RESEARCH ARTICLE Indian Journal of Animal Research, Volume 60 Issue 4: 720-727 (April 2026)

Prevalence and Phylogenetic Analysis of *E. coli* in Diarrhoeic Goats in Eastern Plain Zone of Uttar Pradesh

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ABSTRACT

Background: The pathogens associated with diarrhoea in small ruminants include *Escherichia coli*, Rotavirus, PPR virus and *Salmonella spp.* either singly or in combination. Many researchers have worked on *E. coli* strains of goats almost in all agro-climatic zones of India but most of the studies were limited to only small study areas and no study has been conducted on *E. coli* prevalence in goats in Eastern Plain Zone of Uttar Pradesh.

Methods: The study was conducted in eight district of Eastern Plain zone of Uttar Pradesh including goat farm of ANDUAT, Kumarganj, Ayodhya which comes under Agro-climatic zone IV (Middle Gangetic Plain region). Faecal samples were collected for one year (September, 2021 to August, 2022) from dead goats having history of diarrhoea.

Result: The overall prevalence of *E. coli* isolates on PCR analysis was 52.27%. The prevalence was highest in Varanasi district as 63.63% (7/11) while lowest was reported in Ghazipur district as 36.36% (4/11). The occurrence of *E. coli* isolates was higher in Age Group I (79.31%) followed by Age Group II (41.17%) and Age Group III (36.00%). The *E. coli* isolates revealed higher positivity in female (57.69%) than male (44.44%). The occurrence of *E. coli* isolates was highest in summer (75.86%) followed by rainy (42.42%) and lowest in winter (38.46%) season. The obtained PCR amplicons on sequencing was identified as *Escherichia coli* strain U 5/41 16S ribosomal RNA as it showed highest similarity of 98.96 % with accession no. NR_024570.1

Key words: *E. coli*, Goat, Phylogenetic analysis, Prevalence.

INTRODUCTION

India accounts for the largest livestock sector in the world with 27.80% goat population of the total livestock in India and thus playing a key role in the rural economy (20th Livestock Census). Goats are considered to be one of the earliest domesticated animals and constitute 14.28 million populations in Uttar Pradesh (10% goat population in UP). Infectious diseases of caprine and ovine results in heavy loss in national economy. The major clinical signs associated with intestinal disorders are diarrhoea, anorexia, weight loss, anaemia, general weakness, retarded growth, reduction in meat and milk yield, digestive inefficiency, severe debility and even death in severe cases. Diarrhoea is a major problem in small ruminants affecting the profitability of goat husbandry practices in most countries including India. It's etiology is complicated by the detection of multiple pathogens.

The most important enteropathogens associated with diarrhoea in small ruminants include: Rotavirus, PPR virus, enterotoxigenic *Escherichia coli* (ETEC), *Salmonella spp.* and Cryptosporidium either singly or in combination (Shabana *et al.,* 2017). Other pathogens may also have a role in enteric diseases including: *Clostridium perfringens, Giardia spps, Eimeria* species, *Campylobacter*, *Klebsiella* and *Proteus* (Shabana *et al.,* 2017). Caprine Rota Virus have been commonly implicated in diarrhoea in 2-3 days old kids and the identified goat RV strains are RVA, RVB or RVC (Shabana *et al.,* 2017) but Wani *et al.* (2004) considered that *E. coli* mainly affect animals during first week while rotavirus affects between 7 and 30 days' age group.

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How to cite this article: Gupta, R.K., Niyogi, D., Joshi, R.K., Yadav, V., Jaiswal, V., Shrivastava, D.P., Singh, S.V., Jaiswal, S., Singh, J. and Alam, K. (2026). Prevalence and Phylogenetic Analysis of *E. coli* in Diarrhoeic Goats in Eastern Plain Zone of Uttar Pradesh. *Indian Journal of Animal Research*. **60(4):** 720-727. doi: 10.18805/IJAR.B-5179.

Submitted: 19-06-2023 **Accepted:** 26-02-2024 **Online:** 03-07-2024

E. coli is a Gram-negative, motile, facultative anaerobe, non-spore forming bacteria belong to family Enterobacteriaceae. Most *E. coli* strains are part of gastrointestinal tract flora, but some strains possess virulence factors that enable them to cause diarrhoea in neonatal farm animals and humans (Nguyen *et al.,* 2005). Enterotoxigenic *E. coli* (ETEC) stick to the intestinal microvilli and producing enterotoxins acting on enterocytes. The important virulence factors of enterotoxigenic *E. coli* are enterotoxins and adhesins (Wani *et al.,* 2003). The main adhesins of animal-origin ETEC are the fimbriae (pili): F4 (K88), F5 (K99), F6 (987P), F42, F41, F165, F18 and F17. ETEC produce either heat-stable (ST) or heat-labile (LT) (Gaastra and Svennerholm, 1996).

