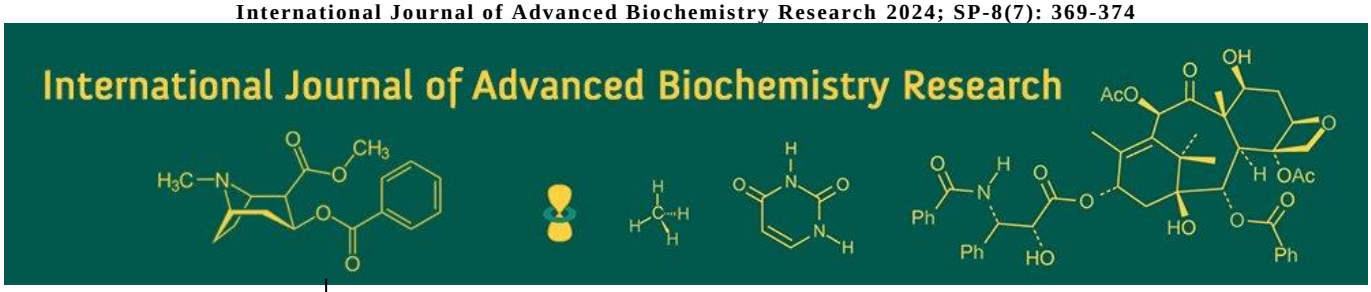

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Proteus mirabilis: A cause of enteric infection in goats

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Abstract
The present investigation was carried out to study the association of *Proteus mirabilis* to enteric infections in goats of Chhattisgarh. A total of 274 dead goats were screened for presence of enteric lesions. Among these, 114 goats showed prominent enteric lesions, from which, 96 faecal swabs and 18 swabs of mesenteric lymph nodes were collected for bacteriological examination. Moreover, representative tissue samples were also taken for histopathology. Overall, 54 bacterial isolates were recovered from 114 samples. Amongst 54 bacterial isolates, 02 (3.63%) isolates were identified as *Proteus mirabilis* by using Gram's staining, colony as well as biochemical characteristics, Matrix-assisted laser desorption ionization-time of flight mass spectrometry analysis and 16s rRNA gene sequencing. Further, phylogenetic analysis was performed. On postmortem examination, gross changes observed as enteritis, congestion and hemorrhages in intestinal mucosa, mucosal thickening, ballooning of intestine, presence of necrotic debris and exudate in lumen and enlarged mesenteric lymph nodes. Histopathology of intestine revealed necrosis of superficial epithelium and lamina propria of small intestine with infiltration of inflammatory cells, villous atrophy and haemorrhagic crypts and typical inflammatory lesions in the large intestine. It could be concluded that, *Proteus mirabilis* causes enteric infections in goats even though it is a common intestinal flora in these animals.

Keywords: Goat, intestine, *Proteus mirabilis*, enteric infections

1. Introduction
Livestock rearing is one of the most important economic activities in the country's rural areas, with a significant contribution to the national economy. It provides a significant source of income for landless and marginal farmers. It also acts as source of protein in form of milk, egg and meat.

Since ancient times, raising goats has been a vital part of agriculture and a primary source of livelihood. The higher demand for meat and skin in the local and foreign markets, focused goat enterprise as extremely important to the vulnerable group of farmers in the country's existing socioeconomic conditions. The success of goat farming depends on the health of the flock. Good health of the animals not only increases the productivity but also the profitability (Khadda *et al*., 2020) [17]. Diseases have a negative impact on livestock productivity because they reduce feed intake, alter digestion and metabolism, increase morbidity and mortality and reduce reproduction, weight gain and milk production rates.

Among goats, the gastrointestinal tract may be the most susceptible to illness. This system's drawback is that animals are exposed to a wide range of pathogens. Of all the diseases, digestive system infections can result in significant losses for goat farmers. Higher rate of kid mortality in the current production system has also been attributed to low birth weight, inadequate milk production of does shortly after kidding, inadequate care and general poor husbandry practices. Because of these, public health officials, veterinarians, and farmers are all interested in enteric pathogen infections. These agents cause substantial financial loss to producers due to their high rates of morbidity and mortality, decreased production efficiency and therapy-related costs. Furthermore, animals that have diarrhoea can harbour these pathogens and infect humans and other healthy animals. Enteritis continues to be the most prevalent and expensive disease affecting small ruminants, even with advancements in management techniques, prevention and treatment methods. Resistance strains have emerged as a result of the indiscriminate use of antibiotics in animals to treat a variety of bacterial infections (Meshram *et al*., 2009) [20].

