Seasonal Effect on the Shedding Pattern of *Salmonella*, *Escherichia coli* and *Campylobacter* in Poultry

Renu*, A.S. Yadav, V. Tripathi and R.P. Singh
Central Avian Research Institute, Izatnagar-243122

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**ABSTRACT**

In the present study, a total of 150 cloacal samples of layers were collected from an organized poultry farm and were screened for the presence of *Salmonella*, *E. coli* and *Campylobacter* and for studying their shedding pattern during different seasons (summer, rainy and winter) of the year 2007. Out of 150 cloacal samples, 11 (7.3%), 12 (8%) and 9 (6%) were found positive for *Salmonella*, *E. coli* and *Campylobacter*, respectively. Prevalence rate of pathogens in rainy season was found to be 34%, which was the highest amongst the seasons studied. Summer and winter seasons showed prevalence rates of 18% and 12%, respectively. High shedding rate in rainy season of these pathogens may be due to stressful and contaminated poultry environment.

**Keywords**: *Campylobacter*, cloaca, *E. coli*, layer, *Salmonella*, season, shedding pattern.

**Introduction**

Foodborne diseases have major public health significance across the world. It is estimated that each year in the United States, there are approximately 76 million foodborne illnesses (Mead *et al*., 1999). Poultry and poultry products have been implicated as a major source of *Salmonella*, *E. coli* and *Campylobacter* (Bailey, 1998; Corry and Atabay, 2001; Adesiyun *et al*., 2005). The incidences of these foodborne diseases have increased dramatically over the past few years in different countries across the world (Notermans and Hoogenboom-Verdegaal, 1992).

Poultry may become infected with these pathogens due to environmental contamination. Contaminated poultry environment leads to transmission of these pathogens either by vertical or horizontal routes. Drinkers, feeders, litter, and air are critical source of horizontal transmission (Hoover *et al*., 1997). Because of the adaptability of *Salmonella*, *E. coli* and *Campylobacter* to survive in different environmental conditions, wide seasonal variations have been reported in shedding pattern of these pathogens in poultry. In the host system also, high environmental temperatures induce stress and depress immune response in poultry (Thaxton and Siegel, 1970) and stress has been reported to increase the shedding of *Salmonella* in chickens (Holt, 1992; 1993). The prevalence rate of *Salmonella*, *E. coli* and *Campylobacter* increases during warm season than during winter. A higher incidence of *Campylobacter* was observed in adverse climatic conditions (Doyle, 1984; Wallance *et al*., 1997). However, available information on the subject, particularly on seasonal shedding of *Salmonella*, *E. coli* and *Campylobacter* in live poultry birds is scanty. Therefore, the present study was designed to know the seasonal effect on the shedding
pattern of *Salmonella*, *E. coli* and *Campylobacter* in live poultry birds.

**Materials and Methods**

**Sample collection**

A total of 150 cloacal samples (50 samples in each season, winter: December-January, summer: April-May and rainy: July-August) were collected from selected 50 White Leghorn layers of an organized farm during different seasons of the year 2007 near Bareilly, Uttar Pradesh. The collected samples were transported to laboratory under aseptic conditions in sterile swabs and processed immediately for isolation of *Salmonella*, *E. coli* and *Campylobacter*.

**Procedure for isolation and identification of pathogens**

For the isolation of *Salmonella* and *E. coli*, cloacal swabs were taken, immersed into tube containing 10 ml of BPW and incubated at 37°C for 18 h. Pre-enriched culture (0.1 ml) from cloacal swab samples were added to 10 ml of Rappaport Vassiliadis (RV) broth for the isolation of *Salmonella* and 1 ml portion of the pre-enriched culture was added to 10 ml of MacConkey broth for the isolation of *E. coli* and then incubated at 43°C for 24 h. Sample from RV and MacConkey enrichment broths were plated on hektoen enteric agar (HEA) and eosin methylene blue (EMB) agar for the isolation of *Salmonella* and *E. coli*, respectively. Further these plates were incubated at 37°C for 24 h for the selective isolation of *Salmonella* and *E. coli*. Suspect *Salmonella* and *E. coli* colonies were selected and subjected to standard biochemical tests.

For the isolation of *Campylobacter*, cloacal swab base along with with 2% agar, 5% sterile defibrinated lysed sheep blood and reconstituted contents of Park and Sander's selective supplement I and Park and Sander's selective supplement II and incubated in McIntosh and Flide's jar assembly for 48 h at 42-43°C under micro-aerophilic conditions. Suspect *Campylobacter* colonies were selected and subjected to standard biochemical tests.

**Polymerase chain reaction**

The DNA was extracted from pre-enrichment cultures using hot-cold lysis method as described by Surendran et al. (2003). Two µl of DNA template was added to a mixture consisting of 2.5 µl 10x PCR-reaction-buffer with 1.5 mM MgCl₂ (Bangalore GENIE), 1 µl 10 mM dNTP mix (Bangalore GENIE), 0.5 µl of each of forward and reverse primers (10 pmol), 1 U of Taq DNA polymerase (Bangalore GENIE) and made to final volume of 25 µl using sterile triple glass distilled water. Primers used in this study were *hisJ* gene (F-5’-ACTGGCGTTATC CCTTTCTCTGGT G-3’, R-5’-ATCTTGTCCTGCCCCTGGTAAGAGA- 3’, Cohen et al., 1993) for *Salmonella*, virulent gene (*Vt*) (F-5’-CAGTTAATGTGGTGGAGAAG 3’ R-5’CTGCTAATAGTTCTGCGCATC3’, Blanco et al., 1996) for *E. coli* and 16s rRNA gene (F-5’-GGATGACACTTTTCGGAGC3’, R-5’CATTGTAGCACGTGTGTC3’, Linton et al., 1996) for *Campylobacter* spp. The PCR was conducted for the detection of these pathogens. Amplified (10 µl) product was analysed on 1.5% agarose gel and photographed using gel documentation system (Syngene, USA).