Many researchers have worked on *E. coli* strains of goats almost in all agro-climatic zones of India but most of the studies were limited to only small study areas and no study has been conducted on *E. coli* prevalence in goats in Eastern Plain Zone of Uttar Pradesh in general and none report so far has been published from the current study areas. Therefore, the objective of this study was to record the prevalence of *E. coli* infection in goats and thereby, design effective disease control and prevention strategies accordingly.

MATERIALS AND METHODS

Sample area and study period

The Study was conducted in eight district of Eastern Plain zone of Uttar Pradesh (Ayodhya, Ambedkar Nagar, Azamgarh, Mau, Ballia, Ghazipur, Jaunpur and Varanasi) which comes under Agro-climatic zone IV (Middle Gangetic Plain region). Faecal samples were collected for one year (September, 2021 to August, 2022) from dead goats having history of diarrhoea from organized and unorganized goat farms of eight district of Eastern Plain zone of Uttar Pradesh including goat farm of ANDUAT, Kumarganj, Ayodhya.

Sample collection

The date of sampling, sex, age and identification number were recorded for each dead goat. A total of 88 faecal samples of which 33 in rainy season, 29 in summer and 26 in winter season were collected. The study included 36 males and 52 females. Depending upon age, dead goats were categorized into 3 groups *viz.* Age Group I (0-3 month), Age Group II (3-12 month) and Age Group III (>12 months).

Collection of faecal samples

Eighty-eight faecal samples/intestinal contents were collected from intestine while doing post-mortem using a disposable latex gloves. All standard precautionary measures were taken to avoid contamination of samples and transported to the clinical pathology laboratory in ice (refrigerated conditions- 40°C) and stored at -200°C until processing. All the samples were processed and screened for *E. coli,* Rotavirus and gastrointestinal parasites. Samples were immediately transported to the Bacteriology Laboratory, Department of Veterinary Microbiology in ice and were immediately cultured or stored at 4°C until cultured.

Isolation of *E. coli*

The samples were processed for the isolation of *E. coli*. as follows:

Plating

Following incubation, a loopful of inoculum was streaked onto Mac-Conkey Lactose Agar (MLA) containing 2 mg/ml of cefotaxime and incubated at 37°C for 18-24 hours. Colonies with rose pink coloration were selected and single colonies were picked up and taken onto Eosine Methylene Blue (EMB) agar and incubated at 37°C for 24 hours. The colony exhibiting distinctive metallic sheen on EMB agar plates were considered as *E. coli* (Cruickshank *et al*., 1975). All the suspected colonies were taken onto nutrient agar slant and stored at 4χC for further identification.

Identification of *E. coli*

The identification of *E. coli* was done on the basis of morphology, growth and biochemical characteristics as per the method described by Edwards and Ewing (1972). The isolates were grown overnight at 37°C in nutrient broth and morphological characteristics were considered for preliminary identification.

Gram's staining

For Gram's staining, a smear was prepared from the culture of nutrient agar slants on a microscopic glass slide with a loopful of culture, air dried, heat fixed and stained with Gram's staining procedure.

Biochemical characterization

Catalase test

A loopful of overnight grown culture of the isolates was mixed with a drop of 3% hydrogen peroxide over a clean glass slide. The production of gas bubbles or effervescence within a few seconds was considered as catalase positive.

Indole test

The isolates were inoculated in 2.0 ml of peptone water and incubated at 37°C for 24 hours. About 0.2 ml of Kovac's reagent was then added and observed for change of color. Development of pink color ring on the top of culture was considered as positive for indole production.

Methyl red test

The isolates were inoculated in MR-VP media and incubated at 37°C for 24 hours. Five drops of MR reagent were then added. Development of cherry red color of the medium was considered as positive test.

Voges-proskauer test

A loopful of test culture was inoculated in 2.0 ml of MR-VP media and incubated at 37°C for 48 hours. After that 1.0 ml of KOH (40% w/v) and 3.0 ml of α-naphthol (Barritt's Reagent) was added. Development of cherry red color was considered as positive.

Citrate test

The culture was inoculated upon Simmon's Citrate Agar slant and incubated for 48 to 72 hours at 37°C. If the original

green color of the slant turned to blue, the test isolates were considered as positive.

Urease test

The culture was inoculated upon urea agar slant and incubated for 48 to 72 hours at 37° h in positive cases while no change in color was considered as negative.

