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Pathomorphological and Microbiological Diagnosis of Pneumonic Pasteurellosis in Goats of Assam, India

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ABSTRACT

Background: Pneumonia is a major cause of economic losses in the global ruminant industry. Pasteurellosis, a bacterial infection, is a leading cause of pneumonia in sheep and goats. It is frequently reported in goats in Assam, India, causing significant economic losses for marginal farmers. There is a lack of scientific data on the incidence of pasteurellosis in goats in the northeast region of India.

Methods: The present study was aimed to study the pneumonic infection due to *Pasteurella* species in goats in and around Guwahati. In the present study 139 animal carcasses were examined, among the samples collected from 62 pneumonic lungs, 21 came positive for *Pasteurella* species. The samples were screened based on gross, microscopic, bacteriological, and molecular assays.

Result: The main clinical signs showed were respiratory distress, depression, anorexia, loss of body condition, high febrile state and lacrimation. Different types of pneumonia recorded based on pathological alterations were bronchopneumonia, interstitial pneumonia, sero-fibrinous pneumonia, haemorrhagic pneumonia and suppurative pneumonia. In microbiological assays, the infection of *Pasteurella* was established by greyish lustrous isolated colonies on BHI agar plate, and non-haemolytic colonies on sheep BA plate. The bipolar organisms were also confirmed from the colonies with help of Gram's and methylene blue staining. Final confirmation was done by PCR based detection, revealing a total of 21 isolates were positive for the *kmt1* gene of *Pasteurella species* isolate.

Key words: Histopathology, Isolation, *Pasteurella multocida*, PCR, Pneumonia.

INTRODUCTION

Goat plays a valuable role in our economy because of their significant contribution to meat and milk production. The great Indian leader Mahatma Gandhi designated goats as "poor man's cow," emphasizing the importance of small ruminants in developing countries. According to 20th Livestock Census, the total goat population in India is about 148.88 million and goat population in Assam is about 4.31 million (Anonymous, 2019).

Respiratory diseases of small ruminants are composite (Lacasta *et al.,* 2008)and there are numerous etiological agents responsible for causing respiratory disease complex. Respiratory diseases represent 5.6 per cent of all these diseases in small ruminants (Hindson *et al.,* 2002). Pneumonia is the most common respiratory illness in goats throughout the world (Ackermann and Brodgen, 2000). Although pneumonia more frequently occurs in kids, it also infects adult goats. Among the infectious agents, *Pasteurella multocida* and *Mannheinmia haemolytica* are more frequently associated with acute pneumonia and death in all age groups of goats (Falade, 2002). Poor managemental condition, transportation stress, over crowding pens, sudden environmental changes, delayed marketing, poor housing conditions, viral infection, lung parasites and other stressful conditions increase goats' susceptibility to pneumonia. Pneumonia caused by *Pasteurella multocida* and *Mannheinmia haemolytica* can lead to widespread financial losses because of death, reduced live weight, treatment cost and un-thriftiness among survivors (Davies *et al.,* 1997; Daniel *et al.,* 2006). Though the pneumonia is a widespread problem, there is not much published documents of pasteurellosis pneumonia from Assam available in public domain. So, in this present work the pathomorphological study of pneumonia due to Pasteurella and its species isolation and identification from lesions was attempted.

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MATERIALS AND METHODS

This study comprised of 62 carcasses of goat that had died of respiratory distress. A total of 139 goat carcasses were admitted to the Department of Veterinary Pathology at the College of Veterinary Science in Khanapara, Guwahati, Assam, India, between February 2020 and October 2021 for postmortem examination.

A detailed post mortem examination of the carcasses of clinically affected cases was conducted for the submitted carcasses. The carcasses were thoroughly examined for the presence of any external lesions. Then all the visceral organs were examinedand the visible gross lesions were recorded systematically. Representative tissue samples of 4-7 mm thickness were collected from the lungs showing pneumonic lesions for histopathological examination. The samples were collected from lungs for bacterial isolation and polymerase chain reaction.

For histopathological examination, the formalin-fixed samples were processed by the standard procedure adopted by Luna (1968) and sectioned at 4-5-micron thickness using a rotary microtome (Thermo Standard TM Finesse TM 325 manual microtome). Processing was done by routine alcohol-xylene dehydration and clearing. The paraffin embedding was done by low melting paraffin wax (56°C to 60°C). The tissue sections were then stained with routine haematoxylin and eosin stain, mounted with DPX. Whenever necessary, duplicate sections were stained with special stain Brown and Brenn for demonstration of the bacterial organism in the tissue following the standard protocol (Culling, 1974).

