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Isolation, Molecular Characterization, and Pathological Findings of Important Bacterial Respiratory Infections in Goats of Eastern Plain Zone of Uttar Pradesh, North India

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Abstract

Small ruminants, particularly goats are susceptible to respiratory diseases. Respiratory diseases, especially pneumonia and associated mortality causes significant threat to the goat farming due to substantial economic losses in the eastern plain zone of Uttar Pradesh, India. Among the respiratory infections in goats, *Pasteurella* spp., *Escherichia coli* (*E. coli*), *Klebsiella* spp. and *Staphylococcus* spp. infections in eastern plain zone of Uttar Pradesh had been studied limited. To determine the occurrence of respiratory diseases among goats, the current study was designed with an objective of isolation, identification and molecular characterization of organisms responsible for respiratory infections, especially *Pasteurella* spp., *E. coli, Klebsiella* spp. and *Staphylococcus* spp. A total of 150 samples were collected during postmortem examination of goats and processed for isolation of respiratory pathogens, genomic characterization, pathological lesions, localization of organisms in tissues by immunohistochemistry, and scanning electron microscopy (SEM). Out of 150 samples, 40.66% of *E. coli*, 26% *Pasteurella* spp., 20% *Klebsiella* spp., and 13.33% *Staphylococcus* spp. were identified in the respiratory tract of goats based on their typical morphology, colony characteristics, bio-chemical properties, and motility. The samples crucial for diagnosis of respiratory bacterial pathogens were lungs, followed by heart blood, nasal swabs, and tracheal swabs. Gross pathological lesions were consolidation in cranio-ventral and cranio-lateral lobes of lungs, congestion, hemorrhages, and fibrinous pleuritis. Histopathological lesions were bronchopneumonia with infiltration of neutrophils, hemorrhages, and necrotic regions contained spindle-shaped/elongated "oat cells or streaming leukocytes". Loss of cilia was noticed by SEM. In lungs and intestine, *Pasteurella* spp. and *E. coli* antigen were localized in the cytoplasm of neutrophils, alveolar macrophages, bronchiolar epithelial cells, and intestinal mucosa. Extraintestinal pathogenic *E. coli* was found in the dead goats with pneumonia. Most of the isolated Pasteurella strains were having similarities with *Pasteurella multocida* based on the phylogenetic analysis targeting *16S rRNA* gene. Results of the present study confirmed the circulation of *Pasteurella* spp., *E. coli, Klebsiella* spp. and *Staphylococcus* spp. among goats in field conditions in eastern plain zone of Uttar Pradesh and necessary control measures should be formulated with effective vaccination strategies in small ruminants for the control of respiratory infections to reduce the economic losses to goat farmers.

Keywords: Goats, Respiratory Infections, *Pasteurella multocida, Escherichia coli,* Phylogenetic Analysis, Isolation, Pathology

INTRODUCTION

India has a rapidly expanding animal husbandry industry that accounts for 11.6% (528 million) of the world's livestock population and working towards becoming self-sufficient in the production of livestock products.[1,2] Among farm animals, goats are considered as "poor farmer's mobile bank" and India is second in the world in terms of population of goats (148.88 million, as per the 20th Livestock Census-2019). The economy of farmers in the arid, semiarid, and hilly areas of India are greatly influenced by goats. Goats are the most valuable asset due to their rapid growth rate as well as their considerable contribution to the meat, milk, and fiber production.[3]

Goats are highly vulnerable to respiratory ailments, which account for roughly 50% of their fatalities.[4] Respiratory diseases due to infectious agents in sheep and goats account for 5.6% of all small ruminant illnesses, regardless of their underlying causes.[2] According to Lacasta *et al.*,[5] the respiratory disease complex in small ruminants is caused by several different etiological factors. Due to their significant clinical manifestations, bacterial diseases have drawn particular attention.[4]

Both humans and animals can get a range of intestinal and extra-intestinal diseases from pathogenic *Escherichia coli* (*E. coli*) and *Pasteurella* spp.[6] Analogous to intestinal diseases, respiratory diseases pose a significant risk to the goat breeding sector.[7] Gram-negative bacterium *Pasteurella*

multocida (*P. multocida*) is pathogenic and causes pasteurellosis. *P. multocida* is categorized into five capsular serogroups, 3 subspecies and 16 serotypes.[8] Worldwide, in ruminants of all ages, *P. multocida* causes bronchopneumonia, which has a cranio-ventral lung distribution. It is an opportunistic pathogen and frequently reside in the upper respiratory tract as commensals, but they can also invade the tissues of animals with impaired immune system.[2] According to Chakraborty *et al.*,[9] animals with compromised immune system or under stress such as pregnancy, nursing, young, or aged animals are more susceptible to respiratory pathogens like *P. multocida*, *Mannheimia haemolytica* (formerly *Pasteurella haemolytica*), and other species.

Klebsiella spp. is a Gram-negative, rod-shaped, and capsulated bacterium belongs to the family *Enterobacteriaceae*. Recently, *Klebsiella pneumoniae* (*K. pneumoniae*) is receiving increased attention worldwide due to severe disease, biofilm formation, and antibiotic resistance.[10,11] *K. pneumoniae* is a virulent bacterium and significant cause of pneumonia in sheep and goats. Clinical signs are pyrexia, loss of appetite, nasal discharge, difficulty breathing, rapidly progressing course, and complication of lung abscess with involvement of multiple lobes. *K. pneumoniae* can also be transmitted to humans from goats.[10-12] *Staphylococcus* spp. normally found in the upper respiratory tract of healthy sheep and goat; however, during stress conditions like overcrowding, inadequate ventilation, poor nutrition and transportation, or in association with other pathogens causes pneumonia.[13,14] Clinical signs are pyrexia, sudden death without warning signs, and increased respiratory rate with abdominal efforts. *Staphylococcus aureus* (*S. aureus*) can be resistant to antibiotics and can be transmitted between animals and humans.[13-15]

Pneumonic animals exhibit a loss of appetite or anorexia, lethargy, coughing, fever, dyspnea, and mucopurulent nasal discharge.[2,4] Young animals are more susceptible to infection compared to adults, which might result in rapid death with or without any prior clinical indications.[12,15] Animals and birds that died of respiratory diseases due to bacterial infections exhibit ventral consolidation in the cranial lobes of the lungs, as well as fibrinous pericardial and pleural effusions.[12,15,16] The most accurate way to diagnose respiratory infections is to isolate the bacteria from the lungs and nasopharyngeal swabs of suspicious animals, followed by biochemical confirmation of the isolates. Using bacteriological techniques, it is frequently difficult to identify *Pasteurella* spp., *E. coli*, *K. pneumoniae*, and *S. aureus* during various circumstances, including antibiotic therapy, frozen tissue, autolytic material, and other situations. However, immunohistochemistry (IHC) technique, overcomes some difficulties with earlier methods of detecting *Pasteurella* antigens on formalin-fixed and paraffin-embedded tissues.[17,18] Molecular methods like polymerase chain reaction (PCR) have recently been found to be incredibly sensitive and specific.[19] Caprine pasteurellosis, *E. coli*, *K. pneumoniae*, and *S. aureus* infections have been given less attention and only a small number of investigations have been conducted. The current study was undertaken to determine the occurrence of the respiratory diseases with isolation, identification and molecular characterization of the causative organisms especially *Pasteurella* spp., *E. coli, K. pneumoniae*, and *S. aureus* among goats of Eastern Uttar Pradesh. The results of the current study could serve as useful information for the concerned authorities in designing the proper therapeutic and control measures and to reduce the economic losses of goat farmers.

MATERIALS AND METHODS

Sample collection
The samples for the present study were collected from the natural cases of respiratory infections in goats from September 2021 to August 2022. Postmortem examinations of 150 goat carcasses suspected of respiratory diseases were conducted. Among 150 goats, 36 were from Azamgarh, 40 from Ghazipur, 24 from Mau, 34 from Ballia, 14 from Bhadohi, and 2 from Varanasi districts of the eastern plain zone of Uttar Pradesh. A total of 51 samples were collected from ≤6 months, 39 samples from >6 months to ≤1 year, 32 samples from >1 year to ≤2 years, 17 samples

from >2 year to ≤3 years, and 11 samples from >3 years. Samples were collected from 109 female and 41 male goats. Further, 76 samples were collected from non-descript goats, 43 samples from Jamunapari goats and 31 samples from Barbari goats. The died goats were not vaccinated and dewormed against any diseases. Among 150 goats, 67 samples were collected during winter (mid-November to February), 28 samples during summer (March to mid-June), 36 samples during monsoon (mid-June to September), and 19 samples during post-monsoon (October and mid-November). The died goats were from unorganized farm, extensive system of rearing with free grazing in pasture lands and tree foliage, and housed in open housing system or thatched roof with wooden/bamboo sticks with an earthen floor. The goats belonged to the landless, small and marginal farmers had the flock size between 5 to 25. Heart blood, nasal and tracheal swabs, and lungs were collected in the sterile container for bacterial isolation. The tissue samples were collected from the cases showing gross lesions in the lungs, trachea, intestine, and lymph nodes in 10% neutral buffered formalin (NBF; Cat No. F1635-4L, Sigma-Aldrich, St. Louis, Missouri, USA) for histopathology and immunohistochemistry. Lungs were collected in 2.5% glutaraldehyde (Cat No. G5882-100ML, Sigma-Aldrich, St. Louis, Missouri, USA) for electron microscopy.