Sugar fermentation reaction

The sugars used for fermentation reaction included Glucose, Lactose, Sucrose, Fructose, Maltose, Mannitol and Sorbitol. The appearance of pink color of the medium and production of gas in Durham's tube was considered as positive for sugar fermentation.

Molecular characterization of *E. coli*

Extraction of genomic DNA

For confirmation, the pure cultures of *E. coli* were subjected to amplification. The DNA templates were prepared using snap-chill method as described by Rawat *et al.* (2018) with slight modification.

PCR analysis

The PCR reactions were performed in 20 μl volumes containing 2 μl of the genomic DNA sample, 1× PCR buffer containing; 0.16 mM dNTP Mix; 20 pmol of forward and reverse primers and 0.75U Taq DNA polymerase (MBI, Fermentas, Lithuania). The mixes were overlaid with 2 drops of mineral oil. Amplification was carried out in a thermal cycler (Eppendorf Mastercycler nexus 230 V/50-60 Hz) with the PCR conditions as follows: an initial denaturation at 95°C for 6 min, 40 cycles of denaturation at 95°C for 30 s, annealing at 50°C for 1 min and extension at 72°C for 1 min. A final extension was performed at 72°C for 10 min. PCR products were analyzed using 2% agarose gel electrophoresis. Sizes of the amplified 16S rDNA segments using primers were estimated using molecular weight markers NEX-GEN DNA Ladder (100 bp) and the gel was run at 100 volts for 45 minutes at room temperature. The PCR products was stained with ethidium bromide and visualized on Gel Documentation System (Zenith, Gel Documentation System; Model No. Gel. LUMINAX-312), the samples were then sequenced using Sanger's Method.

Sequencing of PCR amplicons

Sequencing of PCR amplicons were carried out by Cytogene lab, Lucknow. The obtained forward and reverse sequences were aligned using online pairwise alignment tool BioEdit. The query sequences were identified considering *E* value as $<1 \times 10^{-5}$ and maximum hits (99 or 100%) with a species in the reference database NCBI. In addition to BLAST, MEGA X was used for phylogenetic tree analysis employing Maximum Likelihood (ML) method.

RESULTS AND DISCUSSION

The present study was conducted to isolate and characterize *E. coli* from faecal samples collected from dead diarrhoeic

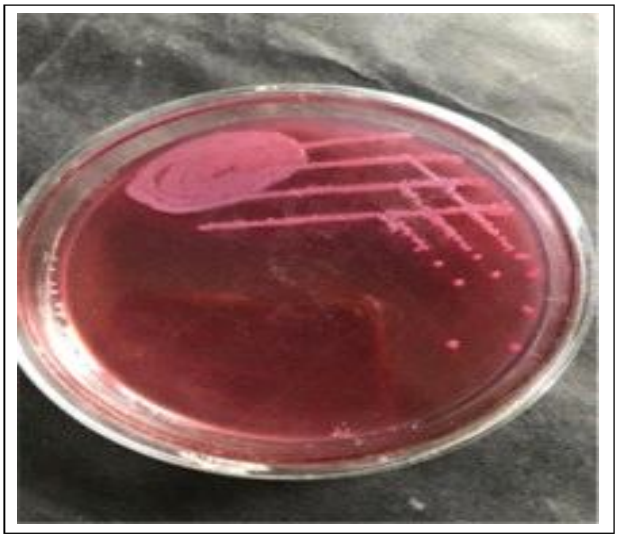

Fig 1: Typical rose pink colonies of *E. coli* on MLA media.

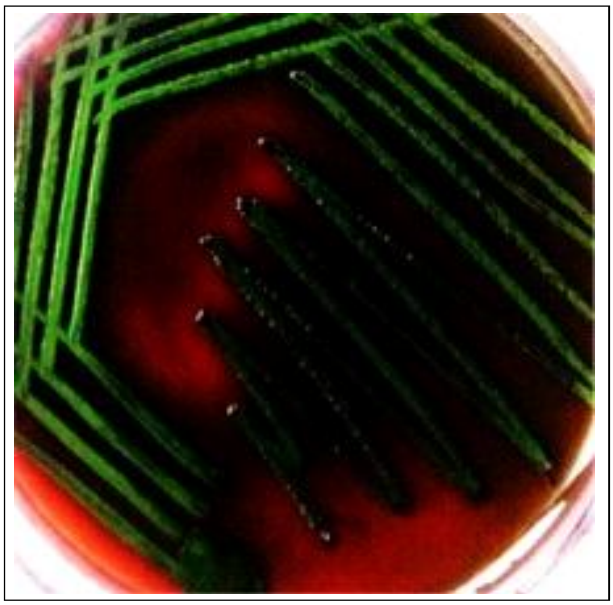

Fig 2: Green metallic sheen of *E. coli* on EMB agar.