For bacterial examination sheep blood agar (5%) and Brain heart infusion Agar (BHI) media were used for primary isolation, purification and preliminary characterization. The plates were incubated at 37°C for 18-24 hours aerobicallyand then examined for bacterial growth. The detected colonies were checked according to (Cruickshank *et al.,* 1975), (Edward and Ewing, 1972) for identification of morphology and colony characteristics. In addition, suspected colonies were stained by Gram's and methylene stain to detect bipolarity (Markey *et al.,* 2013).

All the presumptive *Pasteurella* isolates were tested for haemolysis on Sheep Blood Agar. Firstly, the isolates were streaked onto Blood Agar plates and then the inoculated plates were incubated at 37°C in an incubator for 24 hours and examined for haemolytic zones around the colonies.

The PCR was performed in a thermocycler (Applied Biosystems). Template DNA was prepared from each isolate of *Pasteurella species* for their molecular characterization in terms of detection of *kmt1* gene by simplex PCR. The used forward primers had the sequence of (KMT1T7) 5′-ATCCGCTATTTACCCAGTGG-3′ while the sequence of the reverse one is (KMT1SP6) 5′-GCTGTAAACGAACTCGCCAC-3′ (Townsend *et al.,*1998)**.** The reaction mixture (PCR mastermix, template DNA, forward primer, reverse primer and nuclease free water) which make the final reaction volume to 25 ul was added to a PCR tube and the tube was placed in a thermal cycler for amplification of the *kmt1* gene. The resulting mixture was exposed to a particular thermal profile as follows: an initial denaturation cycle at 94°C for 5 minutes; followed by 35 cycles of denaturation at 95°C for 1 minute, Primary annealing at 55°C for 1 minute and extension at 72°C for 30 seconds; followed by one final extension cycle of 72°C for 10 minutes. The amplified products were confirmed by agarose gel electrophoresis, using 1.5% agarose containing ethidium bromide in 1X Tris-Acetic acid- EDTA (TAE) buffer (40 mM Tris-HCl, 1 mM EDTA and 0.1 per cent glacial acetic acid with pH 8). Electrophoresis was carried out at 80-100 V for 1 hour. The gel was visualized under UV light in Gel Doc System (BioRad, USA)and images were captured by Image lab software. Positive reactions were determined by detection of *kmt1* gene of size (460 bp) in length.

RESULTS AND DISCUSSION

In the present endeavour, a total of 139 goat carcasses were investigated in and around Guwahati between February 2020 and October 2021. Among them, 62 goats showed pneumonia positive gross lesions during post-mortem examination. Out of 62 carcasses, *Pasteurella multocida* was isolated from 21 samples (33.8%) of pneumonia cases. The major clinical signs showed were respiratory distress, dullness, depression, anorexia, emaciation, high rise of body temperature, lacrimation, dyspnoea, coughing and sneezing. In many cases, lethargy, salivationand open-mouth breathing were also prominent. Along with serous, purulent and mucopurulent exudates in the nasal cavity.

Table 1: Types of pneumonia in our study.

Types of pneumonia	Per cent positivity
Bronchopneumonia (5)	23.8
Interstitial pneumonia (3)	14.28
Sero-fibrinous pneumonia (9)	42.85
Haemorrhagic pneumonia (4)	19.04

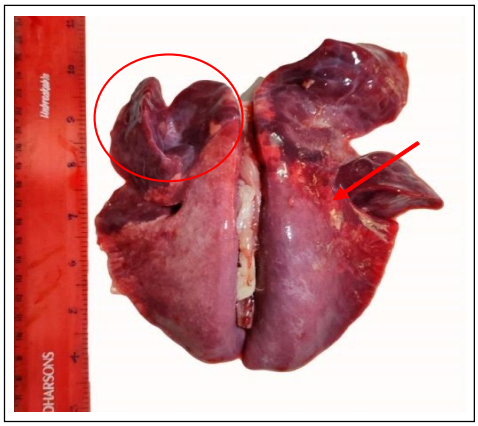


Fig 1: Photograph showing congestion (arrow) and consolidation in the apical and cardiac lobes of lung (circle).

In the gross examination the pneumonic cases were categorized into four groups *viz*. bronchopneumonia, interstitial pneumonia, sero-fibrinous pneumonia and haemorrhagic pneumonia based on the gross lesions found during post-mortem examination and further supported by microscopic examinations of the affected lung samples (Table 1).