**Results and Discussion**

Gastrointestinal pathogens are the principal cause of human foodborne infections in many countries. In our study, occurrence of *Salmonella*, *E. coli* and *Campylobacter* in cloacal samples were detected during different seasons in the year 2007. Out of 150 cloacal swabs screened, 11 (7.3%) were found positive for *Salmonella* spp., 12 (8%) were found positive for *E. coli* and 9 (6%) for *Campylobacter* (Table 1). We have further confirmed our results by amplifying
Seasonal effect on the shedding pattern of *Salmonella*, *Escherichia coli* and *Campylobacter* specific genes for all these bacteria (Fig. 1) through PCR. Our results are in agreement with the previous studies that *Salmonella*, *E. coli* and *Campylobacter* are often present in meat and poultry environment (Todd, 1997). Amongst all the isolated bacterial strain, *Salmonella* spp. infection was predominant over others. This may have been due to the frequent presence of *Salmonella* spp. in the poultry sheds and food processing environments.

The prevalence of these bacteria in cloacae was highest during rainy season (34%), followed by 18% and 12% in summer and winter seasons, respectively (Table 1, Fig. 2). The USDA – Animal and Plant Health Inspection Service (APHIS) – Veterinary Services (VS) reported that the prevalence of *Salmonella* in bovine faecal samples was higher after May than before May in a year-long survey (USDA/APHIS VS, 1994). This pathogen has been reported to have peak shedding in the summer months during June, July and August (Kanistanon, 1997). A number of other factors such as age, environmental stress, moulting and breed of the bird have also been identified and suggested to play role in on-farm population dynamics of pathogens, but the season has been reported as the major factor (Edrington *et al*., 2006). Fecal shedding of *E. coli* is typically low in winter and increases in the spring (Van Donkersgoed *et al*., 1999). Survival of airborne microorganisms depends on atmospheric variables such as temperature and relative humidity (Hoover *et al*., 1997). Microbial infections increase during rainy season due to favourable conditions like high relative humidity. Temperature and relative humidity during the study period are depicted in Table 2. The season having highest temperature and relative humidity favored the maximum occurrence of these pathogens. This may be due to the thermal stress and subsequent reduction in immunity as reported earlier by Thaxton and Siegel (1970), APHIS (2001) and Wallance *et al*. (1997). During the summer season, the elevation of ambient

![Fig. 1. Gel electrophoresis of PCR amplified products of *Salmonella* and *Campylobacter* in 1.5% agarose gel.](image)

### Table 1: Prevalence of *Salmonella*, *E. coli* and *Campylobacter* during different seasons of the year 2007

<table>
<thead>
<tr>
<th>Season</th>
<th>Number of cloacal samples</th>
<th>Number positive Salmonella</th>
<th>Number positive E. coli</th>
<th>Number positive Campylobacter</th>
<th>Frequency of pathogens in season</th>
</tr>
</thead>
<tbody>
<tr>
<td>Winter (December-January)</td>
<td>50</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>6 (12%)</td>
</tr>
<tr>
<td>Summer (April-May)</td>
<td>50</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>9 (18%)</td>
</tr>
<tr>
<td>Rainy (July-August)</td>
<td>50</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>17 (34%)</td>
</tr>
<tr>
<td>Total</td>
<td>150</td>
<td>11</td>
<td>12</td>
<td>9</td>
<td>32 (21.3%)</td>
</tr>
</tbody>
</table>
Table 2: Temperature and relative humidity of the year 2007

<table>
<thead>
<tr>
<th>Month</th>
<th>Temperature (°C)</th>
<th>RH (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Minimum</td>
<td>Maximum</td>
</tr>
<tr>
<td>January</td>
<td>3.31</td>
<td>26.82</td>
</tr>
<tr>
<td>April</td>
<td>16.15</td>
<td>39.38</td>
</tr>
<tr>
<td>May</td>
<td>19.48</td>
<td>41.92</td>
</tr>
<tr>
<td>June</td>
<td>21.82</td>
<td>42.04</td>
</tr>
<tr>
<td>July</td>
<td>23.03</td>
<td>36.96</td>
</tr>
<tr>
<td>August</td>
<td>23.27</td>
<td>35.27</td>
</tr>
<tr>
<td>December</td>
<td>4.99</td>
<td>24.09</td>
</tr>
</tbody>
</table>

Fig. 2: Prevalence of *Salmonella, E. coli and Campylobacter* during summer, rainy and summer seasons.

Temperature may provide more conducive environment for *E. coli* 0157:H7 outside the host in soil, bedding material, feed, and water thereby resulting more constant source of infection or re-infection for cattle (Edrington *et al.*, 2006). It is evident from the available literature that there is a peculiar seasonality in the prevalence of the above enteropathogens. The same pattern was also observed in the current study. Thus, understanding the seasonality will enable to formulate suitable control measures to safeguard the public health.

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References