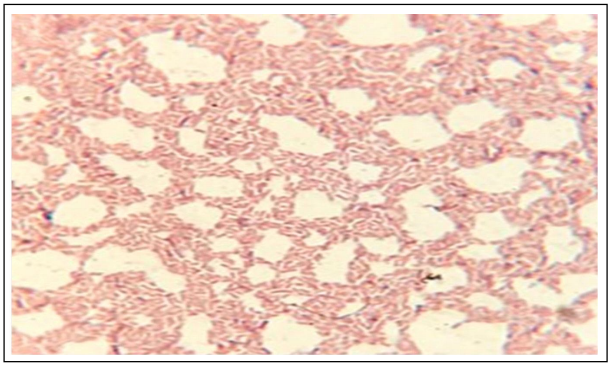

Fig 3: Shows Gram staining characteristics of *E. coli* as rod shaped pink colour stain (1500X).

goats in Eastern plain zone of Uttar Pradesh. A total of 88 samples were examined for prevalence of *E. coli* that were collected from eight districts of Eastern plain zone of Uttar Pradesh.

Isolation and cultural identification of *E. coli*

For isolation of *E. coli*, all 88 faecal samples were initially screened on MLA plates. Colonies with rose pink colouration were suspected as *E. coli*, (Fig 1). Lactose fermenting pink coloured colonies were selected for identification. A single rose pink colony was picked up and streaked on EMB plate. Colonies with greenish metallic sheen (Fig 2) on EMB agar plates were tentatively considered to be of *E. coli*. Fifty-eight (58) isolates of *E. coli* were obtained out of total 88 faecal samples were presumptively positive, which were further characterized on the basis of Gram's staining, standard biochemical tests and molecular detection by PCR analysis using specific oligonucleotide primers.

Biochemical and molecular characterization of *E. coli* isolates

The isolates which showed cultural characteristics similar to *E. coli* were further confirmed by Gram's staining, standard biochemical tests (Table 1). Gram negative isolates (Fig 3) showing IMViC pattern (+ + - -) (Fig 4), catalase (+ve) (Fig 5), urease test (-ve) (Fig 6) and fermented glucose, lactose, mannitol, sucrose and sorbitol by production of acid and gas (Fig 7) were taken as final presumptive *E. coli* isolates. Out of 88 isolates, 49 were considered as final presumptive *E. coli* after biochemical analysis (Table 1). Analysis for 16S rRNA on PCR revealed 46 isolates as confirmed *E. coli* (Table 2) (Fig 8).

Sequencing of PCR amplicons and data analysis were carried out by Cytogene Laboratory, Lucknow. The obtained forward and reverse sequences were aligned using online pairwise alignment tool BioEdit. The query sequences were identified considering *E* value as $<1 \times 10^{-5}$ and maximum hits

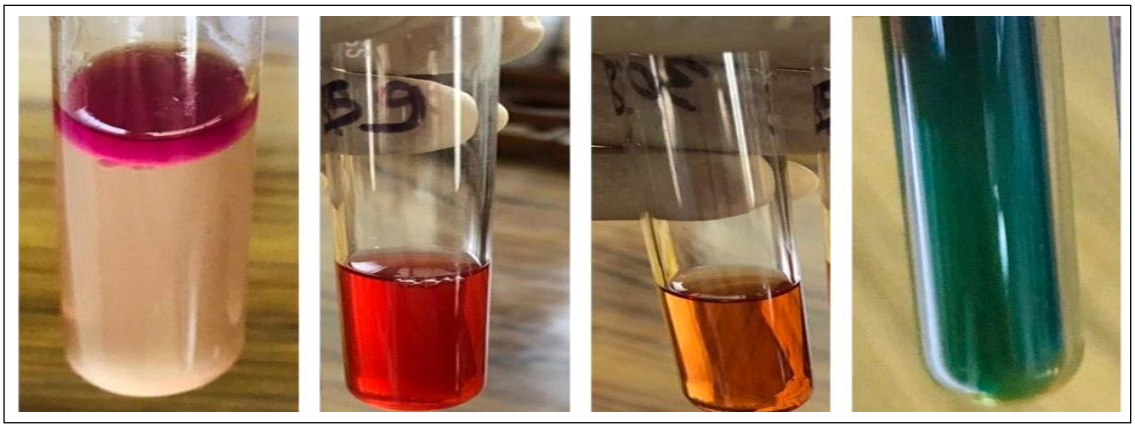

Fig 4: IMViC patern of *E. coli* (+ + - -).

