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Pathomorphological studies of respiratory mannheimiosis in goats

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Abstract

The study investigated an outbreak of respiratory mannheimiosis in goat maintained at the Goat Research station, AAU, Burnihat during September- October, 2019. Out of the total 215 nos. of animals at risk, 48 nos. were affected and 16 died with a morbidity and cause specific mortality of 22.32% and 33.3% respectively. The clinical signs exhibited by the affected animals were offed, high rise of temperature (104°-105°F), thick mucoid nasal discharge and coughing. During necropsy, pneumonia was diagnosed among all the cases and the most frequently affected lobe was right cranial lobe. In most of the cases lung showed fibrinous bronchopneumonia. Consolidated areas of the lungs were swollen and dark red in color. In some cases presence of whitish fine foamy fluid or creamy suppuration could also noticed.

Sometimes, the fibrin was adhered in the ribs with or without hydrothorax. The tracheal rings were hemorrhagic and the lumen was filled with the mucus and froth. Microscopically, three different types of pneumonia viz. Fibrinous, suppurative and fibrinopurulent could be seen. Alveolar lumen was filled with fibrinous exudate, erythrocytes, and polymorphonuclear cells. During isolation, the results observed were typical to those exhibited by *Mannhemia* spp.

Keywords: Assam, goat, *Mannhemia* spp, *Pasteurella* spp., pathology, pneumonia

Introduction

Pneumonic pasteurellosis is also known as respiratory mannheimiosis, is most common among the respiratory tract infections with a wide prevalence in ruminants. Small ruminants such as goat and sheep are fairly susceptible and contract the disease due to exposure to physical stress or unfavourable environmental conditions [1]. Acute infection in small ruminant frequently occurs during transportation and the term shipping fever was adopted. The disease is highly infectious, fatal and results in huge economic losses in small ruminant farming. *Mannhemia haemolytica* is categorised as an opportunistic pathogen that particularly inhabits the nasopharynx and tonsils of goat and sheep and has the ability to produce disease when the immunity of the body is compromised [2]. The severity of the disease had greatly led to the reduction of one of the most lethal forms of pneumonia with bronchopneumonic and lobar pattern. *Mannhemia haemolytica* substantiates infection in the lungs by disrupting the innate mucosal defence mechanism which is the main cause of acute pulmonary infection [3]. *Pasteurella multocida*, an opportunistic pathogen, is analogous to *M. haemolytica* [4] and is one of the most important respiratory pathogens of domestic ruminants in which it causes serious outbreaks of acute pneumonia [5]. This syndrome is caused by a complex interaction between the environmental stress, microorganisms, and immunity of the host. The acute events tend to form haemorrhagic and fibrino-necrotic bronchopneumonia that causes fibrinous pleural adhesion and abscess [6].

In Assam, a North Eastern part of India, livestock resources is highly livelihood oriented. Goat Farming is predominantly practiced across the state as a source of livelihood. In such situation outbreak of respiratory mannheimiosis in goat causes severe economic losses. The present communication was aimed to study the pathomorphology and isolation of *Mannhemia* spp. from a natural outbreak.

Materials and Methods**Ethical approval**

The approval from the Institutional Animal Ethics Committee (IAEC) was not required for the present study since the samples were collected from the animals without animal experimentation and dead animals during necropsy.

Study area

The study was conducted in Burnihat area of Assam, which is located at 26°6'N, 91°34'E, 72 m above the sea level. Climatically, the region is humid with hot subtropical summer and receives an annual rainfall of about 1722 mm. The temperature is highest in June to August. During summer, the maximum temperature is about 31.9 °C and minimum temperature is about 25.2 °C. Relative humidity is lowest during March (53%) and highest in July (83%). The meteorological data were collected from the Regional meteorological Centre, Barjhar, Guwahati.

Sample collection

The materials were collected from the Goat Research station, AAU, Burnihat, during September- October, 2019 during post- mortem examination at the Department of Veterinary Pathology, CVSc, AAU, Khanapara, Ghy-22. Clinical signs exhibited by the affected animals were obtained from the history provided by the Farm officials.

Gross and Histopathological examination

The carcasses were examined systematically following standard protocol. The physical conditions of the animals and gross alterations in different organs were recorded. For histopathological examination, representative tissue samples were collected in 10% formol saline solution. After proper fixation, paraffin embedded tissue sections of 4-6µ were prepared and stained by routine H & E Technique [7].

