



Investigating respiratory health challenges in Ganjam goats: An in-depth study in Odisha, India

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Abstract

At the core of Odisha, India, respiratory illnesses loom large over communities dependent on goat rearing, endangering their livelihoods and economic stability. This research explores the frequency, consequences, and origins of these incapacitating respiratory conditions in Ganjam goats, aiming to untangle the intricacies of this health issue and lay the groundwork for successful prevention and control measures. In detail the present research, involving 32 affected Ganjam goats out of 880 animals, aimed to assess prevalence, clinical manifestations, hemato-biochemical alterations, and pathological changes linked to respiratory diseases. Ganjam goats had 3.64% respiratory disease prevalence, with higher rates in females (59.4%) than males (40.6%). Winter had the highest occurrence (68.75%), and kids were most affected (31.25%). Clinical signs included dullness, depression, nasal discharge, anorexia, pyrexia, lachrymation, coughing, sneezing, tachycardia, tachypnea, and peracute mortality. Elevated neutrophil and monocyte levels, along with decreased lymphocyte and eosinophil values, were observed. Biochemical parameters (total protein, globulin, A/G ratio) were below normal, and SGPT values increased. Significant variations in serum glucose, albumin, A/G ratio, and SGOT values were noted by age. Gross examination revealed dehydrated, pale animals with congested organs. Histopathology indicated various abnormalities, and microbiological analysis identified specific bacteria which were further confirmed by PCR analysis to be *P. multocida*.

Keywords Respiratory illnesses · Ganjam goats · Hemato-biochemical alterations · Histopathology · Molecular markers

Introduction

Goats play a crucial role in the socioeconomic life of rural communities, particularly in less favored regions globally. Known for their resilience to heat stress, goats thrive in diverse agroclimatic conditions. The practice of goat farming significantly contributes to the economies of Africa, the Mediterranean, and Southeast Asian countries. Historically, goats were the first domesticated ruminant animals, providing essential protein in regions with lower-quality plant sources (Monteiro et al. 2017). In India, goat farming focuses on meat and milk production, especially among

marginal farmers. The total population of goat is around 148.88 million, with 6.39 million from Odisha (AHD 2018; NDDDB 2019; DES 2020). The “Ganjam goat” from Odisha is among the 37 recognized goat breeds in India (NBAGR 2022). These medium-sized goats have a dark tan body coat with white patches on their face and body. Thriving in hot and humid climates of south-eastern agroclimatic zone of Odisha, these dual-purpose goats have an adult body weight of 30–40 kg. They are distributed across Ganjam, parts of Gajapati, Nayagarh, and Khordha districts in Odisha. As per 20th Livestock Census, (DAHDF 2019), the population of pure breed Ganjam goats is 2,35,336 comprising 3.68% and 0.15% of the total goat population in Odisha and India respectively (DES 2020).

Diseases pose a significant challenge to profitable goat farming, often leading to farmer disinterest due to economic losses from respiratory and enteric diseases, particularly fatal respiratory ailments affecting goats of all ages. These illnesses, stemming from diverse pathogens in the nasal passage, pharynx, and tonsils, lead to significant economic

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losses (Karthik et al. 2021). Viruses, bacteria, nasal irritants, throat or tracheal injuries, flies, and parasites serve as causative agents. Increased susceptibility to diseases is attributed to physical stress and environmental conditions, including transportation, weather, vaccination, and malnutrition. Small ruminant respiratory diseases are complex, with bacterial infections being particularly harmful due to diverse symptoms, evolving strains, disease severity, lack of vaccines, and antibiotic resistance (Abera and Mossie 2023). Common bacterial agents include *Mannheimia haemolytica*, *Streptococcus pneumonia*, *Klebsiella pneumonia*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus species*, *Pseudomonas* spp., *Proteus* spp., etc. (Kumar et al. 2020; Hashem et al. 2022). These respiratory diseases constitute 5.6% of all goat diseases, and the severity is often influenced by environmental stress, immunosuppression, poor management practices, and other predisposing factors (Kumar et al. 2014). Goats with respiratory diseases exhibit clinical symptoms such as fever (104 to 106 °F), breathing difficulties, rapid breathing, thick nasal discharge, coughing, sneezing, and tears. Lungs auscultation may reveal crackling sounds, and additional signs include loss of appetite, depression, weight loss, and potential fatality. Gross findings, dependent on the disease cause and respiratory tract involvement, range from congested to edematous lungs with varying textures, hepatization, congestion, atelectasis, frothy exudates, pleural adhesion, tracheal bleeding, and rib imprints on lung surfaces. Microscopic examination reveals neutrophilic and lymphocytic infiltration, severe pulmonary blood vessel congestion, evident fibrin strands in alveoli, and an edematous appearance of alveolar spaces (Bahta et al. 2022; Bukar et al. 2023).

In Odisha, goats are the second most crucial livestock after cattle, supporting a significant portion of the farming community for income and nutritional improvement. The goat population in Odisha correlates positively with forest cover and the proportion of scheduled tribes (Bahta et al. 2022). The state government has prioritized goat farming through various programs, and the central government funds projects like the AICRP project in OUAT, Bhubaneswar, focusing on Ganjam goats' production and reproduction. Odisha's total goat population is 6.39 million, ranking 9th in India, with a 1.84% decrease from previous census as per 20th livestock census (DAHDF 2019). This decline poses economic challenges for the goat industry and landless and tenant farmers in Odisha, with mortality being a critical factor. Considering the limited literature on Ganjam goats, particularly regarding diseases, it was an imperative to conduct thorough clinicopathological investigations for early diagnosis and effective clinical management to an important cause of mortality in kids *i.e.* respiratory diseases. In light of these considerations, the current study is designed with specific objectives. This involves screening for respiratory

diseases in Ganjam goats, incorporating an assessment of previous information and clinical signs. Additionally, there is an examination of haemato-biochemical alterations resulting from respiratory infections. The study extends to identifying and characterizing bacterial etiological agents responsible for these infections. Moreover, an analysis of gross and microscopic changes in tissue samples from postmortem cases was undertaken to advance our understanding of the respiratory health of Ganjam goats.

Materials and methods

Source of animals

The research aimed to assess respiratory diseases in Ganjam goats across Ganjam, Nayagarh, and Khordha districts in Odisha over ten months (January to October 2021) (Fig. 1). Field visits covered Rambha, Khallikote, Odagaon, and Tangi, studying goats aged 1 month to 5 years, categorized into age groups (Group-I to IV) to account for age-related immunity and disease susceptibility. Respiratory disease cases were identified through history, clinical signs, hematological analysis, serum biochemistry, post-mortem examinations, and microbiological tests. Thirty-two blood samples and eight nasal swabs were collected from suspected Ganjam goats in Odisha, with data on age, sex, and clinical signs recorded.

Thirty-two serum samples from sick goats were examined for hematological and biochemical parameters, compared with normal values. Clinical signs of respiratory diseases included dullness, depression, thick nasal discharge, lachrymation, coughing, sneezing, tachycardia, and peracute mortality. Post-mortem examinations of deceased goats studied macroscopic and microscopic organ changes, and microbiological tests on nasal swabs and lung tissue samples confirmed etiological agents.

