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Prevalence of caprine diarrhea due to *Escherichia coli* in the Vindhya region (Rewa) of Madhya Pradesh

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Abstract
The study was conducted for the estimation of prevalence of goat diarrhoea due to *E. coli* in and around Rewa district of Madhya Pradesh. A total of 200 faecal samples were collected from diarrhoeic goat and were screened for the presence of *E. coli.* The microbiological samples were processed for isolation of *E. coli* and isolates were identified on the basis of cultural, morphological, biochemical characteristics and were also subjected to antibiotic sensitivity test for determining antimicrobial sensitivity/ resistance pattern. Maximum prevalence of *E. coli* associated diarrhoea in kids was recorded in the age group of 0-14 days (35%), followed by age group 15- 30 days (25.5%). The *E. coli* isolates from diarrhoeic samples, were highly sensitive to Gentamicin (97.7%) and Amikacin (97.7%) followed by Chloramphenicol (92.5%), Meropenem (82.7%), Ciprofloxacin (78.1%) and Tetracycline (70.6%).

Keywords: *E. coli*, diarrhoea, goat, prevalence, antibiotic resistance

Introduction
Small ruminants occupy an important economic and ecological niche in agricultural systems throughout developing countries (Devendra, 2005) [5]. Diarrhoea is documented as a frequent cause of neonatal mortality among animals including goats throughout the world. *Enterotoxigenic Escherichia coli* (ETEC) and *Cryptosporidium parvum* are considered among the most prevalent causative agent of enteritis in goats. Bacterial enteritis is the most important cause of diarrhoea in lambs and goat kids. It is caused by pathogenic serotypes of *Escherichia coli*. *E. coli* is causative agent of white scour in goat (Bhat *et al.*, 2008) [2]. The mortality due to diarrhoea may go as high as 60%, and *E. coli* scours (colibacillosis) is the single major cause of death in neonatal goat kids.
In enterotoxic colibacillosis potent enterotoxin are produced by the pathogenic *E. coli* after adhering the mucosa and proliferation which encourages excessive secretion of fluid from intestinal mucosa. Enterotoxigenic *E. coli* produce severe diarrhoea in goat kids mainly during the first two weeks of life with highest frequency of pathogenicity in kid younger than three days. Animals suffering from white scour have severe colitis characterized by abdominal pain, pasty faeces, severe enteritis which may culminates into death due to severe dehydration (Radostis *et al.*, 2016) [13]. The study has been designed with the objective of estimation of prevalence of goat diarrhoea due to *E. coli* in and around Rewa district of Madhya Pradesh and study its antibiotic sensitivity pattern.

Material and Methods
Diarrhoeic goats were identified on the basis of apparent clinical signs of diarrhoea e.g. yellowish watery diarrhoea along with dehydration and depression, at the earliest possible and information with respect to age and sex were recorded. 200 faecal samples were taken from diarrhoeic goats, from various villages around Rewa district of Madhya Pradesh (Table 01), using sterilized swab directly from rectum and were carried to laboratory in chilled condition for bacteriological examination. These samples were dissolved in phosphate buffer saline, then the samples were vortexed and centrifuged at 3000 rpm for 15 minutes. Then loopful of supernatant were streaked on Tryptone Bile X-Glucoronide Agar (TBX) and incubated at 42° C overnight (18-24 hours) (Shrivastava *et al.*, 2016) [16].

Table 1: Various sources for collection of fecal sample for bacteriological examination

S. No.	Place	No. of animals
1.	Veterinary Clinical Complex	04
2.	Neem chouraha, Rewa	15
3.	Bodabagh, Rewa	12
4.	Village Kuthuliya, Rewa	17
5.	Village Garh, Rewa	37
6.	Village Ramnai, Rewa	24
7.	Village Ajgaraha, Rewa	15
8.	Village Rampur naikin, Rewa	28
9.	Village Sagra, Rewa	48
	Total	200

Identification and characterization of *E. coli*

For the identification and characterization of *E. coli*, samples were processed. The growth obtained from TBX agar were streaked on MacConkey agar and incubated for 24 hours at 37 ^{0}C, on which *E. coli* gives pink colored colonies, the growth obtained were then streaked on EMB (Eosin Methylene Blue) agar and incubated for 24 hours at 37 ^{0}C, typical metallic sheen were seen and the growth was transferred to nutrient agar (NA), incubated at 37 ^{0}C for 18-24 hours to obtain pure colonies which were subjected to standard morphological and biochemical tests. Microscopic examination for *E. coli* was done by Gram's staining.

