Prevalence of *Salmonella* Serovars of Zoonotic Importance and their Molecular Characterization

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**ABSTRACT**

A total of 50 *Salmonella* isolates belonging to different serovars viz., *S. Typhimurium* (21), *S. Weltevreden* (12), *S. Ughelli* (5), *S. Essen* (3), *S. Elisabethville* (2), *S. Lagos* (2), *S. Drogana* (2), *S. Enteritidis* (1), *S. London* (1) and un-typable *Salmonella*-isolate (1) were recovered from 1,132 samples originating from man, animals and foods of animal origin. The most prevalent serovar was *S. Typhimurium* followed by *S. Weltevreden*. The emerging *Salmonella* serovars viz., *S. Elisabethville*, *S. Essen*, *S. Lagos*, *S. Ughelli* and *S. Drogana* were recovered for the first time from different sources from Pantnagar and its vicinity. *S. Drogana* was recovered from human stool sample, which is possibly the first isolate from human source in India. Multiple *Salmonella* serovars (*S. Typhimurium*, *S. Weltevreden* and *S. Essen*) were recovered from a single cattle dung sample while, mixed infection was also recorded in single sample source of cattle dung (*S. Weltevreden* and *S. Ughelli*), poultry droppings (*S. Essen* and *S. Ughelli*), pig faeces (*S. Weltevreden* and *S. London*), sheep faeces (*S. Typhimurium* and *S. Drogana*) and pig faeces (*S. Weltevreden* and *S. Ughelli*). The high prevalence rate of *Salmonella* was recorded in facces of sheep, poultry, pig, buffalo, cattle, autopsied poultry tissues, poultry eggs, pork, poultry meat and human beings as 27.27%, 8.33%, 3.7%, 2.91%, 2.86%, 26.67%, 2.04%, 1.28%, 0.94% and 0.65%, respectively. Overall prevalence (in animals and human beings) of *Salmonella* was 3.8% in Pantnagar and its vicinity.

**Keyword**: Molecular charcterization, *Salmonella*, zoonotic importance

Salmonella organism is a major cause of food-borne disease throughout the world (WHO, 2005). Salmonellosis is one of the most common and widely distributed food-borne disease and become an important public health problem throughout the world (Srifuengfung et al., 2005). The foods particularly those of animal origin have been identified as common vehicles for transmission of *Salmonella* organisms to humans and spreading them to processing and kitchen environment. Raw and inadequately cooked meat, eggs, milk and especially poultry are the most commonly implicated vehicle for *Salmonella* infection (Rabsch et al., 2001).

There are more than 2,579 serovars of *Salmonella* has been recorded throughout the world (WHO, 2007) and of these, the most common non-typhoidal serovar isolated from human is *S. Typhimurium* followed by *S. Enteritidis*, whereas, the most common serovar of non-human origin is *S. Typhimurium* followed by *S. Newport*. Among all the serovars of *Salmonella enterica*, the *Salmonella Typhimurium* is most commonly associated with enteric infections in man and animals. This serovar has diverse host range, which includes humans, cattle, pigs, sheep, horses, rodents and birds (Kingsley and Baumler, 2000).

The polymerase chain reaction (PCR) based *Salmonella* detection was first carried out by Widjjoatmodjo et al. (1991). Swamy *et al* (1996) established the presence of invasive A (*invA*) gene in nearly all *Salmonella* irrespective of serovar or source. The *invA* gene is highly conserved in both species, i.e., *S. enterica* and *S. bongori* (Malorny et al., 2009).

The increase in the demand of foods of animal origin throughout the world has resulted in intensive practices in animal husbandry and food processing industries. Many factors such as increase in international travel and trade, inadvertent introduction of pathogens into new geographic areas, microbial adaptation and changes in the
food production system as well as human demography and behaviour have resulted in the emergence of salmonellae along with other pathogens world-wide (Knabel, 1995 and Altekruse et al., 1997).