Proteus mirabilis is a rod-shaped, gram-negative bacterium that exhibits rapid, coordinated multicellular activity as well as a unique "swarming" behaviour on agar plates. Peritrichous flagella, which are necessary for its movement across surfaces results in production of the identifiable "bull's-eye" pattern. Notably, *Proteus mirabilis* is present in a variety of environments, such as sewage, water and soil (Armbruster *et al*., 2018) [2]. Still, it is primarily found in the digestive tracts of animals and humans because it is regarded as an essential component of the faecal flora (Chakkour *et al*., 2024) [5].

Proteus mirabilis is one of the main pathogens of the urinary tract and primary infectious agents in patients with indwelling urinary catheters (Armbruster *et al*., 2018) [2]. This sp most frequently isolated from human clinical material. However, several recent investigations in human identifies the *Proteus* spp as potential gut pathogens. *Proteus* spp possess many virulence factors potentially relevant to gastrointestinal pathogenicity, including motility, adherence, fimbriae, the production of urease, hemolysins, metallophores, biofilm formation and IgA proteases and the ability to acquire antibiotic resistance (Hamilton *et al*., 2018) [14]. Besides urinary tract infections, *Proteus mirabilis* have been described as opportunistic etiological agents in infections of the respiratory tract and of wounds, burns, skin, eyes, ears, nose and throat as well as in gastroenteritis (Rozalski *et al*., 1997) [24].

In addition to human, this bacterium has been isolated from a variety of animal species, including chickens, ducks, dogs, foxes, goats, mink and others (Hu *et al*., 2020) [15]. Previous researchers have described it as an opportunistic pathogen of intestines in calves, sheep and dogs (Botes 1964; Chatzopoulos *et al*., 2016; Hu *et al*., 2020) [3, 6, 15].

It has been observed that *Proteus mirabilis* had developed severe drug resistance to common antibiotics. Drug-resistant strains have thus caused clinical challenges and have emerged as a possible threat to public health (Cernohorska and Chvilova, 2011) [4].

Various studies had been performed relating to the isolation of *Proteus* spp in urinary and digestive tract infection of human and few animal species. But there is a meager information regarding enteric infection related to *Proteus* spp in veterinary science. Keeping in-view the aforementioned facts, the present study was carried out to investigate the *Proteus mirabilis* associated enteric diseases in goats of Chhattisgarh.

2. Materials and Methods

2.1 Place of work

The research was carried out from December 2020 to July 2023 in the Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Dau Shri Vasudev Chandrakar Kamdhenu Vishwavidyalaya, Anjora, Durg, Chhattisgarh.

2.2 Collection of the samples

The samples were collected from goat carcasses that had been submitted for post mortem examination to the department or during field visits to several organized and unorganized goat farms in Durg, Rajnandgaon, Balod and Raipur districts of Chhattisgarh.

Throughout the study period, a total of 274 deceased goats of either sex were randomly screened for the enteric illnesses during post mortem examination. Among them, 114 goats had the most prominent enteric lesions from which samples were collected for various pathological investigation.

Faecal swabs and swabs from the mesenteric lymph nodes were collected from dead goats with suggestive lesions of enteric disease. The swabs were suspended in phosphate buffer saline (PBS) and were stored at 4 °C for bacterial culture and at -20 °C for their molecular characterization. Moreover, representative tissue samples from mesenteric lymph node, small intestine and large intestine were collected in separate container and preserved in 10% neutral buffer formal saline solution for histopathological examination.

2.3 Isolation and identification of bacteria

For isolation of *Proteus* spp, the swabs were streaked onto 5% calf blood agar and Mc Conkey agar and they were aerobically incubated at 37 °C for 24 h. Bacteria were identified based on Gram's staining, colony and biochemical characteristics. The identity of bacteria was further confirmed by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDl-TOF MS). For this analysis, bacterial isolates were outsourced to National Centre for Microbial Resource, NCCS, Pune, Maharashtra, India.

