Study on Prevalence of *Salmonella* Serotypes among Poultry and Cattle in-and-around Pantnagar

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**ABSTRACT**

Present study was undertaken to determine the prevalence of *Salmonella*, for which a total of 597 samples comprising poultry droppings (400), intestinal contents of necropsied birds (60) and cattle dung (137) were collected from various locations in and around Pantnagar and analyzed for the presence of *Salmonella* species. A total of 8 isolates were recovered from different faecal samples and serotypes identified as *Salmonella Typhimurium*, *S. Billa*, *S. Virchow* and *S. Enteritidis*. The overall prevalence of Salmonellae was 1.52% in poultry (0.75% in poultry droppings and 6.66% in intestinal contents of necropsied birds) and 0.72% in cattle. Confirmed isolates were further analyzed by PCR where amplification of a 449 bp fragment of invasive sipA gene was evident in all the 8 serovars, suggestive that these isolates possessed invasive property. The results obtained in this study indicated serovars *Salmonella Typhimurium*, *S. Billa* and *S. Virchow* confining to the poultry population while *S. Enteritidis* among cattle.

**Keywords:** PCR, prevalence, *Salmonella* Billa, S. Virchow, *Salmonella invasive pathogen*

**Introduction**

*Salmonella enterica* subsp. *enterica* is one of the leading causes of zoonotic and food-borne disease worldwide. Animals are the principal reservoirs of this pathogen and foods with animal sources such as beef, poultry (meat and egg) and milk have been found to carry the pathogen (Gillespie et al., 2003). Poultry products are frequent vehicles in the transmission of *Salmonella*, dominating other foods of animal origin as potential source of infection. The most common route of transmission of *Salmonella* is the faecal-oral route, where humans get infected through ingestion of the bacteria from contaminated food or water, or following direct or indirect contact with the faeces of an infected human, bird or animal. Continuous exploration and identification of *Salmonella* throughout the food production process is important for surveillance and improving prevention and control of the disease.

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The process of isolation and identification of *Salmonella* employing conventional methods is laborious and time consuming. It may take 5 to 7 days to complete and may show poor sensitivity for samples with low levels of contamination (Gouws et al., 1998). Further, increased public awareness related to health and economic impact of food-borne contamination and illnesses has resulted in the greater efforts to develop more sensitive methods of pathogen detection and identification. Therefore, efforts have been made by several workers to reduce the time required for detection and to increase the sensitivity of the methods to detect *Salmonella* (Carli et al., 2001).

Advances in molecular biology particularly advent of the polymerase chain reaction (PCR), has allowed more reliable microbial identification and surveillance (Hassan et al., 2004). The PCR has also become a valuable tool for investigating food-borne outbreaks and identifying the responsible etiological agent by virtue of its increased sensitivity, rapidity and has thus enhanced the likelihood of detecting bacterial pathogens. In addition to analysis of foods, the PCR has also been successfully applied to the detection and identification of pathogenic organisms in the clinical and environmental samples (Simon, 1999).
The present study was therefore undertaken to determine the prevalence of *Salmonella* serotypes among poultry and cattle in-and-around Pantnagar.

**Materials and Methods**

**Sample collection**

A total of 597 samples comprising poultry droppings (400), intestinal contents of necropsied birds (60) and cattle dung (137) were collected from various locations in-and-around Pantnagar from October 2010 to April 2011 and analyzed for the presence of *Salmonella* species following the standard methods described in ISO 6579:2002.

**Bacterial isolation**

Samples collected from various locations were enriched in RV broth and incubated at 42°C for 24 h. The inoculums from RV broth culture were then streaked on BGA and XLD and incubated at 37°C for 18-24 h. The plates were examined for the presence of typical colonies of *Salmonella*, i.e. moderately large, moist, smooth and colourless colonies with pink background on BGA and red colonies with black centre on XLD. Suspected colonies were picked up from each selective media and transferred onto MLA plates. The MLA plates showing development of colourless colonies were then subjected to various biochemical tests and serological tests for further identification.

**DNA extraction**

One typical colony from each of the isolates was picked and inoculated to 5 mL of brain heart infusion (BHI) broth and incubated overnight at 37°C. The broth culture was then subjected for DNA extraction using the Hi-Pura Bacterial and Yeast genomic DNA purification kit (Hi-Media, Mumbai) as per the instructions of manufacturer. The DNA samples were diluted to a concentration of 50 ng/µl and stored at -20°C for further use.