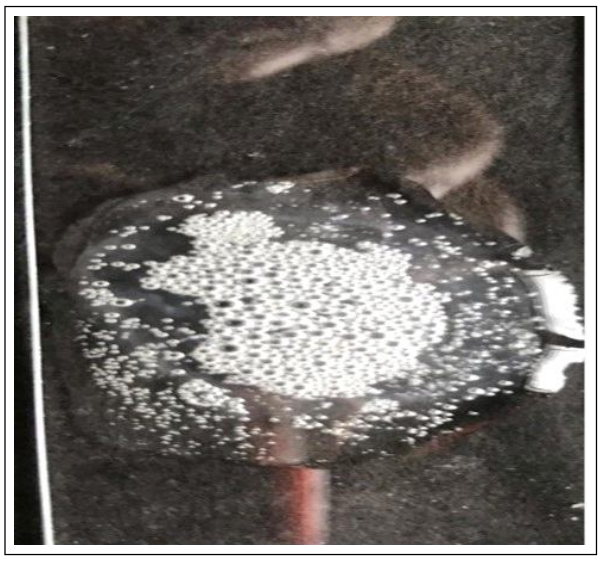

Fig 5: *E. coli* shows catalase test positive

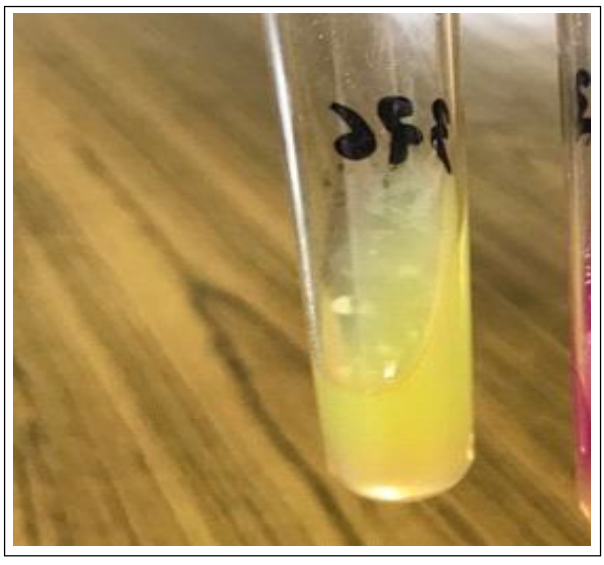

Fig 6: *E. coli* shows urease test positive.

(99 or 100%) with a species in the reference database NCBI and query sequences was identified as *Escherichia coli* strain U 5/41 16S ribosomal RNA as it showed highest similarity of 98.96 % with accession no. NR_024570.1 (Phylogenetic tree is attached in Fig 9 and Fig 10).

Overall and district-wise prevalence of *E. coli* isolates

Overall prevalence of presumptive positive isolates was 55.68% (49) in 88 faecal samples obtained from dead diarrhoeic goats while on PCR analysis, prevalence was 52.27% (46/88) (Table 2). The prevalence of presumptive *E. coli* was highest in Ayodhya District (68.75%) and lowest in Ambedkar Nagar district (40%). The occurrence of confirmed positive isolates on PCR analysis was highest in Varanasi district (63.63%) while lowest was reported in Ghazipur district (36.36%) (Table 2). The overall prevalence of presumptive *E. coli* positivity corroborated with the findings of Wani *et al*. (2004) who reported 58.83% *E. coli* isolates



Fig 7: *E. coli* shows positive sugar fermentation test.