2.4 Biofilm forming ability

Biofilm forming ability of bacteria was detected by Congo Red Agar (CRA) method. Briefly, CRA media were prepared using 50 g/L of sucrose, 37 g/L of brain heart infusion agar and 0.8 g/L of Congo Red indicator. First Congo red stain was prepared as a concentrated aqueous solution and autoclaved (121 °C for 15 min) separately from the other medium constituents. Then it was added to the autoclaved brain heart infusion agar with sucrose at 55 °C. CRA plates were inoculated with test organisms and incubated at 37 °C for 24-48 h aerobically. Black colonies with a dry crystalline consistency indicate biofilm production (Karthik *et al*., 2018) [16].

2.5 Nucleotide sequencing

A bacterial isolate was sent to the National Center for Microbial Research (NCMR), National Center for Cell Science, Pune, Maharashtra, for partial nucleotide sequencing of the 16s rRNA gene in order to obtain both forward and reverse sequences and create consensus sequences. The sequences were initially analysed using NCBI online BLAST server to identify the sequence specificity. Based on the BLAST results, sequences were further compared with other nucleotide sequences of *Proteus mirabilis* in GenBank database.

2.6 Phylogenetic analysis

The comparative sequence analysis of 16s rRNA gene of bacterial isolate was carried out along with chosen sequences from the Genbank databases. The CLUSTALW feature of MEGA11 software was used to analyse and align sequence identities and compared with other publicly available sequences of isolates from various countries i.e. India (MN749808.1; MH718833.1; OK083595.1), China (CP046048.1; CP053683.1; CP053615.1; MF576130.1), Brazil (CP049753.1; CP086377.1), Canada (CP048404.1), Pakistan (MN956898.1; MK494931.1), Nigeria (MN120786.1), United Kingdom (LR134205.1), Vietnam

(AP026827.1) and Spain (CP096775.1). Moreover, Phylogenetic tree was prepared by using MEGA11 software tool through neighbor-joining algorithm with 1000 bootstrap replicates (Tamura *et al.*, 2021) [27].

2.7 Pathomorphological study
For pathomorphological studies, formal saline fixed tissue samples of intestine and mesenteric lymph node were subjected to routine processing, paraffin embedding, sectioning at 4-5 µm thickness followed by staining with haematoxylin and eosin (Gridley, 1960) [13].

2.8 Data analysis
In MALDI TOF analysis, to identify the isolates and visualize the mass spectra, Bruker's MALDI biotyper software 3.0 was used. The strain showing score value >1.7 were considered valid to the genus level identification, whereas scores >2.0 were considered valid to the species level identification. Strains with score values ranging from 1.7-1.99 indicate strains with more genus-level identification and the strains with score values less than 1.7 could not be identified by MALDI biotyper database.

3. Results and Discussion
3.1 Occurrence
In present study, out of 114 samples with enteric lesions in dead goats, 55 bacterial isolates were found, in which 02 (03.63%) swabs were positive for *Proteus mirabilis*. Despite the seemingly small number, it is clear that these opportunistic bacteria were implicated in enteric infection in goat. This result corroborates with the findings of Botes (1964) [3], Munsi *et al*. (2015) [23], Chatzoupoulos *et al*. (2016) [6], Singh *et al*. (2017) [25], Hu *et al.* (2020) [15] and Sun *et al*. (2020) [26]. They also isolate the *Proteus mirabilis* from several species of animals (cattle, sheep, dog, emu, pig, horse, mink, fowl) suffering from diarrhoea and described the *Proteus mirabilis* as intestinal pathogen. Munsi *et al*. (2015) [23] reported the 60% prevalence of *Proteus mirabilis* in sheep whereas Sun *et al*. (2020) [26] recorded the prevalence rate as 32.76%, 28.7%, 23.26% and 22.5% in dog, mink, cattle and fowl respectively, with overall prevalence 28.66%.

3.2 Isolation and identification of bacteria
The bacteria appeared as Gram's negative rods in Gram's staining. On nutrient agar, irregular, effuse, translucent greyish white colonies were observed. whereas on MacConkey agar, it produced circular, convex, smooth, pale, colourless, transparent colonies (Fig 01). These findings are in accordance with the results of Karthik *et al*. (2018) [16] and Al-Kubaisi and Al-Deri (2022) [01].

