Pathology of Experimental Infection by *Pasteurella Multocida* Serotype A: 1 in Buffalo Calves

P. E. Praveena, S. Periasamy, A. A. Kumar and N. Singh

Vet Pathol published online 9 January 2014
DOI: 10.1177/0300985813516647

Article

Veterinary Pathology
1-4

DOI: 10.1177/0300985813516647
vet.sagepub.com

Pathology of Experimental Infection by *Pasteurella Multocida* Serotype A:1 in Buffalo Calves

P. E. Praveena[1], S. Periasamy[1], A. A. Kumar[2], and N. Singh[1]

Abstract

Pasteurella multocida serotype A:3 has been mostly implicated in pneumonic pasteurellosis in ruminants. In contrast, our previous studies have reported that both serotypes A:1 and A:3 were responsible for respiratory diseases in cattle and buffaloes. However, the pathology and pathogenesis of *P. multocida* serotype A:1 (*Pm* A:1) infection have not been studied in ruminants. In the present study, 12- to 15-week-old buffalo calves (*Bubalus bubalis*) infected by *Pm* A:1 had fibrinous and suppurative bronchopneumonia with focal areas of coagulation necrosis typical of pneumonic pasteurellosis. For the first time, this study reports the lung pathology and pathogenecity of *Pm* A:1 infection in calves.

Keywords

bronchopneumonia, calves, *Pasteurella multocida* serotype A:1, pathology, experimental infection, time course, *Bubalus bubalis*

Introduction

Pasteurella multocida is a Gram-negative commensal bacterium normally found in the upper respiratory tracts of mammals and birds. *P. multocida* has been recognized as one of the primary pathogens associated with bovine respiratory disease (BRD) complex, resulting in loss of productivity and high mortality.[3] Mostly, *P. multocida* serotype A:3 (*Pm* A:3) has been implicated in fatal pneumonia of cattle.[3] Interestingly, our previous studies have reported that *P. multocida* serotype A:1 (*Pm* A:1) was also responsible for respiratory disease in cattle and buffaloes.[6,7] In addition, it has been reported that both serotypes A:1 and A:3 were associated with fatal pneumonia and septicemia of cattle and buffaloes in India.[2] In pasteurellosis, studies on pathogenesis and vaccine trails are hampered by inconsistency in reproducing the experimental infection and poorly known virulence factors.[5] In cattle, lung lesions caused by *Pm* A:3 have been studied in detail.[3] However, pathology and pathogenicity of *Pm* A:1 infection has not been studied experimentally in ruminants. In the present study, we infected buffalo calves with *Pm* A:1 and studied the lung lesions. This is the first report describing the lung lesions and pathogenecity of *Pm* A:1 in buffalo calves.

Pm A:1 (P120-IVRI) strain isolated from a clinically infected cattle was used in this study. The capsular and somatic serotyping of the isolate was done at National Animal Disease Center, Ames (IA), USA.[7] The bacterial culture for inoculum preparation was described previously.[8] Buffalo calves (*Bubalus bubalis*) ($n = 15$), 12 to 15 weeks old, purchased from local farms, and tested negative for *P. multocida, Mannheimia haemolytica* and *Mycoplasma* species were used in this study. All the experimental procedures were approved by the Institute Animal Ethics Committee (IAEC). Twelve calves were infected individually with 50 ml bacterial inoculum containing 10^9 cfu by intratracheal route. Three calves were administered with sterile PBS to serve as uninfected control. Following infection, calves were observed daily for clinical signs, and scheduled necropsy was performed on 3 infected calves each at 2, 4, 6, and 12 days post infection (dpi). Control calves were sacrificed at 12 dpi. On necropsy, visceral organs were examined and gross lesions in the lungs were scored as follows: no lesions (score 0), $\sim 15\%$ of the surface lesion (score 5), $\sim 30\%$ of the surface lesion (score 10), and $>30\%$ surface lesions (score 20). Representative tissue samples from lungs and mediastinal lymph nodes (mLN) were fixed in 10% buffered formalin and processed for histology. The sections were routinely stained with hematoxylin and eosin (HE).[8] The histological lesions in the lungs were scored on the basis of severity of the cellular infiltration and type of the lesions as follows: no lesions (score 0), mild (score 1), moderate (score 2), and severe (score 3).