Prevalence and collection of blood samples and Nasal swabs

Data pertaining to the age and clinical manifestations of the animals were systematically documented during the blood sampling process conducted in the regions of Ganjam, Nayagarh, and Khordha. Subsequently, a comprehensive analysis was undertaken to ascertain the prevalence of respiratory diseases among Ganjam goats, with a meticulous categorization based on age groups observed throughout the study period spanning from January 2021 to October 2021. Blood and serum samples were collected aseptically ensuring minimal pain and distress to the animals. Additionally,



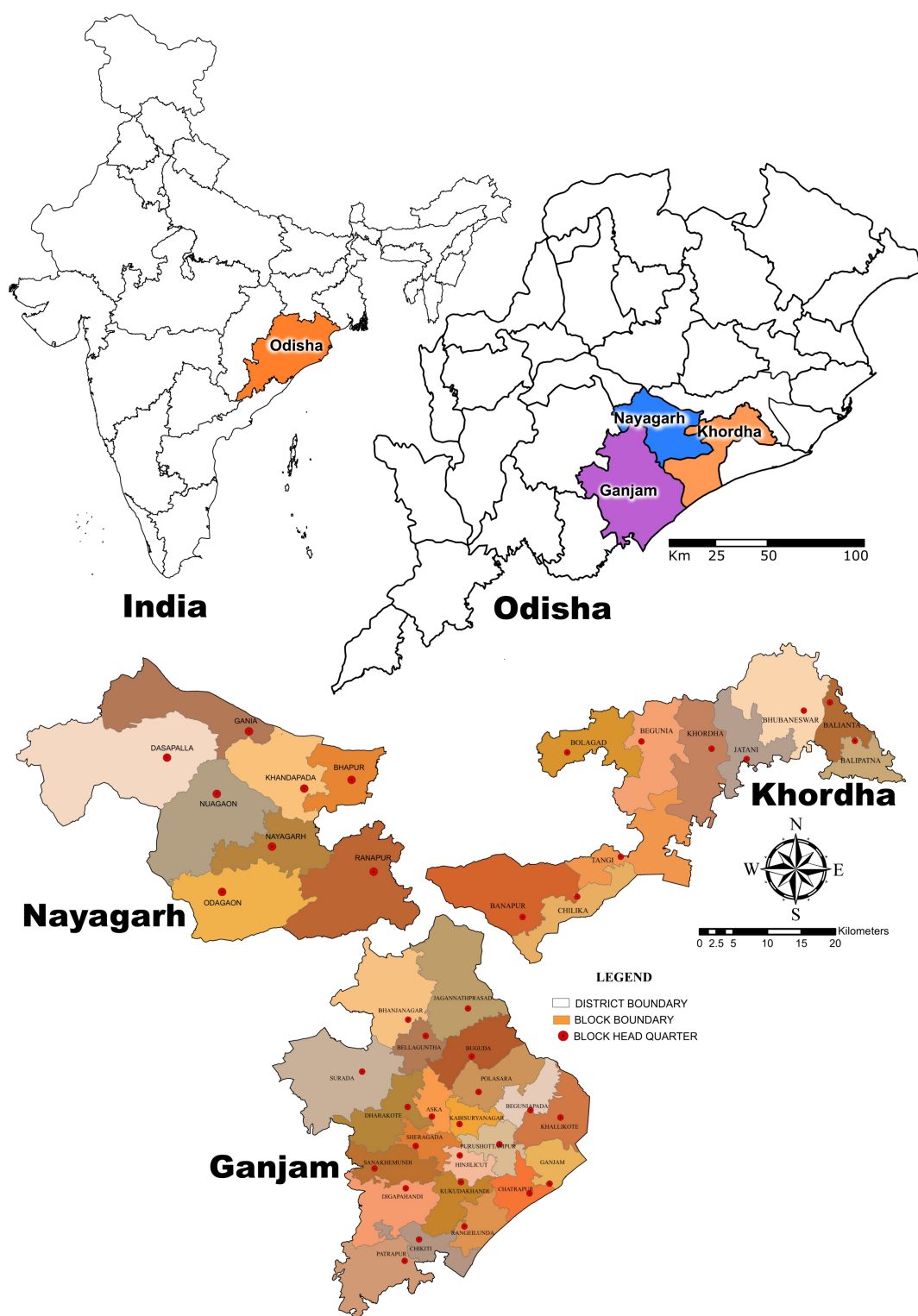


Fig. 1 Map showing three districts of Odisha included in the study

eight nasal swabs were aseptically obtained from clinically affected goat flocks, and screening of these clinical samples was performed to confirm the presence of the etiological agent.

Haematological study

Blood samples were obtained from diverse groups of goats exhibiting respiratory illnesses using two types of test tubes:

an EDTA vial for blood hematology and a clot activator for serum biochemistry analysis. A total of 32 blood samples were gathered in 2 ml EDTA vials for hematological evaluation, while 32 serum samples were collected in 3 ml clot activator vials for serum biochemistry analysis. The aseptic collection of blood samples was performed from the jugular vein of goats using a 22-gauge disposable needle and syringe. Additionally, eight nasal swabs were aseptically obtained from clinically affected goat flocks, and clinical sample screening was conducted to validate the presence of the etiological agent. Haematological parameters like Hb%, PCV, TEC, TLC and DLC etc. by following established methods (Benjamin 1979) were also calculated as part of the comprehensive haematological analysis. The details are provided in the supplementary file.

Serum biochemical analysis

A total of 32 blood samples were gathered in 3 ml clot activator vials, left undisturbed in a slanting position for approximately 2 hrs, and then serum was collected in sterile serum vials. The serum samples were stored at -20°C in the Department of Veterinary Pathology for later serum biochemistry analysis. Using kits supplied by Crest Biosystems, a division of Coral Clinical Systems in Goa, India, and the Eppendorf BioSpectrometer at the Department of Veterinary Pathology, various parameters including serum glucose, total protein, albumin, globulin, A/G ratio, SGOT, SGPT, and calcium were estimated. The detailed biochemical data from 23 cases of the diseased animals were analyzed across different age groups of suspected goats.

Post-mortem and histopathological study

Post-mortem examination

The goats affected by respiratory diseases received treatment from field veterinarians or paravets to facilitate their early recovery. Unfortunately, during the study period, a 2 month-old kid under Veterinary Dispensary Tangi area succumbed to respiratory diseases while undergoing treatment. A comprehensive postmortem examination of the deceased kid was conducted in the Postmortem Hall of the Department of Veterinary Pathology, OUAT. Aseptic swab samples from the trachea, heart, and lungs were collected for bacteriological isolation and identification. Extensive examination of pulmonary tissues was undertaken to identify various pneumonic lesions, and the observed findings were duly recorded. Gross pathological lesions in other internal organs were also documented, with tissue samples collected from both affected and healthy areas. Representative tissue samples were preserved in 10% Neutral buffer formal saline for subsequent histopathological examination. The postmortem

diagnosis was established based on the correlation of gross lesions with the animal's history and observed gross lesions.

Histopathological examination

In the postmortem assessment of goats with a history of respiratory ailments, 2 cm-thick tissue pieces were meticulously collected for histopathological scrutiny. After immersion in a 10% formal saline solution, the tissues underwent a series of procedures including fixation, dehydration, clearing, impregnation, and embedding in paraffin blocks. Subsequently, thin sections measuring 5 microns were cut and stained using haematoxylin and eosin (H & E) to facilitate microscopic examination of histopathological changes. This histopathological analysis aimed to correlate findings with postmortem lesions and discern potential etiological factors contributing to respiratory illnesses in the goats, with additional details provided in the supplementary file.

Microbial examination

A 2 month-old kid suffering from pneumonia died under Veterinary Dispensary Tangi area during our study period and thorough postmortem was conducted. Aseptic swab samples from the trachea, heart, and lungs were collected for bacteriological isolation and identification. Pneumonic lung smears underwent Gram's staining for bacterial differentiation. Heart blood and tracheal swabs were mixed in sterile saline, and then incubated in Brain Heart Infusion Broth. Streaks on BHI, MacConkey, and nutrient agar were made for bacterial isolation. The VITEK-2 Compact system identified isolated colonies, utilizing bacterial suspensions prepared in sterile saline and adjusting turbidity with DensiCHEKplus. Tube-fixed pipetting was performed for gram-negative and gram-positive bacteria, followed by automated incubation and analysis in the VITEK-2 Compact system.