Bacteriological examination

In brief the lung specimens were separately inoculated onto Blood agar containing 5% sheep blood and Mac Conkey's agar. The agar plates were incubated at 37 °C, in aerobic conditions for 24-48 hr. Identification of bacterial species was done on the basis of colony characteristics, morphology and biochemical properties. The suspected colonies were stained by Gram's and Leishman's staining method. For further confirmation, different biochemical tests were performed *viz.* indole, catalase, urease and sugar fermentation tests.

Results and Discussion

Occurrence

During September- October, 2019, out of the total 215 nos. of animals at risk, 48 nos. were affected and 16 died with a morbidity and case fatality rate of 22.32% and 33.3% respectively. Alemneh and Tewodros (2016) reported that overall prevalence of small ruminant pasteurellosis was 32.6% in which 37.1% and 21.9% were recorded in sheep and goats respectively [8]. The finding coincides with Yeshwas *et al.* (2013), who reported 33.1% prevalence [9]. The occurrence of pneumonia might have been predisposing due to environmental or nutritional stress.

Clinical sign

The clinical signs exhibited by the most of the affected animals (n=14) were off fed, rise of temperature (104°-105°F), passing of thick mucoid nasal discharges (Fig. 1), coughing and irregular breathing. Two animals died without manifestation of any clinical sign. The clinical signs observed in the present study were almost similar to those reported earlier [10]. The manifestation of acute respiratory distress usually appears within 10-14 days in adult ruminants after exposed to stressful conditions. Occurrence is observed more young animals than adults and they develop a more severe

illness in which sudden death may occur with or without any previous sign [11].

Gross Pathology

Determination of localization of consolidated areas in the goat lungs, the each lobe has been labelled as A, B, C, D, E & F (Fig. 2). The localization of consolidated area in the lung has been shown in Table 1.



Fig 1: Affected animal passing thick mucoid nasal discharges

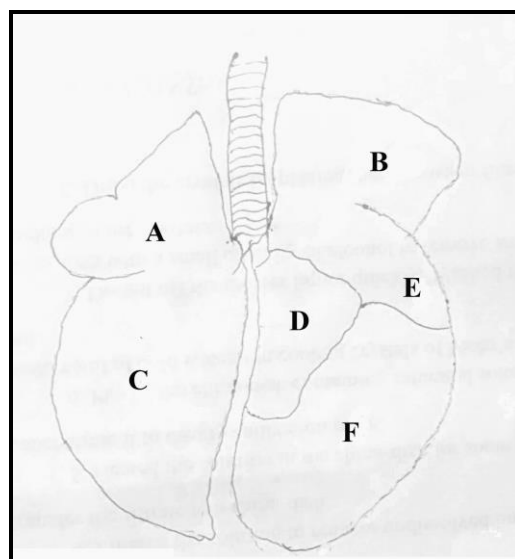


Fig 2: Schematic diagram of lung (Ventral view)

Table 1: Showing localization of lung lesion in different lobes

Location	Number (n)
A	2
B	5
A+B	2
A+C	2
A+B+C	2
A+B+C+E+F	2
A+B+C+D+E+F	1

At necropsy, it was found that most frequently affected lobe was right cranial lobe (B). Other affected lobes were the left cranial lobe (A)/ right and left cranial lobe (A+B)/ left cranial and caudal lobe (A+C)/ both two cranial lobe with left caudal

lobe (A+B+C)/ both right and left cranial and caudal lobe and right middle lobe (A+B+C+E+F)/ all the lobes (A+B+C+D+E+F) respectively. Hazioglu (1994) reported that *Pasteurella pneumonia* is usually indicated lobar distribution, and often fibrinous, purulent and necrotic lung infection [12]. But in the present study, both lobar and lobular pattern of pneumonia could be seen. The consolidated surface was present at the contact surfaces of lobes close to each other like right cranial and medial lobe (B+E), often left cranial and caudal lobe (A+C). This might be due to spread of infection

by endobronchial way or spread by contact to the adjacent lobe. The animal wise distribute of different types of lesions has been presented in Table 2. In most of the cases (n=6) lung showed fibrinous bronchopneumonia that was characterised by focal to diffuse areas of consolidation with presence of fibrinous exudates in varying degrees (Fig. 3a). Consolidated areas of the lungs were swollen and dark red in color (Fig. 3b). Affected lung tissues were mostly palpated as liver and crusty in consistency. In some cases presence whitish fine foamy fluid or creamy suppuration could be noticed (Fig. 3c).

Table 2: Showing animal wise distribution of different types of gross lesions.

