Rapid typing of *Pasteurella multocida* isolates from haemorrhagic septicaemia cases by multiplex PCR for their virulence

S H Somshekhar1*, B M Veeregowda1, V V S Suryanarayana2, Awati Basawaraj3, S Isloor1 and D Rathnamma1

1Department of Veterinary Microbiology, Veterinary college, KVAFSU, Hebbal, Bangalore 560 024, India
2Indian Veterinary Research Institute, Hebbal, Bangalore 560 024, India
3Department of Veterinary Microbiology, Veterinary College, Bidar 585 401, India

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A total of 12 *P. multocida* isolates recovered from field outbreaks of haemorrhagic septicaemia in buffaloes were studied for the presence of 4 virulence genes (*pfhA*, *tbpA*, *hgbB* & *toxA*) in comparison with vaccine strain by multiplex PCR. The study showed the presence of *pfhA* and *tbpA* in all the field isolates and vaccine strain. None of the isolates harboured *toxA* and *hgbB*. The virulence genes *tbpA* and *pfhA* appear to be closely related to bovine *P. multocida* serogroup B isolates and are important for severe form of the disease.

Keywords: Multiplex PCR, *Pasteurella multocida*, virulence genotyping

*Pasteurella multocida* is the causative agent of economically important diseases worldwide like enzootic bronchopneumonia in cattle and sheep, and haemorrhagic septicemia (HS) in cattle and buffaloes. The latter being highly fatal. Acute septicaemic disease of cattle and buffaloes is caused by *P. multocida* serogroup B. Although the molecular basis of the pathogenicity and host specificity of *P. multocida* is not well understood, several studies have reported that a number of virulence factors (VFs) such as, adhesins, iron acquisition proteins and toxins, are associated with the pathogenesis. A PCR based novel method was suggested for epidemiological studies of *P. multocida* where genotyping of 14 virulence associated genes were carried out. The present study was focussed to investigate the distribution of virulence associated genes, viz., *pfhA* (putative pasturella filamentous haemagglutinin), *tbpA* (transferrin binding protein), *hgbB* (haemoglobin binding protein) and *toxA* (dermonecrotic toxin) by multiplex PCR in *P. multocida* strains from HS cases to find the epidemiological associations amongst them.

In the present study, a total of 12 isolates of *P. multocida* recovered earlier from HS cases and confirmed by multiplex PCR as serogroup B were used. Vaccine strain (P52), procured from Institute of Animal Health and Veterinary Biologicals, Bangalore, was used as reference standard strain.

DNA for multiplex PCR analysis was prepared from broth cultures using commercial kit (Qiagen, USA) following manufacturers’ guidelines. Virulence genotyping by multiplex PCR was carried out, using the primers and method described by Atashpaz et al

(1998) (Table 1). Reaction mixture (25 µL) containing 2 µL of template DNA, 3.2 mM each of four primer pairs, 200 µM from four dNTPs, 2.5 µL of 10× PCR buffer and 1 U of Taq-polymerase was prepared and subjected for PCR with initial denaturation of 94°C for 5 min, followed by 30 cycles of 94°C for 45 sec, 54°C for 50 sec, 72°C for 50 sec, and final extension at 72°C for 10 min. Each sample (5 µL) was electrophoresed on a 2% agarose gel in 1× Tris-acetate EDTA (TAE) running buffer at 4 V/cm for 1 h. The DNA in the gel was stained with ethidium bromide (0.5 µg/mL in water), and the bands were visualized under UV illumination in UV transilluminator and photographed.

Despite the economic importance of infections caused by *P. multocida*, the mechanisms by which the pathogen causes different diseases are poorly understood. Therefore, the studies of the present work are crucial for the identification of virulence factors associated with *P. multocida* of *P. multocida* strains from HS cases to find the epidemiological associations amongst them.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequence (5′-3′)</th>
<th>Product size</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>pfhA</em></td>
<td>F- AGC TGA TCA AGT GGT GAA C &lt;br&gt; R- TGG TAC ATT GGT GAA TGC TG</td>
<td>275 bp</td>
</tr>
<tr>
<td><em>hgbB</em></td>
<td>F- TCA TTG AGT ACG GCT TGA C &lt;br&gt; R- CTT ACA GTA ACA CTC G</td>
<td>499 bp</td>
</tr>
<tr>
<td><em>tbpA</em></td>
<td>F- TGG TTG GAA ACA AGT GTA AAG C &lt;br&gt; R- TAA CGT GTA CGG AAA AGC C</td>
<td>728 bp</td>
</tr>
<tr>
<td><em>toxA</em></td>
<td>F- TCT TAG ATG AGC GAC AAG G &lt;br&gt; R- GAA TGC CAC ACC TCT ATA G</td>
<td>846 bp</td>
</tr>
</tbody>
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*Author for correspondence:*
Mobile: +91-9902561957
somuhogtapur@gmail.com

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Table 1—Details of PCR primers and product size
understood. Very few studies have been aimed to detect/determine the frequency of virulence-gene patterns. Also, limited data on the origin of virulent strains of bovine are available. The present study involving multiplex PCR for virulence genotyping resulted in the amplification of 275 bp and 728 bp sized products for all the field isolates and vaccine strain, indicating the presence of pfhA and tbpA virulent genes, respectively. No amplification was observed for hgbB and toxA genes (Fig. 1).

The present results are in agreement with the study conducted by Prabhakar et al. who reported the presence of pfhA and tbpA in all the P. multocida capsular group B isolates of ovine origin. Shayegh et al. reported high prevalence of pfhA among cattle isolates. Ewers et al. also found all the P. multocida capsular group B isolates positive for pfhA and correlated the presence of the gene to the occurrence of pasteurellosis in cattle. Tang et al. working with swine strains reported that only 15% of P. multocida isolates tested positive to the pfhA (35/233). However, pfhA was not found in any strains of rabbits. Thus the prevalence of pfhA gene was high among capsular group B isolates as well as bovine isolates and varied among other hosts.

The tbpA is found closely associated with ruminant strains (cattle, sheep, and buffaloes) of P. multocida. The presence of tbpA in all the 4 isolates of the present study contradicts the findings of Ewers et al., who reported only 70% of capsular group B isolates positive for tbpA. None of the field isolates and vaccine strain in this study harbored hgbB. This further contradicts the observation of Ewers et al who found that 70% of capsular B strains of P. multocida from sheep harboured hgbB. Further, it has been reported that inactivation of hgbB does not affect the ability to bind haemoglobin, nor does it influence the bacterial virulence in a mouse model. The absence of hgbB may, therefore, be compensated by tbpA or any other systems of iron acquisition, a hypothesis which should be clarified by future transcriptional analysis.

None of the bovine P. multocida isolates of the present study revealed the presence of toxA gene, which is usually associated with atrophic rhinitis in swine caused by P. multocida type D. Some researchers opined that the toxA gene encoding the toxin is not inserted into the bacterial chromosome but in a lysogenic bacteriophage that infects the agent.

To conclude, the present study revealed the associations of the virulence factors (pfhA & tbpA) to a specific capsular type, as these factors are commonly distributed among the isolates of capsular type B. These virulence factors can be used as epidemiological markers for HS. However, further work is required to elucidate the mechanisms of pathogenesis and to determine unequivocally the role of these factors in immunity to HS.

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References