Detection of glutamate dehydrogenase gene (gdh) in Streptococcus suis isolated from pigs

S SONOWAL1, A G BARUA2, R A HAZARIKA3, S RAJKHOWA4, C C BARUA5 and D K BHATTACHARYA6

Assam Agricultural University, Khanapara, Asom 781 022 India

Received: 26 November 2013; Accepted: 30 November 2013

Key words: Glutamate dehyrdogenase, Polymerase chain reaction, Subclinical carrier, Streptococcus suis

Streptococcus suis infection, a zoonotic disease (Perch et al. 1968), causes arthritis, pneumonia, septicaemia, endocarditis, polyserositis, and abscesses in pigs with high economic losses. Subclinical carrier animals are important source as bacteria are transmitted to susceptible young pigs (Okwumabua et al. 2003). Detection of S. suis by standard culture methods and serotyping are laborious and results can be inconclusive or ambiguous. This study was conducted keeping in view the emergence of S. suis infections, to detect the presence of gdh gene from S. suis isolated from porcine origin by PCR.

Isolates (15) of Streptococcus suis were isolated from the 126 samples consisting of nasal swabs of apparently healthy (69) and different clinical samples, viz. heart blood, joint fluid and lungs of diseased (57) pigs from AICRP on Pig, Khanapara and NRC on pig, Rani, Guwahati. The non-S. suis isolates, consisting of several Gram-positive bacteria, including streptococcal species and Gram-negative bacteria, were obtained from different clinical samples in the laboratory (Table 1). Identification of all these isolates was confirmed by cultural and biochemical tests as per standard procedures (Facklam 1980, Rosendal et al. 1986, Tarradas et al. 1994).

DNA extraction of S. suis was done using DNeasy blood and tissue kit following the manufacturer’s instruction. PCR was performed to detect gdh gene of S. suis using oligonucleotide primers (Table 2) according to Okwumabua et al. (2003).

The amplification of bacterial DNA for gdh gene was performed in thermal cycler in 50 μl volume containing 5 μl of 10X PCR buffer, 1 μl dNTPs, 1 μl primers, 3 μl of the template DNA and 1 μl of Taq DNA polymerase. The conditions for PCR were 94°C for 5 min for initial denaturation of DNA within the sample followed by 30 cycles of 94°C for 1 min (denaturation), 55°C for 1 min (primer annealing), 72°C for 1 min (DNA extension) and a final extension at 72°C for 7 min. The amplified PCR products of desired size were visualized by submarine gel electrophoresis using 4 μl of PCR product on 1.5% agarose gel in 1 X TBE buffer for 75 min at 80V. The amplified DNA fragments of specific sizes were located by Gel Doc System and the image was captured using Alpha Imager EP software after staining with ethidium bromide (0.5 μg/ml). Molecular size markers were included in each gel.

In the present study the PCR primers obtained for S. suis gdh gene amplified the DNA from all the 15 S. suis strains tested and yielded the expected 688–bp product

Table 1. Non-S. suis isolates used in the study

<table>
<thead>
<tr>
<th>Other streptococcal species (n=5)</th>
<th>Other bacteria (n=5)</th>
<th>Other Gram-positive bacteria (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptococcus dysgalactiae</td>
<td>Escherichia coli</td>
<td>Bacillus subtilis</td>
</tr>
<tr>
<td>Streptococcus zooepidemicus</td>
<td>Klebsiella pneumonia</td>
<td>Clostridium perfringens</td>
</tr>
<tr>
<td>Streptococcus uberis</td>
<td>Pseudomonas aeruginosa</td>
<td>Listeria monocytogenes</td>
</tr>
<tr>
<td>Streptococcus equisimilis</td>
<td>Salmonella species</td>
<td>Staphylococcus aureus</td>
</tr>
<tr>
<td>Streptococcus pyogenes</td>
<td>Aeromonas hydrophila</td>
<td>Enterococcus faecalis</td>
</tr>
</tbody>
</table>

Table 2. Primes used for amplification of gdh gene

<table>
<thead>
<tr>
<th>Primer</th>
<th>Primer sequence (5’ - 3’)</th>
<th>Product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>JP4 F</td>
<td>GCAGCGTATTCTGTCAACGC</td>
<td>688</td>
</tr>
<tr>
<td>JP5 R</td>
<td>CCATGGACAGATAAGATG</td>
<td>688</td>
</tr>
</tbody>
</table>

Present address: 1M.V.Sc. (mysticdunes.sonowal9@gmail.com), 2Associate Professor (chanacin@satyam.net.in), 3Professor and Head (rah1962@rediffmail.com), Department of Veterinary Public Health; 5 Professor (chanacin@gmail.com), Department of Veterinary Pharmacology and Toxicology; 6Associate Professor (dilipbhatta@live.com), Department of Microbiology, College of Veterinary Science. 5Senior Scientist (swaraj.rajkhowa@gmail.com), NRC on Pig, Rani, Asom.
(Fig.1) with no false negative, which is in accordance with the results of Okwumabua et al. (2003). Although, a polymerase chain reaction was evaluated previously for S. suis by Okwumabua et al. (1999), the results showed that the targeted DNA regions were not conserved across capsular types or pathogenic strains. Additionally, the presence or lack of targeted genes in several isolates was influenced by geographical location. Creighton (1984) reported that glutamate dehydrogenase (GDH) are highly conserved and exhibited an extremely low rate of point mutation relative to many other genes and was shown by Lyerly et al. (1991) to be used successfully in the diagnosis of Clostridium difficile. Moreover, Okwumabua et al. (2001) showed that analogous to GDH of C. difficile, the S. suis gdh gene is conserved across S. suis capsular types irrespective of geographic origin. As S. suis gdh-based PCR assay relates to specificity, DNA from non-S. suis bacteria (streptococcal species, n=5; other Gram-positive species, n=5; and other bacteria including Gram-negative species, n=5) were used as the templates for PCR to test the diagnostic efficacy during the present investigation. No amplification product was detected, indicating specificity of the primers. Thus, the present study confirmed that the S. suis isolates can be detected regardless of serotype or geographic origin by targeting the gdh gene which are species-specific, highly conserved and thus, is of diagnostic importance.

SUMMARY

The study was undertaken for the detection of glutamate dehydrogenase gene (gdh) in S. suis from pig isolates. The results showed that the PCR primers obtained for S. suis gdh gene amplified the DNA from all the 15 S. suis strains tested and no amplification product was obtained from the non- S.suis isolates. The PCR technique by targeting the gdh gene was found highly specific and sensitive and can be used successfully for the detection of S. suis isolates regardless of serotype or geographic origin as compared to the conventional biochemical and serological tests, which do not unambiguously differentiate S. suis from other related organisms.

ACKNOWLEDGEMENT

The authors are thankful to the Director, National Research Centre on Pig (ICAR), Rani, Asom for providing necessary facilities to carry out the research work.

REFERENCES