Lesions	Goat number															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Presence of fibrin	++	-	++	-	++	-	-	++	-	-	++	++	-	-	-	-
Presence of pus	-	+++	-	++	-	-	-	-	++	-	-	-	++	-	-	+
Presence of fibrin & Pus	-	-	-	-	-	++	+	-	-	++	-	-	-	+	+	-
Marbling of lungs	+++	-	+	-	+	-	-	-	++	+	+	+	-	-	-	-
Consolidation	+++	+	+	+++	+	+++	+	+++	++	++	+	++	+	++	+	+
Presence of straw coloured fluid	-	-	++	-	-	-	-	+	-	-	+	-	++	-	-	-
Presence of froth in the trachea	-	++	-	-	+	-	-	-	-	++	-	-	+	-	+	+
Petechiaal haemorrhages in the heart	+	+	+	-	-	+	-	+	-	-	+	-	-	+	-	-

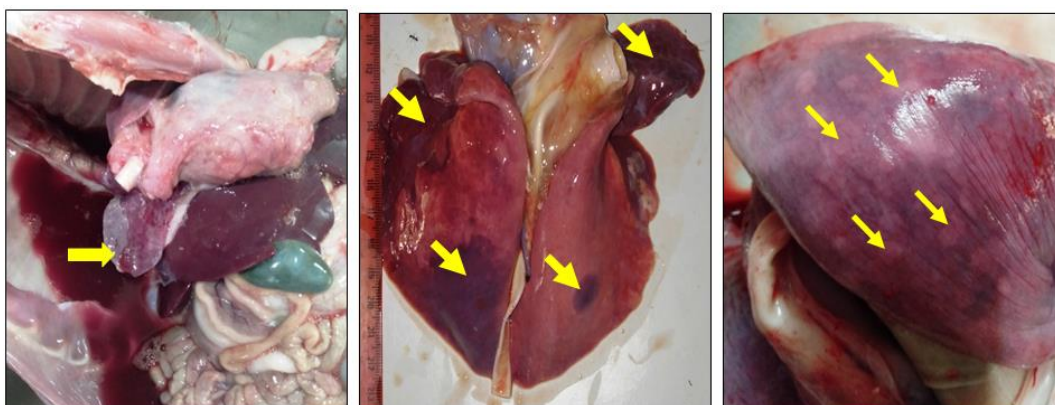


Fig 3: Lung showing (a) accumulation of fibrin in the apical lobe (arrow), (b) consolidation (arrow), (c) whitish fine foamy fluid (arrow)

Marbling of the lobules due to intermixing areas of atelectasis, necrosis and congestion could also be seen (Fig 4a). Sometimes the lung was tightly adhered to the pleura with thick layer of fibrin. In six (6) cases, accumulation of varying degree of froth throughout the trachea could be seen (Fig 4b). Apart from the lesions in the lung, in some cases (n=5), accumulation of straw coloured fluid in the thoracic

cavity could be seen (Fig. 4c). Heart (n=7) showed the presence of petechial haemorrhages in the epicardium. Endotoxins produced by the proliferation of the microbe in the diseased lobules of the lungs cause widespread intravascular thrombosis of pulmonary veins, capillaries, lymphatic and vascular turbulences which lead to ischemic necrosis [13].



Fig 4: (a) Lung showing intermixing areas of atelectasia, necrosis and congestion (arrow), (b) Trachea showing accumulation of froth (arrow), (c) accumulation of straw coloured fluid in the thoracic (arrow).

Similar findings were observed, where the left and right cranio-lateral lobes of lungs were consolidated with the presence of frothy exudates in the trachea, bronchi and cut

surface of the lungs in an adult goat infected with pasteurellosis [5].

Microscopic Pathology

Microscopically, three different types of pneumonia viz. Fibrinous (n=6), suppurative (n=5), and fibrinopurulent could be seen (n=5). Fibrinous pneumonia was characterised by the presence of intra alveolar fibrin in the form of “fibrin balls” within the alveolar spaces (Fig. 5a). Apart from this, interalveolar fibrin accumulation was also predominantly seen. In few cases within the vicinity of the intralveolar fibrin accumulation, an interstitial lymphocytic infiltration was present. Few neutrophils could also be seen but were generally sparse. Like the lymphocytes, the neutrophils were located in the alveolar wall. Type II pneumocytic hyperplasia is prominent. Organizing pneumonia characterised by progressing of fibrinous exudates to fibroblast is also noticed. The traditional ‘oat cells’ were compacted and necrotic

macrophages were inside the damaged alveoli. Lobules undergoing coagulative necrosis emerge as infarcts with thick neutrophilic infiltrates around the margin of the necrotic lobule. Vasculitis with fibrin thrombi was also noticed. Sloughing of the bronchiolar epithelium with the accumulation in the lumen with or without accumulation of inflammatory cells could also be seen (Fig. 5b). Bronchioles were dispensed with fibrin-rich exudates that may spread from alveoli.