In the view of importance of salmonellosis in animals and human beings, the attempts were made to isolate and identify the Salmonella organisms from samples of varied sources in the present study. On the basis of the recovery of the organisms, an attempt was also made to determine the prevalence of Salmonella serovars considering the fact that rate of prevalence would change from time to time due to emergence of new serovars in the same geographical area.

A total of 1,132 samples comprised of poultry meat (212), poultry eggs (49), poultry droppings (60), autopsied poultry tissues (60), pork (156), pig faeces (189), cattle dung (105), buffalo dung (103), sheep faeces (11), goat faeces (31), deer faeces (2) and human stool (154) were aseptically collected from Pantnagar, Nagla, Jawahar nagar, Lalkuan and Rudrapur and processed for isolation of salmonellae using conventional culture method.

Meat samples were aseptically collected in sterile plastic containers while eggs were collected in the sterile polythene pouches. The autopsied poultry tissues (muscle, liver and caeca) were also collected in sterile containers from Necropsy Center, Dept. of Veterinary Pathology, College of Veterinary and Animal Sc, G. B. Pant University of Agriculture & Technology, Pantnagar. Animal faecal samples were collected in sterile containers. For collection of human stool samples, the sample containers were provided to the donors in the evening and samples were collected in the next morning. All the samples collected in sterile containers/pouches following all possible aseptic conditions and brought to the laboratory as soon as possible following cold chain.

O antiserum poly A-I and Vi (Difco) was procured and used for serological identification of Salmonella spp. The oligonucleotide primers of invA genes used in present study was synthesized from Metabion International AG (Germany). The forward primer sequence was ACAGTGCCTGGTTACGACCTGAAT and reverse primer sequence was AGACGACTGTTACTGATCTAT. The biologicals required for molecular works using PCR were procured from Bangalore Genei, (India) and Genetix (India). The Taq polymerase and RNase were procured from Bangalore Genei (India) for use in the molecular works.

The buffered peptone water was used as pre-enrichment media for isolation of Salmonella from meat and autopsied poultry tissue samples. Selective enrichment media used were Tetrathionate broth (Hi-Media, Mumbai) and Rappaport-Vassiliadis broth (Hi-Media, Mumbai) for isolation of non-typhoidal Salmonella, whereas, Rappaport-Vassiliadis broth and Selenite F broth (Hi-Media, Mumbai) were used for isolation of typhoidal Salmonella from human stool samples. Multiple selective plating media viz. brilliant green agar (BGA), bismuth sulphite agar (BSA), xylose lysine deoxycholate (XLD) agar and Hektoen enteric agar (HEA) were used for isolation of salmonellae from faecal samples.

Salmonella organisms were isolated from human stool and animal faecal samples as per the methods described in standard ISO 6579: (2002) and WHO/CDC (2003). Human stool samples were processed for both typhoidal and non-typhoidal Salmonella, whereas, animal faecal samples were subjected to only non-typhoidal Salmonella. The foods of animal origin comprising of pork, poultry meat and eggs were subjected to the isolation of Salmonella organisms. The methods described by USDA/FSIS (2002) and Andrews and Hammack (2003) were followed for isolation of non-typhoidal Salmonella organisms, while the methods described in WHO/CDC (2003) was followed for isolation of typhoidal Salmonella organism. Salmonella organisms were identified using the methods described by Old (1996).

Salmonella isolates identified on the basis of cultural, morphological and biochemical reactions were subjected to agglutination reaction by using O antiserum poly A-I and Vi (Difco, USA) for serological identification. Molecular identification of these isolates was carried out using PCR technique by targeting invA gene fragment (Fig. 1) following the method described by Chiu and Ou (1996). Salmonella isolates identified on the basis of cultural, morphological, biochemical, serological and molecular characterizations were sent for serotyping to Salmonella Typing Center, Division of Bacteriology and Mycology, Indian Veterinary Research Institute, Izatnagar, Bareilly (U. P.).

