RESEARCH ARTICLE

# Molecular Characterization of *Pasteurella multocida* from Spontaneous Outbreaks in Cattle and Buffalo in Punjab

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## Abstract

Haemorrhagic septicemia (HS) is a peracute disease prevalent in India that is affecting cattle, buffalo and pigs. Microscopic and biochemical analysis of isolated bacterial colonies is essential for reliable *Pasteurella multocida* detection in the laboratory along with the pathological findings wherever possible. Present study involved a total of 247 animals from seven outbreaks, out of which 54 animals (21.86%) were affected, with high mortality rate, *i.e.* 48.14% (26/54) in four districts (Ludhiana, Patiala, Moga and Jalandhar) of Punjab. Heart blood, nasal and tracheal swabs collected and processed for bacterial isolation on the blood agar yielded grayish-white colonies of *P. multocida*, which were confirmed microscopically and biochemically in accordance with their established protocols. Molecular characterization was carried out using gene specific and type specific PCR. Histopathological studies on lungs, kidneys, liver and spleen collected from the animals died due to the disease revealed the lesions of HS.

**Key words:** Histopathology, Isolation, *Pasteurella multocida* Type B:2, PCR, Sequencing.

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## Introduction

The livestock sector supports the livelihood of over 200 million rural poor and thus alleviates poverty (Ahuja *et al*., 2008). Infectious diseases of livestock pose a barrier to animal welfare, increased food production and livelihood improvements. One of the key infectious diseases is 'Haemorrhagic Septicaemia (HS)' in cattle and buffalo which cause high mortality and economic losses. HS is a highly contagious, fatal, and septicaemic disease, caused by *Pasteurella multocida* strains especially serotype B:2 (Asian type) among five prevalent capsular serotypes (A, B, D, E, F) and sixteen somatic serotypes (1-16), which belong to the family '*Pasteurellaceae*' (Shivachandra *et al.,* 2011). Numerous animal species are affected by the Gram-negative *P. multocida*, a major opportunistic pathogen of HS in swine and ruminants (Boyce *et al*., 2010). The incubation period ranges from 1 to 3 days leading to sudden death without visible clinical signs. In chronic cases, the incubation period can extend up to 5 days or more. Initially, there is a high fever followed by respiratory distress (rapid and shallow breaths), septicemia, muco-purulent nasal discharge, restlessness, edematous swelling of the throat/brisket region, mild muscular tremors and recumbency leading to death (WOAH, 2023). Buffaloes are highly susceptible to the disease in comparison to other species (Jindal *et al.,* 2002) with high morbidity and mortality. This study was designed to confirm the conventional diagnosis of *P. multocida* by isolation, characterization and histopathlogical studies, including identification of frequent virulence variables to anticipate pathogenic behaviour, viable vaccination candidates and strain circulating in Punjab by molecular technique.

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## Materials and Methods

### History and Collection of Samples

Outbreaks were reported in eight different farms belonging to four districts, *i.e.,* Ludhiana, Moga, Patiala and Jalandhar districts of Punjab (India). Out of total of 247 cattle and buffaloes in these farms, 54 animals were affected and death was reported in 26 animals. A history of recumbency, respiratory distress, ocular and nasal discharge was reported. All the animals had history of vaccination against HS and

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FMD. Nasal swabs and blood samples were collected in nutrient broth from the affected live animals and brought to the laboratory on ice packs for further processing. In case of dead animals, post-mortem was conducted and relevant samples like lungs, spleen, heart blood and tracheal froth were collected both in sterile container for isolation and identification of *P. multocida.* In addition lungs, spleen, liver and heart were collected in 10% neutral buffered formalin and fixed for at least 12 h before histopathological processing.

### Isolation and Identification

Samples from nasal swabs and blood in nutrient broth of the live animals, heart blood and tracheal swabs of the dead animals were used for isolation of pathogen on blood agar. Bacterial isolation was carried out on the blood agar. The plates were incubated aerobically at 37°C for 24 h. The grayish-white color *Pasteurella multocid*a colonies were stained with Gram's staining and observed microscopically. Isolates were confirmed by biochemical tests including catalase, oxidase, indole, triple sugar iron agar, urease, fermentation of mannitol and sucrose (Quinn *et al*.., 1994).

