Salmonella infections in poultry flocks in the vicinity of Tehran

Morshed, R.; Peighambari, S.M.*
Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

Key Words:
Salmonella enteritidis; group C; poultry; broiler; Tehran.

Correspondence
Peighambari, S.M.,
Department of Clinical Sciences,
Faculty of Veterinary Medicine,
University of Tehran, P.O. Box: 14155-6453, Tehran, Iran
Tel: +98(21)61117150
Fax: +98(21)66933222
Email: mpeigham@ut.ac.ir
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Introduction
Salmonellosis is one of the most important food-borne diseases worldwide (Hendriksen, 2003; Valkenburgh et al., 2007; Gast, 2008). In addition to the risks to public health, Salmonella spp. infections impose economical losses to both healthcare systems and the poultry industry (Collard et al., 2008). More than 2,600 serovars of Salmonella have been identified, some of which are responsible for human illness and diseases in a wide variety of animals (Gast, 2008). Humans most often become infected after the consumption of contaminated eggs, poultry meat, pork, or, less frequently, bovine meat (Veige et al., 2005; Soltan Dallal et al., 2007; White et al., 2007; Collard et al., 2008). In order to manage the risk to public health, it is essential to investigate the prevalence of Salmonella infections at the farm level and counteract this problem to reduce the amount of cross contamination which can occur throughout the food chain process. Because animals are most often sub-clinically infected, the disease tends to spread easily within a herd or flock; additionally, because animals can become intermittent or persistent carriers, the prevalence of Salmonella spp. can be detected by routine sampling for bacteriological examination (Waltman et al., 1998; Gast, 2008). In this study, the prevalence of Salmonella infections in different types of flocks around Tehran was investigated and the most prevalent serogroups and serotypes was determined.

Materials and Methods
Sampling
We collected specimens from pullet, layer, and broiler flocks at different ages (Wilks et al., 2000). Each house in a farm was considered as a separate flock. In most cases, 60 freshly dropped fecal samples (not less than 1 g each) were randomly collected from each flock, each of which had at least 5,000 birds. Each set of 10 fecal samples were pooled. Anatomical samples (viscera and intestine) were also taken from dead birds in the aforementioned flocks. Totally, 1,463 of these samples were obtained from 28 flocks (Table 1).

Bacteriological culture
Isolation and identification of Salmonella spp. was performed according to standard procedures as previously
Salmonella infections in poultry flocks in . . .

Table 1: Sampling data and results obtained in bacteriological and serological tests.