Moreover, these bacteria exhibit characteristic swarming growth with fishy odour on blood agar (Fig 02). The swarm colony's edge advanced rather steadily, along with some crowding of the growth behind the edge. The rings appeared to originate from the colony's leading edge, causing a surface ripple that eventually gave rise to a concentric ring. These swarming phenomena also observed by Rozalsky *et al*. (1997) [24], Manos and Belas (2006) [19], Karthik *et al*. (2018) [16], Al-Kubaisi and Al-Deri (2022) [01] and Giri *et al*. (2022) [11] in their studies. The swarming behavior of bacteria has been studied most extensively (Rozalsky *et al*., 1997[24]; Manos and Belas, 2006[19]). *Proteus* bacilli are dimorphic bacteria. When grown in a liquid medium, these cells display swimming behaviour and have a distinct morphology, i.e., they are motile, peritrichously flagellated (06-10 flagella per cell) rods, 1.0- 2.0 mm in length. These bacilli, referred to as swimmer cells. When transferred to a solid medium, *Proteus* bacilli undergo morphogenesis and differentiate into elongated hyperflagellated cells (swarmer cells) and swarm over the surface of solid medium. This kind of growth of *Proteus* on solidified nutrient medium is termed as swarming phenomenon. The swarming phenomenon is periodic in nature and consists of four phases (1) swarmer cell differentiation, (2) the lag period prior to active movement, (3) swarming colony migration and (4) consolidation (where the cells stop moving and dedifferentiate back to swimmer cell morphology). The cycle of swarming and consolidation is then repeated several times, until concentric rings, formed by the swarming bacteria and delineating the phase changes, cover the agar surface.

The biochemical analysis revealed positive results for catalase, methyl red and urease tests, glucose and sucrose fermentation with acid production whereas negative for oxidase test, indole test, Voges–Proskauer (VP) test, citrate utilization test, lactose and mannitol fermentation. The similar findings were reported by Chouduri and Wadud (2013) [07] and Karthik *et al*. (2018) [16].

As regards the identification, MALDI-TOF MS of the bacterial isolate yielded good quality spectra with score of 2.434 and was identified as *Proteus mirabilis* 13210_1 CHB (Fig 03) based on Bruker taxonomy database using Biotyper 3.1 software. The MALDI-TOF MS technique has already been endorsed for the identification of several microorganisms (Moreno *et al*., 2018 and Dalmutt *et al*., 2020) [21, 8]. For routine clinical microbiology, the MALDI-TOF MS is a highly efficient, extremely precise and affordable technique that is widely used. The detection of novel and atypical taxa from various clinical specimens is made possible using MALDI-TOF MS, which has more extensive databases, for bacterial identification. The identification of these isolates more frequently may aid in understanding their distribution and epidemiological context.

3.3 Biofilm forming ability
A biofilm is a collection of microbial cells covered in extracellular matrix that adhere to particular surfaces and nearby cells. It promotes bacterial survival, enabling them to better utilize nutrients and adapt to their environment. Biofilm formation by bacteria related to its pathogenicity. It can develop on numerous surfaces, such as living tissues, implanted medical equipment, water system piping and natural aquatic systems.

In our study, it was found that both isolates of *Proteus mirabilis* produced black colonies on Congo red agar indicating biofilm production, though the isolates were observed as moderate biofilm former. This is consistent with the findings of Kwiecinska-Pirog *et al*. (2016) [18], where it was established that all 20 strains of *Proteus mirabilis* isolates found their research, were biofilm producers, in which, 12 (60%) strains formed a medium biofilm and 08 (40%) strains formed a strong biofilm. Karthik *et al*. (2018) [16] noted that among 150 *Proteus* spp isolates of patients suspected of bacterial infection, *Proteus mirabilis* was the most isolated sp (57.3%), in which, 52.32% were biofilm producers. Sun *et al*. (2020) [26] proved that among 176

Proteus mirabilis isolates collected from diarrhetic animals, 92.05% (N=162) of the isolates were biofilm producers in which 78 (48.15%) isolates showed moderate biofilm formation, whereas 62 (38.27%) isolates showed moderate biofilm formation. On the other hand, 07.95% (N=14) of the isolates were nonproducers.