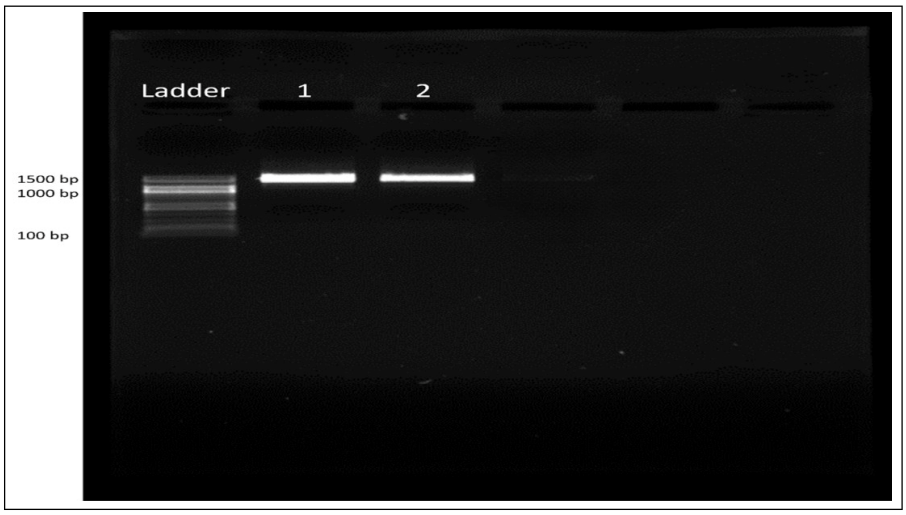

Fig 8: PCR amplification of 16S rRNA gene: product size -1.5 kb.

Table 1: Result of biochemical characterization of *E. coli* isolates.

Biochemical tests			Result
IMViC Pattern		Indole	+
		Methyl red	+
		Voges Proskauer	-
		Citrate utilization	-
Catalase test			+
Urease test			-
	Glucose	Acid	+
		Gas	+
	Sucrose		Acid
	Sugar fermentation		+
		Gas	+
	Mannitol	Acid	+
		Gas	+
	Sorbitol	Acid	+
		Gas	+

Table 2: District wise prevalence of presumptive and confirmed *E. coli* isolates.

District	Sample examined	Presumptive positive isolates (No./%)	Confirmed positive isolates (No./%)
Ayodhya	16	11 (68.75)	10 (62.50)
Ambedkar Nagar	10	4 (40.00)	4 (40.00)
Azamgarh	9	5 (55.55)	5 (55.55)
Ghazipur	11	5 (45.45)	4 (36.36)
Mau	9	6 (66.66)	5 (55.55)
Ballia	10	6 (60.00)	6 (60.00)
Jaunpur	12	5 (41.66)	5 (41.66)
Varanasi	11	7 (63.63)	7 (63.63)
Total	88	49 (55.68)	46 (52.27)

Table 3: Age-wise, sex-wise and seasonal prevalence of *E. coli* isolates.

	Category	Samples examined	Final presumptive positive isolates (No./%)	Confirmed positive isolates (No./%)
Age	Group I	29	25 (86.20)	23 (79.31)
	Group II	34	15 (44.11)	14 (41.17)
	Group II	25	9 (36.00)	9 (36.00)
Sex	Male	36	17 (47.22)	16 (44.44)
	Female	52	32 (61.53)	30 (57.69)
Season	Rainy	33	15 (45.45)	14 (42.42)
	Winter	26	10 (38.46)	10 (38.46)
	Summer	29	24 (82.75)	*E* 75.86)

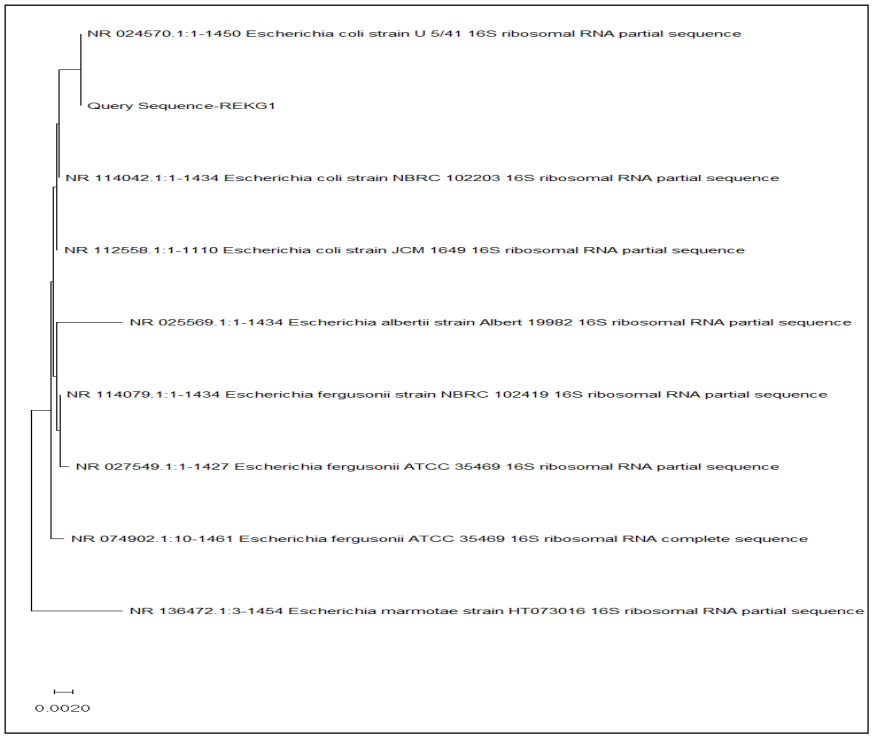

Fig 9: The query sequence was identified as *Escherichia coli* strain U 5/41 16S ribosomal RNA as it showed highest similarity of 98.35% with accession no. NR_024570.1

