer tubes without anticoagulant, and centrifuged at 2000 x g for 5 min. The separated serum samples were collected in screw capped plastic vials and stored at -20°C till further use. The sero-biochemical parameters sodium, potassium and alkaline phosphatases were analyzed with the help of semi-automatic biochemical analyzer using Erba diagnostic kit. Data were analyzed by using SPSS 15.00 for Windows.

Tissue Processing for Histopathology

Tissue pieces from representative portions of the intestine and mesenteric lymph nodes showing definite gross lesions were collected and fixed in 10% neutral buffered formalin for histopathology. Processing of tissue and staining by routine Haematoxylin and Eosin (H&E) stain was done as per the procedure described by Luna (1972).

Results and Discussion

Prevalence of Colibacillosis

Out of 108 intestinal contents collected, only 33 (30.55%) were found positive for the *E. coli* infection. Almost similar prevalence of colibacillosis was reported by Sharma *et al*. (2020) in goats. However a higher prevalence of colibacillosis was reported by Marodia *et al.* (2020) and Shrivastava *et al*. (2022), while Akanbi *et al.* (2011) reported lower prevalence. The differences in the prevalence could be due to the sample size, geographical location and the type of diagnostic tests used for screening.

The prevalence of *E. coli* was higher in 6-12 month of age group (33.87%, 21/62) and decreased with advancement of age (26.08 %, 12/46). Similar findings were reported by Ndegwa *et al*. (2020). Sex wise prevalence of colibacillosis was higher in female goats (36%, 9/25) than male goats (28.91%, 24/83) and concurred with the report of Sharma *et al.* (2020) in goats. Contrary to the present finding, many workers reported higher prevalence in male in comparison to the female goats (Rao *et al*., 2018), while Zaman *et al*. (2018) reported no significant sex difference. The present study was based on the slaughter houses, where more number of male goats was coming for slaughter than female. Therefore, the number of samples collected from female goats was less and only sick, old and sterile goats were sent for slaughter as compared to male.

Heamto-Biochemical Changes in Goats Infected with Colibacillosis

Haemato-biochemical parameters were recorded in 33 goats, which were found positive for colibacillosis along with ten healthy goats free from *E. coli* infection take as healthy control group. The mean haemoglobin (Hb) was significantly ($p<0.05$) decreased in *E. coli* infected group as compared to the corresponding value of the control group (Table 1), which concurred with the report of Maina *et al*. (2015). The mean packed cell volume (PCV), total erythrocyte counts (TEC) and total leukocyte count (TLC) were significantly ($p<0.05$) increased in *E. coli* infected group as compared to control group (Table 1). These findings were in accordance with Zaki *et al*. (2010). However, Jesse *et al*. (2016) reported lower packed cell volume and leucopenia in a Jamunapari goat with colibacillosis. Maina *et al*. (2015) mentioned that the diarrheal goats had a markedly higher haemoglobin concentration but no apparent variation in packed cell volume or red blood cell counts. According to Radostits *et al*. (2007), intestinal infections were primarily linked to an increase in packed cell volume in diarrheal lambs and kids. There was a significant ($p<0.05$) increase in neutrophil counts and decrease in lymphocyte counts in *E. coli* infected goats as

compared to control group (Table 1). Intestinal inflammation and acute enteritis may be the cause of neutrophilia in the present study. Maina *et al*. (2015) recorded neutriplilia, while Hassan *et al*. (2013) reported increased lymphocyte count in *E. coli*. infected animals. Jain (1986) observed that lymphopenia is common in acute inflammatory response because inflammatory mediator stimulate movement of lymphocyte from blood to the inflamed tissue. The eosinophil counts were significantly ($p<0.05$) higher in infected goats.

The current study revealed significantly decreased levels of sodium and increased level of potassium in *E. coli* infected group than healthy group (Table 1). These findings agreed with Zain-Eldin *et al*. (2013) in small ruminants. Pathogenic *E. coli* act on the intestinal villi which secrete excessive amount of Na+ ions, which are lost through the intestinal tract and cause hyponatremia (Radostits *et al*., 2007). Most of bacterial enteropathogens interfere with intestinal function increasing the crypt cell's ability to secrete chloride or making it more difficult for the villus cells to absorb sodium (Hirschhorn and Greenough, 1991). The mean alkaline phosphatase content was significantly greater in infected group, similar to that reported by Zaki *et al*. (2010).