In the present study, a total of 50 isolates were confirmed as Salmonella spp. These isolates were serotyped to 10 different serovars viz., S. Typhimurium (21), S. Weltevreden (12), S. Ughelli (5), S. Essen (3), S. Elisabethville (2), S. Lagos (2), S. Drogana (2), S. Enteritidis (1), S. London (1) and un-typable Salmonella isolate (1) recovered from 1,132 samples (Table 1).

Overall prevalence (in animals and human beings) of Salmonella was 3.8% in Pantnagar and its vicinity. The high prevalence rate of Salmonella was calculated as 27.27% in sheep followed by 26.67% in autopsied poultry tissues, 8.33% in poultry droppings, 3.7% in pig, 2.91% in buffalo, 2.86% in cattle, 2.04% in poultry eggs, 1.28% in pork, 0.94% in poultry meat and 0.65% in human beings. At similar locations, Sharma and Thapliyal (1995)
Table 1: Prevalence of *Salmonella* serovars

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Sample source</th>
<th>Number of samples processed</th>
<th>Number of isolates recovered (%)</th>
<th>Serovar identified (number)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Poultry meat</td>
<td>212</td>
<td>2 (0.94%)</td>
<td>S. Typhimurium (2)</td>
</tr>
<tr>
<td>2</td>
<td>Pork</td>
<td>156</td>
<td>2 (1.28%)</td>
<td>S. Weltevreden (2)</td>
</tr>
<tr>
<td>3</td>
<td>Poultry eggs</td>
<td>49</td>
<td>1 (2.04%)</td>
<td>S. Typhimurium (1)</td>
</tr>
<tr>
<td>4</td>
<td>Autopsied poultry tissues</td>
<td>60</td>
<td>16 (26.67%)</td>
<td>S. Typhimurium (14), S. Lagos (1) and Un-typable <em>Salmonella</em> (1)</td>
</tr>
<tr>
<td>5</td>
<td>Poultry droppings</td>
<td>60</td>
<td>6 (8.33%)</td>
<td>S. Ughelli (2), S. Elisabethville (2), S. Weltevreden (1) and S. Essen (1)</td>
</tr>
<tr>
<td>6</td>
<td>Pig faeces</td>
<td>189</td>
<td>9 (3.7%)</td>
<td>S. Weltevreden (6), S. Ughelli (2) and S. London (1)</td>
</tr>
<tr>
<td>7</td>
<td>Cattle dung</td>
<td>105</td>
<td>6 (2.86%)</td>
<td>S. Weltevreden (3), S. Typhimurium (1), S. Essen (1) and S. Ughelli (1)</td>
</tr>
<tr>
<td>8</td>
<td>Buffalo dung</td>
<td>103</td>
<td>3 (2.91%)</td>
<td>S. Typhimurium (2) and S. Lagos (1)</td>
</tr>
<tr>
<td>9</td>
<td>Sheep faeces</td>
<td>11</td>
<td>4 (27.27%)</td>
<td>S. Typhimurium (1), S. Enteritidis (1), S. Essen (1) and S. Drogana (1)</td>
</tr>
<tr>
<td>10</td>
<td>Goat faeces</td>
<td>31</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>Deer faeces</td>
<td>2</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>Human stool</td>
<td>154</td>
<td>1 (0.65%)</td>
<td>S. Drogana (1)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>1,132</strong></td>
<td><strong>50</strong></td>
<td></td>
</tr>
</tbody>
</table>

reported the prevalence rate of salmonellae as 3.29% in cattle and 6.49% in poultry, which is almost similar to the present findings. However, relatively higher prevalence (8.33%) of *Salmonella* organisms was recorded in the poultry which might be due to poor hygienic and managemental practices adapted at the poultry farms.