Isolation of bacteria from Nasal swab and bipolar staining

Nasal swabs from affected goats were cultured on brain–heart infusion agar at 37°C overnight. Subsequent steps involved streaking samples on nutrient agar and MacConkey agar, followed by incubation at 37°C for 24 h. Pure cultures from nutrient agar were streaked on blood agar slant tubes and incubated aerobically at 37°C for 24–48 h. Whole cells from single colonies on blood agar were used for PCR amplification reactions. Culture isolates were then subjected to Gram's staining for differential identification. This widely used microbiological method employs basic and acidic dyes, highlighting a distinct staining pattern where the ends of bacilli stain more intensely than the middle portion. The method is invaluable in veterinary medicine and public



health for precise identification of this gram-negative bacterium associated with animal respiratory infections, offering a reliable tool for studying its pathogenic effects.

Molecular diagnosis by PCR method

The amplification reactions utilized the whole cell isolates grown on blood agar as templates, following the methodology outlined by Townsend et al. (1998). Initially, stock and working primers were prepared, with forward and reverse primers obtained from Integrated DNA Technologies (USA) (The Forward and reverse primers of *Pasteurella multocida* had the sequences (5'-3', 21 nt) ATC CCG CTA TTT ACC CAG TGC and (5'-3', 20 nt) GCT GTA AAC GAA CTC GCC AC, respectively). After reconstitution, stock primers were centrifuged and stored at 4 °C, while working primers were created by diluting stock primers in DNase-free water. Subsequently 16 µl of PCR master mix and 4 µl of template were combined to form a 20 µl volume for PCR reactions. The amplified DNA products underwent agarose gel electrophoresis (1%) and were visualized using a gel documentation system. The gel preparation involved boiling agarose in 0.5× TAE buffer, cooling, adding ethidium bromide, and casting the gel at 4 °C. Loading the gel included mixing PCR mixture with gel loading dye and DNA ladder, followed by electrophoresis for 50 min at 110 V. The desired bands were observed under the gel documentation system.

Statistical analysis

The average hematological values were juxtaposed with established normal values (Benjamin 1979), while the mean biochemical data were compared against normal values (Omid et al. 2018). Data across various age groups underwent analysis through one-way analysis of variance (ANOVA), followed by post hoc examination employing Duncan's multiple comparison tests using SPSS 22 software. The results were presented as mean ± SE, and statistical significance was considered at $P < 0.05$.

Results and discussion

This research investigates the prevalence and clinical effects of respiratory diseases in Ganjam goats in its home tract. Conducted over a ten-month period from January to October 2021, field visits covered Rambha, Khallikhote, Odagaon, and Tangi. Cases were identified through history and clinical signs, followed by comprehensive analyses including hematological, serum biochemistry, post-mortem examinations, and microbiological testing. Data regarding age, sex, season, clinical signs and district wise distribution were recorded (Supplementary Table 1). A total of 32 blood samples, 32

serum samples, and eight nasal swabs were collected from clinically suspected Ganjam goats. Treatment by local veterinarians was administered, but one goat succumbed due to the severity of respiratory diseases and inadequate care facilities. Post-mortem examinations aimed to ascertain causes of death and gross lesions, with collected tissues utilized for histopathological studies. Microbiological examinations of nasal swabs, lung tissues, tracheal swabs, and heart blood swabs were conducted to isolate and confirm etiological agents, and bacterial colonies were used as templates for PCR molecular diagnosis. The study provides valuable insights into the respiratory health of Ganjam goats in the specified regions.

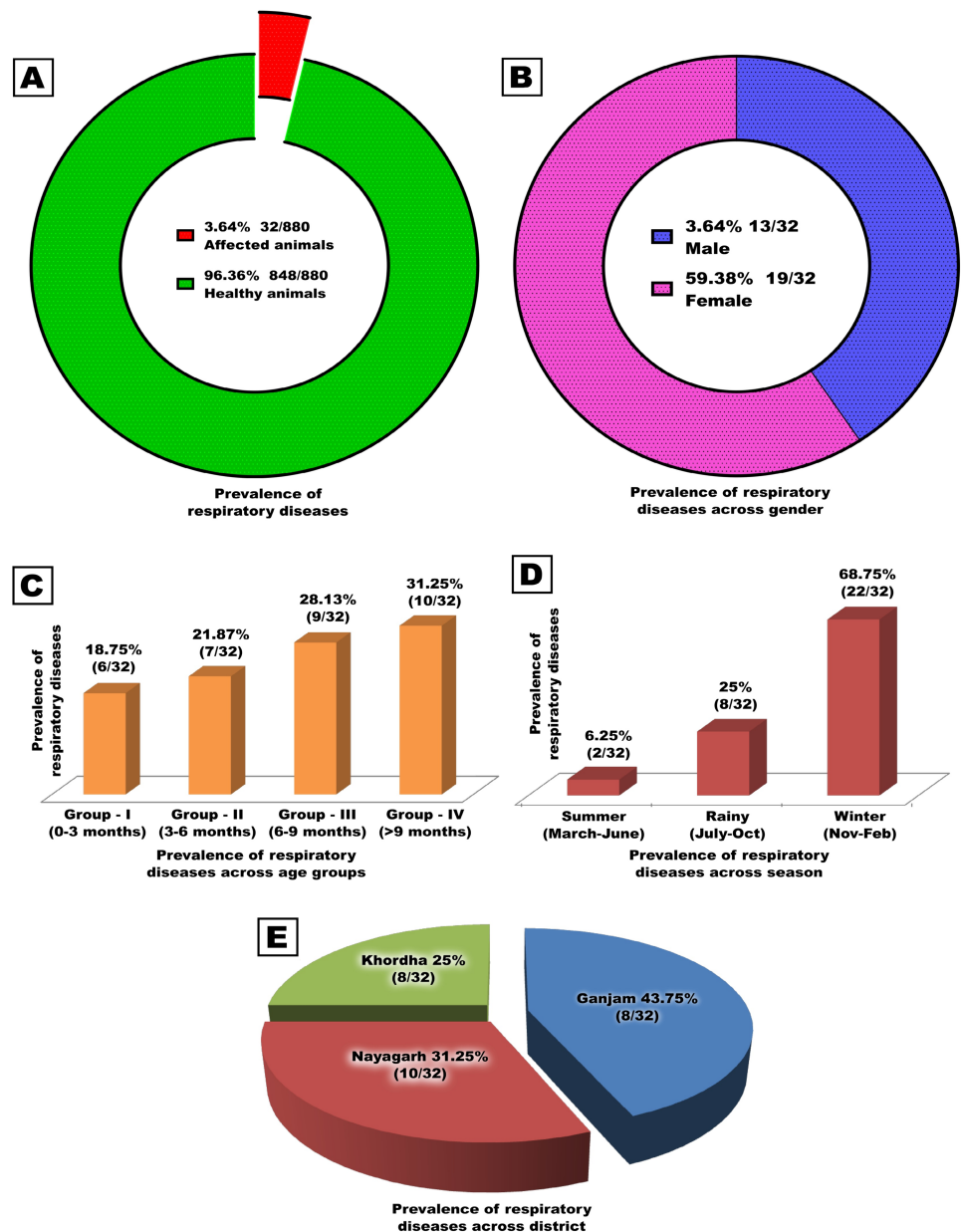
Prevalence study

In this study, the prevalence of respiratory diseases was identified at 3.64% (32/880), aligning with reported rates ranging from 3 to 21% (Tahamtan et al. 2014; Rawat et al. 2019) (Fig. 2A). Throughout the study period, the overall prevalence was 59.4% (19/32) in females and 40.6% (13/32) in males (Fig. 2B). Female goats showed a higher prevalence of pneumonia, as observed by Kashyap et al. (2020), while the prevalence of contagious caprine pleuropneumonia (CCPP) was higher in males compared to females, as reported by Suryawanshi et al. (2015). Among kids under 3 months, the prevalence of respiratory diseases was highest at 31.25%, decreasing with age to 28.13% in the grower stage, 21.87% in the sub-adult stage, and 18.75% in the adult stage. This age-related pattern is in line with Mohamed and Abdel-salam (2008) observation that the prevalence of pasteurellosis is higher in younger animals than in adults (Fig. 2C). The majority of respiratory infection cases were reported in winter (68.75%), followed by rainy season (25%), and summer season (6.25%) (Fig. 2D). This seasonal pattern aligns with the findings of Verma et al. (2014), who observed the highest percentage of respiratory infections in December, January, and February. Additionally, the occurrence of respiratory diseases was highest in winter (42.93%), followed by rainy (38.13%) and summer seasons (18.93%), consistent with the observations of Kattimani et al. (2020). The prevalence of distribution of respiratory infection was highest in Ganjam district compared to other districts (Fig. 2E).