Isolation of *Escherichia coli*

A total of 150 nasal swabs and lung tissues from dead goats were processed for bacterial isolation. A loop of inoculum from the nutrient broth (Cat No. M002-500G, HiMedia Laboratories Private Limited, Thane, Maharashtra, India) was streaked onto MacConkey agar (MCA; Cat No. MH081-500G, HiMedia Laboratories Private Limited, Thane, Maharashtra, India) plates to isolate the lactose-fermenting and non-lactose-fermenting bacteria. A bright red or pink color colony was used to culture in eosin methylene blue (EMB) agar (Cat No. M317-500G, HiMedia Laboratories Private Limited, Thane, Maharashtra, India). The colonies of red or pink and greenish black in color with metallic sheen were considered

as positive for *Escherichia coli* in EMB agar. The positive colonies were subculture into EMB agar to obtain pure colonies. Again, the pure colonies were subjected to sugar fermentation and other biochemical tests namely indole, catalase, Voges-Proskauer (VP), methyl red (MR), citrate utilization, nitrate reduction, triple sugar iron (TSI) agar, hydrogen sulfide (H$_2$S) production, and urease production tests as per the procedures outlined by Quinn *et al.*[20]

Isolation of *Pasteurella multocida*

Blood agar (BA; Cat No. M1301-500G, HiMedia Laboratories Private Limited, Thane, Maharashtra, India) and MacConkey agar were used as primary culture media for the initial isolation of organisms from the samples as per the procedures outlined by Quinn *et al.*[20] Initial speculation suggested that *Pasteurella multocida* was present in the isolates that failed to grow on MCA. A single non-haemolytic colony of these isolates was selected from the primary culture and re-streaked on a fresh BA plate. The cultures were stained with Gram's staining (Cat No. K001-1KT, HiMedia Laboratories Private Limited, Thane, Maharashtra, India) to determine whether the growth and morphology of the organisms were pure. The cultures were inoculated into the nutrient agar slant and stored at 4 °C for further identification. Identification of *P. multocida* was done based on growth morphology and biochemical characteristics as per the method described by Edward and Ewing.[21]

Isolation of *Klebsiella* spp. and *Staphylococcus* spp.

A loop of inoculum from the nutrient broth was streaked onto MacConkey agar plates to isolate the lactose-fermenting and non-lactose-fermenting bacteria. A lactose-fermenting, large, mucoid, and red colony was used to culture in EMB agar to isolate the *Klebsiella* spp. The mannitol salt agar (Cat No. MH118-500G, HiMedia Laboratories Private Limited, Thane, Maharashtra, India) plates were streaked with a loopful of inoculum from nutrient broth to isolate the *Staphylococcus aureus* as per the procedures outlined by Quinn *et al.*[20]

Demonstration of bipolar organisms

The smears of the colonies from blood agar were prepared and stained with Giemsa stain (Cat No. TC232-5G, HiMedia Laboratories Private Limited, Thane, Maharashtra, India) for demonstration of bipolar organisms.

Isolation of bacterial genomic DNA

Genomic DNA was isolated from the bacterial samples after biochemical characterization. The samples were homogenized with extraction buffer (Cat No. 56304, QIAamp® DNA Micro Kit, Qiagen, Maryland, USA). Equal volume of phenol, chloroform, and isoamyl alcohol (25:24:1, respectively) were added and mixed well. The tubes were centrifuged at 14,000 rpm for 15 min at room temperature (RT). After collecting the upper aqueous phase in a fresh tube, the mixture of equal parts of chloroform:isoamyl alcohol (24:1) was added. After centrifuging for 10 min at 14,000 rpm in RT, the upper aqueous phase was collected and transferred into a fresh tube. By adding 100 µL of 3 M sodium acetate, pH 7.0 and 700 µL of isopropanol to the mixture, the DNA was isolated and precipitated. The tubes were incubated for 15 min at RT, and centrifuged for 15 min at 14,000 rpm on 4 °C. After twice washing, the DNA pellet in 70% ethanol and quickly rinsed again in 100% ethanol and allowed to air dry. In Tris-EDTA (TE) buffer (Tris-Cl, 10 mM, pH 8.0; EDTA, 1 mM), the DNA was dissolved. In order to remove the RNA, 5 µl of DNAse free RNAse (10 mg/mL, Cat No. FEREN0531, Thermo Fisher Scientific, Carlsbad, California, USA) was added to the DNA as per the procedures described by Ingale *et al.*[22]

PCR amplification of *16S rRNA* gene

Isolated bacterial genomic DNA was amplified using universal bacterial 16S ribosomal RNA (*16S rRNA*) gene primers 27F (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1392R (5'-GGT TAC CTT GTT ACG ACT T-3'),[23] which covers nearly full length of *16S rRNA* gene with expected amplicon of >1,350 bp. Polymerase chain reaction (PCR) was performed to amplify the *16S rRNA* gene using 10 ng of genomic DNA extracted from each bacterial isolate and 10 pM concentrations of each forward and reverse primers as per the procedures described by Srinivasan *et al.*[23] The amplified PCR products were visualized on a 1% agarose gel stained with ethidium bromide under UV light to confirm the presence of a >1,350 bp band. *Pasteurella multocida* specific PCR (PM-PCR) was performed using published primers[24] and protocols described by Townsend *et al.*[24] The serotype of the *E. coli* isolates was identified by O-genotyping PCR using published primers[25] and protocols described by Iguchi *et al.*[25]

Sequencing of bacterial isolates

The PCR products were sequenced Bi-directionally.[26] The amplified products of *16S rRNA* by PCR with universal primer 27F-1392R were purified and submitted for sequencing.

Phylogenetic analysis

The sequence data was aligned and analyzed to identify the bacterial species and its closest neighbours. The obtained forward and reverse sequences were aligned using the online pairwise alignment tool BioEdit.[27] The query sequences were identified by considering E-value 1×10^{-5} and maximum hits (99% or 100%) with a species in the reference database National Center for Biotechnology Information (NCBI; https://www.ncbi.nlm.nih.gov/). In addition to Basic Local Alignment Search Tool (BLAST; https://blast.ncbi.nlm.nih.gov/), Molecular Evolutionary Genetics Analysis Version 7.0 (MEGA7) software was used for phylogenetic tree analysis to conduct the evolutionary analyses[28] by employing Maximum Likelihood (ML) method.[26]

Necropsy examination

Systematic necropsy examinations of 150 goat carcasses were carried. The gross pathological lesions in different visceral organs were recorded.

Histopathology

After proper fixation in 10% NBF, tissue samples from the lungs, trachea, intestine, and lymph nodes were processed for routine paraffin wax embedding. Tissue sections of 4-5 µ thickness were cut and stained with hematoxylin and eosin (H&E) method for histopathological examination as per the standard procedure.[29]

Immunohistochemistry

Tissue sections were taken on the (3-aminopropyl)triethoxysilane (APES; Cat No. 440140-100ML, Sigma-Aldrich, St. Louis, Missouri, USA) coated glass slides to carry out IHC. Immunohistochemical staining was done using polyclonal antibodies as per the method described by Saminathan *et al.*[30] Further, the intensity of the IHC reaction was determined based on the antigen-positive cells and graded as mild, moderate, or marked. Scoring was done by the panel of two Pathologists. Tissue sections were examined in low power (10X objective lens) and high power (40X objective lens) fields and the entire tissue sections were examined for scoring purposes. If no staining, it was considered as negative, if 5 to 10% stained cells as mild, if more than 10 to 25% stained cells as moderate, and if more than 25% stained cells as marked IHC reaction.

Special staining

Special staining such as Leishman's staining (Cat No. GRM949-25G, HiMedia Laboratories Private Limited, Thane, Maharashtra, India) for *Pasteurella* bipolar organisms, Von Kossa stain for the demonstration of calcium, and Masson's trichrome stain for connective tissue fibers were done as per the standard procedure.[29]

Scanning electron microscopy

For scanning electron microscopy, specimens were fixed with 3.5 N hydrochloric acid (HCl) for 3 weeks at RT. Specimens were treated with 0.5% tannic acid solution. Post-fixation was done by immersing in 1% osmium tetroxide (OsO_4) solution for 10 min. Specimens were dehydrated with a graded series of ethanol (70-100%). Dehydrated specimens were freeze-dried with a t-butyl alcohol freeze-drying method. Specimens were coated with platinum (Pt)-palladium (Pd) and observed under scanning electron microscope (S-800, Hitachi-Hi-Technologies, Japan).