Suppurative pneumonia was characterised by multiple focal areas of necrosis in the lung parenchyma. The area were characterised by central caseonecrotic mass surrounded by pyogenic membrane with infiltration of polymorpho nuclear cells, mononuclear cells, lymphocytes, plasma cells and macrophages (Fig. 6a),

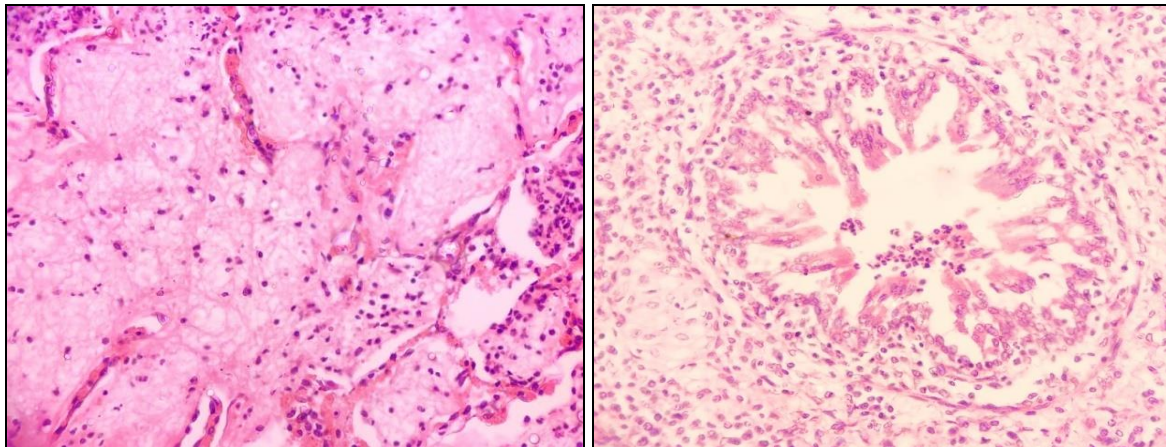


Fig 5: Photomicrograph of lung showing (a) presence of intra alveolar fibrin in the form of “fibrin balls” within the alveolar spaces, (b) Sloughing of the bronchiolar epithelium with accumulation inflammatory cells in the bronchiolar lumen.

Vesicular congestion and inflammatory exudates were seen in the alveoli of the affected part of the lungs. The purulent materials were also present in the bronchi and bronchioles with infiltration of neutrophils. Sometimes the caseonecrotic materials were admixed with the bacterial colonies surrounded by a thick connective tissue capsule and fibrovascular reaction.

Microscopically, fibrinopurulent pneumonia was characterised by suppurative and fibrinous pneumonia (Fig.

6b) with pronounced acute congestion and multifocal desquamation of type II pneumocyte and alveolar macrophages in the alveolar lumen. Adjacent to the bronchioles, there were focal infiltrations of large numbers of neutrophil and few lymphocytes. Sometimes fibrinous exudates were admixed with neutrophilic infiltration. Formation of syncytia with sloughing bronchiolar epithelium and accumulation in the lumen could be noticed (Fig. 7).