Table 1: Haemato-biochemical parameters in control and *E. coli* infected group (Mean ± SE)

Blood parameters	Control group (n=10)	*E. coli* infected group (n=33)
Hematological Parameters		
Hb (g/dL)	10.15 ± 0.22	8.31 ± 0.17*
PCV (%)	31.50 ± 0.34	38.81 ± 0.39*
TEC (10^6/µL)	10.31 ± 0.15	13.97 ± 0.11*
TLC (10^3/µL)	7.35 ± 0.11	10.77 ± 0.16*
Neutrophil (%)	35.60 ± 0.49	48.12 ± 0.86*
Lymphocyte (%)	57.50 ± 0.45	42 ± 1.13*
Monocyte (%)	3.80 ± 0.32	3.72 ± 0.26
Eosinophil (%)	2.90 ± 0.31	6.30 ± 0.2*
Biochemical Parameters		
Sodium (mEq/L)	142.11 ± 0.27	126.15 ± 1.24*
Potassium (mEq/L)	4.31 ± 0.004	4.60 ± 0.05*
Alkaline phosphatase (U/L)	222.36 ± 1.38	319. 84 ± 4.69*

*Significantly ($p<0.05$) different as compared to corresponding control values

Pathomorphological Changes in Intestine

Out of 33 slaughtered goats observed during the study on the basis of microscopic examination of intestine, nine goats showed the lesions of catarrhal enteritis, six cases were of haemorrhagic enteritis, eight cases of necrotizing enteritis, six cases of necro-haemorragic enteritis and four cases of fibrinous enteritis (Table 2).

In most of the cases of catarrhal enteritis, the mucosa of small intestines was congested and covered with mucus exudates. Mesenteric lymph nodes were swollen, congested and edematous. Microscopically, the intestinal villi were sloughed and lamina propria was severely congested and

Table 2: Prevalence of different types of enteritis in *E. coli* infected goats

Types of enteritis	No. of cases	%
Catarrhal enteritis	9	27.27
Hemorrhagic enteritis	6	18.18
Necrotizing enteritis	8	24.24
Necro-haemorragic enteritis	6	18.18
Fibrino-necrotic enteritis	4	12.12
Total	**33**	100.0

infiltrated with few inflammatory cells. Goblet cell hyperplasia was observed in all cases of catarrhal enteritis.

In haemorrhagic enteritis grossly, serosa of the intestine was edematous and dotted with petechial to ecchymotic haemorrhage. Microscopically, marked haemorrhages in the mucosa, sloughing of the villi with infiltration of mononuclear cells in the lamina propria, around cryptic glands and submucosa of the jejunum and ileum was noticed (Fig. 1a). These types of lesions in catarrhal and haemorrhagic enteritis due to *E. coli* infection were in accordance with Tiwari *et al.* (2018) in goats.

In necrotizing enteritis grossly, pin point size small necrotic foci were observed in the intestinal mucosa. Microscopically, cryptic glands showed necrosis, engorged blood vessels with severe infiltration of mononuclear cells in the lamina propria of jejunum (Fig. 1b). Edema and infiltration of mononuclear cells was present in submucosal layer of the jejunum, distancing of muscle fiber in mucosal layer. Microscopic lesions such as necrosis of enterocytes, desquamation of the lining epithelium have been reported by several workers (Jesse *et al.,* 2016) in *E. coli* infected kids. These types of changes in intestine were also seen due to *E. coli* infections (Tiwari *et al*., 2018).

In the necro-haemorragic enteritis grossly, mucosa of small intestine was congested, haemorrhagic and thick. Histopathologically in these cases, ileum showed necrosis at the tip of villi with mild congestion and haemorrhages with sever mononuclear cell infiltration in mucosa. Necrosis and severe congestion in the muscular layer of submucosa with deposition of necrotic debris was observed (Fig 2a). Focal lesions of necrosis with severe infiltration of lymphocytes in the crypts of ileum were seen. In mesenteric lymph nodes, the connective tissue capsule was loosened and follicles in the cortex were necrosed along with vascular engorgement. These lesions in the present study of necro-haemorragic enteritis are very closely similar to the lesion reported by Singh *et al.* (2023) in bovine.

In fibrino-necrotic enteritis there was necrosis with deposition of fibrinous material in the mucosa and submucosa of the jejunum and some part of ileum (Fig. 2b). In mesenteric lymph node, there was deposition of fibrin in outer and inner cortex areas. Similar lesions in small intestine were reported by Singh *et al.* (2023) in calves and. Fibrinous enteritis, in the present study might be due to mixed bacterial infection such as *E. coli* pathotypes, *Clostridium perfringens* type C and *Salmonella* spp.

