Prevalence of *Salmonella* in Foods of Animal Origin and its Public Health Significance

C. Shekhar*, A.K. Upadhyay and S.P. Singh

Department of Veterinary Public Health and Epidemiology, College of Veterinary and Animal Sciences, G. B. Pant University of Ag. & Tech., Panthnagar, U. S. Nagar (U. K.)-263 145

(Received 17.06.2012; accepted 30.03.2013)

ABSTRACT

Total 417 samples from foods of animal origin comprising poultry meat (212) poultry eggs (49) and pork (156) were aseptically collected for the isolation of *Salmonella* from Pantnagar, Nagla, Lalkuan and Rudrapur. Total 5 *Salmonella* isolates were recovered from poultry meat (2), poultry eggs (1) and pork (2). The maximum prevalence was observed in poultry eggs (2.04%), followed by pork (1.28%) and poultry meat (0.94%). *Salmonella* isolates recovered from different samples were sent for serotyping and were identified as *Salmonella* Typhimurium and *Salmonella* Weltevreden. The most prevalent serovar was *Salmonella* Typhimurium, followed by *Salmonella* Weltevreden.

Keywords: Poultry meat, poultry eggs, pork, *Salmonella*, prevalence

Introduction

Salmonellosis constitutes a major public health burden and represents a significant cost to society in many countries (WHO, 2005). Salmonellosis caused by different non-typhoidal *Salmonella* serovars has become an important public health problem throughout the world (van der Klooster and Roelofs, 1997; Workman *et al.*, 1999; Srifuengfung *et al.*, 2005). Salmonellosis as emerging foodborne disease is showing an increasing trend in recent years (Bhattacharya and Dash, 2000; Shahane *et al.*, 2007). Salmonellosis in humans is generally contracted through the consumption of contaminated foods of animal origin (mainly meat, poultry, eggs and milk), although many other foods, including green vegetables contaminated from manure, have been implicated in its transmission.

Materials and Methods

Sampling

Total 417 samples from foods of animal origin comprising poultry meat (212) poultry eggs (49) and pork (156) were aseptically collected from Pantnagar, Nagla, Lalkuan and Rudrapur. Meat samples were aseptically collected in sterile plastic containers and brought to the laboratory as soon as possible, while eggs were collected in the sterile polythene bags.

Isolation procedure

*Salmonella* organisms were isolated from foods of animal origin viz. pork, poultry meat and eggs as per the methods described by USDA/FSIS (2002) and Andrews and Hammack (2003). Isolation of *Salmonella* organisms from foods of animal origin was carried out in the following steps-

Pre-enrichment

The samples from pork, poultry meat and eggs were pre-enriched with the buffered peptone water (Hi-Media, Mumbai) as pre-enrichment medium in order to overcome the organisms from stresses due to adverse environmental conditions like heat, cold etc. and some mechanical forces like trituration, homogenization etc. The samples were taken in a
quantity of 25 g/25 ml, homogenized properly and transferred to a flask having 225 ml of sterilized buffered peptone water. The inoculated pre-enrichment broths were incubated at 37°C for 20-24 h.

Selective enrichment
Two selective enrichment media viz., tetrathionate broth and Rappaport-Vassiliadis broth (Hi-Media, Mumbai) were used for isolation of *Salmonella* from foods of animal origin. One ml of culture from pre-enriched broth was inoculated into each of the tube containing 10 ml sterile tetrathionate broth and Rappaport Vassiliadis broth. Inoculated tetrathionate broth and Rappaport-Vassiliadis broth tubes were incubated for 24 h at 37°C and 43°C, respectively.

Selective plating
A loop-full culture for each selective plating medium was taken from selective enriched broth cultures and streaked onto selective plating media. Simultaneously directly streaking onto selective plating media was also attempted from meat and egg homogenates. Streaking was also carried out from selective enrichment culture after 6 h and 48 h of incubation in order to ensure the recovery of salmonellae. For isolation of *Salmonella* from foods of animal origin, the multiple selective plating media used were brilliant green agar (BGA) and Hektoen enteric agar (HEA) in order to ensure the recovery of *Salmonella* organisms. The inoculated plates were incubated at 37°C for 24 h.

Differential plating
The colony showing characteristics of *Salmonella* spp. from selective plating media was streaked onto the differential plating medium like MacConkey’s lactose agar. Plates were incubated at 37°C for 24 h. The culture plates showing lactose non-fermenting colony was transferred onto nutrient agar slant for identification and confirmation of *Salmonella* organisms.

Identification/confirmation
*Salmonella* isolated from different samples were subjected to cultural, morphological, biochemical and serological characterization for their identification as per the methods described by Old (1996). Colonies of *Salmonella* appeared as moderately large, moist, smooth and colourless with pink background on brilliant green agar (BGA), blue green color colony with a black center on Hektoen enteric agar (HEA) and medium size, opaque and colourless colonies on MacConkey’s lactose agar (MLA). All *Salmonella* isolates showed motility when observed under microscope by hanging drop method. All *Salmonella* isolates on Gram staining appeared as Gram-negative rods. Serological test was carried out by slide agglutination test using O antiserum poly A-I and Vi (Difco, USA).

*Salmonella* isolates were confirmed using PCR technique by targeting *invA* gene fragment following the method described by Chiu and Ou (1996). The oligonucleotide primers of *invA* gene used in this study was synthesized from Metabion International AG (Germany). The forward primer sequence was ACAGTGCTCGTTACGACCTGAAT and reverse primer sequence was AGACGACTGCTACGTATCC. 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.