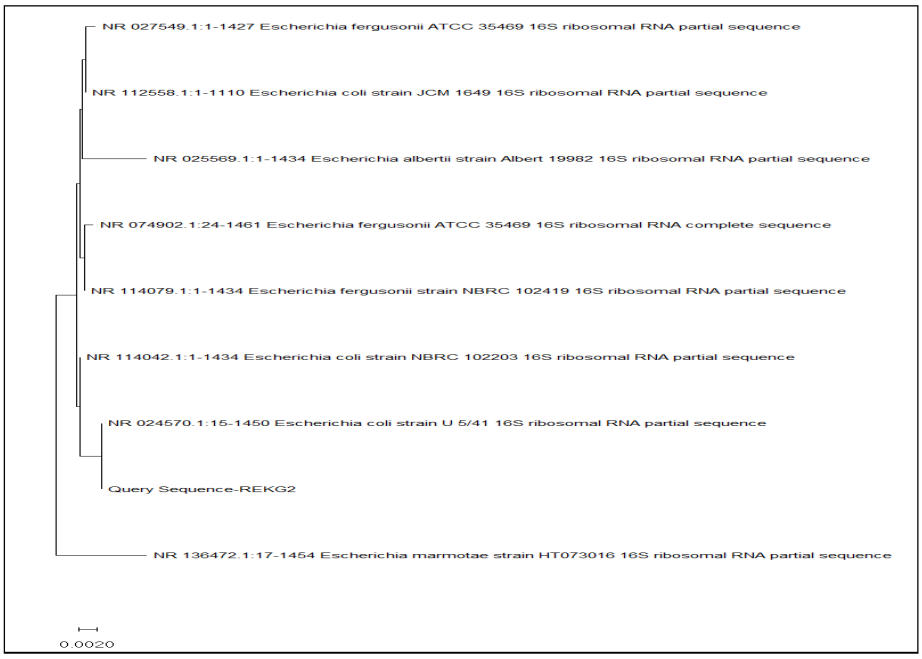

Fig 10: The query sequence was identified *as Escherichia coli* strain U 5/41 16S ribosomal RNA as it showed highest similarity of 98.96% with accession no. NR_024570.1

in lambs in Kashmir Valley but the results was inconsistent with the findings of Sharma *et al.* (2020) who found only 17.46% (22/126) prevalence in goat kids in Udaipur, Rajasthan. The present findings were also inconsistent with reports of Hardik *et al.* (2017) and Younis *et al.* (2009) who reported very high prevalence of *E. coli* isolates as 72.81% (75/103) in Junagarh, Gujarat and 87.72% (193/220) in Egypt, respectively.

Age-wise, sex-wise and seasonal prevalence

The prevalence of presumptive *E. coli* isolates was highest in Age Group I (86.20%) followed by Age Group II (44.11%) and Age Group III (36.00%) (Table 3). On PCR analysis, order of prevalence was same but percentage was changed in Age Group I and Age Group II as 79.31% and 41.17% respectively (Table 3). Since no earlier study conducted in this zone with respect to age group, the results of present study was compared to earlier study of Sharma *et al*. (2020) who reported similar findings as higher prevalence in age group (0-1 month) as 45.45% followed by 1-2 months (31.81%) and 2-3 months (22.72%) in Udaipur, Rajasthan and Shabana *et al.* (2017) whose results were corroborating with the present findings as they also reported higher prevalence of ETEC in younger age group (17.90%) than adult (7.70%) in goats in Medina, Saudi Arabia. The higher prevalence in goat kids is attributed to less intake of colostrum, immature immune system and lack of specific antibodies.

The occurrence of presumptive *E. coli* isolates was higher in female (61.53) when compared to male (47.22%) goats (Table 3). The prevalence of confirmed *E. coli* isolates by PCR analysis also revealed higher positivity in female (57.69%) than male (44.44%). The present results corroborated with the reports of Sharma *et al.* (2020) who reported higher prevalence in female (63.64%) than male (36.36%) goats in Udaipur district of Rajasthan. Higher occurrence in females might be due to reduced immune system, breeding stress and lactation period which exposes them to micro-organisms invasion. The present study contradicted with report of Shabana *et al.* (2017) who reported higher prevalence in male goats and was difference of 50% positivity in male (20.50%) and female animals (10.30%) in Medina Saudi Arabia.