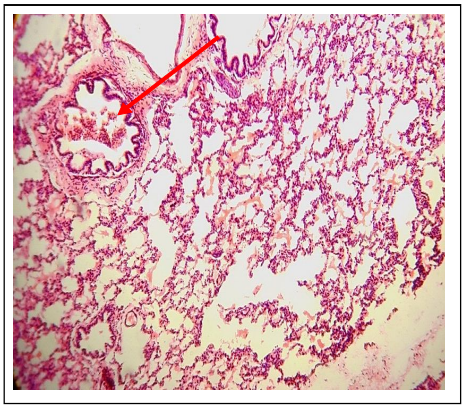


Fig 2: Photomicrograph showing mild denudation of bronchiolar epithelium (arrow) (H and E 10X).

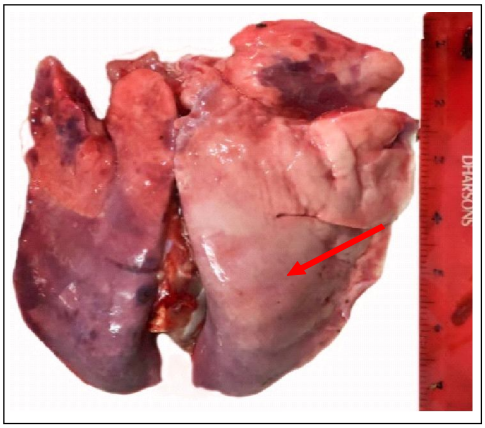


Fig 3: Photograph of lungs showing pale, swollen and rubbery in consistency along presence of rib imprints on the pleural surface (Arrow).

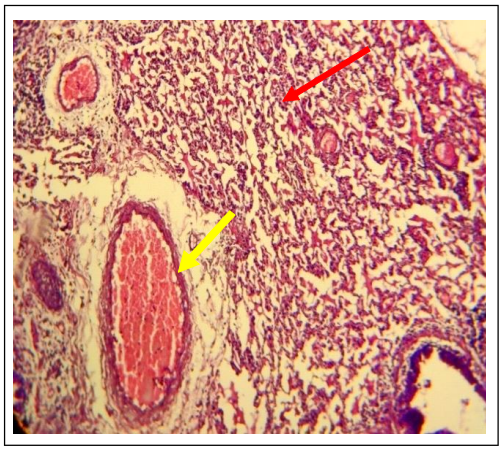


Fig 4: Photomicrograph showing interstitial pneumonia with leucocytic infiltration (Red arrow) and congestion of blood vessels (Yellow arrow) (H and E 10X).

Five samples (23.8%) showed evidence of bronchopneumonia depicting lesions of frothy and purulent exudates in the lumen of the trachea. The surface of the apical lobes of the lungs was dark, congested and consolidated (Fig 1). The microscopic lesions revealed accumulation of mononuclear cells in the alveolar lumen, interstitium and bronchiolar lumen along with mild denudation of the bronchial epithelium (Fig 2). Diffuse infiltration of inflammatory cells in the interlobular septa was also seen.

Three samples (14.28%) revealed interstitial pneumonia as the predominant cause of death with diffuse gross lesions in the affected lungs. The lungs appeared pale, swollen and rubbery in consistency (Fig 3). The lung parenchyma revealed the lesions of interstitial pneumonia characterized by the presence of thickened interalveolar septa with infiltration of mononuclear cells, engorgement of alveolar capillaries, along with congested blood vessels (Fig 4).

Nine samples (42.85%) showed serofibrinous pneumonia where the lung parenchyma revealed focal to diffuse areas of consolidations involving the right apical lobe in most cases either alone or with cardiac and anterolateral borders of the diaphragmatic lobes. In some cases, the affected lobes were covered by a layer of fibrinous membrane (Fig 5). Microscopically, the lung parenchyma showed presence of sero-fibrinous and sometimes sero-fibrinopurulent or only fibrinous pneumonia. There was presence of intra alveolar fibrin in the form of “fibrin balls” within the alveolar spaces (Fig 6). Mild to moderate number of polymorphs, mononuclear cells and occasionally a few macrophages were also seen.