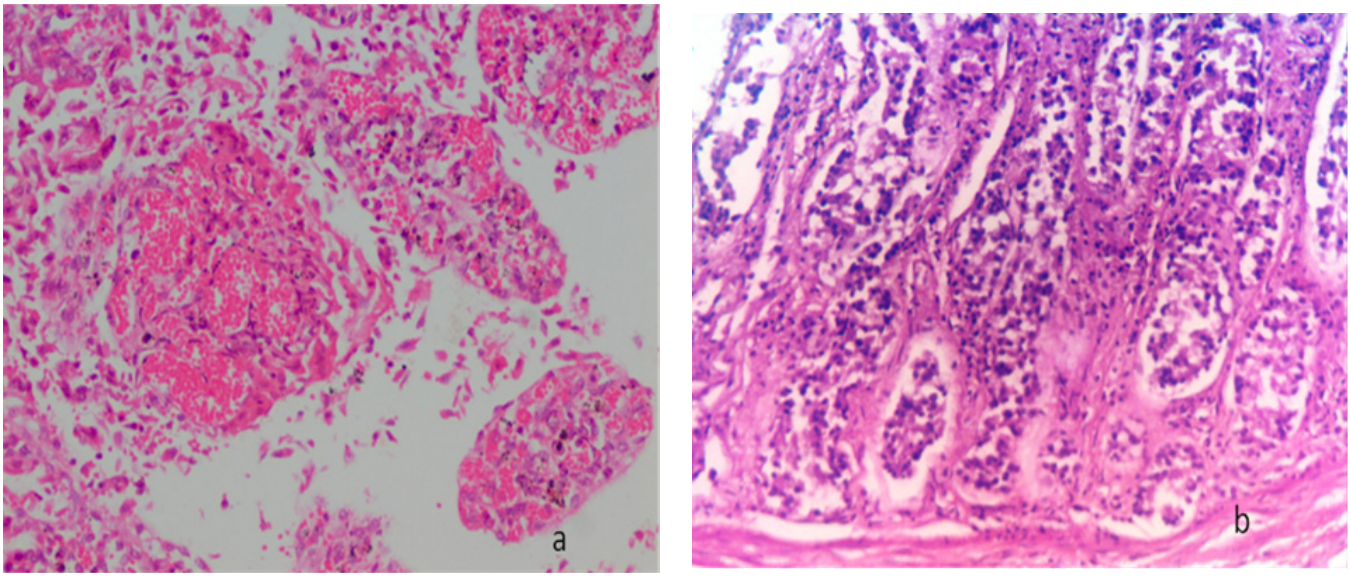

Fig. 1: Microphotographs: **(a)** Jejunum showing sever congestion and haemorrhages in the mucosa with desquamated villi., **(b)** Ileum showing necrosis cryptic glands (H&E, 20X)

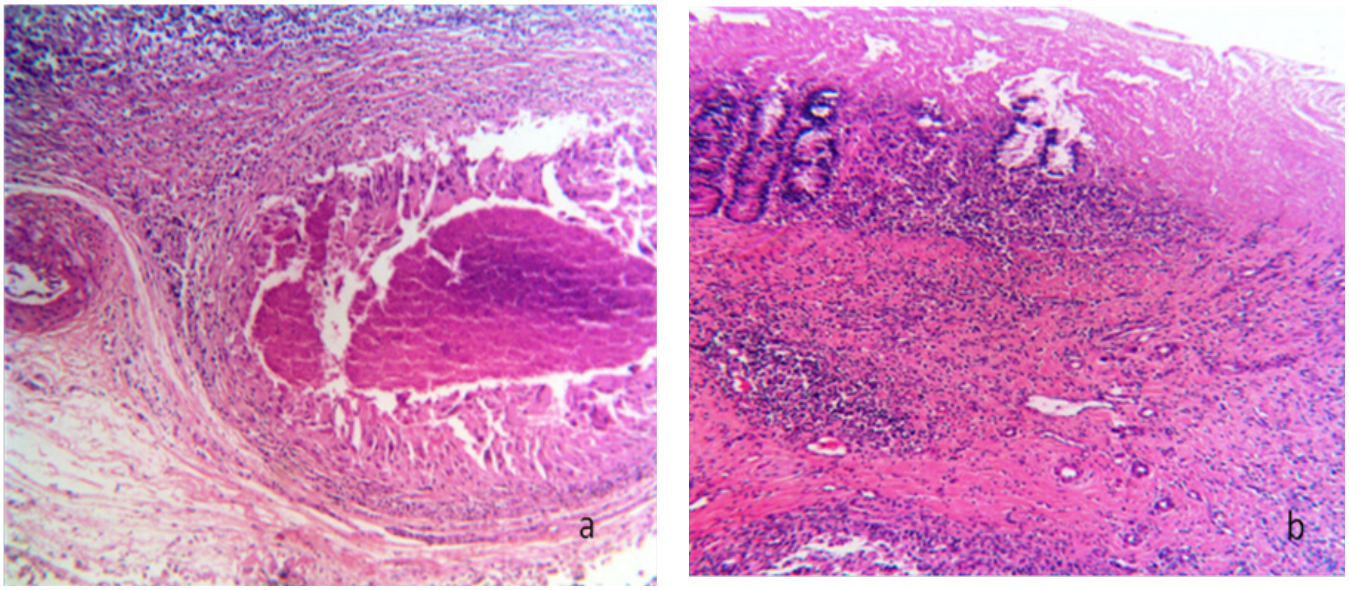

Fig. 2: Microphotographs: **(a)** Small intestine showing necrosis and sever congestion in the muscular layer of submucosa with accumulation of necrotic debris, **(b)** Small intestine showing extensive necrosis and fibrinous materials in the mucosa and submnucosa pf the ileum (H&E, 20X).

Conclusions

From the present study it is evident that some predisposing factors like age and sex of goats might be contributing factors for the colibacillosis. Goats with colibacillosis had significantly lower level of haemoglobin and lymphocyte count and significantly higher levels of PCV, TLC, TEC, neutrophils and eosinophils percentage. Histopathlogical lesions are indicated that *E. coli* causes catarrhal, haemorrhagic, necrotizing, necro-haemorragic and fibrinous enteritis. To understand the patho-epidemiology of colibacillosis in goats, more research with a larger area and a larger number of animals of all ages is required.

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