The prevalence of presumptive *E. coli* isolates was highest in summer (82.75%) followed by rainy (45.45%) and lowest in winter (38.46%) season (Table 3). On PCR analysis, pattern of order of prevalence was same of confirmed *E. coli* isolates as 75.86% and 42.42% in summer and rainy season, respectively (Table 3). The results of this study simulated the reports of Pralhad *et al.* (2018) who also reported higher positivity in summer when compared to winter and rainy season. This is might be due to fact that warmer weather provides favourable conditions for *E. coli* growth or animal movement in summer between pasture and housing, diet change may lead to stress in animal (Pralhad *et al.,* 2018).

CONCLUSION

It is concluded from this study that *E. coli* infections are prevailing throughout the year in districts (under study) of Eastern Plain zone of Uttar Pradesh. However, further epidemiological studies in relation to the strains, antigenic structures with reference to serotypes and zoonotic potential

of these organisms are necessary. It is required to evaluate the possible variation in infection in diverse geographic zones which might help to propose an effective control and prevention methods against these infections.

Conflict of interest

The authors report no conflict of interest.

REFERENCES

Cruickshank, H.R., Duguid, J.P., Marmion, B.P. and Swain, R.H.A. (1979). Medical Microbiology. 12th Edn. pp. 641

Edwards, P.R. and Ewing, W.H. (1972). Identification of enterobacteriaceae. Burgess Publication, Minneapolis, 3rd edn. pp. 362.

Gaastra, W. and Svennerholm, A.M. (1996). Colonization factors of human enterotoxigenic *Escherichia coli* (ETEC). *Trends in Microbiology.* **4:** 444-452.

Hardik, P., Kalyani, I.H., Akshay, B., Vaibhav, D., Pramod, S. and Kishan, S. (2017). Isolation, characterization, serotyping and antibiogram studies of *E. coli* collected from diarrhoeic neonatal kids. *International Journal of Microbiology Research*. **9(2):** 854-856

Nguyen, D., LeVan, P., Le Huy, C., Gia, K.N. and Weintraub, A. (2005). Detection and characterization of diarrheagenic *Escherichia coli* from young children in Hanoi. *Vietnam Journal of Clinical Microbiology*. **43:** 755-760.

Prahlad, Awati, B., Ramesh, B.K., Patil, N.A., Kumar, P., Rao, J., Tikare, V., Mallinath, K.C., Kharate, A., Suryakanth, P., Paramesh, N.B. and Revappayya, M. (2018). Influence of age, sex and Season on the occurrence of *Escherichia coli* O157: H7 in sheep and goats of Hyderabad-Karnataka Region, India. *Int. J. Curr. Microbiol. App. Sci.* **7(6):** 2896-2904.

Rawat, N., Maansi, Kumar. and Upadhyay, A.K. (2018). Virulence typing and antibiotic susceptibility profiling of thermophilic Campylobacters isolated from poultry, animal and human species. *Veterinary World*. **11(12):** 1698-1705.

Shabana, I.I., Bouqellah, N.A. and Zaraket, H. (2017). Investigation of viral and bacterial enteropathogens of diaarheic sheep and goats in Medina, Saudia Arabia. *Tropical Biomedicine.* **34(4):** 1-12.

Sharma, S.K., Manat, N. and Joshi, M. (2020). Prevalence of colibacillosis in goat kids in Udaipur district of Rajasthan. *The Indian Journal of Veterinary Science and Biotechnology*. **6(2):** 98-100.

Wani, S.A., Bhat, M.A., Samanta, I., Ishaq, S.M., Ashrafi, M.A. and Buchh, A.S. (2004). Epidemiology of diarrhoea caused by rotavirus and *Escherichia coli* in lambs in Kashmir Valley, India. *Small Ruminant Research*. **52:** 145-153.

Wani, S.A., Bhat, M.A., Samanta, I., Nishikawa, Y. and Buchh, A.S. (2003). Isolation and characterization of shiga toxin producing *Escherichia coli* (STEC) and enteropathogenic *Escherichia coli* (EPEC) from calves and lambs with diarrhoea in India. *Letters in Applied Microbiolog*. **37:** 121-126.

Younis, E.E., Ahmed, A.M., El-Khodery, S.A., Osman, S.A. and El-Naker, Y.F. (2009). Molecular screening and risk factors of enterotoxigenic *Escherichia coli* and *Salmonella* spp. in diarrheic neonatal calves in Egypt. *Research in Veterinary Science*. **87(3):** 373-379.