Haemorrhagic pneumonia was evident in four samples (19.04%) where the lung parenchyma revealed the presence of haemorrhagic patches throughout the lobes (Fig 7). The cut surface was severely congested and the bronchi/bronchioles showed clotted blood or froth, either blood-tinged or greenish-white with flakes of pus. Microscopically, the lung parenchyma revealed diffused areas of haemorrhage with congested blood vessels characterized by diffuse infiltration of erythrocytes in the alveoli, interalveolar and interlobular septa (Fig 8).

Out of the total 62 bacterial isolates, *Pasteurella* species was isolated from 21 samples (33.8%). The organisms showed greyish lustrous isolated colonies on BHI agar plate and non-haemolytic colonies on sheep BA plate (Fig 9, 10). Colonies were confirmed by molecular detection of *Pasteurella multocida* specific *kmt1* gene (460 bp) by simplex PCR (Fig 13). In Gram’s as well as methylene blue staining, the presence of gram-negative bipolar organisms was observed (Fig 11, 12).

The goal of the current project was to thoroughly research the pneumonia caused by *Pasteurella species* in

goats. In this investigation 21 of the 62 carcasses examined died from pneumonia, tested positive for *Pasteurella* species. Based on the macroscopic lesions discovered during post-mortem inspection and microscopic abnormalities shown during histological analysis, four primary forms of pneumonia-bronchopneumonia,

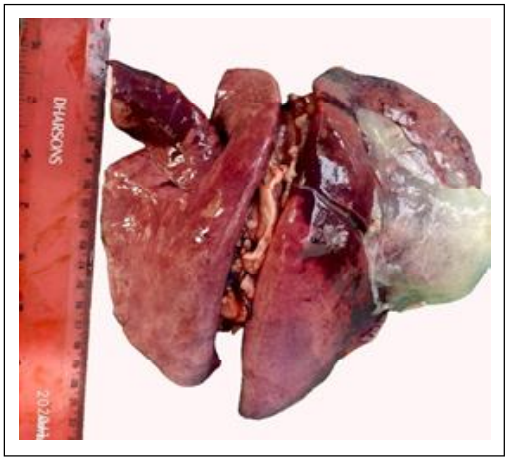


Fig 5: Photograph of lung showing presence of mass of fibrin in the apical and cardiac lobes with areas of haemorrhages.

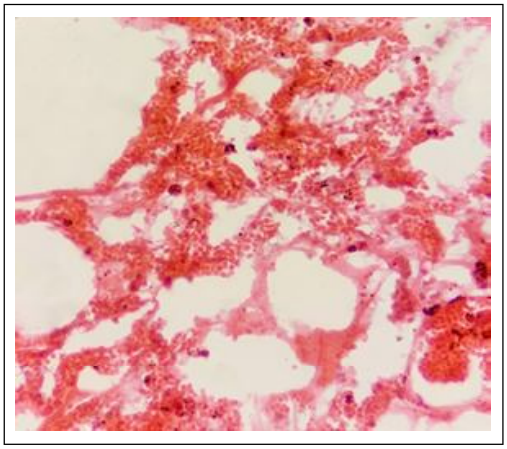


Fig 8: Photomicrograph showing presence of free RBC's inside alveoli and the interalveolar septa (H and E 40X).

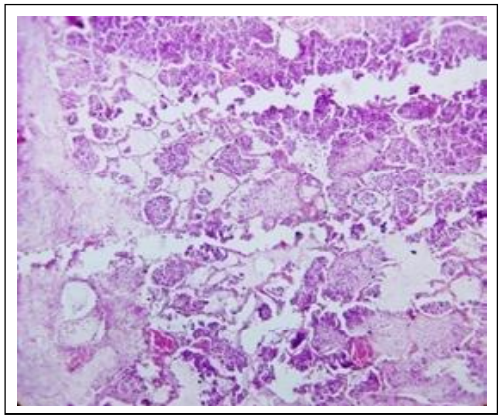


Fig 6: Photomicrograph of lung showing presence of fibrin inside alveolar spaces and areas of suppuration (H and E 10X).

Fig 9: *Pasteurella spp.* showing greyish lustrous isolated olonies on BHI agar plate.

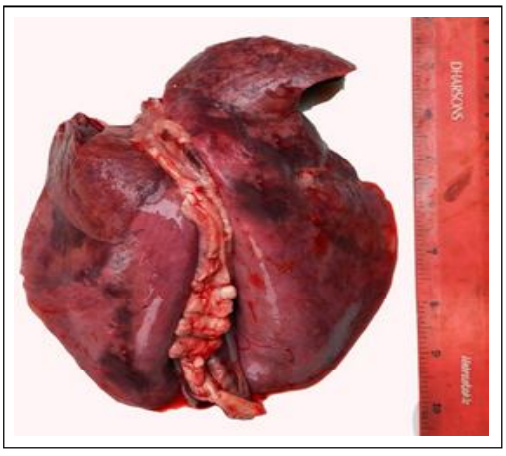


Fig 7: Photograph showing presence of haemorrhagic patches throughout the dorsal surface of the lung.