Risk factor analysis

Binary logistic regression (Table 1) was carried out to analyse the potential risk factor (age, sex, season and locality) for prevalence of respiratory disease in Ganjam goats (Sobeih et al. 2022). Sex and location were statistically significant ($p < 0.05$) in multivariable logistic regression model where as age and season was statistically insignificant. The probability of occurrence of respiratory

Fig. 2 **A** Prevalence of respiratory diseases in Ganjam goats, **B** Sex-wise prevalence of respiratory diseases in Ganjam goats, **C** Season-wise prevalence of respiratory diseases in Ganjam goats, **D** Age group wise prevalence of respiratory diseases in Ganjam goats, **E** District-wise prevalence of respiratory diseases in Ganjam goats



disease was insignificant between different age groups. The likelihood of occurrence of respiratory disease was 1.316 times higher in age group below 3 month (18.75%, $p = 0.623$, $\beta = 0.275$, $OR = 1.316$, 95%CI 0.440–3.932) as compared to the age group above 9 month. Similarly the likelihood of occurrence of respiratory disease was 1.168 times higher in age group 3–6 month (21.86%, $p = 0.772$, $\beta = 0.155$, $OR = 1.168$, 95%CI 0.409–3.337) as compared to the age group above 9 month. The probability of occurrence of respiratory disease was significantly lower in male (40.63%, $p < 0.05$, $\beta = -2.859$, $OR = 0.057$, 95%CI 0.007–0.438) as compared to female. The likelihood of occurrence of respiratory disease was 4.019 times higher

in rainy season (34.5%, $p = 0.55$, $\beta = 0.724$, $OR = 4.019$, 95%CI 0.973–16.603) as compared to the winter season. The probability of occurrence of respiratory disease was significantly 8.045 times higher in Ganjam district (59.5%, $p < 0.05$, $\beta = 2.085$, $OR = 8.045$, 95%CI 1.229–52.661) as compared to Khordha district. The likelihood of occurrence of respiratory disease was 1.328 times higher in Nayagarh district (31.25%, $p = 0.566$, $\beta = 0.284$, $OR = 1.328$, 95%CI 0.505–3.496) as compared to Khordha district. According to Mekuria and Asmare, (2010) adult age, sedentary production system and high land agro-ecology were having significant association with CCPP seropositivity in study districts.



Table 1 Results of binary logistic regression analysis to test the occurrence of respiratory disease and associated risk factors (age, sex, season and locality)

Variable	Categories	Number of animals (%)	Number of clinically affected animals (%)	OR	p value	95%CI	S.E	β
Age	0–3 months	24.8	18.75	1.316	0.623	0.440–3.932	0.559	0.275
	3–6 months	24.4	21.86	1.168	0.772	0.409–3.337	0.536	0.155
	6–9 months	23.8	28.14	0.842	0.735	0.310–2.286	0.510	-0.172
	> 9 months	27	31.25	–	–	–	–	–
Sex	Male	35.8	40.63	0.057	0.006*	0.007–0.438	1.038	– 2.859
	Female	64.2	59.37					
Season	Summer	6.1	6.25	3.396	0.209	0.505–22.855	0.973	1.223
	Rainy	34.5	25	4.019	0.055	0.973–16.603	0.724	1.391
	Winter	59.4	68.75	–	–	–	–	–
Location	Ganjam	59.5	43.75	8.045	0.030*	1.229–52.661	0.959	2.085
	Nayagarh	25.2	31.25	1.328	0.566	0.505–3.496	0.494	0.284
	Khordha	15.2	25	–	–	–	–	–

S.E standard error * $p < 0.05$, CI confidence interval, OR odds ratio, β regression coefficient

Clinical symptoms

Clinical symptoms played a crucial role in the preliminary diagnosis of respiratory diseases in Ganjam goats, guiding further confirmatory diagnostic tests. Infected goats exhibited various clinical signs, including dullness, depression, thick nasal discharge, anorexia, pyrexia, lachrymation, coughing, sneezing, tachycardia, tachypnea, and peracute mortality. Additional signs such as crusts around the nasal orifice, lethargy, and open-mouth breathing were prevalent in most cases. Among the 880 goats in Ganjam, Nayagarh, and Khordha districts, only 32 animals were suspected of respiratory diseases based on history and clinical signs. Similar clinical manifestations were reported by Halium et al. (2019), Ahmad et al. (2021), Ma et al. (2023), Emikpe et al. (2010) and Jatav et al. (2020).

Haematological study

A total of 32 blood samples were obtained from infected goats, with 2 ml collected in EDTA vials for hematological evaluation. Hematological parameters, including Hb%, PCV, TEC, TLC, DLC, MCV, MCH, and MCHC, were examined and compared to normal values (Constable et al. 2016). Average haematological values in Ganjam goats ($n = 32$) affected with respiratory diseases is represented in Supplementary Table 2. The average hematological values obtained were 11.13 ± 0.23 g/dl for Hb, 10.8 ± 0.35 thousands/ μ l for TLC, 12.18 ± 0.19 millions/ μ l for TEC, $34.11 \pm 0.68\%$ for PCV, 28.17 ± 0.63 fl for MCV, 9.21 ± 0.25 pg for MCH, and 32.86 ± 0.73 g/dl for MCHC. The percentage of leukocytes in DLC showed values of $54.47 \pm 0.69\%$, $2.94 \pm 0.24\%$, $37.47 \pm 0.97\%$, and $4.81 \pm 0.39\%$ for neutrophil, eosinophil,

lymphocytes, and monocytes, respectively (Table 2, Fig. 3). MCH exhibited an increase from the normal range (5.2–8 pg), while other parameters were within the normal range, though with variations. Lymphocytes and eosinophils showed averages below the normal range, while monocytes slightly exceeded it. Neutrophil levels were higher than the normal value (30–48%). However, a significant difference ($p < 0.05$) emerged in the average values of neutrophils and lymphocytes between the age groups (Fig. 3A), with Group-I having significantly higher neutrophils and lower lymphocytes than Group-IV. Average hematological values did not significantly differ for parameters such as Hb%, TLC, TEC, PCV%, MCV, MCH, MCHC, eosinophil, basophil, and monocyte among different age groups (Fig. 3B–H). This finding aligns with similar reports on neutrophilia and lymphocytosis in respiratory diseases by Ghanem et al. (2015), Rehman et al. (2022), Mondal et al. (2004), Yatoo et al. (2019) and Dutta et al. (2017).

