Comparative Efficacy of Cultural and PCR Methods for Detecting *Salmonella* in Livestock Products

A. Vijaya Kumar*, N. Krishnaiah and L.Venkateswara Rao
Department of Veterinary Public Health, College of Veterinary Science, Sri Venkateswara Veterinary University, Rajendra nagar, Hyderabad, Andhra Pradesh, India.

**ABSTRACT**

Livestock products were screened for *Salmonella* to compare the efficacy of cultural and PCR methods. A set of primers derived from invA gene were employed to standardize the PCR assay for detection of *Salmonella* from livestock products, which gave specific amplification of a 389 bp fragment. Buffered peptone water as pre enrichment broth followed by four different selective enrichment broths were used in this study. This two-step enrichment protocol gave good results both in cultural and PCR assay. Screening of 220 livestock products viz., milk, meat, chicken, egg swabs and fish revealed 48 and 61 samples positive for *Salmonella* by cultural and PCR assay, respectively.

**Keywords:** Cultural method, invA gene, livestock products, PCR, *Salmonella*.

**Introduction**

*Salmonella* is one of the most important pathogen involved in human foodborne illness. *Salmonella* grows at temperatures between 7°C and 46°C, in a wide range of cooked or raw foods (Golden *et al.*, 1993) and storage at non-refrigeration temperatures has been shown to be important in many outbreaks. Human salmonellosis mainly occurs due to the consumption of contaminated eggs, poultry, pork, beef and dairy products (Guerra *et al.*, 2002). Livestock products must be tested for the presence of *Salmonella*, due to its potentially low infective dose (Bennett *et al.*, 1998). The standard laboratory procedure to culture and identify *Salmonella* serovars is laborious and can take up to seven days for confirmation. The PCR represents a major advance in terms of the speed, sensitivity and specificity of diagnostic methods and has been increasingly used to identify several bacterial species from food and clinical samples (Stone *et al.*, 1994). Thus PCR has become a powerful tool in microbiological diagnostics during the last decade (Sachse, 2003).

Several workers have used PCR with varied success for detection of *Salmonella* from foods using specific gene sequences for targeting (Bennett *et al.*, 1998; Chao *et al.*, 1998; Tsen and Chen, 2001). Of these, invA gene and fliC gene have been the most frequently targeted genes (Gado *et al.*, 2000; Chen *et al.*, 2001; Soumet *et al.*, 1999; Olivera *et al.*, 2002). The present study was envisaged to compare the efficacy of cultural and PCR methods for detecting *Salmonella* in livestock products.
Materials and methods

Sample collection

Milk (20 ml), meat (50 g), chicken (50 g), fish (50 g) and eggs were collected from local markets, vendors and slaughterhouses.

Conventional isolation (cultural) method

About 10 g each chicken, meat, fish samples were inoculated into 90 ml buffered peptone water (BPW) in individual sterile polythene bags homogenized thoroughly in a stomacher for 3 to 5 min and incubated at 37°C for 16 h. Similarly 10 ml of each milk sample was also inoculated and incubated. Yolk part of eggs and swabs from egg shells were inoculated in BPW in test tubes (50 ml) and incubated (37°C, 16 h).

One ml of pre-enrichment inoculum was transferred to 10 ml of Rappaport Vassiliadis (RV), tetrathionate (TT), selenite cysteine (SC), and selenite F (SF) broths for selective enrichment and incubated at 42°C for 18 h, whereas SC broth was incubated at 37°C. The enriched inoculum was streaked onto different selective agar plates like xylose-lysine deoxy cholate (XLD), bismuth sulphate (BS), brilliant green (BG), Salmonella-Shigella (SS), hektoen enteric (HE) and Mac Conkey agar and incubated at 37°C for 24 h. The presumptive colonies of *Salmonella* were picked up and subjected to biochemical tests for confirmation.

PCR method

Template preparation: About 1000 µl of the 24 h old broth culture was centrifuged at 6000 rpm for 5 min and resuspended in 50 µl of molecular grade water. It was then kept in a boiling water bath for 10 min and immediately transferred onto ice, later it was centrifuged at 13000 rpm for 5 min. For PCR technique, 5 µl of supernatant was used as template.