### DNA Extraction and PCR Amplification

Genomic DNA isolation was carried out from the isolated colonies using snap chill method followed by polymerase chain reaction tests with specific primers for the determination of *P. multocida* serotype B KMT1SP6-KMT1T7 (Townsend *et al*.., 1998). PCR reaction was prepared in final volume of 25 µL reaction consisting of 12.5 µL of 2x Taq PCR Master mix (Qiagen), 1 µL each (10 pmol) of forward and reverse primer, 3 µL of DNA template and 7.5 µL of nuclease free water. PCR reaction was performed with initial denaturation at 94°C for 7 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 2 min. Finally, the amplification was ended by final extension at 72°C for 5 min. A volume of 5 µL of sample was electrophoresed on a 1.5 % agarose gel and visualized under UV transilluminator (Fig. 1, 2).

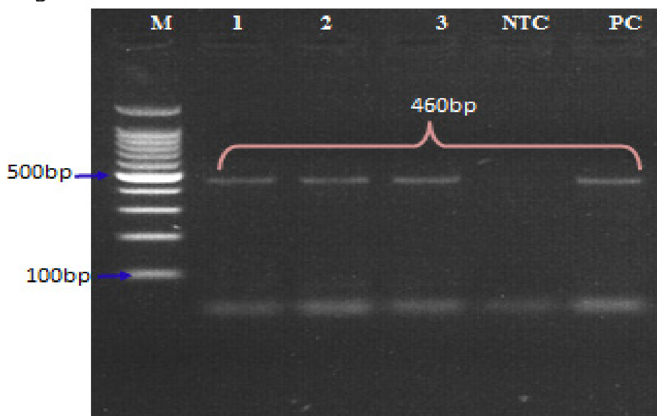


**Fig. 1:** Confirmation of isolate using specific primers KMT1SP6-KMT1T2 for *p. multocida* serotype B. Lane M: 100 bp DNA lader, Lane 1: HSB GAD-1, Lane 2: HSB GAD-2, Lane 3: HSB GAD-3, Lane 4: Negative templete control (NTC), Lane 5: positive control (PC).

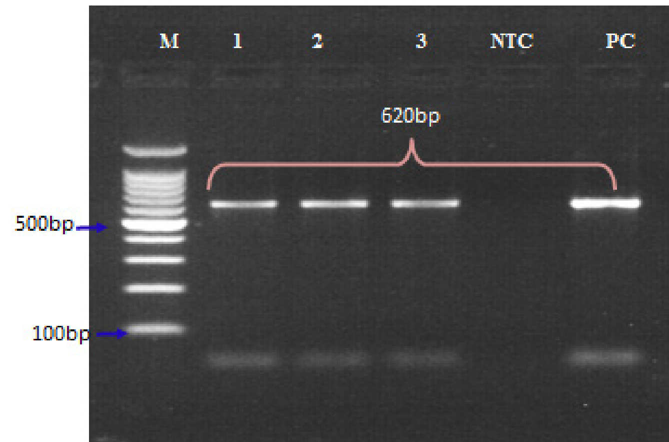


**Fig. 2:** Confirmation of isolate serotype using specific primers KTSP61 and KTT72 for the *p. multocida* serotype B. Lane M: 100 bp DNA lader, Lane 1: HSB GAD-1, Lane 2: HSB GAD-2, Lane 3: HSB GAD-3, Lane 4: Negative templete control (NTC), Lane 5: positive control (PC).

### Confirmation of Amplified Genome by Sanger's Sequencing

The specific bands of PCR amplified product on 1.5 % agarose gel electrophoresis were cut for gel extraction. The gel extraction purified product was used for commercialized Sanger sequencing using *Pasteurella mulocida* KTSP61 and KTT72 specific primers. Only *P. multocida* B: 2, B: 5, and B: 2, 5 produced amplification products.

### Histopathological Evaluation

The samples were processed for histopathological examination using the paraffin embedded technique, sectioned at 4-5 µm and stained with hematoxylin and eosin (Luna, 1968). The histological sections were examined at five to eight random fields under microscope and histopathological changes were recorded. (Fig3, 4)