Serum biochemical analysis

The mean values for various biochemical parameters in affected goats were as follows: 69.06 ± 1.91 mg/dl for glucose, 5.39 ± 0.20 g/dl for total protein, 2.90 ± 0.14 g/dl for albumin, 2.48 ± 0.13 g/dl for globulin, 1.27 ± 0.09 for A/G ratio, 178.56 ± 9.67 IU/l for SGOT, 26.19 ± 1.14 IU/l for SGPT, and 9.58 ± 0.21 mg/dl for calcium. The details are provided in the supplementary Table 3. Biochemical parameters such as total protein, globulin, and A/G ratio exhibited average values below their normal reference range, while SGPT showed an increased average value, indicating potential hepatocellular injury, inflammation, infection, and toxemia. This aligns with findings reported by Jarikre

Table 2 Mean values of haematological parameters in different age groups of affected Ganjam goats

Parameters	Group-I (0–3 months)	Group-II (3–6 months)	Group-III (6–9 months)	Group-IV (above 9 months)
Hb%	10.48 ± 0.45	10.95 ± 0.40	11.08 ± 0.48	11.68 ± 0.43
TLC (thousands/ μ l)	10.8 ± 0.91	9.98 ± 0.72	11.44 ± 0.63	11.07 ± 0.63
TEC (millions/ μ l)	12.5 ± 0.56	12.51 ± 0.26	11.92 ± 0.42	11.99 ± 0.35
PCV (%)	34.8 ± 1.22	8.52 ± 1.84	34.66 ± 1.13	8.90 ± 1.38
MCV (fl)	27.60 ± 0.91	26.81 ± 1.44	29.20 ± 0.96	28.54 ± 1.49
MCH (pg)	8.53 ± 0.76	8.76 ± 0.32	9.8 ± 0.38	9.84 ± 0.51
MCHC (gm/dl)	30.81 ± 2.14	8.02 ± 1.53	32.09 ± 1.27	34.6 ± 1.14
N (%)	57.83 ± 1.88 ^a	55.14 ± 1.71 ^{ab}	54.11 ± 0.82 ^{ab}	52.30 ± 0.92 ^b
E (%)	3.16 ± 0.70	2.85 ± 0.55	3.11 ± 0.42	2.70 ± 0.42
B (%)	00	00	00	00
L (%)	34.17 ± 2.13 ^a	34.86 ± 3.10 ^a	38.00 ± 1.17 ^{ab}	40.80 ± 0.90 ^b
M (%)	4.83 ± 0.94	5.71 ± 0.99	4.78 ± 0.66	4.20 ± 0.69

NB means with different superscripts differ significantly ($p < 0.05$)

et al. (2016) in pneumonic goats, where there was slight decrease in plasma protein. Serum biochemistry analysis revealed a significant reduction in total protein, albumin, and albumin-globulin ratio, with an elevated globulin value, SGOT, SGPT enzyme levels, and serum glucose level, consistent with Mondal et al. (2004). Statistical comparison of biochemical parameters among different age groups showed no significant difference in serum values of total protein, globulin, SGPT, and calcium (Table 3). However, the serum glucose level in Group-IV (above 9 months) was significantly ($p < 0.05$) lower than in Group-I, Group-II, and Group-III, but in case of calcium level a negligible change was observed (Fig. 4A). While no significant difference was observed in serum SGOT values between Group-I, Group-II, and Group-III, the serum SGOT value of Group-IV was significantly ($p < 0.05$) higher than in Group-I, Group-II, and Group-III (Fig. 4B). Additionally, the serum albumin level in Group-IV was significantly ($p < 0.05$) higher than in Group-II (3–6 months) (Fig. 4C), and the A/G ratio was significantly higher in Group-III compared to Group-I (Fig. 4C). These findings are consistent with findings reported by Mondal et al. (2004). SPSS 22 software analysis indicated that kids below 3 months of age were more affected by respiratory disease compared to adults (Emikpe et al. 2013).

Post-mortem examination

A post-mortem examination was conducted on a kid that succumbed to respiratory disease (Fig. 5A). External inspection revealed weakness, emaciation, and a thick purulent crust on the nasal orifice. Peripheral renal parenchyma appeared pale (Fig. 5B). The skin appeared dry and dehydrated, and the oral and cutaneous mucous membranes were

pale (Fig. 5C). Abdominal examination revealed pale viscera (Fig. 5D).

Extensive examination of pulmonary tissues documented congested and edematous lungs with pleural thickening, emphysema in some lobules, and pneumonic and congested apical lobes (Fig. 6A). Diaphragmatic, cardiac, and accessory lobes exhibited congestion with edema, indicating pulmonary diseases. Macroscopic lesions in other visceral organs were recorded, including notable epicardial congestion in the heart, congested tracheal mucous membrane with frothy exudates, and pale mesentery with undigested feed materials in the stomach (Fig. 6B–H). These findings align with Al-Karawi and Al-Shammari (2019), Mohamed and Abdelsalam, (2008) and Abera and Mossie, (2023).

Histopathological studies

Microscopic examination of lungs tissue revealed alveolar exudates, mainly neutrophils, within the lumen, along with notable interalveolar congestion. Necrosis of alveolar epithelium accompanied the infiltration of inflammatory cells such as mononuclear cells and plasma cells. Pulmonary blood vessels exhibited marked congestion. The microscopic lungs appearance displayed adjacent areas of atelectasis and emphysema. Tracheal and bronchial epithelium denudation occurred with inflammatory cell infiltration (Fig. 7A–D).

Necrotic debris and exudates were present around the villous space of the intestine, showing severe congestion, epithelial desquamation, mild ulceration, and submucosal edema (Fig. 7E–H). The liver exhibited periportal necrosis, hepatocyte cytoplasm dissolution with fibrotic proliferation, and sinusoidal congestion. Disorganization of hepatic