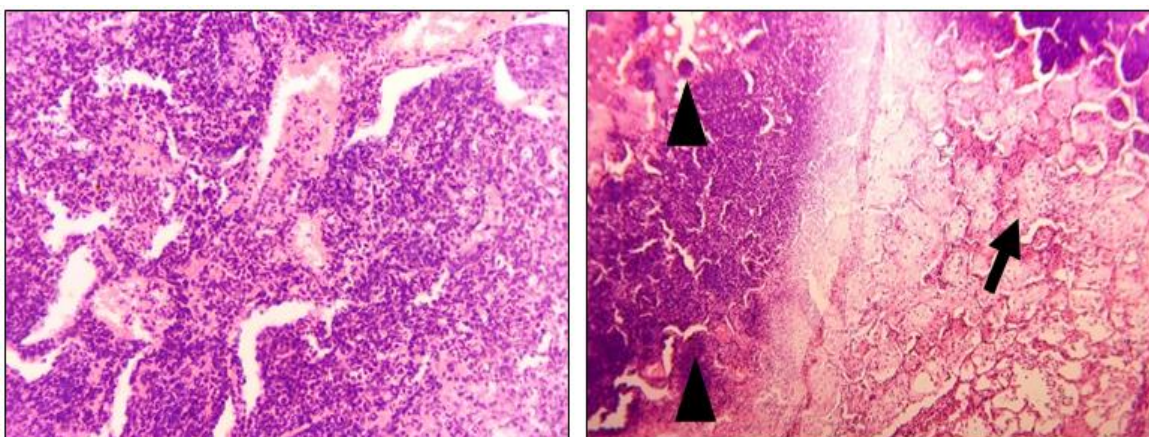


Fig 6: Photomicrograph of lung showing (a) infiltration of polymorpho nuclear cell, mononuclear cells, lymphocytes, plasma cells and macrophages, H&E, X20 (b) suppuration (arrow head) and accumulation of fibrin (arrow), H&E, X10.

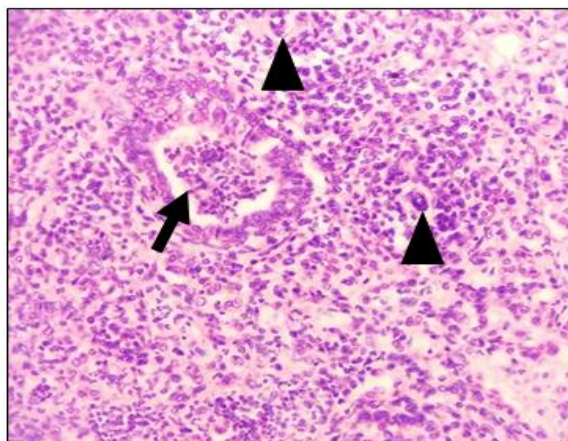


Fig 7: Photomicrograph of lung showing formation of syncytia with sloughing bronchiolar epithelium (arrow), H&E, X40

The histopathological findings reported in the present study corroborates with the earlier findings ^[14, 15]. During an infection, mannheimiosis causes increased infiltration of neutrophils, seroproteinaceous fluid, fibrin and blood into the bronchi, bronchioles, and alveoli within hours. The compensatory mechanism such as lipopolysaccharide, leukotoxin and inflammatory factors are released by neutrophils and other cells in the acute inflammation to combat infections, but these may cause extensive parenchymal necrosis ^[16]. Infection and inflammation of the respiratory tract in particularly the bronchial, bronchiolar and lung tissues were found to secrete high concentration of interleukin-1 β , tumour necrosis factor alpha and Interleukin-8 during post-infection ^[17].

Culture and Biochemical characteristics

Small, round, mucoid, hemolytic colonies were evident on Blood agar while mild growth was observed on MacConkey's agar 48 hrs. post inoculation. Staining revealed presence of Gram negative coccus bacilli with bipolar appearance. Further the isolates could ferment lactose, sucrose and maltose, and were found to be urease negative, indole negative and catalase positive. The results observed were typical to those exhibited by *Mannheimia* spp. Involvement of *Pasteurella multocida* and *Mannheimia haemolytica* in pneumonic goats has also been reported from Chhattisgarh ^[10] and Western Maharashtra ^[18]. Pneumonia in small ruminants is primarily caused by parainfluenza-3 virus and respiratory syncytial virus and mycoplasma infection and is predisposed by variable weather conditions ^[14]. Respiratory viral infections affect mucociliary clearance mechanisms in lungs for removing the pathogens that reach the lower respiratory tract and thus increase the susceptibility of sheep and goats to secondary bacterial infections ^[19]. The respiratory viral agents create a favorable environment in the lungs supporting the bacterial growth by interfering with the mucociliary clearance mechanism of the respiratory tract and by downregulating the phagocytosis by the pulmonary macrophages ^[20]. However, *M. haemolytica*, the most frequently isolated bacterial pathogen from the lungs, is considered as the main cause of the pneumonic pasteurellosis/respiratory mannheimiosis ^[21].

Conclusion

The occurrence of goat pneumonia might have been predisposed due to environmental and nutritional stress. Fibrinous, suppurative and fibrinopurulent pneumonia was

predominantly recorded. Isolation of *Mannheimia* spp. confirmed the occurrence of respiratory mannheimiosis in goats. Further study for other aetiology and molecular understanding of *Mannheimia* spp. is required to characterize the causative agent.

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