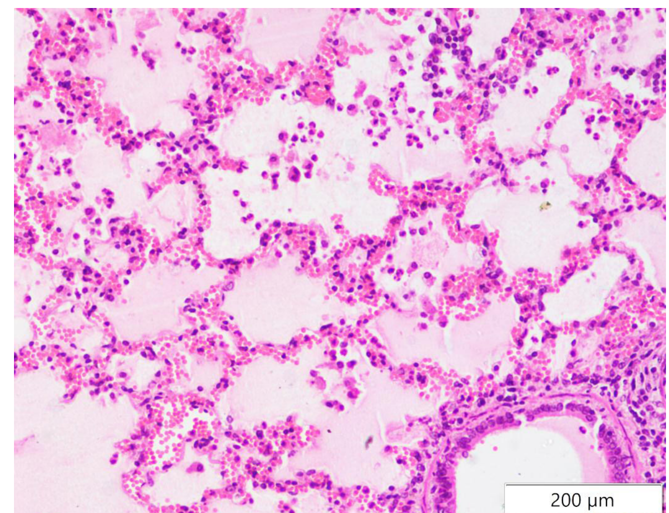


**Fig. 3:** Lung section reveals marked congestion of alveolar capillaries with multifocal hemorrhages. Alveolar lumen are filled with fibrin, seroproteinaceous fluid, and numerous infiltrating neutrophils indicative of acute inflammation (H&E).

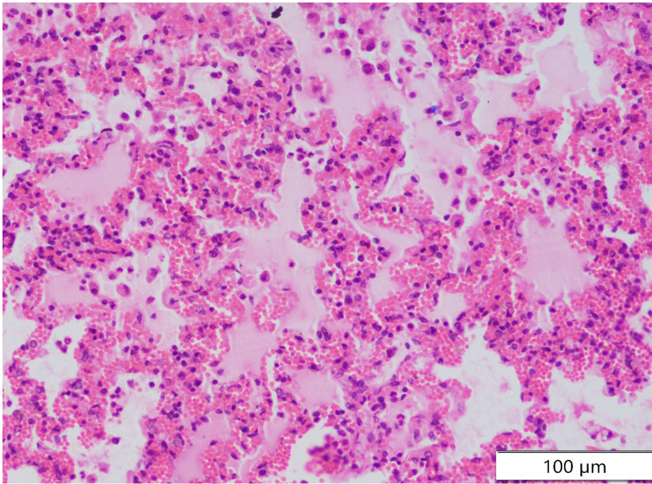


**Fig. 4:** High-power view of the lung shows extensive infiltration of alveolar spaces with neutrophils and seroproteinaceous fluid. (H&E)

## Results and Discussion

Pasteurellosis has been recognized as a disease of major economic importance. Confirmation of the disease by isolation and identification is considered as a gold standard. Three Pasteurella isolates were obtained in the present study. In case of outbreaks from Ludhiana and Moga districts *Pasteurella multocida* isolates having characteristic 'sweetish' odour and greyish-white non-haemolytic colonies were isolated on blood agar. No growth was observed in MacConkey lactose agar. The bacterial isolates were non-motile, catalase and oxidase positive. The isolates were indole positive and urease negative. As for growth on triple sugar iron agar, the slant and butt were yellow and no gas or $H_2S$ production was observed. Similar results were obtained by Cheesbrough (2006) and Rahman *et al.* (2016). Karimkhani *et al.* (2011) obtained six isolates of *P multocida* from cows and buffaloes in a slaughter-house which were confirmed by biochemical tests. Ara *et al.* (2016) isolated two isolates from suspected cases and primarily identified as *P. multocida* based on morphological study, staining properties, cultural and biochemical characteristics. PCR is a rapid and highly specific molecular technique for confirmatory detection of many species of bacteria including *P. multocida* (OIE, 2009).

In the present study, both the buffalo and cattle isolates of *P. multocida* exhibited species specific amplification of 460-bp size using primers KMT1SP6 and KMT1T7 (Fig. 1). These findings confirmed the results obtained by Townsend *et al.*. (2001), who reported the specificity of this primer pair for all *P. multocida* isolates. The serotype of *Pasteurella multocida* was carried out with specific product size 620 bp serotype B primers KTSP61 and KTT72 (Fig. 2). The raw sequences obtained from Eurofins Scientific were analysed using BLAST, DNAstar, and Mega X. All these three isolates (Accession number PP981520, PP981521, PP981522) were confirmed to have 99-100 % identity at nucleiotide level through NCBI BLAST. The serotyping and phylogenetic analysis of *Pasteurella multocida* were carried out using Maximum Likelihood method with Neighbor-Joining bootstraps value 1000 replicates. Pasteurella based on the Timura 3 parameter model using Mega X other reference sequences were obtained from NCBI Genbank database (Fig. 5). The analysis of sequences by comparing with references AJ421513.1 found insertion at position 151 of A and three point deletion at position C84, A112, and C167, respectively and these substitution leads to nonsynonymous