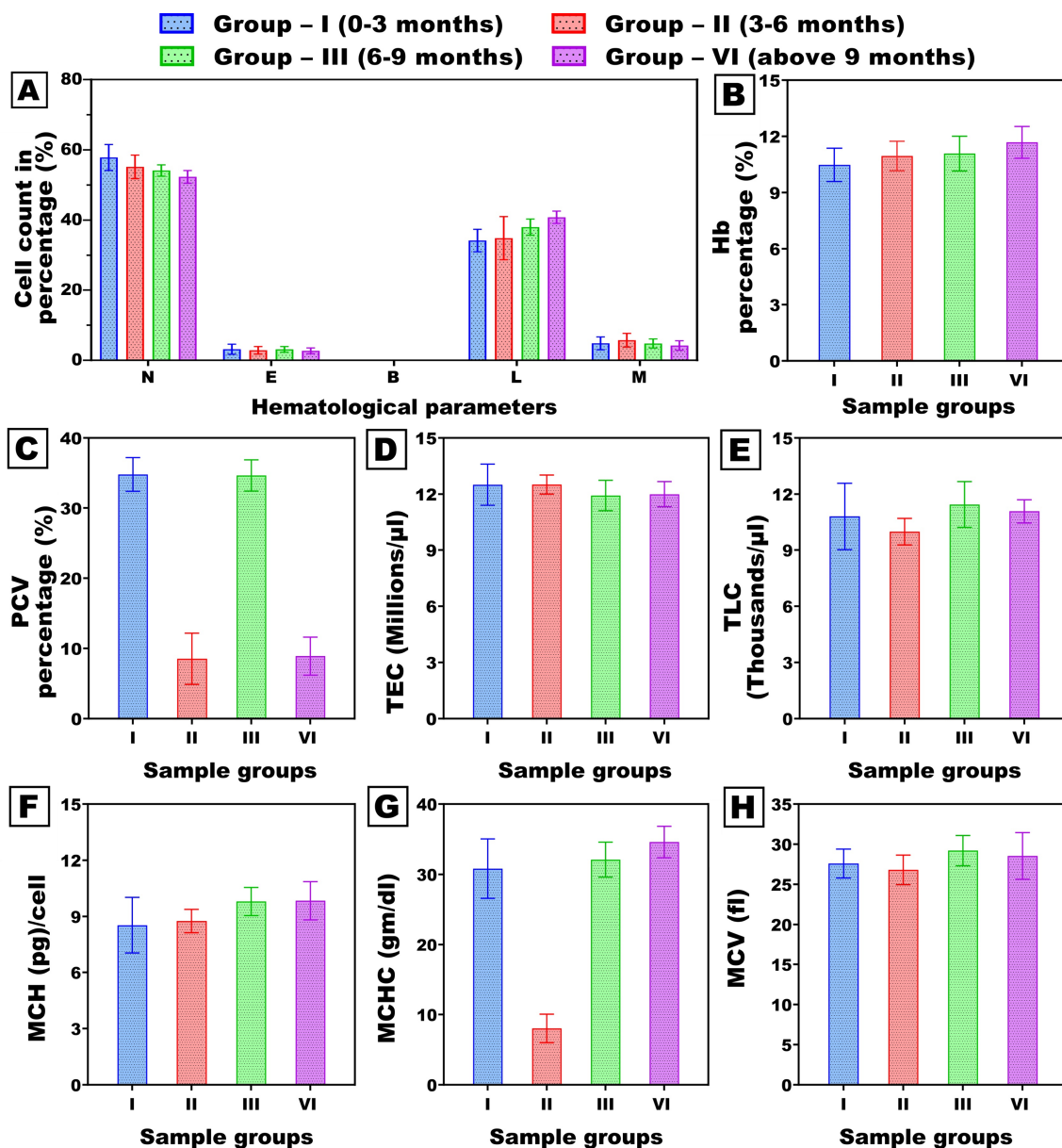


Fig. 3 **A** Cell count percentage in blood sample Ganjam goats with respiratory diseases, **B** Haemoglobin (Hb) percentage in blood sample Ganjam goats with respiratory diseases, **C** Packed Cell Volume (PCV) percentage in blood sample Ganjam goats with respiratory diseases, **D** Transient Erythroblastopenia of Childhood (TEC) in blood sample Ganjam goats with respiratory diseases, **E** Total Leu-

cocyte Count (TLC) in blood sample Ganjam goats with respiratory diseases, **F** Mean Corpuscular Hemoglobin (MCH) in blood sample Ganjam goats with respiratory diseases, **G** Mean Corpuscular Hemoglobin Concentration (MCHC) in blood sample Ganjam goats with respiratory diseases, **H** Mean Corpuscular Volume (MCV) in blood sample Ganjam goats with respiratory diseases

cords and hepatocyte necrosis were evident (Fig. 7I, J). Kidneys displayed glomerular congestion and mild interstitial glomerular congestion with tubular epithelial cell swelling (Fig. 7K, L). Heart tissue presented myocardial congestion with haemorrhagic lesions in the epicardium. These findings align with Mohamed and Abdelsalam (2008) and (Mondal et al. 2004).

Pathogen identification, characterization and etiological diagnosis

Non-hemolytic colonies on blood agar, which didn't grow on MacConkey agar, showed gram-negative Cocobacilli and bipolar organisms suggesting *P. multocida* (Fig. 8A–D). These findings were consistent with Rawat et al. (2019), who, in their study on bacterial examination

Table 3 Serum biochemical changes in different age groups of Ganjam goats

Parameters	Group-I (0–3 months)	Group-II (3–6 months)	Group-III (6–9 months)	Group-IV (above 9 months)
Glucose (mg/dl)	79.67 ± 4.10 ^a	71.29 ± 3.20 ^a	71 ± 2.75 ^a	59.40 ± 2.8 ^b
Total protein (g/dl)	5.18 ± 0.34	4.81 ± 0.42	5.52 ± 0.55	5.81 ± 0.22
Albumin (g/dl)	2.50 ± 0.22 ^{ab}	2.47 ± 0.26 ^a	3.11 ± 0.34 ^{ab}	3.27 ± 0.21 ^b
Globulin (g/dl)	2.68 ± 0.17	2.34 ± 0.31	2.41 ± 0.36	2.54 ± 0.14
A/G ratio	0.93 ± 0.07 ^a	1.16 ± 0.17 ^{ab}	1.52 ± 0.23 ^b	1.8 ± 0.12 ^{ab}
SGOT (IU/l)	138.83 ± 4.82 ^a	137.86 ± 7.69 ^a	162.22 ± 10.74 ^a	245.60 ± 11.95 ^b
SGPT (IU/l)	29.17 ± 2.72	25.86 ± 2.19	27.8 ± 2.55	23.60 ± 1.74
Calcium (mg/dl)	9.11 ± 0.50	9.70 ± 0.53	9.63 ± 0.37	9.75 ± 0.39

NB Values carrying different superscripts are significantly different ($p < 0.05$) among various groups

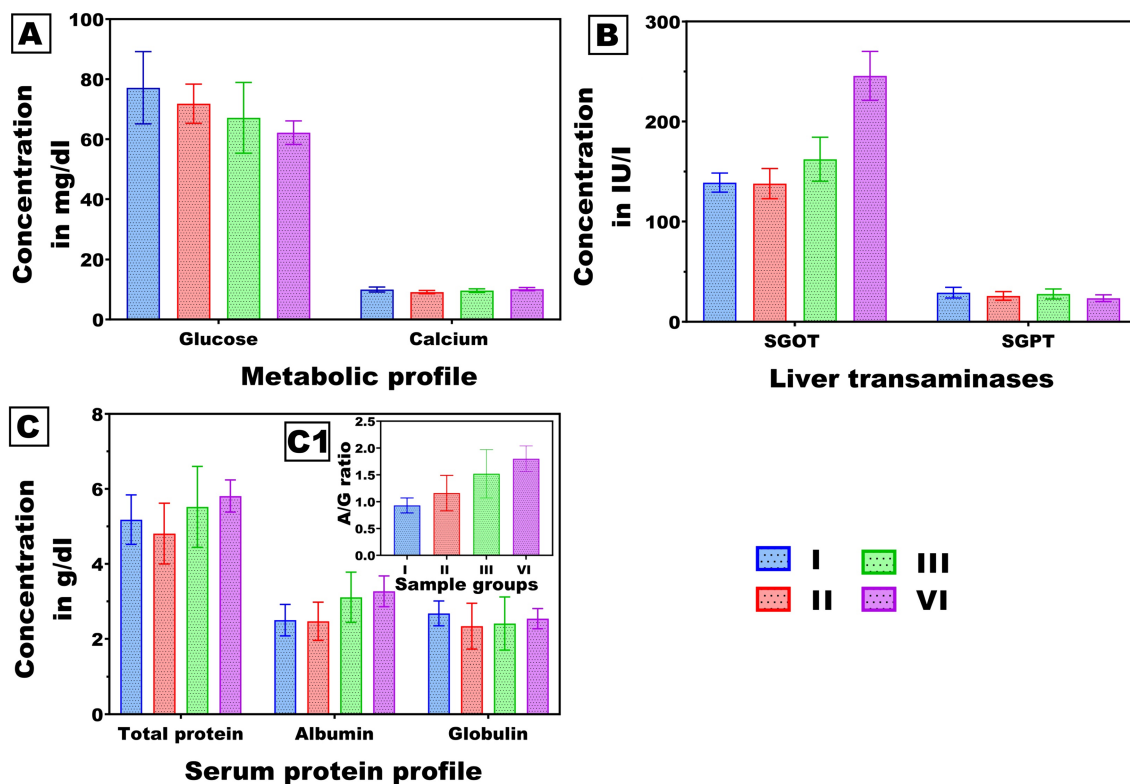


Fig. 4 **A** Metabolic profile of the serum sample of Ganjam goats with respiratory diseases, **B** liver transaminases of Ganjam goats with respiratory diseases, **C** serum protein profile of the Ganjam goats with respiratory diseases

of lungs tissue samples and nasal swabs from affected goats, reported the isolation and identification of *M. hemolytica* and *P. multocida* organisms. The compact analysis using VITEK-2 demonstrated the identification of *Pseudomonas mendocina* with an 89% probability and the organism *S. gallolyticus*. The results align with the research of Funke et al. (1998) and Delmas et al. (2008), supporting the notion that both *Streptococcus* and

Pseudomonas spp. are implicated in causing respiratory infections in animals.