Statistical analysis

Statistical Package for the Social Sciences (SPSS) software (version 17.0) was used to perform the standard statistical procedures on the data obtained on various parameters. The chi-square test was utilized according to the WEB AGRI STAT PACKAGE programme, which was created at the ICAR Research Centre, Goa, India.[31]

RESULTS

Clinical signs

Goats affected with respiratory infections exhibited pyrexia with a temperature of 40-41 °C, anorexia, wet cough with crackling sounds, dyspnea, serous to mucopurulent nasal and ocular discharges, increased respiration, abdominal breathing, and sudden death without clinical signs.

Isolation and identification of bacteria from respiratory infections

Among 150 samples, based on the typical colony characteristics (motility, morphology, and bio-chemical properties), 61 (40.66%) samples were identified as positive for *E. coli*, and 39 (26%) samples for *Pasteurella* spp., 30 (20%) samples for *Klebsiella* spp., and 20 (13.33%) samples for *Staphylococcus* spp. in the respiratory tract of goats. A total of 96 lung samples, 84 heart blood, 46 nasal swabs, and 29 tracheal swabs from the goats that were clinically suspected and later died of respiratory infections were showed positivity for the respiratory pathogens by bacteriological screening. On MacConkey agar, *E. coli* isolates produced big (2-3 mm) and pink colonies that were fermented lactose (Figure 1A). On EMB agar, *E. coli* produced the distinctive "metallic shine" colonies (Figure 1B). The rose-pink colonies with a mucoid appearance and also colonies of light purple mucoid appearance on EMB agar were construed as *Klebsiella* spp. (Figure 2A). *Staphylococcus* spp. colonies on solid media (Mannitol salt agar) were round, smooth, and glistening with shining surface (Figure 2B). No growth on MacConkey agar is indicative of *Pasteurella* spp. (Figure 3A).

Morphology of bacteria

The isolated non-hemolytic and hemolytic individual colonies were cultured on a blood agar plate, and the smears of colonies from the blood agar plate were stained with Gram stain. A total of

130 isolates were Gram-negative and 20 isolates were Gram-positive by Gram staining. *Pasteurella* spp. showed coccobacilli by Gram staining and in Leishman's staining, *Pasteurella* spp. appeared as rod-shaped with bipolar appearance (hairpin-like shape), because the ends of the bacteria stained more intensely than the middle (Figure 3B). *E. coli* and *Klebsiella* spp. showed rod shaped, and *Staphylococcus* spp. showed cocci in clusters, pairs and occasionally in short chains of organisms by Gram staining.

Biochemical characterization of *Pasteurella* spp.

All 39 isolates of *Pasteurella* spp. were subjected to biochemical tests. The percent positivity for *Pasteurella multocida* was 74.35% (29); while, the percent positivity of *Mannheimia haemolytica* was 25.64% (10). The results of biochemical tests were shown in Table 1.

Biochemical characterization of *Escherichia coli*

All the *E. coli* isolates showed typical biochemical reactions that were positive to the indole, MR, TSI agar, and nitrate reduction tests; while, negative to the VP and H_2S production tests. *E. coli* isolates were negative for the citrate utilization and urease activity tests. All the *E. coli* isolates were motile and isolates fermented D-glucose, lactose, and mannitol.

Molecular confirmation of bacterial isolates

Genomic DNA of *Pasteurella* spp. (39) and *E. coli* (61) isolates identified by bacteriological methods were used for amplification and confirmation using PCR by targeting the *16S rRNA* gene. All the tested *Pasteurella* spp. (Figure 4A) and *E. coli* (Figure 4B) isolates yielded positive results. Out of 39, 31 isolates yielded positive results by *Pasteurella multocida* specific PCR (PM-PCR). Out of 61 *E. coli* isolates, 52 were identified as O serotype by O-genotyping PCR.

Nucleotide sequence and phylogenetic analysis

Sequencing of *16S rRNA* gene of *Pasteurella* spp. and *E. coli* isolates, which were confirmed as *P. multocida* and *E. coli* by specific PCR were done. The *P. multocida* isolates were showed more than 99% similarity with those *P. multocida* strains submitted in GenBank (accession numbers: NR_041809.1, NR_041811.1, NR_041810.1, and NR_115138.1), according to the results of BLAST analysis. The sequences of some isolates showed highest identity (99.69%) with those of the *16S rRNA* of *P. multocida* strain CCUG 17976 (Figure 5). The sequences of some isolates showed close similarity (98%) with the *P. multocida* subsp. *gallicida* strain CCUG 17978 (Figure 6). The sequences of some isolates showed close similarity (99.05%) with the *P. multocida* subsp. *septica* strain PM24 (accession no. NR_115138.1)
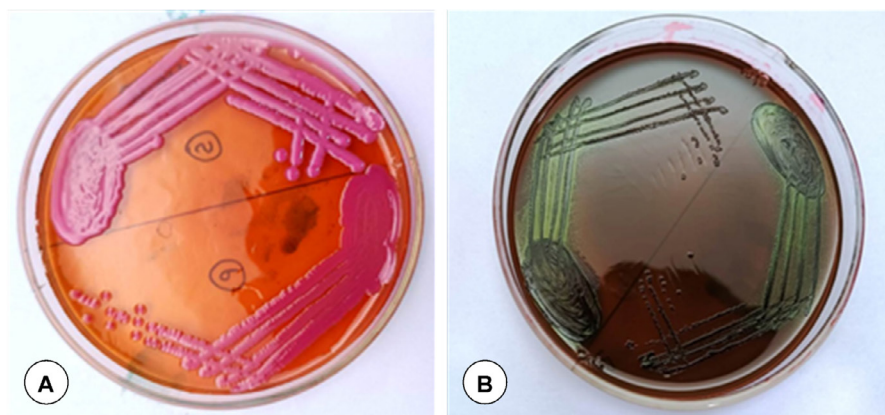

Figure 1. (A). Growth of *E. coli* on MacConkey lactose agar visible as lactose-fermenting pink color colonies. (B). Growth of *E. coli.* on eosin methylene blue (EMB) agar visible as blue-black with a dark center and metallic sheen

and strain CCUG 17977 (Figure 7). The sequences of *E. coli* isolates were showed similarity with the *E. coli* (Figure 8).

Occurrence of respiratory pathogens in eastern plain zone of Uttar Pradesh

The overall occurrence of *Pasteurella* spp. and pathogenic *E. coli* in the goats of eastern plain zone of Uttar Pradesh was found to be 26% (39 samples) and 40.66% (61 samples), respectively. District-wise occurrence of *Pasteurella* spp. was found to be 27.77%, 32.5%, 20.58%, 33.33%, 0%, and 50% in the districts of Azamgarh, Ghazipur, Ballia, Mau, Bhadohi, and Varanasi, respectively. Occurrence of *Klebsiella* spp. and *Staphylococcus* spp. in the goats of eastern plain zone of Uttar Pradesh were presented in the Table 2. The

Klebsiella spp. showed significant difference (p < 0.01) in the occurrence of respiratory pathogens between the various districts of the eastern plain zone of Uttar Pradesh, India (Table 2 and 3).

Sex-, age-, breed-, and season-wise occurrence of respiratory pathogens

The overall sex-, age-, breed-, and season-wise occurrence of *Pasteurella* spp., *E. coli*, *Klebsiella* spp. and *Staphylococcus* spp. in the goats of eastern plain zone of Uttar Pradesh were presented in the Table 4-11.

Gross pathology of *P. multocida* infection

In most cases, pneumonic lesions with consolidation and firm consistency, dark red areas in various lobes especially cranio-ventral
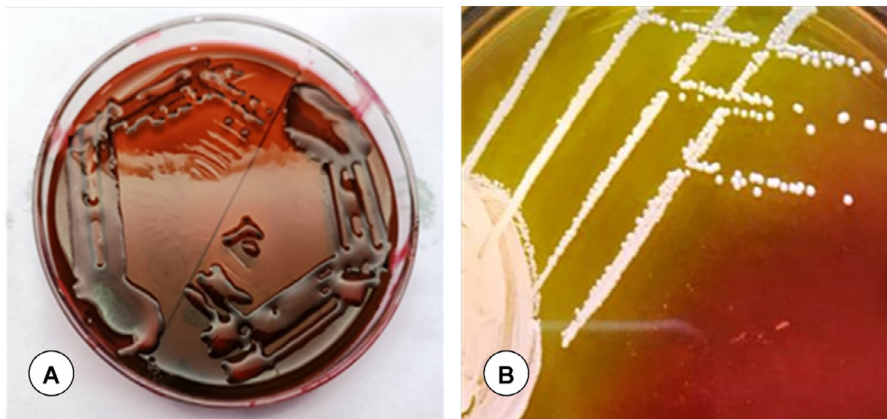
Figure 2. (A). Dark purple mucoid colonies on eosin methylene blue (EMB) agar indicating *Klebsiella* spp. (B). Yellow colour in the region of medium where the *Staphylococcus* grown due to mannitol fermentation on mannitol salt agar
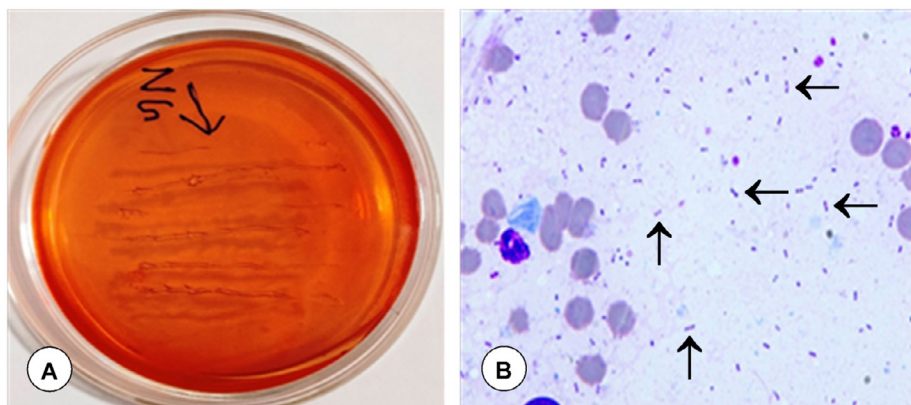