**Detection of virulence gene in *Salmonella* isolates by PCR**

*Salmonella* isolates were confirmed by PCR amplification of *Salmonella* pathogenicity island 1 specific primers. Product of 449 bp amplified targeting *sipA* gene (*Salmonella* invasion protein) was carried out as described by Wang et al. (2009). The primers [TTC GAC TAA CAG CAG CA and CCG TCG TAC CGG CTT TAT TA] got custom synthesized by Metabion International AG, Germany.

**Serotyping**

After cultural, morphological, biochemical and molecular confirmation, the *Salmonella* isolates were transferred to semisolid agar medium (0.8%) and sent to National *Salmonella* Centre, Division of Bacteriology and Mycology, Indian Veterinary Research Institute, Izatnagar, Bareilly (U.P.) for serotyping.

**Results and Discussion**

Of the total 597 samples processed, eight isolates were recovered with 4 serotypes (*Salmonella Typhimurium*, *S. Bsilla*, *S. Virchow* and *S. Enteritidis*) as shown in Table 1. *Salmonella enterica* was confirmed by PCR amplification of a 449 bp fragment of invasive *sipA* gene in all the 8 serovars (Fig. 1). These findings were in accordance with the observation of Hapfelmeier et al. (2004) who reported carriage of *Salmonella* pathogenicity island 1 (SPI 1) among *Salmonella* serovars. This region codes for *sipA* gene which is required for initiation of intestinal and systemic infection in the host.

This study revealed an overall prevalence of 1.52% among poultry (0.75% in poultry droppings and 6.66% in intestinal contents of necropsied birds) which was in agreement with the findings of Suresh et al. (2011), who reported 1.4% *Salmonella* in cloaca and 6.9% in crop. Higher prevalence of 14.7% of *Salmonella* has been reported in live poultry birds by Murugkar et al. (2003). Most common serovar found was *S. Typhimurium* and this observation is in accordance with the findings of Nagappa et al. (2007) who reported *S. Typhimurium* as most prevalent serovar in poultry eggs and meat of Tarai region of Uttaranchal.

In cattle, prevalence rate of 0.72% was observed and it was significantly lower than the earlier reports stated by Murugkar et al. (2005) who reported a prevalence rate of 9.6% in the north-eastern India, however similar prevalence (0.71%) has been reported by Bisht (2010) in the study area.

**Table 1. Distribution of *Salmonella* serovars in poultry and cattle**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Sample type</th>
<th>No. of sample processed</th>
<th>Serovars identified</th>
<th>No. of isolates</th>
<th>No. of positive samples (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Poultry droppings</td>
<td>400</td>
<td>S. Virchow, <em>S. Typhimurium</em></td>
<td>1,2</td>
<td>3 (0.75)</td>
</tr>
<tr>
<td>2.</td>
<td>Poultry intestinal content</td>
<td>60</td>
<td>S. Bsilla, <em>S. Typhimurium</em></td>
<td>2,2</td>
<td>4 (6.66)</td>
</tr>
<tr>
<td>3.</td>
<td>Cattle dung</td>
<td>137</td>
<td>S. Enteritidis</td>
<td>1</td>
<td>1 (0.72)</td>
</tr>
</tbody>
</table>
Present study revealed two new serovars among the poultry in the pantnagar region i.e. S. Bsilla and S. Virchow. Padungtod and Kaneene (2006) considered Salmonella Virchow as a frequently encountered isolate from poultry and Willocks et al. (1996) showed that human salmonellosis caused by S. Virchow was mainly related to consumption of poultry products. In the present study, S. Virchow was recovered from poultry which was similar to the observations made by Sumner et al. (2004) and Capita et al. (2007).

There exist limited reports on isolation of S. Bsilla from poultry, however, Singh et al. (2007) reported this serovar from mint, coriander, radish and carrots from the Northern parts of India.

S. Typhimurium serovar was found to be the most prevalent in the poultry population, which could pose great risk to the human health. The present investigation illustrates importance of poultry in the transmission of Salmonella to the humans and also to the increasing occurrence of uncommon Salmonella serovars in the study population.

References