[1]Division of Veterinary Pathology, Indian Veterinary Research Institute (IVRI), Izatnagar, India
[2]Division of Bacteriology, Indian Veterinary Research Institute (IVRI), Izatnagar, India

Corresponding Author:
N. Singh, Division of Veterinary Pathology, Indian Veterinary Research Institute (IVRI), Izatnagar-243112 India.
Email: nemjdresearch@yahoo.co.in

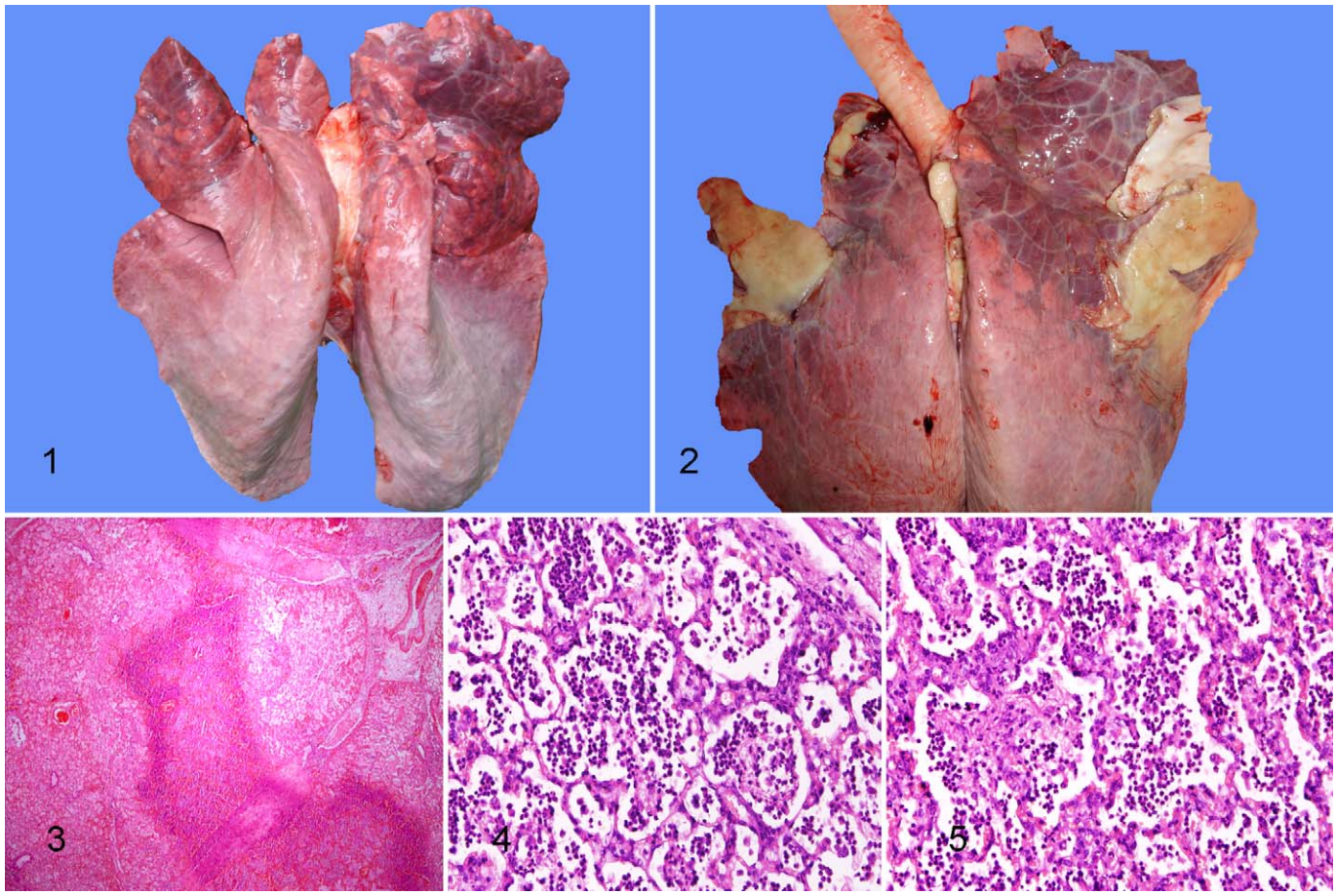

Figure 1. Lung; buffalo calf, 2 days post infection (dpi). Congestion and consolidation of the cranial lobes. **Figure 2.** Lung; buffalo calf, 6 days post infection (dpi). Consolidation, with sheets of fibrin on lung surface. **Figure 3.** Lung; buffalo calf, 4 days post infection (dpi). Necrotizing bronchopneumonia with multifocal and irregularly shaped areas of coagulation necrosis. HE. **Figure 4.** Lung; buffalo calf, 6 days post infection (dpi). Fibrinous bronchopneumonia consisted of fibrinous exudates and infiltration by macrophages and neutrophils in the alveoli. HE. **Figure 5.** Lung; buffalo calf, 6 days post infection (dpi). Intra-alveolar fibrin clumps and infiltration by macrophages and neutrophils. HE.

Pathological Observations

Buffalo calves infected by *Pm* A:1 were found to be dull and anorectic as early as 1 dpi and had a higher rectal temperature on 2 dpi onwards. On necropsy, infected calves had prominent pneumonic changes in lungs. The pneumonic changes were bilateral and cranioventrally distributed. The cranial and middle lobes were consistently affected in both sides of lungs. At times, the accessory lobes also had pneumonic changes. The affected lobes were severely congested, consolidated, and firm in consistency at 2 dpi (Fig. 1). The gross lesions were more severe at 4 and 6 dpi than at 2 dpi. In 2 calves examined at 6 dpi, bilateral fibrinous pleuritis with fibrin adhesions of the pleura were observed (Fig. 2). Calves examined at 12 dpi showed consolidation and nodularity due to purulent bronchitis and abscesses. The mLN were enlarged in infected calves and were prominently observed in the thoracic cavity. Control calves had no appreciable lesions in any organs. The summarized results of gross and histopathology are given in Table 1.