Biochemical tests for identification of *E. coli*

A single colony of *E. coli* from Nutrient Agar was subjected to IMViC (Indole reaction, Methyl Red reaction, Voges Proskauer, Citrate utilization test) test and Triple Sugar Iron (TSI) agar slant reactions for identification of suspected *E. coli*, as per the method described by Markey *et al*. (2013) [9, 10].

Table 2: Biochemical tests for identification of *E. coli*

Biochemical test	Indole production	MR test	VP test	Citrate	TSI Butt	TSI Slant	H_2S Production	Gas production	Biochemical test
Reaction	+	+	-	-	yellow	yellow	-	+	Reaction

Kirby- Bauer disk diffusion method for antibiotic sensitivity test (CLSI, 2012)

Inoculums were prepared by direct colony suspension method, using a sterile loop a single colony from an 18-24 hours old tryptone soya agar plate was transferred to a tube with sterile physiological saline to make a direct colony suspension. The inoculums were adjusted to 0.5 McFarland standards.

Kirby- Bauer disk diffusion method

1. A sterile cotton swab on a wodden applicator (HiMedia) was soaked by dipping into the standardized inoculums. The soaked swab was rotated firmly against the upper inside wall of the tube to express excess of the fluid.
2. The entire surface of Muller- Hinton (MH) agar plates were streaked 3 times by turning at 60^0 angles between each streaking.
3. Using aseptic technique the anti-microbial discs were applied to the 90mm MH agar plates at least 24mm apart.
4. Plates were incubated at 37^{0}C for 24 hours.
5. The zone of inhibition was measured using a transparent scale and diameter of the zone to the nearest millimeter (mm) was recorded.

The antimicrobial discs (table no. 03) were procured from HiMedia laboratories Pvt. Ltd., Mumbai for determination of antibiogram of *E. coli* strains of diarrhoeic kids.

Table 3: Antibiotic disc used, their symbol, concentration and interpretation

S. No.	Antimicrobial Disc	Symbols	Concentration (mcg)	Interpretation (mm)		
				R	I	S
1.	Tetracycline	TE	30	<11	12-14	>15
2.	Ciprofloxacin	CIP	5	<15	16-20	>21
3.	Gentamicin	GEN	10	<12	13-14	>17
4.	Amikacin	AK	30	<14	15-16	>17
5.	Meropenem	MRP	10	<19	20-22	>23
6.	Chloramphenicol	C	30	<12	13-17	>18

Results and Discussion

Isolation and Identification of *E. coli* from the Faecal Sample

Morphology of *E. coli* colony in different agar media

Typical growth of *E. coli* colonies in TBX agar were seen as dark blue green round colonies (Fig. 01) after incubation at 42 ^{0}C for 24 hours which was an indication of β-glucuronidase activity. Similar findings have been reported by various workers in food samples (Verhaegen *et al*., 2015) [19], in cecal swabs of broilers (Shrivastava *et al*., 2016) [6], in wheat and rye flour (Made *et al*., 2017) [8] and in fecal samples in calves (Mishra, 2018) [11] as well as those isolated from sub-clinical mastitis in cattle (Tiwari, 2019) [18]. Glucuronide allows specific detection of *E. coli* through the formation of blue colonies that are the result of rapid conversion of the liberated aglycone to indigo.

When a single colony from TBX was further streaked on MacConkey agar (Fig.02) and EMB agar (Fig.03) pink lactose fermenting colonies were obtained on MacConkey and typical metallic sheen was observed on EMB agar, respectively and were similar to the findings reported in faecal swabs of diarrhoeic kids (Sahoo *et al*., 2015) [14], in faecal samples of goats and in diarrhoeic sheep (Islam *et al*., 2016 and Shabana *et al*., 2017) [6, 15].