Standardization of PCR Protocol

The primers derived from *inv A* gene for detection of *Salmonella* spp. were custom synthesized by integrated DNA technologies (IDT). The nucleotide sequence of the primers used were *Salm*-3 (20): 5'- GCT GCG CGC GAA CGG CGA AG-3', *Salm*-4 (19): 5'- TCC CGG CAG AGT TC CAT T-3' (Cocolin *et al.* 1998). The PCR protocol was initially standardized by optimizing the concentration of the components of the reaction mixture in the PCR assay and by varying annealing temperatures and cycling conditions. The reaction mixture consisted of 5 µl of the bacterial lysate, 2.5 µl of 10x assay buffer for Taq polymerase containing 1.5 mM MgCl₂, 1 µl of 25 µM each dNTP mix, 1 µl each of forward and reverse primer (4 pmol) and 0.9 U/µl of Taq DNA polymerase made up to 25 µl using molecular grade water. Optimal cycling conditions included initial denaturation at 95°C for 5 min. followed by 35 cycles of denaturation (95°C for 1 min), annealing (58°C for 80 sec.), extension (72°C for 45 sec) and final extension at 72°C for 7 min. The PCR products were analyzed by agarose gel electrophoresis using 1.5% agarose gel containing 0.5 µg/ml ethidium bromide at constant voltage 5 V/cm in 1x TAE.

Results and Discussion

Screening of 220 samples revealed a wide variation in detection of *Salmonella* in livestock products. The cultural method detected 48 samples as positive, whereas PCR detected *Salmonella* in 61 samples (Table 1 and Fig. 1). Out of the 50 milk samples screened for *Salmonella*, PCR assay detected two samples as positive, whereas the cultural method detected *Salmonella* only in one sample. In meat samples, out of 50, PCR assay detected 10 samples positive for *Salmonella*, whereas cultural method gave seven positive. A similar trend was observed with different types of poultry samples screened. Among fish samples, 18 samples were positive by PCR, whereas only 14 samples were positive by cultural method. A similar higher positivity of PCR has been reported earlier by several workers (Scuderi *et al*., 1996; Cason *et al*., 1997; Bailey, 1998; Gonclaves *et al*., 1998). The percentage of positive
Table 1: Results of detection of *Salmonella* by cultural and PCR methods

<table>
<thead>
<tr>
<th></th>
<th>No. of samples tested</th>
<th>No. of samples positive for <em>Salmonella</em></th>
<th>% of cultural method compared to PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk samples</td>
<td>50</td>
<td>1</td>
<td>50</td>
</tr>
<tr>
<td>Meat samples</td>
<td>50</td>
<td>7</td>
<td>70</td>
</tr>
<tr>
<td>Fish samples</td>
<td>50</td>
<td>14</td>
<td>77.7</td>
</tr>
<tr>
<td>Chicken meat</td>
<td>50</td>
<td>12</td>
<td>80</td>
</tr>
<tr>
<td>Eggs</td>
<td>20</td>
<td>14</td>
<td>87.5</td>
</tr>
</tbody>
</table>

confirmation by cultural method as compared to PCR assay ranged from 50 to 90% for different types of samples. Aabo *et al.* (1995) has reported a sensitivity of 92% by the PCR method, whereas the routine cultural method had a sensitivity of 50% in detecting naturally contaminated food samples. The combination of both pre-enrichment and selective enrichment is recommended by all regulatory agencies for the conventional cultural isolation method and this protocol was used successfully by many workers (Miyamoto *et al.*, 1999; Soumet *et al.*, 1999, Gado *et al.*, 2000). Low sensitivity of cultural method as compared to PCR technique, when testing raw food samples may be due to overgrowth of competing micro flora (Bailey *et al.*, 1991; Aabo *et al.*, 1995). In the present study PCR assay gave better results when compared with cultural method in detection of *Salmonella* in various methods.

Acknowledgements

The authors are thankful to Sri Venkateswara Veterinary University, Tirupathi for providing necessary facilities and financial support to carry out this work.

References