Microscopically, lung sections of 2 dpi calves had classical lesions of bronchopneumonia characterized by infiltrations with polymorphonuclear (PMN) cells and widespread edematous changes in the alveolar and bronchiolar lumen. Other lesions included scattered infiltrates of mononuclear cells hemorrhages into alveolar spaces, severe congestion of pulmonary blood vessels, and peri-bronchiolar and peri-vascular cellular infiltrates. The thickened alveolar septa had cellular infiltrates and edematous changes.

The 4 dpi group had lesions of severe bronchopneumonia with necrosis. Focal or multifocal, often coalescing, and irregularly shaped areas of coagulation necrosis were seen in the lung parenchyma (Fig. 3). The necrotic areas were surrounded by a zone of edema and infiltrates of PMN and mononuclear cells. Marked thickening of the alveolar septa and dilated lymphatics were observed. Lymphocyte infiltrates around bronchioles were present.

Calves examined at 6 dpi showed lesions of necrotizing- and fibrinous-bronchopneumonia. The histological lesions were characterized by the loosely packed cellular infiltrates of predominantly mononuclear cells with some PMN cells, interspersed with fibrinous exudates into the alveoli

Table 1. Pathological Observations in *P. multocida* Serotype A:1–Infected Buffalo Calves.

Animal Group	Gross Pathology	Histopathology[a]	Pathology Scores (mean)	
			Gross	Histology
Control	No lesions	No lesions	0	0
2 dpi	Congestion and consolidation	Bronchopneumonia (3)	5	2
4 dpi	Extensive consolidation and firm appearance	Bronchopneumonia (1), necrotizing bronchopneumonia (2)	15	3
6 dpi	Extensive consolidation, fibrin attachment, and pleuritis	Fibrino-necrotizing bronchopneumonia (3), pleuritis (2)	20	3
12 dpi	Small abscesses and lumpy consolidation	Suppurative bronchopneumonia (2), chronic bronchopneumonia (1)	15	3

Abbreviation: dpi, days post-infection.
[a]Number of calves showing the specified lesions

(Figs. 4, 5). Fibrin clumps streaming through pores of Kohn were observed in the alveolar septa. Cellular infiltrates were also seen in bronchioles and lymphatic vessels. Hyperplastic and well-developed BALT structures were seen in the lungs. Pleural thickening with fibrin and cellular infiltration were noticed.