3.4 Nucleotide sequencing and phylogenetic analysis

The sequencing of 16S rRNA gene of the bacterium yielding a final consensus sequence with 1184 positions in the final dataset, that was further used for alignment. Based on BLAST (Basic Local Alignment Search Tool) analysis, the nucleotide sequence showed 99.02-99.11% homology with other previously available sequences. The nucleotide sequences of present study showed 99.11% similarity with *Proteus mirabilis* 16S rRNA gene sequences identified in India, Canada, Pakistan, Nigeria, United Kingdom, Vietnam and Spain whereas 99.02% with Brazil. On the other hand, the isolate of this study displayed similarity ranged between 99.02-99.11% with *Proteus mirabilis* 16S rRNA gene sequences identified in China.

Furthermore, the phylogenetic analysis revealed that *Proteus mirabilis* isolate detected in this study was most similar to strains of *Proteus mirabilis* isolated from China and Brazil (Fig 04).

3.5 Pathomorphological study:

In present study, postmortem examination of goats revealed gross changes as enteritis, congestion and hemorrhages in intestinal mucosa, mucosal thickening, ballooning of intestine, presence of necrotic debris and exudate in lumen and enlarged mesenteric lymph nodes. In microscopic examination, the histological damages were characterized by necrosis of superficial epithelium and lamina propria of small intestine with infiltration of inflammatory cells (Fig 05), villous atrophy and haemorrhagic crypts (Fig 06). In addition, typical inflammatory lesions in the large intestine (Fig 07) were also observed. These findings are consistent with the previous literatures of *Proteus mirabilis* associated gastroenteritis in calves and mice (Botes 1964; Gong *et al*., 2019) [3, 12].

Proteus spp represent a significant opportunistic pathogen that can be found in soil, water as well as in the intestinal tracts of mammals. These bacteria are frequently used as markers of faecal pollution in soil and water environments (Drzewiecka *et al*., 2016) [9]. Consuming contaminated water or seafood posing a threat of infection with these organisms. The bacteria primarily infect individuals with weakened immune systems and the majority of them can cause nosocomial infections as well as complex wound and urinary tract infections.

Proteus spp are considered an undesirable component of intestinal microbiota because they have the potential to cause diarrhoea. In earlier studies, it was found that *Proteus mirabilis* strains were statistically more often isolated from the faeces of diarrhoea patients than from healthy individuals. The author hypothesized that the bacteria might cause intestinal disorders on their own or that they might become opportunists when the illness is caused by other intestinal pathogens (Muller, 1989) [22]. As a result, the presence of this bacteria in the gastrointestinal tract may also be considered as carriers, as in certain circumstances, it may result in cross-infections and autoinfections, particularly in the urinary tract.

Similar to humans, *Proteus* spp bacteria are found in the intestines of a wide variety of domestic and wild animals, including birds, reptiles, amphibians, insects and seafood. Animal intestines containing *Proteus* spp may pose a threat of autoinfection and cross-infection. Gaastra *et al*. (1996) [10], observed that *Proteus mirabilis* strains from the urine and faeces of dogs with recurrent urinary tract infections has resulted from autoinfection.

Fig 1: *Proteus mirabilis* showing pale, colourless (non-lactose fermenting) colonies on Mac Conkey's agar.

Fig 2: *Proteus mirabilis* showing characteristic swarming colonies on blood agar.