Pathogen identification at the molecular level

To further identify *P. multocida* bacteria, PCR analysis was conducted using a set of ruminant-origin *P. multocida* primers, as outlined by Kumar et al. (2009). Townsend et al. (1998) adopted capsular and somatic typing methods





Fig. 5 **A** Dead body of goat infected with respiratory disease, **B** Pale conjunctiva from infected Ganjam goat, **C** Pale oral mucous membrane of the Ganjam goats with respiratory diseases, **D** Pale colour of Visceras of the Ganjam goats with respiratory diseases

to isolate *P. multocida* for subsequent serological characterization. Complementing this, PCR assays were utilized, underscoring the versatility of PCR in differentiating and characterizing bacterial strains. The synergy between these methods contributes to a comprehensive understanding of the targeted bacteria. A noteworthy aspect of the study is the direct application of PCR amplification on bacterial colonies. This not only expedites the detection process but also enhances the sensitivity of *P. multocida* identification (Fig. 8E). The use of specific primers, KMT1T7 and KMT1SP6, further refines the method, ensuring a reliable

and accurate outcome. The KMT1 gene was sequenced and was submitted to the NCBI database with Accession No. OM222694 (Fig. 8F).

Conclusion

In conclusion from the clinicopathological study of respiratory diseases in Ganjam goats in Odisha, a 3.64% prevalence was identified. Clinical signs included dullness, depression, nasal discharge, anorexia, pyrexia,

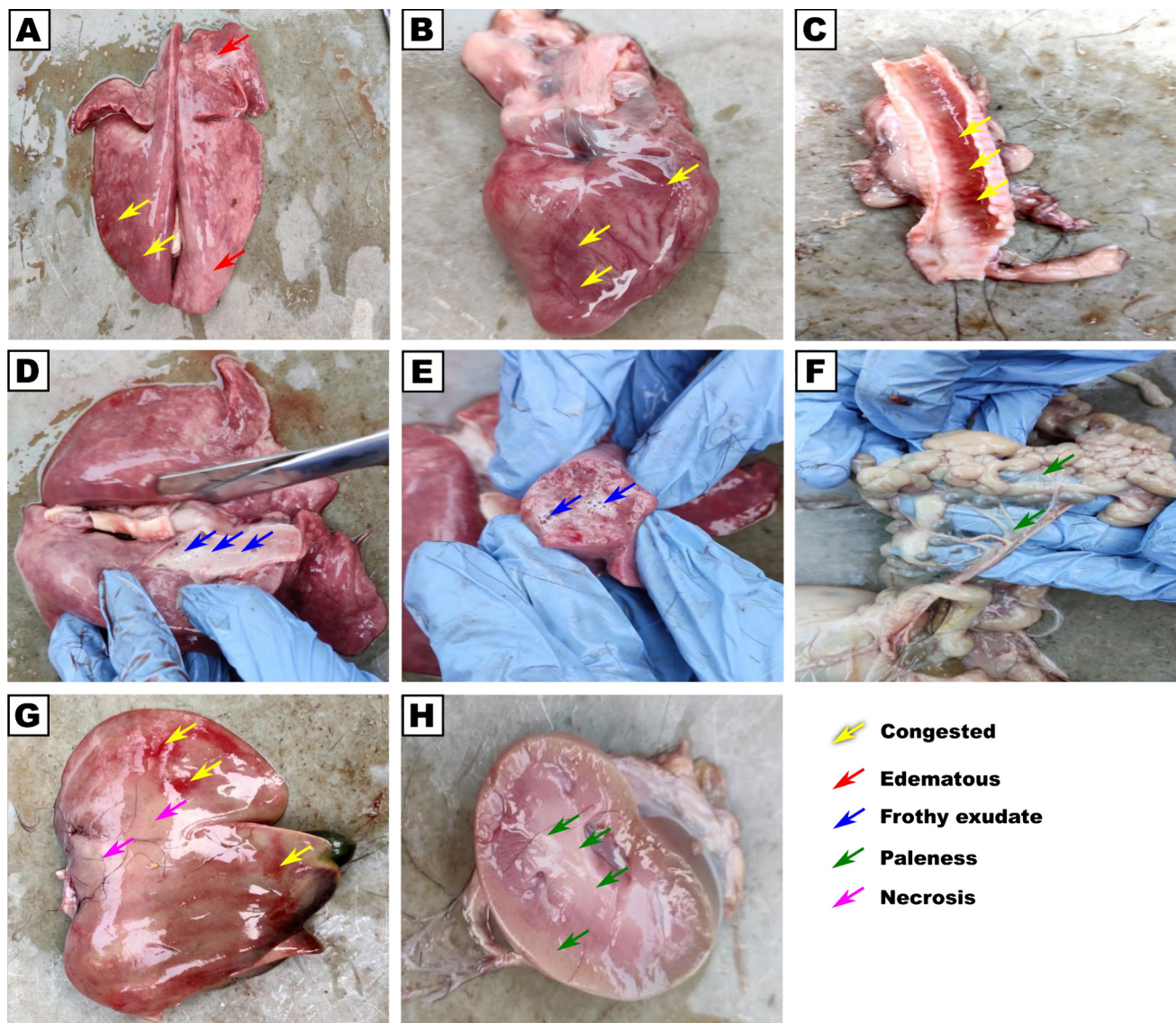


Fig. 6 **A** Congested and edematous lungs, **B** Epicardial congestion of heart, **C** Congested tracheal mucous membrane, **D** Frothy exudate in the tracheal lumen, **E** Frothy exudate in the lower respiratory tract,

F Pale mesentery in the Ganjam goat, **G** Enlarged liver with marked congestion and necrosis, **H** Pale colour of renal parenchyma

lachrymation, coughing, sneezing, tachycardia, tachypnea, and peracute mortality, with specific indicators like crusts around the nasal orifice, lethargy, and open-mouth breathing. Blood analysis revealed absolute neutrophilia and lymphopenia, with significant age-related variations. Gross lesions in organs, such as lungs, trachea, liver, kidney, and intestine, provided insights into the anatomical

impact of respiratory diseases. Microscopic examinations uncovered specific pathological changes, while microbiological and molecular analyses confirmed the presence of pathogens like *Streptococcus*, *Pseudomonas*, *Pasteurella* spp., including *P. multocida*. This marks the first record of molecular diagnosis for identifying the etiological agents of respiratory infection in Ganjam goats. Furthermore, this

Fig. 7 Photomicrograph of Lungs (A–D), **A** Alveolar exudates predominant with neutrophils and mononuclear cells (400×), **B** Necrosis of alveolar epithelium and alveoli lumen filled with cellular exudates predominant with neutrophils (400×), **C** Congestion of alveoli with infiltration of inflammatory cells (400×), **D** Emphysema of alveoli and congestion of blood vessels (100×); Photomicrograph of Intestine (E–H), **E** Congestion with sloughing of mucosal membrane and necrotic debris filled in the lumen (400×), **F** Submucosal edema with infiltration of neutrophils (100×), **G** Submucosal edema in mucosal folding with congestion (100×), **H** Ulceration and desquamation of mucosa of intestine along with infiltration of inflammatory cells (400×); Photomicrograph of Liver (I and J), **I** Sinusoidal congestion with fibroblast proliferation (400×), **J** Disorganization of hepatic cords and necrosis of hepatocytes (400×); Photomicrograph of Kidney (K and L), **K** Coagulative necrosis with mild congestion (400×), **L** Mild interstitial and glomerular congestion and swelling of tubular lining epithelial cells (400×)