Figure 3. (A). No growth on MacConkey lactose agar indicating *Pasteurella* spp. (B). *Pasteurella* spp. appeared as rod-shaped with bipolar appearance (hairpin-like shape) in tissue impression smears by Leishman's staining

lobes were found in the lungs on both sides during necropsy. Focal haemorrhages, congestion, emphysematous patches, fibrinous pleuritis and foamy exudate on cut sections were noticed (Figure 9A). Lungs were grossly heavier than normal with focal or multifocal emphysematous areas and inflated. Hepatized lungs showed diffuse and thickening of the pleura. Further, in majority of the cases, there was noticeable congestion, haemorrhage, and scattered necrotizing lesions in other visceral organs.

Histopathology of *P. multocida* infection

Microscopically, lung parenchyma of animals showed variable degrees of bronchopneumonia characterized by severe necrosis with nuclear debris and infiltration of neutrophils. Bronchiolar and alveolar lumens were filled with eosinophilic fibrinous and inflammatory cellular exudates, thickened inter-alveolar septa, and engorgement of blood vessels (Figure 9B and C). Bronchiolar epithelium showed degenerative changes. Lungs had severe hemorrhages into the perivascular and alveolar spaces (Figure 9D). The necrotic regions contained spindle-shaped/ elongated degenerated leukocytes especially neutrophils and macrophages, which exhibited a streaming pattern of basophilic chromatin known as "oat cells or streaming leukocytes". Oat cells are also infiltrated in the lumen of alveoli (Figure 9E). In cases of chronic pneumonia, areas of calcification were noticed.

Scanning electron microscopy

The lung tissues from *Pasteurella* confirmed cases were subjected to scanning electron microscopy (Figure 9F). Walls of the alveoli were lined by a single layer of cells. The affected lungs showed loss of cilia and alveoli were filled with exfoliated cells. Bronchiolar lumen was also filled with exfoliated cells.

Gross pathology in *E. coli* infection

Varied degrees of pulmonary consolidation and pneumonic lesions were evident in the cranio-ventral and cranio-lateral lobes of the lungs on either side along with congestion and hemorrhages. Frothy exudate was observed

Table 1. Biochemical test results of *Pasteurella multocida* and *Mannheimia haemolytica*

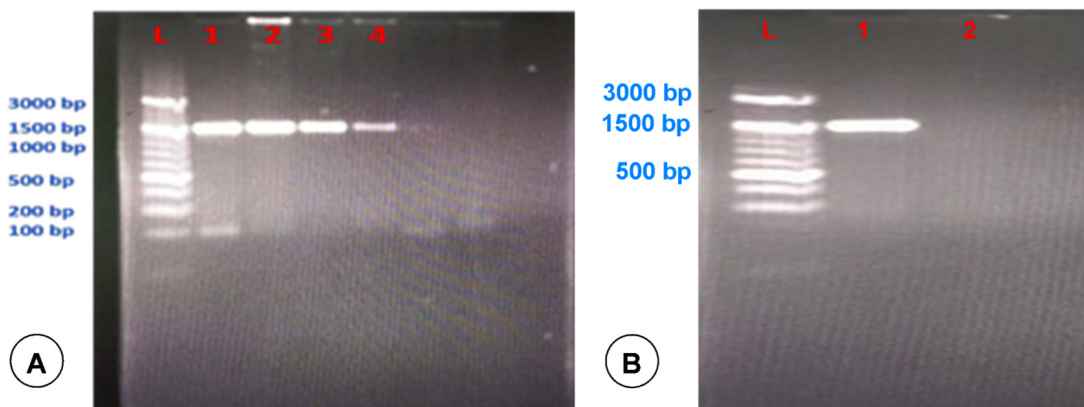| Biochemical test	Species of *Pasteurella* isolated/suspected	
	P. multocida (29)	*Mannheimia haemolytica* (10)
Catalase	++	+
Oxidase	+	+
Urease	-	-
Voges-Proskauer (VP)	+	-
Methyl red	+	-
Sucrose	+	+
Indole	-	+
Hemolysis	-	+
Citrate	-	+

Figure 4. PCR amplification of *16S rRNA* gene of *Pasteurella* spp. (A) and *E. coli* (B). Lane L: 100 bp DNA ladder and L1-L4: Samples

Table 2. Occurrence of respiratory pathogens among different districts of eastern plain zone of Uttar Pradesh

Districts	Number of samples tested	*Pasteurella* spp.		*Escherichia coli*		*Klebsiella* spp.		*Staphylococcus* spp.	
		Number of positive	Percent positivity	Number of positive	Percent positivity	Number of positive	Percent positivity	Number of positive	Percent positivity
Azamgarh	36	10	27.78	16	44.44	8	22.22	2	5.56
Ghazipur	40	13	32.50	20	50.00	2	5.00	5	12.50
Ballia	34	7	20.59	13	38.24	10	29.41	4	11.76
Mau	24	8	33.33	10	41.67	2	8.33	4	16.67
Bhadohi	14	0	0	1	7.14	8	57.14	5	35.71
Varanasi	2	1	50.00	1	50.00	0	0	0	0
Total	150	39	26.00	61	40.67	30	20.00	20	13.33

Table 3. District-wise comparison of respiratory pathogens by chi-square test

Parameters	*Pasteurella* spp.			*Escherichia coli*			*Klebsiella* spp.			*Staphylococcus* spp.		
	Value	df	Asymptotic significance (2-sided)	Value	df	Asymptotic significance (2-sided)	Value	df	Asymptotic significance (2-sided)	Value	df	Asymptotic significance (2-sided)
Pearson chi-square	2.087[a]	4	0.720	8.343[b]	5	0.138	21.508[c]	4	0.000	8.183[d]	4	0.085
Likelihood ratio	2.103	4	0.717	9.961	5	0.076	21.118	4	0.000	7.129	4	0.129
Linear-by-linear association	0.035	1	0.852	3.547	1	0.060	3.793	1	0.051	6.092	1	0.014
N of valid cases	136			150			148			148		

Note: df - Degrees of freedom; Superscript 'a' indicate 2 cells (20.0%) have expected count less than 5. The minimum expected count was 0.57; 'b' indicates 2 cells (16.7%) have expected count less than 5. The minimum expected count was 0.81; 'c' indicates 2 cells (20.0%) have expected count less than 5. The minimum expected count was 2.84; 'd' indicates 4 cells (40.0%) have expected count less than 5. The minimum expected count was 1.89. Except *Klebsiella* spp. (p < 0.01), there was no significant difference among districts regarding respiratory pathogens

in the trachea (Figure 10A), bronchi, bronchioles, and cut surfaces of lungs.

Histopathology of *E. coli* infection

Lungs showed variable degrees of bronchopneumonia. The alveolar lumens were filled with fibrinous exudate, hemorrhages and inflammatory cells especially neutrophils, and severe engorgement of the blood vessels in lungs. Bronchiolar epithelium showed degenerative changes and lumen displayed the infiltration of inflammatory cells and hemorrhages (Figure 10B and C). Alveolar septa showed thickening due to infiltration of inflammatory cells, hemorrhages, and edema. Small intestine showed necrosis, congestion, hemorrhages, and infiltration of inflammatory cells in lamina propria (Figures 10D).

Special staining

In *Pasteurella* positive cases, the lungs showed the presence of calcium deposits with Von Kossa stain. The mineralized tissues showed an amorphous and black appearance. In *E. coli* positive cases, the necrotic intestine showed mild to moderate fibrosis with Masson's trichome stain (Figure 10E).