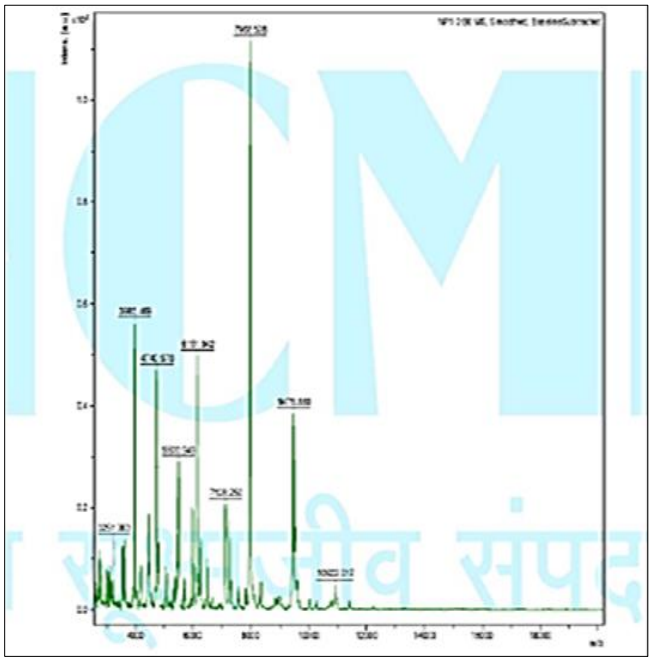

Fig 3: MALDI TOF MS spectra of *Proteus mirabilis*.

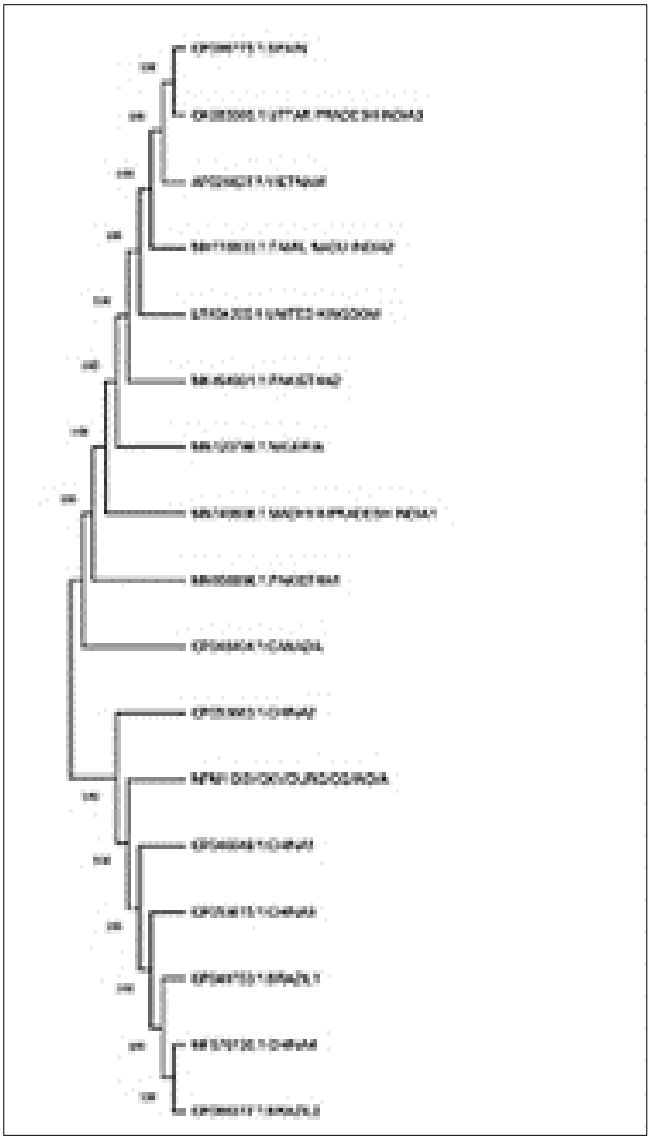

Fig 4: Cladogram of *Proteus mirabilis* isolate (NPM1 DSVCKV/DURG/CG/INDIA) using neighbour-joining algorithm with 1000 bootstrap replicates