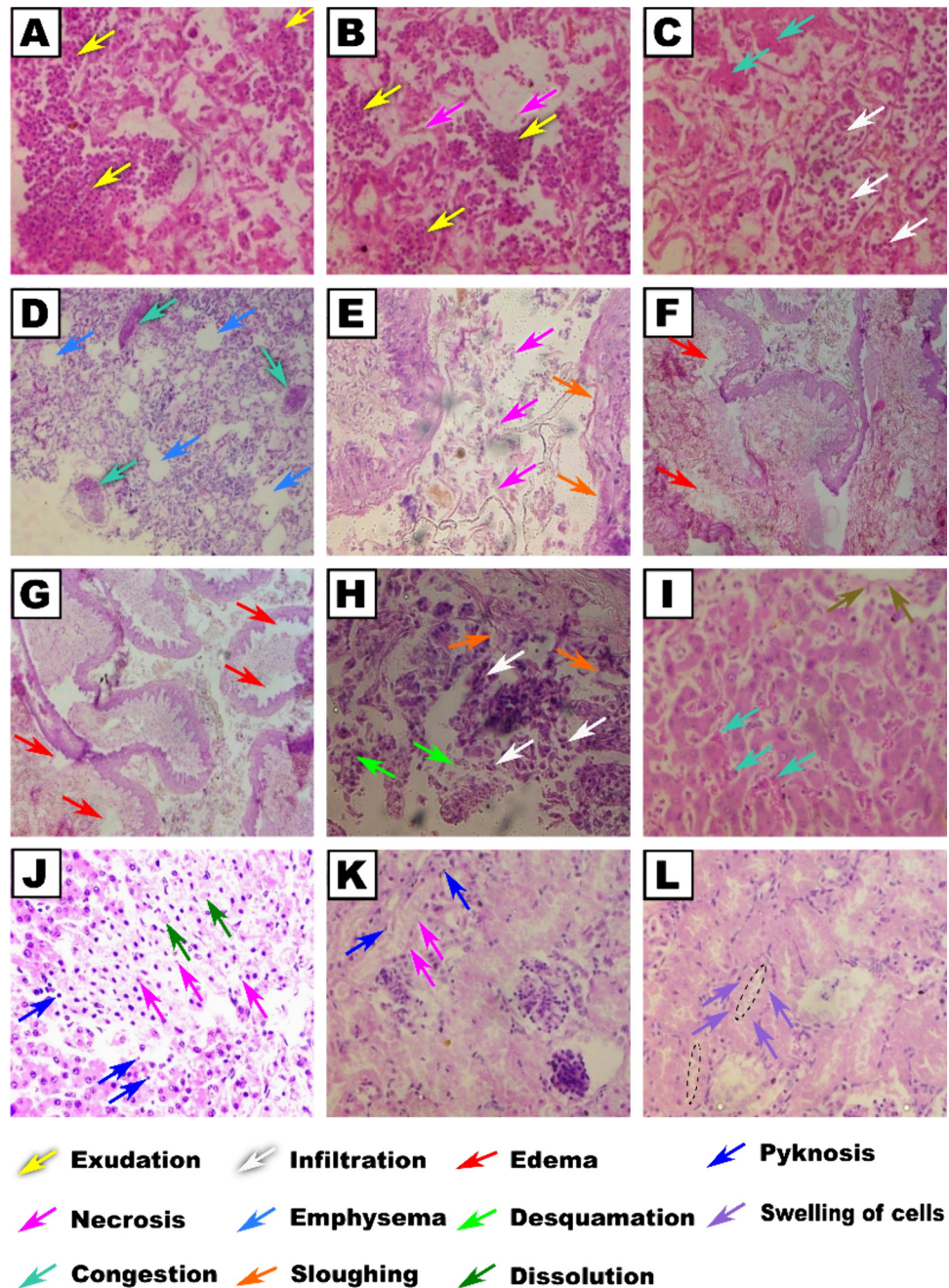
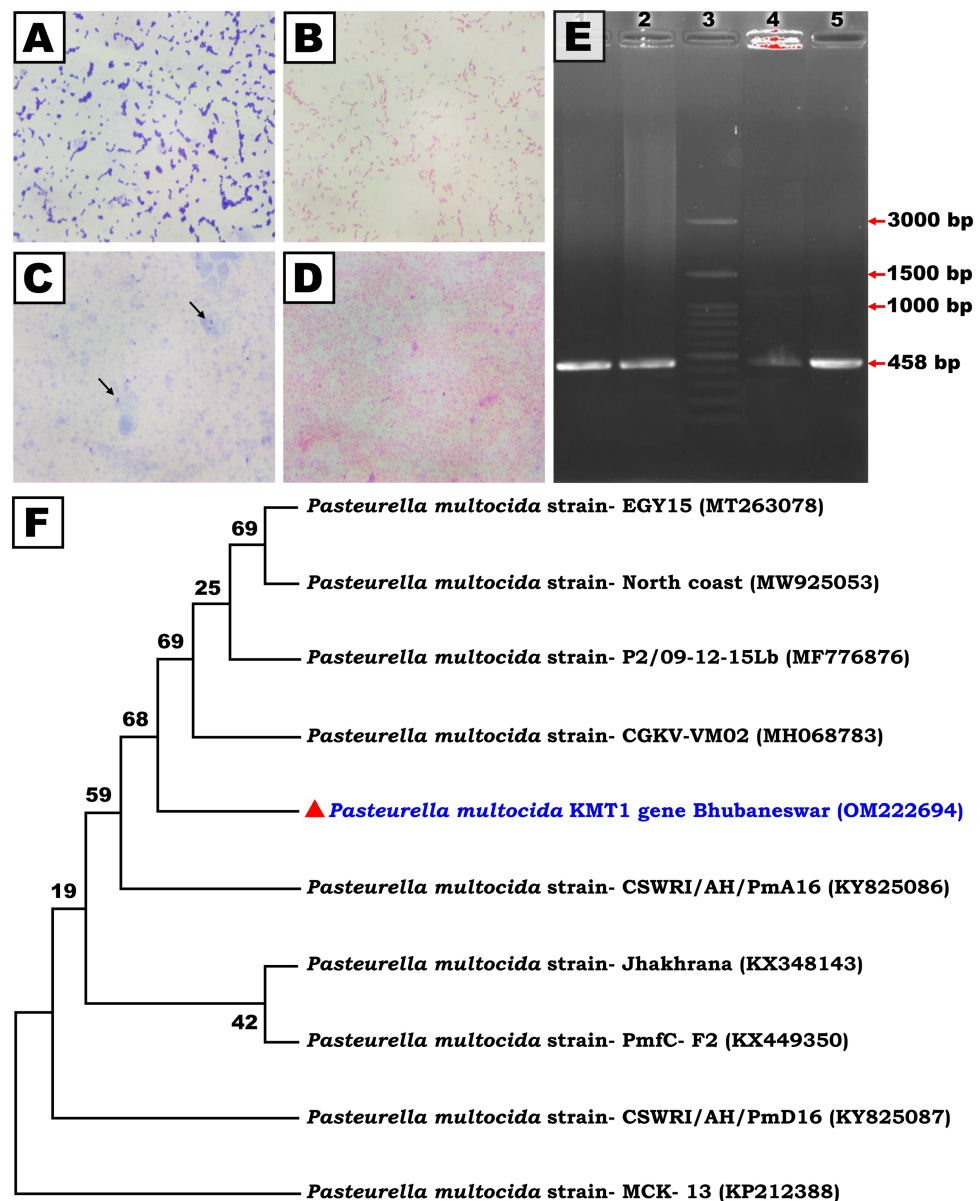


Fig. 8 **A** Gram positive Streptococcus showing chain of cocci, **B** Gram negative *Pseudomonas* showing pink bacilli, **C** Bipolar organism *P. multocida* in Methylene blue staining, **D** Gram negative coccobacilli and *P. multocida* in gram staining, **E** Gel electrophoresis of PCR product (Well no: 1, 2, 4 and 5 are positive for *P. multocida* and 3 is 100bp ladder), **F** Phylogenetic tree was constructed using *P. multocida* KMT1 gene and red triangle indicates the sequence obtained in the present study



comprehensive approach offers valuable insights for effective control measures and enhances the understanding and management of respiratory diseases in Ganjam goats.

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Declarations

Conflict of interest The authors declare no conflict of interest.

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