Immunohistochemistry

In various tissues, including the lungs and intestine, *Pasteurella* and *E. coli* antigens were localized as brown positive immunoreaction. Varied degrees of IHC reaction were observed, which were graded either as mild, moderate, or high, depending on the antigen distribution. In the cases of *E. coli* infection, most frequently the

Table 4. Occurrence of respiratory pathogens among different sex in eastern plain zone of Uttar Pradesh

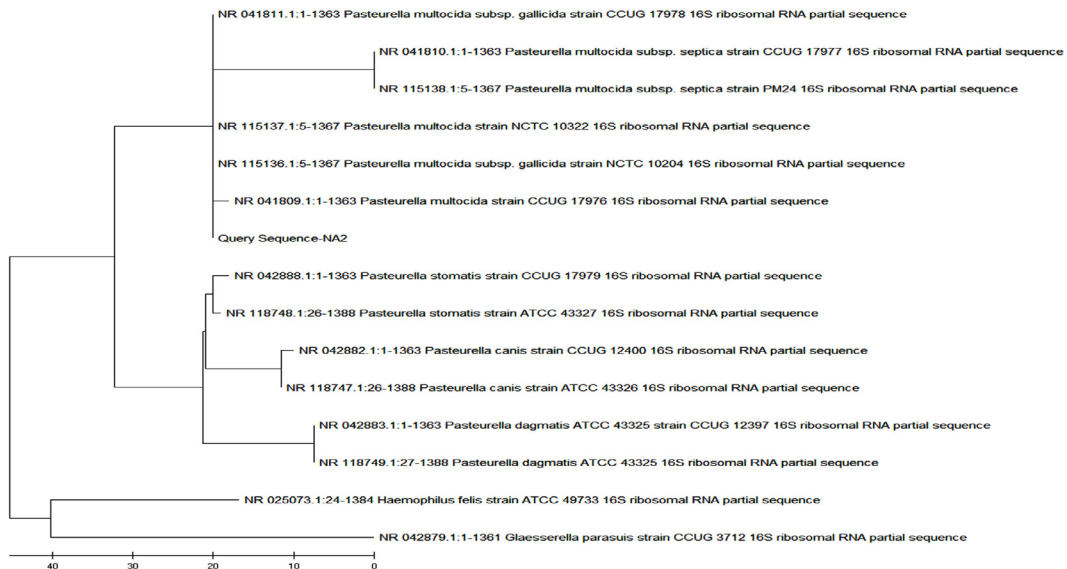| Sex	No. of samples tested	*Pasteurella* spp.		*Escherichia coli*		*Klebsiella* spp.		*Staphylococcus* spp.	
		No. of positive	Percent positivity	No. of positive	Percent positivity	No. of positive	Percent positivity	No. of positive	Percent positivity
Male	41	9	21.95	22	53.66	6	14.63	4	9.76
Female	109	30	27.52	39	35.78	24	22.02	16	14.68
Total	150	39	26.00	61	40.67	30	20.00	20	13.33

Figure 5. Phylogenetic analysis of *16S rRNA* gene of *Pasteurella* spp. positive sample from goat showed highest similarity (99.69%) with *P. multocida* strain CCUG 17976 *16S rRNA*

Table 5. Sex-wise comparison of respiratory pathogens by chi-square test

Parameters	*Pasteurella* spp.					*Escherichia coli*					*Klebsiella* spp.					*Staphylococcus* spp.				
	Value	df	Asymptotic significance (2-sided)	Exact significance (2-sided)	Exact significance (1-sided)	Value	df	Asymptotic significance (2-sided)	Exact significance (2-sided)	Exact significance (1-sided)	Value	df	Asymptotic significance (2-sided)	Exact significance (2-sided)	Exact significance (1-sided)	Value	df	Asymptotic significance (2-sided)	Exact significance (2-sided)	Exact significance (1-sided)
Pearson chi-square	0.481[a]	1	0.488			3.947[b]	1	0.047			1.015[c]	1	0.314			0.625[d]	1	0.429		
Continuity correction[e]	0.235	1	0.628			3.241	1	0.072			0.606	1	0.436			0.271	1	0.602		
Likelihood ratio	0.492	1	0.483			3.902	1	0.048			1.067	1	0.302			0.660	1	0.416		
Fisher's exact test				0.538	0.319				0.062	0.036				0.367	0.221				0.592	0.310
Linear-by-linear association	0.478	1	0.490			3.921	1	0.048			1.009	1	0.315			0.621	1	0.431		
N of valid cases	150					150					150									

Note: df - Degrees of freedom; Superscript 'a' indicate 0 cells (0.0%) have expected count less than 5. The minimum expected count was 16.67; 'c' indicates 0 cells (0.0%) have expected count less than 5. The minimum expected count was 5.47; 'e' indicates computed only for a 2x2 table. Except *Escherichia coli* (p < 0.05), there was no significant difference among male and female regarding respiratory pathogens; 'b' indicates 0 cells (0.0%) have expected count was 10.66; 'b' indicates 0 cells (0.0%) have expected count less than 5. The minimum expected count was 8.20; 'd' indicates 0 cells (0.0%) have expected count less than 5. The minimum expected count was 5.47; 'e' indicates computed only for a 2x2 table.

immunoreaction was found in the cytoplasm of cellular exudates especially in neutrophils and alveolar macrophages, bronchiolar epithelial cells, alveolar interstitial tissues of lungs (Figure 10F), and pulmonary blood vessels. Moderate positive reaction was also seen in the intestinal mucosa.

Strong positive immunoreaction was observed in the *Pasteurella*-infected lungs in the cellular exudates especially in neutrophils and alveolar macrophages and bronchiolar epithelial cells (Figure 11A). The tissue sections from uninfected animals were treated similar to the infected slides

Figure 6. Phylogenetic analysis of *16S rRNA* gene of *Pasteurella* spp. positive sample from goat showed highest similarity (98.83%) with *P. multocida* subsp. *gallicida* strain CCUG 17978 *16S rRNA*
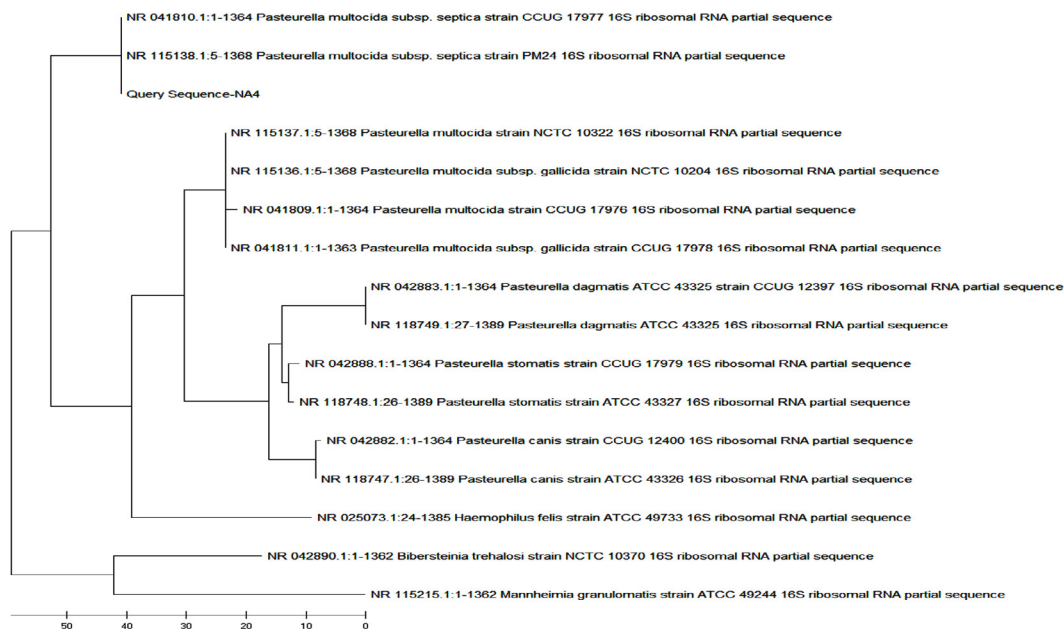

Figure 7. Phylogenetic analysis of *16S rRNA* gene of *Pasteurella* spp. positive sample from goat showed highest similarity (99.05%) with *P. multocida* subsp. *septica* strain CCUG 17977 *16S rRNA*

and did not show any positive signals (Figure 11B). Antigen distribution typically had a substantial correlation with incidence of pathological lesions. It was interesting to note that there was a correlation between the results of PCR, bacterial isolation, and IHC.