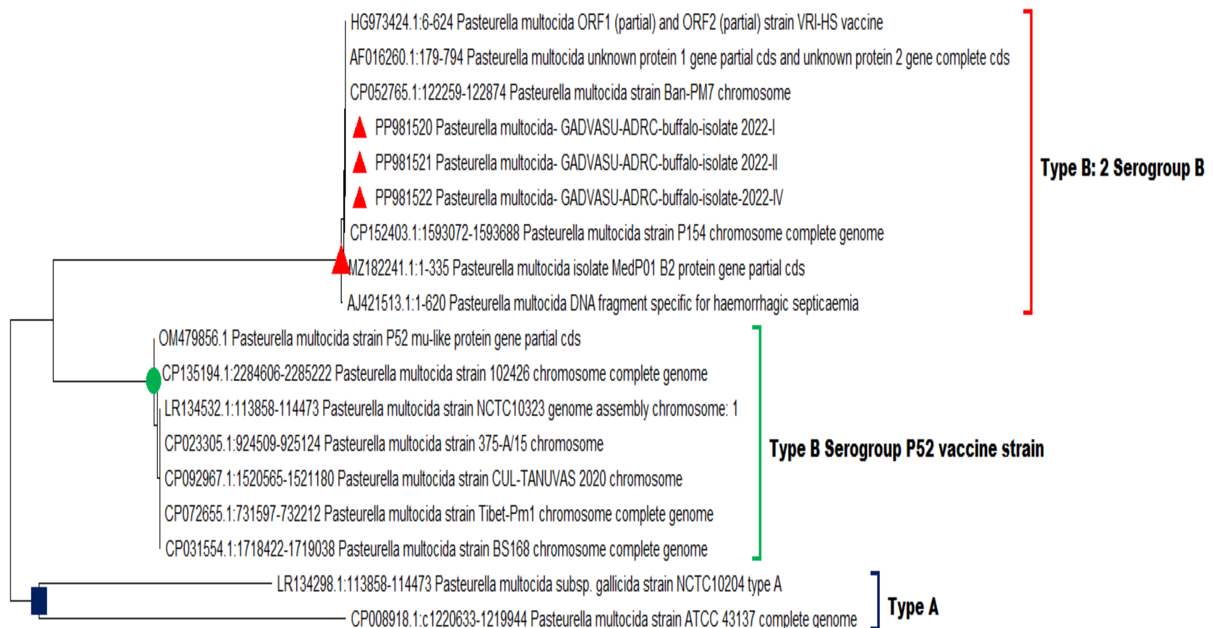


**Fig. 5:** Phylogenetic analysis of *P. multocida* using Maximum Likelihood method with Neighbor-Joining bootsteps value 1000 replicates Pasteurella based on the Timura 3 parameters model using Mega X.

changes. The clade of these isolates was closely related to CP152403.1 and AJ421513.1 of serotype 2 B subgroup B. Similar studies were carried out by Narcana *et al.* (2020), who reported 99% similarities of the isolates obtained with *P. multocida*.

Based on the post-mortem examination of the dead animals, significant pathological changes were observed. The spleen was markedly enlarged and haemorrhagic, indicating a severe systemic infection. The intestines displayed haemorrhagic enteritis, and there was froth present in the trachea, suggesting respiratory involvement. Consolidation of the apical lobe of the lung was noted, which is consistent with pneumonia. Histopathologically, the affected lung tissue showed alveolar edema, haemorrhage, infiltration of inflammatory cells, primarily neutrophils, consistent with bronchopneumonia. The kidneys showed tubular degeneration and mild interstitial nephritis, indicating renal involvement. The liver exhibited signs of chronic venous congestion. In the intestines, necrotic enteritis with lympho-mononuclear cell infiltration was observed, reflecting severe gastrointestinal infection. The spleen showed depletion of lymphoid tissue, a typical sign of immune response suppression. Heart revealed sarcocysts along with degeneration of muscle fibers. Similar findings have been reported by Annas *et al.* (2015) and Balena *et al*. (2016).

## Conclusion

In brief, combination of clinical signs, laboratory diagnostic tests such as bacterial culture and PCR, gross pathological examination and histopathological examination are essential for confirmation of the causative agent responsible for causing high mortality in cattle such as *Pasteurella multocida*.