At 12 dpi, 2 calves showed lesions of suppurative bronchopneumonia and 1 calf had the lesions of chronic bronchopneumonia. At places, bronchitis, bronchiolitis, and alveolar wall thickening or fibrosis were seen. The sections of mLN showed lesions of lymphadenitis consisting of PMN and mononuclear cells in the subcapsular and medullary sinuses.

Discussion

Observation of typical lesions of bronchopneumonia in the present study confirms that *Pm* A:1 establishes a primary lung infection in calves and *Pm* A:1 is pathogenic to ruminants. Although the bacterial number (10^9 cfu) and inoculum volume (50 ml) used in this study seem to be higher than is required in natural infection by *P. multocida*, a successful induction of reproducible and pathologically consistent experimental infection was achieved only at this larger inoculum as reported previously for *Pm* A:3.[4,5] This could be due to the lowered virulence of *P. multocida* when compared to *M. haemolytica.*[1] Further, lack of potential toxic components in *P. multocida* cell wall may inherently reduce the bacterial virulence in comparison to leukotoxin-producing *M. heamolytica.*[1,3] However, it is possible that a heightened bacterial interaction that takes place in mucosal surface due to a larger bacterial inoculum might break the mucosal barriers allowing better colonization and replication of bacteria. This enabled a rapid spread of infection locally within the pulmonary system and caused lung pathology.

Microscopically, lesions of bronchopneumonia or its variations (necrotizing-, fibrinous-, or suppurative-bronchopneumonia) were observed, suggesting that infected calves showed a varying susceptibility to *Pm* A:1. A predominantly PMN cellular infiltrates and edematous changes observed at 2 dpi were changed into mixed cellular infiltrates containing predominantly mononuclear cells and sero-fibrinous exudates at 6 dpi. It is notable that focal areas of coagulation necrosis were induced by experimental infection with Pm A:1.

Acknowledgements

Authors are thankful to Dr Abhijit Pawde, Principal Scientist, Department of Surgery, for helping in bacterial infection.

Authors' Note

Ezhil Praveena and Sivakumar Periasamy contributed equally.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship and/or publication of this article: This study was supported by All India Network Project on Hemorrhagic Septicemia funded from Indian Council of Agricultural Research (ICAR) to NS and AAK.

References

1. Ames R, Markham RJ, Opuda-Asibo J, et al. Pulmonary response to intratracheal challenge with *Pasteurella haemolytica* and *Pasteurella multocida*. *Can J Comp Med*. 1985;**49**:395–400.
2. Anon. Haemorrhagic septicaemia. In: *OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*. 2012; Chapter 2.4. 12, pp 1v13.
3. Dabo SM, Taylor JD, Confer AW. *Pasteurella multocida* and bovine respiratory disease. *Anim Health Res Rev*. 2007;**8**:129–150.
4. Dagleish MP, Finlayson J, Bayne C, et al. Characterization and time course of pulmonary lesions in calves after intratracheal infection with *Pasteurella multocida* A:3. *J Comp Pathol*. 2010;**142**: 157–169.
5. Dowling A, Hodgson JC, Schock A, et al. Experimental induction of pneumonic pasteurellosis in calves by intra tracheal infection with *Pasteurella multocida* biotype A:3. *Res Vet Sci*. 2002;**73**: 37v44.

6. Gautam R, Dutta TK, Kumar AA, et al. Molecular typing of Indian isolates of *Pasteurella multocida* serogroup-A from different animal species. *Indian J Anim Sci*. 2006;**76**:867–872.
7. Kumar AA, Shivachandra SB, Biswas AV, et al. Prevalent serotypes of *Pasteurella multocida* isolated from different animal and avian species in India. *Vet Res Commun*. 2004; **28**:657–667.
8. Praveena PE, Periasamy S, Kumar AA, et al. Cytokine profiles, apoptosis and pathology of experimental *Pasteurella multocida* serotype A:1 infection in mice. *Res Vet Sci*. 2010;**89**(3):332–339.