DISCUSSION

Respiratory infections are significantly responsible for the mortality of small ruminants, especially goats.[2,32] Respiratory infections must be promptly prevented and controlled to minimize the financial losses to the goat farmers and enhance animal welfare.[2,33] The aim of the present study was to report the respiratory infection-causing bacterial pathogens in goats as well as to undertake a molecular and pathological analysis of *Pasteurella* spp. and *E. coli* infections. Yun *et al.*[7] reported the high mortality among goats due to respiratory infections and mainly caused by *Pasteurella* and extraintestinal pathogenic *E. coli*. But, there are no detailed information regarding the respiratory infection-causing

Table 6. Occurrence of respiratory pathogens among different age groups in eastern plain zone of Uttar Pradesh

| Age groups	No. of samples tested	*Pasteurella* spp.		*Escherichia coli*		*Klebsiella* spp.		*Staphylococcus* spp.	
		No. of positive	Percent positivity	No. of positive	Percent positivity	No. of positive	Percent positivity	No. of positive	Percent positivity
≤6 months	51	15	29.41	25	49.02	6	11.76	5	9.80
>6 months to ≤1 year	39	10	25.64	16	41.03	8	20.51	5	12.82
>1 year to ≤2 years	32	8	25.00	11	34.38	9	28.13	4	12.50
>2 year to ≤3 years	17	4	23.53	6	35.29	3	17.65	4	23.53
>3 years	11	2	18.18	3	27.27	4	36.36	2	18.18
Total	150	39	26.00	61	40.67	30	20.00	20	13.33
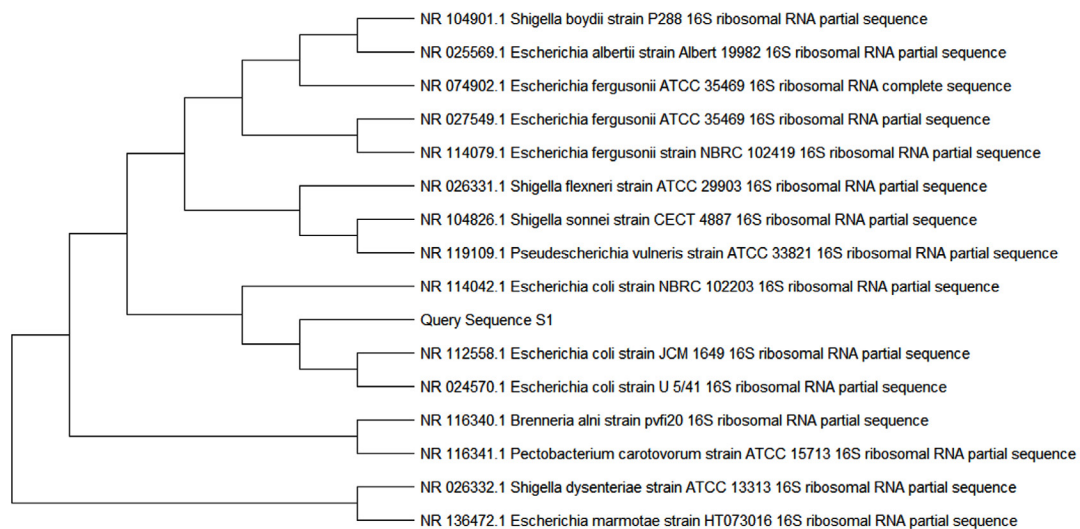
Figure 8. Phylogenetic analysis of *16S rRNA* gene of *Escherichia coli* positive sample from goat showed highest percentage of identity with *E. coli*

Table 7. Age-wise comparison of respiratory pathogens by chi-square test

Parameters	*Pasteurella* spp.			*Escherichia coli*			*Klebsiella* spp.			*Staphylococcus* spp.		
	Value	df	Asymptotic Significance (2-sided)	Value	df	Asymptotic Significance (2-sided)	Value	df	Asymptotic Significance (2-sided)	Value	df	Asymptotic Significance (2-sided)
Pearson chi-square	0.731[a]	4	0.947	3.023[b]	4	0.554	5.388[c]	4	0.250	2.331[d]	4	0.675
Likelihood ratio	0.752	4	0.945	3.054	4	0.549	5.307	4	0.257	2.120	4	0.714
Linear-by-linear association	0.661	1	0.416	2.750	1	0.097	3.439	1	0.064	1.596	1	0.206
N of valid cases	150			150			150			150		

Note: df - Degrees of freedom; Superscript 'a' indicate 2 cells (20.0%) have expected count less than 5. The minimum expected count was 2.86; 'b' indicates 1 cell (10.0%) have expected count less than 5. The minimum expected count was 4.47; 'c' indicates 2 cells (20.0%) have expected count less than 5. The minimum expected count was 2.20; 'd' indicates 3 cells (30.0%) have expected count less than 5. The minimum expected count was 1.47. There was no significant difference (p > 0.05) among age groups regarding respiratory pathogens.

bacterial pathogens in goats of eastern plain zone of Uttar Pradesh, India, available.

The results of bacterial isolation were consistent with the findings reported by Edward and Ewing.[10-15] Initial speculation suggested that *P. multocida* was present in the isolates because that failed to grow on MacConkey agar. However, *Mannheimia haemolytica* was capable of growing on MacConkey agar, and it was haemolytic, similar results were reported by Quinn *et al.*[20] In the present study, *Pasteurella* spp. produced non-haemolytic medium-sized, grey, and mucoid colonies with a sweetish odour on blood agar. These findings were in accordance with the reports of Tigga *et al.*[34] and Desam *et al.*[35]

In the present study, results of the biochemical test were consistent with the findings of Rawat *et al.*,[36] who reported the positivity for catalase, oxidase, and sucrose tests for both *M. haemolytica* and *P. multocida*. *P. multocida* did not produce hemolysis; whereas, *M. haemolytica* produces. Voges-Proskauer test was positive for *P. multocida*; whereas, negative for *M. haemolytica*. Rawat *et al.*[36] reported that indole test was negative for *P. multocida*, but positive for *M. haemolytica*, which was similar to the results of present study. Similar biochemical test findings for *E. coli* isolated from the respiratory tract of goats were reported by Mahmoud *et al.*[37]

The 16S RNA gene sequences of *P. multocida* isolates showed close similarity (99.69%) with *P. multocida* strain CCUG 17976, which was identical to the report of Li *et al.*[38] Similar to the report of Davies,[39] *Pasteurella* isolate in the present study showed similarity with *P. multocida* subsp. *gallicida* strain CCUG 17978. In the present study, *Pasteurella* isolate showed similarity with *P. multocida* subsp. *septica* strain, which was similar to the earlier reports of Davies[39] and Chen *et al.*[40]

The percentage prevalence of respiratory infections in different districts of Uttar Pradesh was remarkably comparable to those of Ahmed *et al.*,[41] who reported 24.30% of *Pasteurella* prevalence. Assefa and Kelkay[42] reported 28.4 to 31.4% prevalence of pasteurellosis in goats. Momin *et al.*[43] reported 20% prevalence of *P. multocida* from black Bengal goats; while, Rashid *et al.*[44] recorded 15% prevalence of *P. multocida* from the lungs of goats, which were contrast to the present findings. This could be due to variations in the sampling methods, goats with pneumonic diseases, better healthcare and laboratory facilities, ecology of the research locations, and predisposing variable.[45] Variation in the results of the current study, compared to the findings of others could be due to most of the researches included both clinically infected and dead animals in their study, but in the present study, only dead goats were included and animals might have been treated with antibiotics for respiratory infections during the clinical course.

In the present study, 20% of samples were positive for *Klebsiella* spp., and 13.33% of samples were positive for *Staphylococcus* spp. in the respiratory tract of goats. *Klebsiella* spp. produces various virulence factors such as lipopolysaccharide, antiphagocytic capsules (K antigen) which aid the bacterium for uptake of iron from the host, and pili for adhesion to host cells. Ibraheim *et al.*[10] reported 35% of positivity to *Klebsiella* spp. including 30% positivity in trachea, 33.33% in nasal swabs, and 40% in lungs from pneumonic goats in Iraq. Sen *et al.*[12] reported *Staphylococcus* spp. was the major organism

Table 8. Occurrence of respiratory pathogens among different breeds in eastern plain zone of Uttar Pradesh