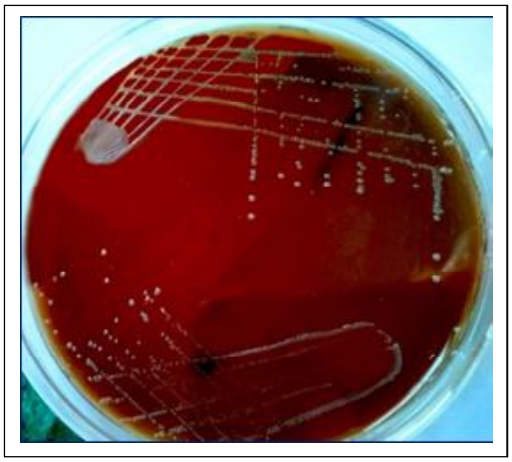


Fig 10: *Pasteurella spp.* showing non-haemolytic colonies on blood agar plate.

interstitial pneumonia, sero-fibrinous pneumonia and haemorrhagic pneumonia were identified which was also found by different workers working with *Pasteurella* species in different species (Doaust, 1989); (Zamri- saad *et al.,* 1996); (Amin, 2020)

Sharma *et al.* (1991) showed that the apical lobe of the lung was affected most frequently (85.71%), which was in congruence with the lesions noticed. In another study, Tijjani *et al.* (2012) and Filioussis *et al.* (2015) characterised bronchopneumonia by heavy and severe neutrophilic infiltrations into the bronchus and the alveoli. The proliferative types of lesions observed were in consonance with the reports of Sharp and Nettleton (2000); Pawaiya *et al.* (2004) and Abraham *et al.* (2005). (Ugochukwu *et al.,* 2017) also observed lungs with interstitial pneumonia being characterised by interalveolar space infiltrated mainly with lymphocytes, macrophagesand a few neutrophils. The proliferative changes suggest a chronic irritation of the air passages by environmental pollutants and allergens. Excessive amounts of serous or serofibrinous fluid were frequently observed in the pericardial, pleural and peritoneal cavities reported by Dungworth (1993). Similar changes observed in this study were also described by earlier workers (Dutta *et al.,* 2020); (Mohamed and Abdelsalam, 2008)and (Abdullah *et al.,* 2014). Nair (1982) observed a similar pattern of gross and microscopical features and opined these lesions as pathological changes caused by *Pasteurella* species in goats. Rashid *et al.* (2013) and Ferdausi *et al.* (2008) observed severe haemorrhage and congestion in the lungs along with excessive haemorrhages within the alveoli and interalveolar septa associated with leukocytic infiltration, which was in resemblance with our findings.

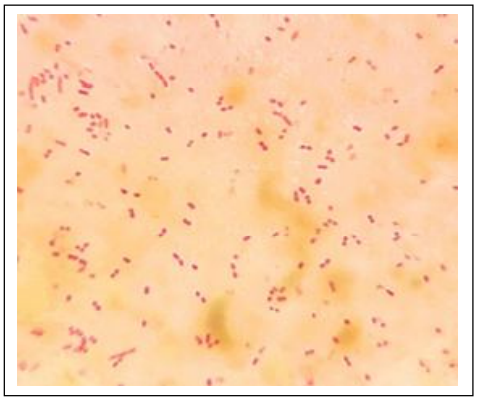


Fig 11: *Pasteurella spp.* showing bipolar staining in Gram's stained smear 100X.