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## References

Ahuja, V., Rajasekhar, M., & Raju, R. (2008). Animal health for poverty alleviation: A review of key issues for India. *Background Paper Prepared for "Livestock Sector Review"* of the World Bank.

Annas, S., Zamri-Saad, M., Jesse, F.F.A., & Zunita, Z. (2015). Comparative clinicopathological changes in buffalo and cattle following infection by *Pasteurella multocida* B:2. *Microbial Pathogenesis, 88,* 94-102.

Ara, M.S., Rahman, M.T., Akhtar, M., Rahman, M., Nazir, K.H., Ahmed, S., Hossen, M.L., Khan, M.F.R., & Rahman, M.B. (2016). Molecular detection of *Pasteurella multocida* type B causing haemorrhagic septicemia in cattle and buffaloes of Bangladesh. *Progressive Agriculture, 27*(2), 175-179.

Balena, V., Patel, S.K., Kumar, R., Rana, J., Agrawal, A., Singh, S., & Singh, R. (2016). Recent trends in spontaneous cases of hemorrhagic septicemic diseases of bovines. *Journal of Pure and Applied Microbiology*, *10*(4), 2913-2917.

Boyce, J.D., Harper, M., Wilkie, I.W., & Adler, B. (2010). Pasteurella. In: *Pathogenesis of Bacterial Infections in Animals.* 4th edn., Gyles, C.L., Prescott, J.F., Songer, J.G., Thoen, C.O., Eds.; Wiley-Blackwell: Hoboken, NJ, USA, pp. 325-346.

Cheesbrough, M. (2006). *District Laboratory Practice in Tropical Countries*. Part 2. 2nd edn., Cambridge University Press, New York. USA, pp. 45-62.

Jindal, N., Kumar, S., Narang, D., Chaturvedi, G.C., Tomer, P., & Garg, D.N. (2002). Some epidemiological observations on haemorrhagic septicaemia in buffaloes and cattle in Haryana state of India. *Buffalo Journal, 18*, 273-280.

Karimkhani, H., Salehi, Z., Zali T., S., Karimkhani, M.H., & Lameyi, R. (2011). Isolation of *Pasteurella multocida* from cows and buffaloes in Urmia's slaughter house. *Archives of Razi Institute*, *66*(1), 37-41.

Luna, L.G. (1968). *Manual of Histologic Staining Methods the Armed Forces Institute of Pathology*. 3rd edn., McGraw Hill Book Company, New York, USA.

Narcana, I.K., Suardana, I.W., & Besung, I.N.K (2020). Molecular characteristic of *Pasteurella multocida* isolates from Sumba Island at East Nusa Tenggara Province, Indonesia, *Veterinary World*, *13*(1), 104-109.

OIE (2009). *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*. World Organisation for Animal Health, Paris, France.

Quinn, P.J., Carter, M.E, Markey, B.K., & Carter, G.R. (1994). *Clinical Veterinary Microbiology*. Wolfe Publication, London, UK, p. 254-258.

Rahman, M.H., Shahiduzzaman, A.N.M., Haque, M.E., Nazir, K.H., & Rahman. (2016). Development of experimental oil based inactivated HS vaccine from field isolates of *Pasteurella multocida* from cattle in Bangladesh. *International Journal of Vaccines & Vaccination, 2*(2), 27.

Shivachandra, S.B., Viswas, K., & Kumar, A. A. (2011). A review of haemorrhagic septicemia in cattle and buffalo. *Animal Health Research Reviews, 12*(2), 67-82.

Townsend, K.M., Boyce, J.D., Chung, J.Y., Frost, A.J., & Adler, B. (2001). Genetic organization of *Pasteurella multocida* cap loci and development of a multiplex capsular PCR typing system. *Journal of Clinical Microbiology, 39*(3), 924-929.

Townsend, K.M., Front, A.J., Lee, C.W., Papadimitrious, J.M., & Dawkins, H.J.S. (1998). Development of PCR assays for species and type specific identification of *Pasteurella multocida* isolates. *Journal of Clinical Microbiology*, *36*(4), 1096-1100.

WOAH (2023). Haemorrhagic septicaemia (*Pasteurella multocida serotypes 6:B and 6:E)*. Chapter 3.4.10. In: *Terrestrial Manual of Diagnostics Tests and Vaccines for Terrestrial Animals.* 12th edn., Paris, France, p. 739-750.