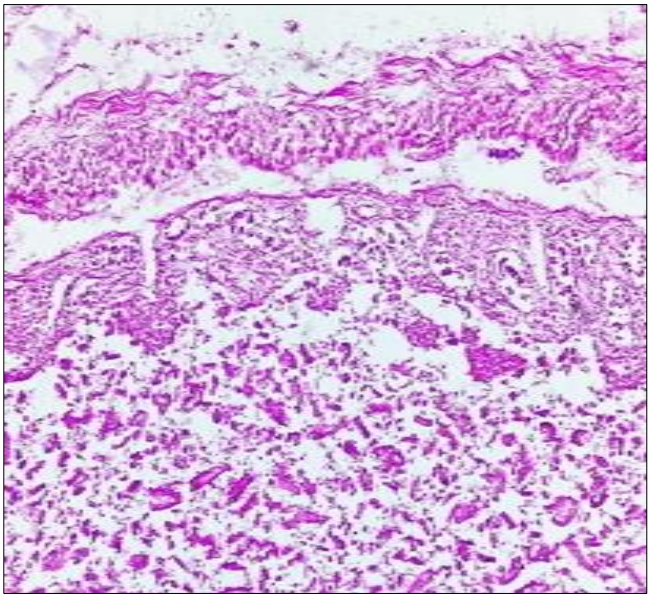

Fig 5: Photomicrograph of small intestine of goat showing necrosis of lamina propria with infiltration of inflammatory polymorphonuclear cells and accumulation of necrotic debris in lumen of intestine. (H & E x40).

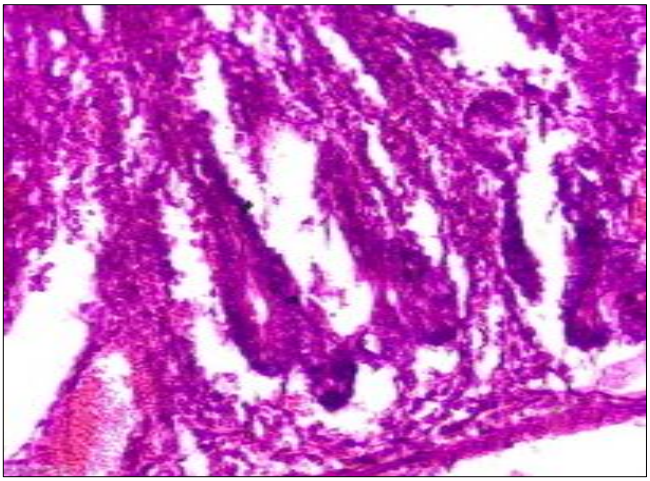

Fig 6: Photomicrograph of small intestine of goat showing degeneration in lamina propria along with mononuclear cell infiltration and haemorrhagic crypts. (H & E x400).

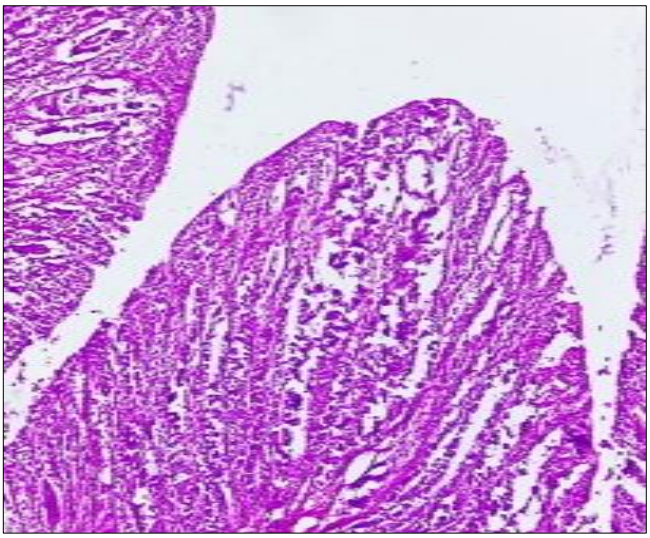

Fig 7: Photomicrograph of large intestine of goat showing degeneration in lamina propria with infiltration of inflammatory polymorphonuclear cells. (H & E x100).

Conclusion

The isolation of *Proteus mirabilis* from the gastrointestinal tract in the current study could be attributed to unsanitary housing and a lack of appropriate management practices. As *Proteus* species are known to colonize the urinary tract, bacterial excretion can contaminate the food, water or soil in that area, potentially leading to enteritis in goats. As it is evident that, the bacteria exist as normal flora in the intestinal tracts of animals, they may cause infection whenever the immune system of animals is compromised. Moreover, infection could be attributed to autoinfection and cross-infection due to presence of other intestinal pathogens. It could be concluded that, despite being a normal flora of the intestine, *Proteus mirabilis* can be considered a cause of enteric infections in goats. These bacteria should not be overlooked because they have the potential to cause serious infections in animals due to their diverse virulence factors. The author suggested keeping a closer watch on animal cases of gastroenteritis linked to *Proteus mirabilis*.

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Conflict of Interest

Authors declares no conflict of interest in this study.

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