| Breeds	No. of samples tested	*Pasteurella* spp.		*Escherichia coli*		*Klebsiella* spp.		*Staphylococcus* spp.	
		No. of positive	Percent positivity	No. of positive	Percent positivity	No. of positive	Percent positivity	No. of positive	Percent positivity
Non-descript	76	21	27.63	30	39.47	17	22.37	8	10.53
Jamunapari	43	11	25.58	19	44.19	8	18.60	5	11.63
Barbari	31	7	22.58	12	38.71	5	16.13	7	22.58
Total	150	39	26.00	61	40.67	30	20.00	20	13.33
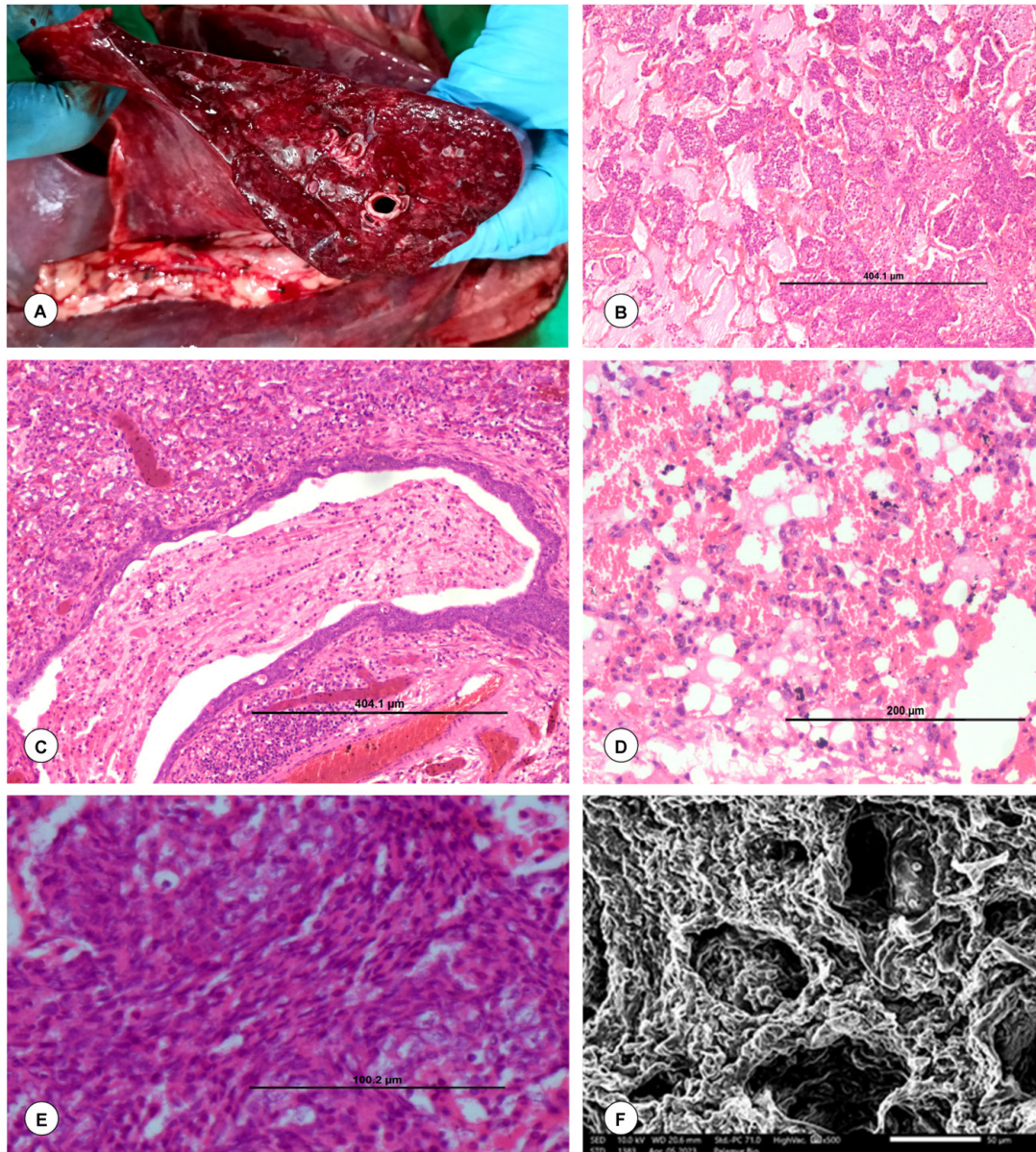
Figure 9. Gross and histopathological lesions in *P. multocida* infection in goats. (A). The cut surface of the lungs showed marked congestion and hemorrhages. (B). Alveolar lumen filled with eosinophilic fibrin and cellular exudates. H&E x100. (C). Bronchiolar lumen filled with eosinophilic fibrin and cellular exudates. H&E x100. (D). Severe hemorrhages in the alveolar lumen and perivascular areas of lungs. H&E x200. (E). Spindle-shaped/elongated necrotic or degenerated leukocytes especially neutrophils and macrophages known as "oat cells". H&E x400. (F). Lungs showed denudation of alveolar and bronchiolar cells. SEM x50
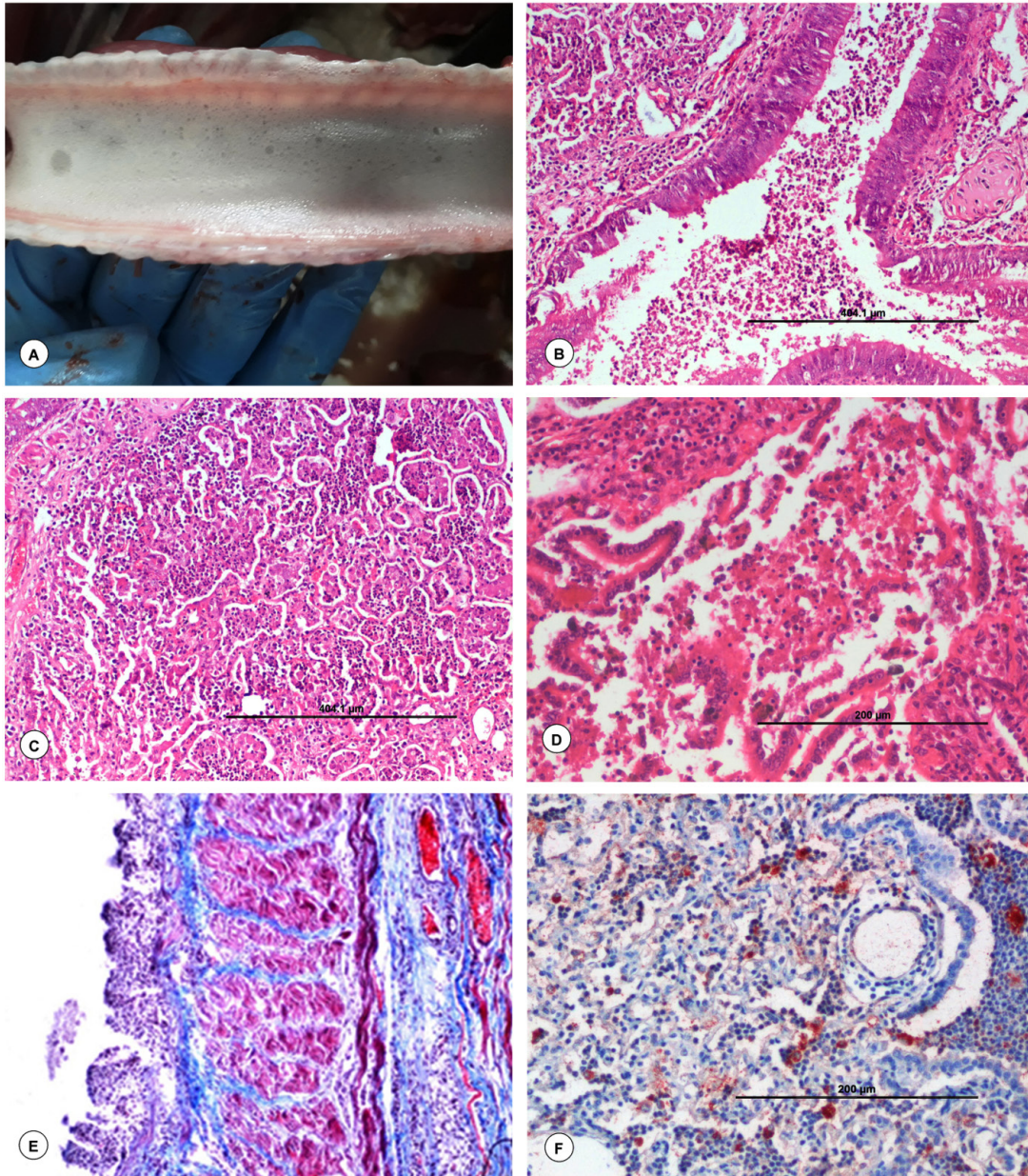
Figure 10. Gross and histopathological lesions in *Escherichia coli* infection in goats. (A)*. Trachea* showed frothy exudate in their lumen. (B). Bronchiole showed infiltration of inflammatory cells and RBCs, along with degenerative changes in the bronchiolar epithelium. H&E x100. (C). Alveolar lumen contained infiltration of inflammatory cells. H&E x100. (D). Small intestine showed necrosis and infiltration of inflammatory cells in lamina propria. H&E x200. (E). *E. coli* positive necrotic small intestine showed fibrosis. Masson Trichrome x200. (F). Presence of *Escherichia coli* antigen in the cytoplasm of alveolar macrophages and neutrophils. IP-DAB-MH, x200
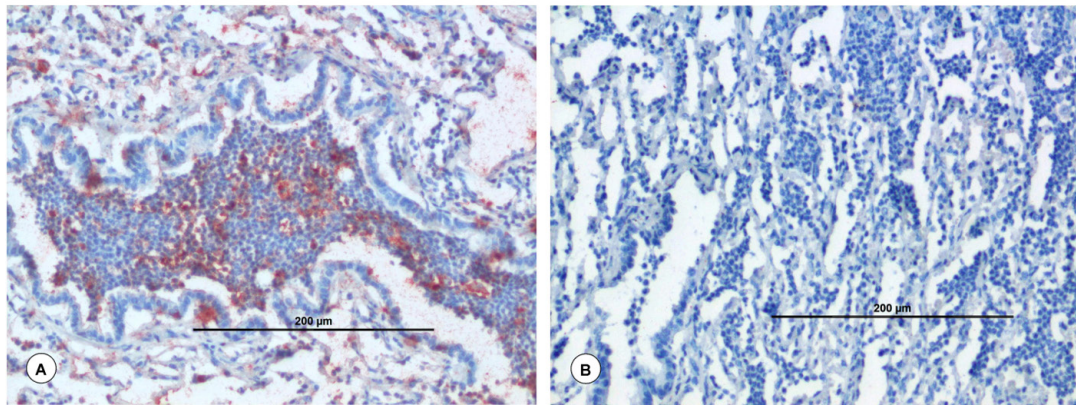
Figure 11. Immunohistochemical and histopathological lesions in *P. multocida* infection in goats. (A). Presence of *Pasteurella* antigen in the cytoplasm of alveolar macrophages and neutrophils. IP-DAB-MH, X200. (B). Negative antibody control: Lungs showed no positive immunolabelling for *Pasteurella* antigen. IP-DAB-MH, X200

followed by *Escherichia coli* and *Pasteurella* spp. in the pneumonia in black Bengal goats.