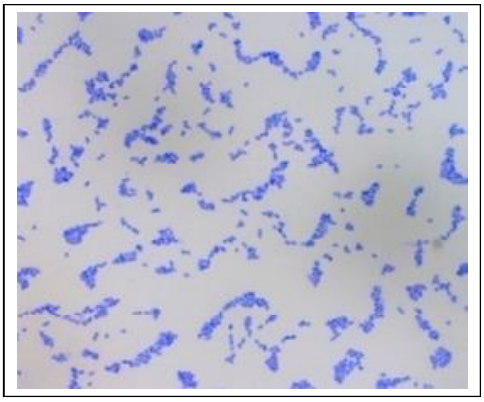
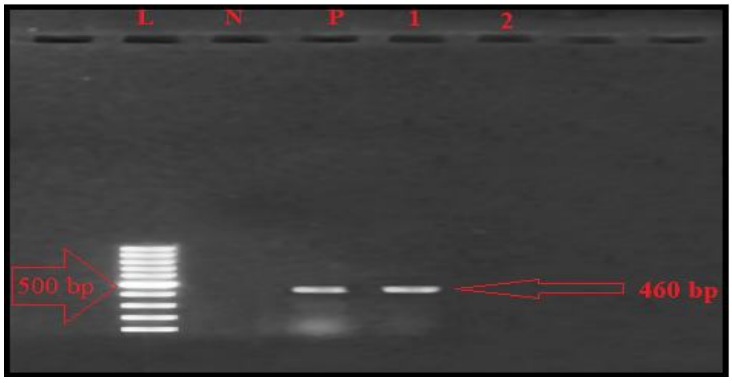


Fig 12: *Pasteurella spp.* showing bipolar staining in methylene blue staining smear 100X.

Bronchopneumonia and intestinal pneumonia are the feature of infection due to *Pasteurella multocida*. Also, haemorrhagic pneumonia and sero-fibrinous pneumonia is seen in pneumonia caused by *Pasteurella multocida*. Ferdausi *et al.* (2008) found a similar result in which *Pasteurella multocida* was found in haemorrhagic pneumonia and interstitial pneumonia. Upadhyaya (1981) described pneumonic pasteurellosis as the cause of sero-fibrinous pneumonia, which agrees with our findings.

Out of the total 62 pneumonic lung samples collected for microbiological examination to detect the aetiological agent. *Pasteurella multocida* was isolated from 21 samples (33.8%). (Hailu *et al.,* 2017), (Elsheikh and Hassan, 2012)



L= Ladder 100 bp, Lane N= Negative control, Lane P=Positive control, Lane 1= Test isolate, Lane 2= Negative isolate

Fig 13: PCR amplification of *kmt1* gene (460 bp) of *Pasteurella multocida* isolates.

and (Emikpe *et al.,* 2013) isolated *Pasteurella spp.* from similar cases. On blood agar plate, whitish, opaque circular non-haemolytic colonies were observed, confirming the involvement of *Pasteurella species* in pneumonia which showed a similar colony characteristic in the previous study by (Momin *et al.,* 2011) and (Rawat *et al.,* 2019).

Molecular methods are very useful and sensitive tools for detection of different bacteria in clinical samples. However, PCR has significantly influenced the identification and characterization of *P. multocida* (Hunt *et al.,* 2000). *Pasturella multocida* specific PCR assay is a suitable technique for specific detection of *Pasturella multocida* compared with traditional bacteriological methods (Hassan *et al.,* 2016). In the present study 21 Pasteurella suspected cultures were screened to detect virulence-associated Kmt1 genes and found to be positive for the virulence gene. The amplification of 460 bp fragment of Kmt1 gene of *Pasteurella* spp. was done using specific primer sets as described by (Townsend *et al.,* 1998). The present observation simulates the results obtained by (Prabhakar *et al.,* 2010) and (Balakrishnan *et al.,* 2012) where they identified *Pasturella multocida* isolated from sheep, by amplification of Kmt1gene using the primers KMT1SP6 and KMT1T7.

CONCLUSION

The results of the current study indicates that the pneumonia due to pasteurellosis is prevalent among the goat population of Assam. The clinical, gross and microscopic changes indicated various types of pneumonia *viz.* bronchopneumonia, interstitial pneumonia, sero-fibrinous pneumoniaand haemorrhagic pneumonia. The organism associated with pneumonia was established as *Pasteurella* species by bacteriological and molecular methods. There is need of further studies to understand the proper epidemiology of the disease condition. So, the effort can be centralized to establishing effective prevention and control measures after screening animals at farms, implementing good husbandry practices, segregating animals and eliminating infected animals

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Authors contribution

Deepjyoti Saharia, Abhijit Deka and Churchis Villee Phangcho envisage the idea of research and execute the research activities, Mousumi Namasudra and Naba Jyoti Deka helps in sampling and sample processing. Dhruba Jyoti Kalita provide the laboratory facilities and Sushanta Goswami helps in manuscript preparation.

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Conflict of interest

No potential conflict of interest was reported by the authors.

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