<table>
<thead>
<tr>
<th>Flocks</th>
<th>Type</th>
<th>Age</th>
<th>Sample</th>
<th>No. of samples (pooled)</th>
<th>Positive samples</th>
<th>Serogroup (serotype)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1, H3</td>
<td>Layer</td>
<td>70 W</td>
<td>Feces</td>
<td>60 (6)</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>F1, H4</td>
<td>Layer</td>
<td>70 W</td>
<td>Feces</td>
<td>60 (6)</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>F1, H5</td>
<td>Layer</td>
<td>70 W</td>
<td>Feces</td>
<td>60 (6)</td>
<td>1</td>
<td>Not A to D</td>
</tr>
<tr>
<td>F1, H6</td>
<td>Layer</td>
<td>70 W</td>
<td>Feces</td>
<td>60 (6)</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>F2</td>
<td>Layer</td>
<td>44 W</td>
<td>Feces</td>
<td>20 (2)</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>F3, H6</td>
<td>Layer</td>
<td>66 W</td>
<td>Feces</td>
<td>60 (6)</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>F3, H7</td>
<td>Layer</td>
<td>66 W</td>
<td>Feces</td>
<td>60 (6)</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>F3, H10</td>
<td>Layer</td>
<td>151 W</td>
<td>Feces</td>
<td>60 (6)</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>F3, H11</td>
<td>Layer</td>
<td>152 W</td>
<td>Feces</td>
<td>60 (6)</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>F3, H12</td>
<td>Layer</td>
<td>128 W</td>
<td>Feces</td>
<td>60 (6)</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>F3, H13</td>
<td>Layer</td>
<td>94 W</td>
<td>Feces</td>
<td>60 (6)</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>F4, H1</td>
<td>Pullet</td>
<td>50 D</td>
<td>Feces</td>
<td>60 (6)</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>F4, H2</td>
<td>Pullet</td>
<td>50 D</td>
<td>Feces</td>
<td>60 (6)</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>F4, H3</td>
<td>Pullet</td>
<td>80 D</td>
<td>Feces</td>
<td>60 (6)</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>F5, H1</td>
<td>Broiler</td>
<td>8 D</td>
<td>Feces</td>
<td>60 (6)</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>F5, H2</td>
<td>Broiler</td>
<td>8 D</td>
<td>Feces</td>
<td>60 (6)</td>
<td>4</td>
<td>D (SE)</td>
</tr>
<tr>
<td>F5, H3</td>
<td>Broiler</td>
<td>11 D</td>
<td>Feces</td>
<td>120 (12)</td>
<td>9</td>
<td>C</td>
</tr>
<tr>
<td>F5, H4</td>
<td>Broiler</td>
<td>12 D</td>
<td>Liver - ceca</td>
<td>6 (2)</td>
<td>1</td>
<td>Not A to D</td>
</tr>
<tr>
<td>F5, H5</td>
<td>Broiler</td>
<td>12 D</td>
<td>Liver - ceca</td>
<td>6 (2)</td>
<td>1</td>
<td>Not A to D</td>
</tr>
<tr>
<td>F5, H6</td>
<td>Broiler</td>
<td>15 D</td>
<td>Feces</td>
<td>60 (6)</td>
<td>5</td>
<td>C</td>
</tr>
<tr>
<td>F5, H7</td>
<td>Broiler</td>
<td>15 D</td>
<td>Liver - ceca</td>
<td>6 (2)</td>
<td>2</td>
<td>C</td>
</tr>
<tr>
<td>F6</td>
<td>Broiler</td>
<td>45 D</td>
<td>Feces</td>
<td>120 (12)</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>F7, H1</td>
<td>Broiler</td>
<td>15 D</td>
<td>Feces</td>
<td>60 (6)</td>
<td>2</td>
<td>1- C 2- Not A to D</td>
</tr>
<tr>
<td>F7, H2</td>
<td>Broiler</td>
<td>15 D</td>
<td>Feces</td>
<td>60 (6)</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>F8</td>
<td>Broiler</td>
<td>1 D</td>
<td>Viscera - yolk sac</td>
<td>25 (5)</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>F9, H1</td>
<td>Broiler</td>
<td>43 D</td>
<td>Liver - ceca</td>
<td>10 (4)</td>
<td>2</td>
<td>C</td>
</tr>
<tr>
<td>F9, H2</td>
<td>Broiler</td>
<td>43 D</td>
<td>Feces</td>
<td>60 (6)</td>
<td>4</td>
<td>C</td>
</tr>
<tr>
<td>F10, H1</td>
<td>Broiler</td>
<td>1 D</td>
<td>Litter papers</td>
<td>10</td>
<td>0</td>
<td>-</td>
</tr>
</tbody>
</table>

1W = week, D = day

Described (Waltman et al., 1998). Briefly, selective enrichment of samples in selenite F at 41°C for 24 hr was followed by sub-cultivation on Salmonella-Shigella and MacConkey agar at 40-41°C (37°C for tissue samples) for 24 hr. During the next step, the suspect colonies were identified and further characterized by biochemical identification. Positive samples were kept at -70°C and liquid nitrogen for future use.

Determination of serogroups and serotypes

The slide agglutination test was carried out using Salmonella somatic O poly A-S antisera (ProLab, England), as previously described (Waltman et al., 1998). Each suspect Salmonella culture was mixed with a drop of polyvalent antisera and incubated for up to 2 min at room temperature. Positive reactors in the slide agglutination test with polyvalent antisera were then tested separately with different somatic O monovalent (O2, O4, O5, O7, O8, O9, O12) and flagellar H monovalent (H2, H6, HL, Hgm) antisera (ProLab, England) available in our laboratory in order to determine the serogroups and serotypes of the isolates. Controls were run simultaneously in parallel in all tests. All negative results were re-tested by the tube agglutination test (Waltman et al., 1998).

Results

Thirty-one Salmonella isolates were recovered out of 1,463 samples from 28 flocks. In total, 10 infected flocks were found. Nine broiler flocks out of 14 (64.2%) and one layer flock out of 11 (9%) were positive for Salmonella, but all pullet flocks were negative. One isolate was obtained from the layer flock and the other 30 Salmonella isolates were obtained from broiler flocks. The slide agglutination test determined that all isolates belonged to one of A to S serogroups, as tested with somatic O antisera poly A-S. The frequency of serogroups among 30 broiler isolates was found to be 76.6% and 13.3% for groups C and D, respectively. Three (10%) of the broiler isolates and one layer isolate did not belong to any of the A to D serogroups. In five out of 10 Salmonella positive flocks, only group C were identified. One flock only showed the presence of Salmonella group D. Because individual antisera other than A to D were not available in our laboratory, further characterization was not performed. All group D isolates were found to be Salmonella enteritidis. The results are shown in Table 1.