P. multocida induced pathological findings of the current study were consistent with the findings of Emikpe and Akaptvie[46] and Amin.[47] Pneumonic lesions with consolidation, firm consistency and dark red areas in various lobes of lungs were the most frequent and conspicuous gross lesions observed in most of the cases. The microscopic lesions observed in the current study were consistent with the earlier report by Hashemnia *et al.*,[48] who reported the presence of fibrin exudate and accumulation of neutrophils in the lumen of the bronchi, bronchioles, and alveoli and fibrinous pneumonia. A rim of elongated cells known as "oat cells" or "swirling macrophages" that formed whorl-like structures in and around the alveoli, which is the characteristic feature of pasteurellosis. These findings were similar to the reports by Singh *et al.*,[4] Azizi *et al.*[49] and Kumar *et al.*[50] Gross and histopathological lesions found in the *E. coli*-infected lungs were similar to the reports of Singh *et al.*[51] and Sonawane *et al.*[52]

In the *E. coli*-infected intestine, mild to moderate fibrosis was seen and it was evident by Masson's trichrome staining. In cases of suppurative and chronic caprine bronchopneumonia, regions of calcification were documented earlier by Singh *et al.*[4] In the present study, *P. multocida* was

isolated from the lungs of dead goats, which was in accordance with the findings of Amin.[47] *Pasteurella* spp. antigen can be detected in the formalin-fixed tissue sections by immunoperoxidase staining. Interestingly, in the present study, moderate to strong localization of *P. multocida* antigen was noticed in the lungs, which was in accordance with the findings of Amin.[47] However, a modest response to the IHC may occur due to the low bacterial content in the tissues.

Nevertheless, compared to bacterial culture, the PCR and IHC analysis revealed a higher positive rate of bacterial presence. These results might be explained by the fact that in contrast to the culture approach, which requires living organisms, PCR and IHC can identify nucleic acid and antigen from dead organisms, respectively. In the current study, PCR results for detection of *P. multocida* and *E. coli* antigens were inconsistent with the IHC findings. This may be explained by the uneven assimilation of bacteria among the several organs under investigation. Furthermore, presence of lesions often exhibited a substantial association with the localization of *P. multocida* antigens. As a result, distinct diffuse reactions to *P. multocida* are probably caused by the bacterial solubilized antigen that results from bacterial degradation and lysis.[53] Using IHC, PCR, and bacterial isolation, the current study demonstrated the presence

Table 9. Breed-wise comparison of respiratory pathogens by chi-square test

Parameters	*Pasteurella* spp.			*Escherichia coli*			*Klebsiella* spp.			*Staphylococcus* spp.		
	Value	df	Asymptotic Significance (2-sided)	Value	df	Asymptotic Significance (2-sided)	Value	df	Asymptotic Significance (2-sided)	Value	df	Asymptotic Significance (2-sided)
Pearson chi-square	0.297[a]	2	0.862	0.315[b]	2	0.854	0.609[c]	2	0.737	2.920[d]	2	0.232
Likelihood ratio	0.302	2	0.860	0.313	2	0.855	0.618	2	0.734	2.625	2	0.269
Linear-by-linear association	0.292	1	0.589	0.004	1	0.950	0.598	1	0.439	2.298	1	0.130
N of valid cases	150			150			150			150		

Note: df - Degrees of freedom; Superscript 'a' indicate 0 cells (0.0%) have expected count less than 5. The minimum expected count was 12.61; 'b' indicates 0 cells (0.0%) have expected count less than 5. The minimum expected count was 8.06; 'b' indicates 0 cells (0.0%) have expected count less than 5. The minimum expected count less than 5. The minimum expected count was 6.20; 'd' indicates 1 cell (16.7%) have expected count less than 5. The minimum expected count was 4.13. There was no significant difference (p > 0.05) among breeds regarding respiratory pathogens

Table 10. Occurrence of respiratory pathogens among different seasons of eastern plain zone of Uttar Pradesh

Season	No. of samples tested	*Pasteurella* spp.		*Escherichia coli*		*Klebsiella* spp.		*Staphylococcus* spp.	
		No. of positive	Percent positivity	No. of positive	Percent positivity	No. of positive	Percent positivity	No. of positive	Percent positivity
Winter	67	18	26.87	26	38.81	14	20.90	9	13.43
Summer	28	7	25.00	12	42.86	5	17.86	4	14.29
Monsoon	36	11	30.56	18	50.00	3	8.33	4	11.11
Post-monsoon	19	3	15.79	5	26.32	8	42.11	3	15.79
Total	150	39	26.00	61	40.67	30	20.00	20	13.33

Table 11. Season-wise comparison of respiratory pathogens by chi-square test

Parameters	*Pasteurella* spp.			*Escherichia coli*			*Klebsiella* spp.			*Staphylococcus* spp.		
	Value	df	Asymptotic significance (2-sided)	Value	df	Asymptotic significance (2-sided)	Value	df	Asymptotic significance (2-sided)	Value	df	Asymptotic significance (2-sided)
Pearson chi-square	1.459[a]	3	0.692	3.073[b]	3	0.380	8.979[c]	3	0.030	0.276[d]	3	0.965
Likelihood ratio	1.559	3	0.669	3.142	3	0.370	8.644	3	0.034	0.278	3	0.964
Linear-by-linear association	0.230	1	0.632	0.017	1	0.898	0.450	1	0.502	0.000	1	0.988
N of valid cases	150			150			150			150		

Note: df - Degrees of freedom; Superscript 'a' indicate 1 cell (12.5%) have expected count less than 5. The minimum expected count was 4.94; 'b' indicates 0 cells (0.0%) have expected count less than 5. The minimum expected count was 7.73; 'c' indicates 1 cell (12.5%) have expected count less than 5. The minimum expected count was 3.80; 'd' indicates 3 cells (37.5%) have expected count less than 5. The minimum expected count was 2.53. Except *Klebsiella* spp. (p < 0.05), there was no significant difference among season regarding respiratory pathogens

of *Pasteurella* spp., *E. coli*, *Klebsiella* spp. and *Staphylococcus* spp. antigens in spontaneously infected cases of pneumonia in goats.

Loss of cilia and denudation of cells of alveoli and bronchiole in *Pasteurella* infected lungs were evident by scanning electron microscopy. These ultramicroscopic lesions in the lungs lead to the failure of gaseous exchange causing the inability of oxygen-carrying capacity in the animal. Therefore, infected animals experience suffocation, and finally, death of animals.

CONCLUSION

Among the respiratory infections in goats, *Pasteurella* spp., *E. coli*, *Klebsiella* spp. and *Staphylococcus* spp. infections in eastern plain zone of Uttar Pradesh had been studied limited. Extraintestinal pathogenic *E. coli*, *Pasteurella* spp., *Klebsiella* spp. and *Staphylococcus* spp. were found in this study. These findings imply that *Pasteurella* spp. and *E. coli* infections may be significant contributors to respiratory illnesses in goats. Goats may act as reservoirs for extraintestinal pathogenic *E. coli*, threatening both public health and goat rearing. The samples crucial for diagnosis of respiratory bacterial pathogens were lungs, followed by heart blood, nasal swabs, and tracheal swabs. The PCR followed by IHC and bacterial culture were the laboratory techniques for diagnosis of respiratory bacterial pathogens. Based on the phylogenetic analysis, most of the isolated *Pasteurella* strains were having similarities with *P. multocida*. Results of the present study confirmed the circulation of *Pasteurella* spp., *E. coli*, *Klebsiella* spp. and *Staphylococcus* spp. among the population of goats in field conditions in eastern plain zone of Uttar Pradesh and should be considered for effective vaccination strategies in small ruminants for the control of respiratory infections.

ACKNOWLEDGMENTS

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

DN, SS, and VY conceptualized and visualized the study. DPS performed investigation and data curation. MS and KPS assisted in histopathology, immunohistochemistry and PCR. DN, SS, and VY performed formal analysis. NKD, HK and JKC performed statistical analysis. MS and KPS performed data validation. DPS drafted the manuscript. MS, KPS, NKD, HK and JKC reviewed and edited the manuscript. All authors read and approved the final manuscript for publication.

FUNDING

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This study was approved by the Institutional Animal Ethical Committee (IAEC) of Acharya Narendra Deva University of Agriculture & Technology (ANDUA & T), Kumarganj, Ayodhya, Uttar Pradesh, India.

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