These were further incubated on Nutrient agar for 37 ^{0}C for 24 hours, on which colourless, circular and smooth colonies were obtained. Similar types of colonies in nutrient agar have been reported by various workers like from diarrhoeic calves and lambs (Wani *et al*., 2003) [20]; from poultry litter and feed (Islam *et al*., 2014) [7] and from diarrhoeic calves (Ansari *et al*., 2014) [1].

Following Gram's staining technique, smear revealed gram negative rods of different size arranged in single, paired or in short chain manner. This was in agreement to the findings of many other workers like from poultry litter and feed (Islam *et al*., 2014) [7] and in calves (Ansari *et al*., 2014 and Mishra, 2018) [1, 11].

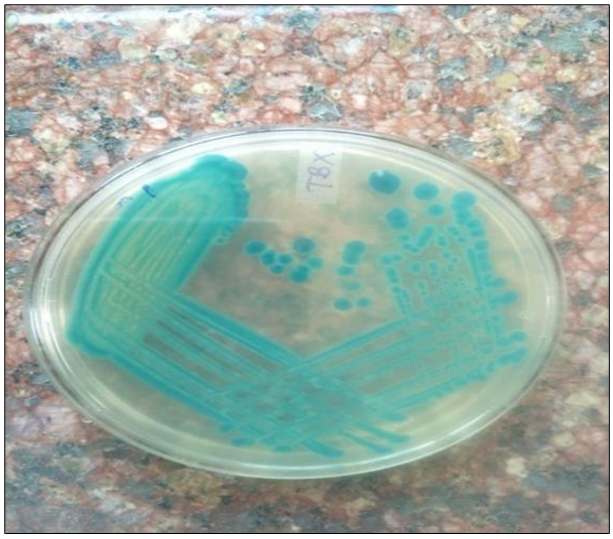

Fig 1: *E. coli* growth on TBX agar plate

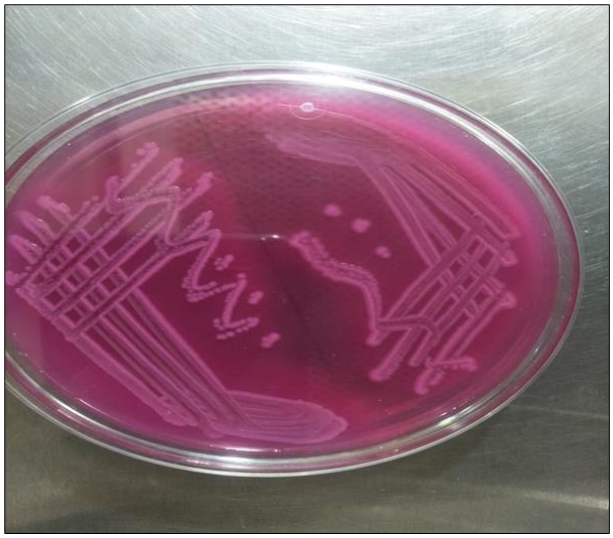

Fig 2: *E. coli* growth on MacConkey agar plate

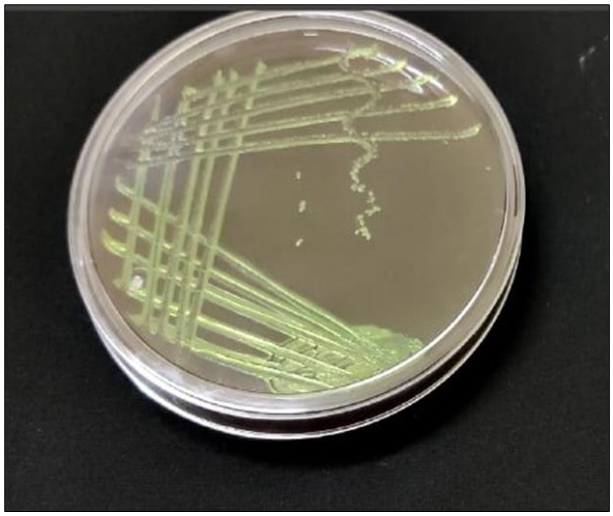

Fig 3: *E. coli* growth on EMB agar plate

Biochemical tests

Following IMViC test, 174 *E. coli* isolates gave positive reaction for indole and methyl red test and negative reaction for Voges Proskauer and citrate utilization test. Hence, giving + + - - IMViC test pattern for all isolates (Fig. 04). The characteristic IMViC pattern obtained was suggestive for presumptive identification of *E. coli* in accordance with the findings of Malik *et al*., 2013 [9, 10].