Discussion

Salmonellosis (the illness caused by the Salmonella bacterium) is one of the most important zoonotic diseases of birds due to its economic impact and public health concerns. It is estimated that in the United States, the annual economic cost of salmonellosis is more than 2.6 billion USD in humans (Anonymous, 2009). Recent studies in the USA have found 4.3% rate of Salmonella positive samples from meat, poultry, and egg products (White et al., 2007). Recent surveys of Salmonella infection in the UK also detected a prevalence of 11.7% (54 out of 454) and 10.7% (41 out of 382) in commercial layer and broiler flock holdings, respectively (Snow et al., 2007; Snow et al., 2008).

In the present study, we sampled 28 flocks of different types and obtained 1,463 tissue samples, of which 31 (2.11%) were found to be positive for Salmonella. Broiler flocks were considerably more implicated in Salmonella infection than layer flocks. The reason for the lower prevalence of Salmonella spp. in layer flocks could be the declining rate of colonization and fecal shedding two weeks post-infection in laying.
chickens. Consequently, it would be difficult to isolate Salmonella from pullet and layer flocks. Interestingly, it has been shown that Salmonella persists in the intestinal tracts or visceral organs of these birds for several months. Moreover, in some farms, the birds may be infected with Salmonella without showing signs of the illness, which means the presence of a sub-clinical infection in the flock. Feces from these flocks may contain Salmonella in low numbers (Hendriksen, 2003).

There are some risk factors for the prevalence and persistence of Salmonella in broiler chicken flocks (Angen et al., 1996; Namata et al., 2009). If a broiler flock is infected by Salmonella, the bacteria can persist within the flock if the prerequisites and procedures for cleaning and disinfection of the house are not adequate (Brown et al., 1992). Important factors in this respect could be if the house standards do not allow for satisfactory cleaning or if bacteria survive in or on beetles that live in the insulation of the building. The ability of Salmonella to resist desiccation allows it to survive for long periods in the environment. It has been shown that Salmonella remains in dust of ventilation filters for several months (Kim et al., 2007). Using well-trained workers for proper cleaning and disinfecting procedures will dramatically reduce such risk factors (Huneau-Salaun et al., 2007).

The exposure of flocks to external contamination is another important risk factor. In one study, there was a significantly increased risk of Salmonella contamination of the broiler flocks if there were more than three houses on a farm (Brown et al., 1992), which might increase the possibilities of transmission of Salmonella between houses. This might be due to the shorter time available for cleaning and disinfection before new stock is introduced to the farm, making it more difficult to follow the 'all in-all out' principle. Animal density might influence the infection pressure within the flock (Martin et al., 1987). Introducing only Salmonella-free chicks, such as by vaccinating the parental flocks against Salmonella, is an effective way to control vertical transmission but will not prevent the infection of birds with Salmonella from environmental sources if no additional hygienic measures are taken simultaneously. Measures to reduce horizontal transmission include ensuring Salmonella-free feed and water, effective cleaning and disinfection of the farm, the use of feed additives, applying 'all in-all out' procedures, appropriate biosecurity measures against animated or unanimated vectors, and other activities (Wales et al., 2007).

One study in Iran showed a high prevalence of group C Salmonella (S. Thompson) in poultry product samples (Soltan Dallal et al., 2007) that corresponds with our findings. Many studies have shown Salmonella group C serotypes such as S. hadar as the most common serogroup and serotype in chicken broiler flocks and chicken carcasses (Caldwell et al., 1995; Uyttendaele et al., 1998; Antunes et al., 2003). Unfortunately, due to lack of some serotype-specific antisera in our laboratory, we did not proceed with the serotyping to determine the predominant serotypes within the group C isolates.

Salmonella enteritidis (SE) has been one of the most common causes of food-borne infections in the last three decades (Velge et al., 2005). This serotype ranked among the top two most frequently isolated serotypes from human sources, as reported to the Center for Disease Control in 2006 (Anonymous, 2006). Poultry and poultry products are considered as major sources of SE infections for humans (Velge et al., 2005). Recent studies in the USA have shown that 1.3% of Salmonella isolates among samples from meat, poultry, and egg products were SE (White et al., 2007). In another US study, it was reported that 4.4% of Salmonella isolates from 51,327 broiler rinses were SE (Altekruse et al., 2006). Studies from the UK and The Netherlands’ poultry flocks have also demonstrated that SE was the predominant serotype (van de Giessen et al., 2006; Snow et al., 2007; Snow et al., 2008). In our study, all group D isolates were identified as SE, which included 13.3% of broiler isolates.

In the present study, we have shown a high incidence of Salmonella spp. in broilers, which might be a potential vehicle for the transmission of drug-resistant Salmonella spp. to humans. These findings are important for the Iranian poultry industry and public health authorities.

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References