Serotyping
After molecular confirmation, *Salmonella* isolates were sent for serotyping at National *Salmonella* Center, Division of Bacteriology and Mycology, IVRI, Izatnagar, Bareilly (U. P.).

Results and Discussion
In the present study, the tetrathionate broth was found most suitable selective enrichment medium, while brilliant green agar as most appropriate selective plating medium for isolation of salmonellae from foods of animal origin. Molecular characterization of *Salmonella* isolates was performed by targeting amplification of *invA* gene primer fragment for confirmation of *Salmonella* spp. On agarose gel electrophoresis, the amplified PCR products of *invA* gene exhibited the desirable PCR product of 244 bp size (Fig. 1).
Table 1. Prevalence of *Salmonella* in foods of animal origin

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Source/Type of sample</th>
<th>Number of sample processed</th>
<th>Number of isolate recovered</th>
<th>Prevalence(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Poultry meat</td>
<td>212</td>
<td>2</td>
<td>0.94</td>
</tr>
<tr>
<td>2</td>
<td>Poultry eggs</td>
<td>49</td>
<td>1</td>
<td>2.04</td>
</tr>
<tr>
<td>3</td>
<td>Pork</td>
<td>156</td>
<td>2</td>
<td>1.28</td>
</tr>
</tbody>
</table>

Table 2. Status of *Salmonella* serovars in different samples

<table>
<thead>
<tr>
<th>Sl.No.</th>
<th>Source/Type of sample</th>
<th>Number of isolate recovered</th>
<th>Serovar identified (number)</th>
<th>Antigenic structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Poultry meat</td>
<td>2</td>
<td>S. Typhimurium (2)</td>
<td>1,4,[5],12:i:1,2</td>
</tr>
<tr>
<td>2</td>
<td>Poultry eggs</td>
<td>1</td>
<td>S. Typhimurium (1)</td>
<td>1,4,[5],12:i:1,2</td>
</tr>
<tr>
<td>3</td>
<td>Pork</td>
<td>2</td>
<td>S. Weltevreden (2)</td>
<td>3,10[15]:r:z</td>
</tr>
</tbody>
</table>

A total of 5 *Salmonella* isolates were recovered from poultry meat (2), poultry eggs (1) and pork (2). The maximum prevalence was observed in poultry eggs (2.04%), followed by pork (1.28%) and poultry meat (0.94%) (Table 1). Sharma *et al.* (1989) examined 446 meat samples of sheep, goat and buffalo collected from retail outlets and found 57 samples (12.78%) positive for *Salmonella* of different serovars viz., S. Typhimurium, S. Senftenberg, S. Weltevreden, S. Anatum, S. Newport, S. Bareilly, S. Stanley, S. Saintpaul, S. Agona, S. Chester and monophasic variant with antigenic formula as 3, 10: r:-. Bajaj *et al.* (2003) reported highest incidence of salmonellosis in pork (78%), followed by chicken (69%), sheep (57%) and beef (56%). Murugkar *et al.* (2005) conducted a study to find the distribution of different serovars of *Salmonella* among the animal species and found the prevalence rate of 14.7% in poultry, 14.25% in piglets and 9.6% in cattle with most common serovar S. Typhimurium. 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. In the present study, comparatively lower prevalence of *Salmonella* was observed in poultry meat which might be due to low endemicity of salmonellosis in this area and better status of managemental and hygienic practices.

*Salmonella* isolates recovered from different samples were sent for serotyping which revealed presence of *Salmonella* Typhimurium and *Salmonella* Weltevreden. The most prevalent serovar was *Salmonella* Typhimurium, followed by *Salmonella* Weltevreden (Table 2). *Salmonella* serovars isolated from different sources in the present study have also
been reported by other researchers. Singh (1976) reported S. Weltevreden, S. Typhimurium and other serovars form goat meat samples. Singh et al. (1980) reported S. Typhimurium and many other serovars form pigs. Sharma et al. (1987) examined 343 samples of pork and pork products for the presence of Salmonella and recovered different serovars of Salmonella viz. S. Oranienburg, S. Senftenberg, S. Heidelberg, S. Weltevreden, S. Infantis, S. Anatum, S. Indiana, S. Newport, S. Bareilly, S. London, S. Stanley, S. Saintpaul, S. Derby and S. Bovismorbidicans from 42 samples. Suressh and Thapliyal (1989) recovered 12 isolates of Salmonella, 6 each of S. Typhimurium and S. Saintpaul from 104 slaughtered buffaloes. Salmonella Weltevreden isolated in the present study have also been reported by Sharma et al. (1987) from samples of pork and pork products. Foley et al. (2008) isolated Salmonella from pork and poultry and identified many serovars viz., S. Typhimurium, S. Derby, S. Choleraesuis, S. Heidelberg, S. Agona, S. Infantis, S. Anatum, S. Worthington, S. Senftenberg and Brandenburg with most prevalent serovar Typhimurium. These finding are similar to the findings of the present study. The findings of above researchers indicated that the most prevalent serovar was Salmonella Typhimurium which is similar to the findings of the present study.

The results of the present study revealed lower prevalence of Salmonella in foods of animal origin with presence of S. Typhimurium and S. Weltevreden.

References