On Triple Sugar Iron (TSI) agar, the isolates gave yellow colour of butt as well as slant with gas production (Fig. 05) and these findings were similar to the findings of Islam *et al*., (2014) [7], in *E. coli* isolated from diarrhoeic calves (Mishra, 2018) [11] as well as those isolated from sub-clinical mastitis in cattle (Tiwari, 2019) [18].

Fig 4: Biochemical test depicting IMViC test pattern of *E. coli* (++--)

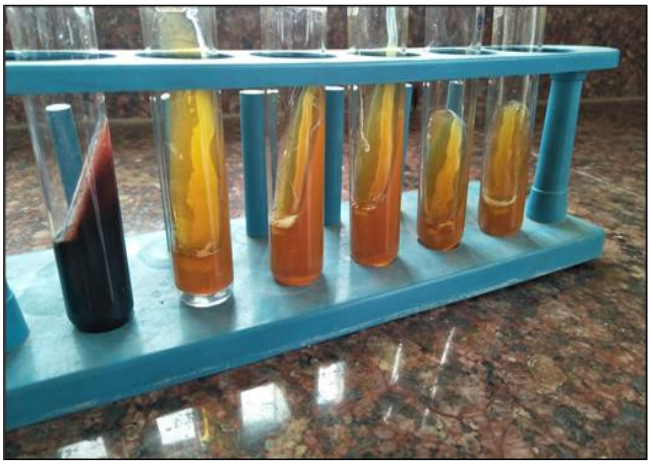

Fig 5: *E. coli* isolates on TSI agar depicting yellow

Prevalence of goat diarrhea due to *E. coli*

The age and sex wise prevalence of undifferentiated diarrhea and *E. coli* associated diarrhea in goats have been presented in the Table no. 04 and 05 respectively. The prevalence was expressed in percent positive by using the following formula (Singh *et al*., 2021) [17]:

$$\text{Prevalence} = \frac{\text{No. of animals positive}}{\text{No. of animals tested}} \times 100$$

Table 4: Age and sex-wise occurrence of undifferentiated diarrhea in goats

Days	0-14	15-30	30-45	45-60	60-90	90-120	3-6 months	Total	Days
Male	15% (30)	12.5% (25)	7.5% (15)	2.5% (5)	3.5% (7)	2.5% (5)	1.5% (3)	45% (90)	Male
Female	21% (42)	15% (30)	7% (14)	4% (8)	3% (6)	3.5% (7)	1.5% (3)	55% (110)	Female
Total	36% (72)	27.5% (53)	14.5% (23)	6.5% (11)	6.5% (13)	6% (12)	3% (6)	100% (200)	Total

Figures in parenthesis indicate number of cases

Table 5: Age and sex-wise occurrence of *E. coli* associated diarrhea in goats

Days	0-14	15-30	30-45	45-60	60-90	90-120	3-6 months	Total
Male	14% (28)	12% (24)	6% (12)	2% (4)	2.5% (5)	1.5% (3)	1% (2)	39% (78)
Female	21% (42)	13.5% (27)	4% (8)	2.5% (5)	2.5% (5)	3% (6)	1.5% (3)	48% (96)
Total	35% (70)	25.5% (51)	10% (20)	4.5% (9)	5% (10)	4.5% (9)	2.5% (5)	87% (174)

Figures in parenthesis indicate number of cases

During the study, the maximum numbers of cases of diarrhoea were reported in age group of 0-14 days (36%) followed by 15- 30 days (27.5%), 30-45 days (14.5%), 45-60 days (6.5%), 60-90days (6.5%), 90-120 days (6%) and 3-6 months (3%). Similarly, majority of the affected kids (35%) with diarrhoea associated with *E. coli* were in the age group 0-14 days (35%), followed by age group 15- 30 days (25.5%), 30-45 days (10%), 45-60 days (4.5%), 60-90days (5%), 90-120 days (4.5%) and 3-6 months (2..5%). The overall prevalence of *E. coli* was 87% in diarrhoeic goats. Incidence of diarrhoea and *E. coli* associated diarrhoea were mostly recorded in females (55% and 48% respectively) as compared to the male kids (45% and 39% respectively).