So far as the prevalence of salmonellosis in pigs are concerned, it is similar to the findings of Barber and co-workers (2002) who reported that 1.4 to 3.1% of swine population on the farms was harbouring *Salmonella* organisms. Although, the sheep maintained at University farm was found free from *Salmonella* infection, but interestingly, the sheep (n=7) kept for experimental purpose showed high positivity of 27.27% with the isolation of one serovar each of S. Typhimurium, S. Enteritidis, S. Essen and S. Drogana. Such a high prevalence of salmonellae in experimental sheep indicates towards the poor hygienic as well as managemental practices prevailing at the experimental house, poor immune status of animals, malnutrition & stressful environment. Kane (1973) monitored the prevalence of sub-clinical *Salmonella* infection in sheep at two slaughterhouses in the southern part of the North

![Agarose gel electrophoresis showing amplified PCR products of invA gene. Lane M: 100 bp Marker, Lane 1-18: *Salmonella* isolates, Lane 19: Negative control and Lane 20: Positive control (*Salmonella* Typhimurium).](image-url)
Island over a 17-month period during 1975 and 1976 and found 4.5% *Salmonella* infection in sheep in which the most common serovar was *S. Typhimurium* (78%).

It is worth mentioning that none of the faecal samples obtained from the goat (31) reared at Nagla and Jawahar nagar revealed the presence of *Salmonella* organisms during the course of present study. On the contrary, in the previous studies from the similar locations, (Sharma *et al*., 1989) reported the isolation of *Salmonella* from goats. The reasons for the absence of *Salmonella* infection in goat could be only attributed to the good managemental practices, less number of samples processed and good immune status of goats in the study area.

In the present study, a very high prevalence of *Salmonella* (26.67%) was observed in autopsied poultry tissues, which was might be due to outbreak of salmonellosis at the poultry farms that resulted high mortality among poultries.

Maharjan *et al*. (2006) reported the prevalence of *Salmonella* spp. in raw meat samples of chicken (14.5%), buffalo (13.5%) and goat (3.3%) with 11.4% in overall samples from the local meat market of Kathmandu metropolitan city during September 2002 to May 2003. Comparatively higher prevalence of *Salmonella* in pigs and other animals has been reported by these researchers which might be due to the different environmental conditions prevailing in different geographical areas as well as different status of managemental and hygienic practices.

The prevalence of 0.65% of *Salmonella* in human faecal samples was observed in the present study. Blaser *et al*. (1982) reported non-typhoidal *Salmonella* in human beings with the prevalence rate of 0.29% and 0.26% in urban and rural areas, respectively. This finding is almost similar to the findings of the present study. Murugkar *et al*. (2005) reported 20.5% prevalence rate of *Salmonella* belonging to 3 serovars, namely *S. Typhimurium*, *S. Enteritidis* and *S. Paratyphi* B in man. The higher prevalence of *Salmonella* in human beings reported by these researchers was probably due to the hyper-endemic zone of salmonellosis selected in the studies.

In the present study, the overall prevalence of *Salmonella* in different samples was found to be 3.8%. According to a surveillance report of different countries of European Union, the prevalence of salmonellosis varied from 0-7.3% (EFSA, 2007) and this variation in the prevalence could be due to either variation in the level of endemicity of salmonellae in the particular geographical areas or varied number of samples processed. Therefore, the findings of the present study are similar to the earlier report of EFSA (2007).

As per the findings of the present study, it may be concluded that there was large number of *Salmonella* serovars recovered from different sources, which are of great zoonotic importance. The most prevalent serovar was *S. Typhimurium*, followed by *S. Weltevreden*. The emerging *Salmonella* serovars viz., *S. Enteritidis*, *S. Typhimurium*, *S. Drogana* were recovered for the first time from different sources from Pantnagar and its vicinity. *S. Drogana* was recovered from human stool sample, which is possibly the first isolate from human source in India. Multiple *Salmonella* serovars recovered from many samples indicates that salmonellosis may involve infection with many *Salmonella* serovars that may complicate the process of pathogenesis.

**References**


Molecular characterization of Salmonella serovars of zoonotic importance