These findings were similar to the findings of Zaki *et al.* (2010) [21] who reported 68.84% and Patel *et al.* (2017) [12] who reported 70% prevalence for *E. coli* in 1-2 weeks of age group in diarrhoeic goat kids and was in contrast to the findings of Cid *et al.* (1994) [3] who observed lesser percentage (36%) of *E. coli* in diarrhoeic kids. The reasons behind higher prevalence in kids might be due to lack of sufficient acquired immunity, susceptibility to different *E. coli* pathotypes, nutritional imbalance, faulty husbandry practices and unhygienic condition of the farms (Zaki *et al*., 2010).

In the present study, the examination of 200 faecal samples revealed that the occurrence of *E. coli* diarrhoea was more in unorganized farms (71.9%) than in organized farm (43.6%) as presented in Table no. 06. Percentage prevalence of infection in unorganized farms may be high due to lack of colostrum feeding, open grazing malnutrition and contamination of bedding material. On the contrary daily cleaning and periodical disinfection of corrals in farms does not provide suitable environment for higher transmission. Lower prevalence of *E. coli* in organized farm may also be due to adaption of better corral hygiene than field flocks.

Table 6: Prevalence in organized and unorganized farm

S. No.	No. of Animals					
	≤50		≤75		≥100	
	Total	Infected	Total	Infected	Total	Infected
Organised (43.6%)	10	4 (40%)	26	12 (46%)	35	15(42.8%)
Unorganised (71.9%)	25	15(60%)	60	41(68%)	150	113(75%)

Antibiotic Sensitivity Test

The percentage antibiotic sensitivity pattern of *E. coli* isolates from diarrhoeic kids have been presented in Table no. 07. From antibiogram study (Fig. 06), it was revealed that among the *E. coli* isolates from diarrhoeic samples, 97.7% isolates were highly sensitive to Gentamicin and Amikacin followed by Chloramphenicol (92.5%), Meropenem (82.7%), Ciprofloxacin (78.1%) and Tetracycline (70.6%). However, maximum resistance was shown against Tetracycline (29.3%) followed by Ciprofloxacin (21.8%) and Meropenem (0.04%). The increased resistance to antibiotics among enteric bacteria might have increased markedly due the excessive use of antibiotics. as the cases had history of prior treatment.

Table 7: Susceptibility pattern of the *E. coli* isolates against antimicrobial agents in Disk Diffusion Assay

S. No.	Antimicrobial Disc	Symbols	Concentration (mcg)	Interpretation (mm)			
				R	I	S	Total
1.	Tetracycline	TE	30	51	0	123	174
2.	Ciprofloxacin	CIP	5	38	0	136	174
3.	Gentamicin	GEN	10	4	0	170	174
4.	Amikacin	AK	30	4	0	170	174
5.	Meropenem	MRP	10	8	22	144	174
6.	Chloramphenicol	C	30	4	9	161	174

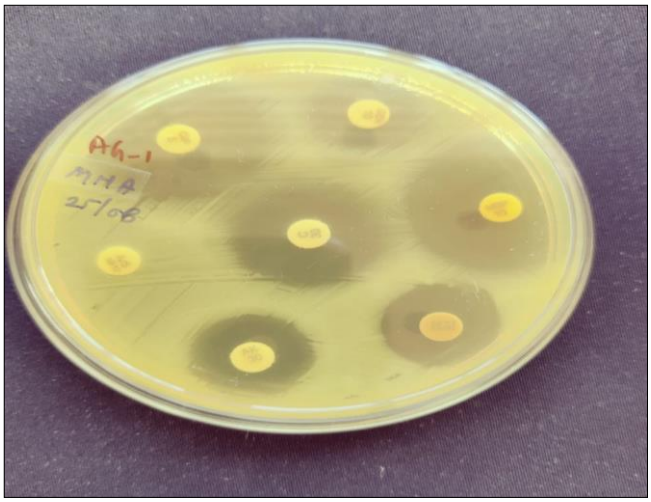

Fig 6: Antibiotic sensitivity test of *E. coli*

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