SEROPREVALENCE OF SALMONELLOSIS IN LAYER CHICKENS WITH ISOLATION, IDENTIFICATION AND ANTIBIOGRAM STUDY OF THEIR CAUSAL AGENTS

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ABSTRACT

Salmonellosis is a common problem in poultry farms of our country. Indiscriminate use of antibiotic to control the disease results drug resistance and limits the therapeutic possibilities in the treatment of the disease. This study was conducted during the period from January to May 2006 at Gobindapur of Dinajpur district. The present study was undertaken to determine the seroprevalence of salmonellosis in layer flocks and antibiogram study following isolation of Salmonella. A total of 225 Star cross 579 brown chickens were studied with rapid serum plate agglutination test. Liver of 200 dead birds was studied for isolation and identification of Salmonella. In vitro antibiotic sensitivity test of isolated Salmonella was performed with commercial sensitivity discs. The overall seroprevalence was recorded 23.11%. The prevalence was varied from age to age. The highest rate was 28% in above 20 weeks of age. The antibiotic study revealed that the isolates were sensitive to ciprofloxacin (80%), nitrofurantoin (100%), sulphamethoxazole/trimoxprim and amoxycillin (50%), tetracycline (60%) but resistant to penicillin-G and erythromycin. Further studies should be conducted on serotyping of the isolated Salmonella, isolation and identification of Salmonella from different feed and environmental sample.

Key words: Salmonellosis, seroprevalence, antibiogram, layer chickens

INTRODUCTION

Salmonellosis in poultry causes heavy economic loss through mortality and reduced production (Khan et al., 1998). The disease is most significant because the causal agents of the disease are transmitted vertically from parent to offspring. Vaccines form local isolates commercially still not available in the market for effective preventive measure. So, the control of the disease mainly relies on the use of antimicrobial drugs. This leads to indiscriminate use of antimicrobial drugs in poultry industry that results antibiotic resistance and limits the therapeutic possibilities in the treatment of bacterial diseases. In Bangladesh, the Salmonella infections in chicks and layer chickens must be evaluated for effective control measures of the diseases (Islam et al., 2006). Considering these facts, a study was undertaken to determine the sero-prevalence and clinical-prevalence of salmonellosis in layer flock as well as antibiotic sensitivity test following isolation of Salmonella from those flocks.

MATERIALS AND METHODS

This study was conducted during the period from January to May 2006 at Gobindapur of Dinajpur district in ‘Star Agro Poultry Farm’. The samples were collected from the birds of selected layer farm and brought to the Department of Microbiology, Dinajpur Government Veterinary College for laboratory analysis. The breed was Star cross 579 brown. The birds and their parents were not vaccinated with any Salmonella vaccine. No antibiotics were used previously in the selected flocks for the prevention of any disease. The birds were divided into three groups as group A (~8 weeks old), group B (9-20 weeks old) and group C (above 20 weeks old).

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Seroprevalence study

Sample collection
A total of 225 blood samples were collected from selected flocks (10% of total flock) from the wing vein of individual birds. Blood were collected aseptically in sterile vial with sterile 5-ml syringe. Then the samples were allowed to clotting in the syringe and kept for 1-2 hours at room temperature, after clotting, sera were separated, centrifuged and poured in sterile vials, labeled individually and stored at -20°C until used.

Rapid serum plate agglutination test
The rapid serum plate agglutination test was performed according to the procedure described by OIE (2000) with crystal violet stained Salmonella antigen (Nobilis® SP antigen). For this test 0.02 ml of antigen and 0.02 ml of chicken sera were placed side by side with a micropipette on a glass plate and was mixed thoroughly by stirring with stirrer stick followed by rocking. The results were observed within 2 minutes. In positive cases granules were formed slowly which was seen during rocking. In the absence of antibody, no such granules were formed within two minutes. All rapid serum plate agglutination test results were recorded.

Isolation and identification of causal agent

Sample collection
Liver of a total of 200 dead birds from different flock (Group A- 70, Group B-60 and Group C-70) were collected and brought to the Department of Microbiology, Dinajpur Government Veterinary College, Dinajpur. Isolation and identification of Salmonella were performed as per procedure described by OIE (2000), Merchant and Packer (1967) and Cowan (1985). Salmonella sample were isolated from the collected liver samples by sterilized inoculation loop. Primary culture was performed in nutrient agar. Subcultures were performed in blood agar, MacConkey (MC) agar and Salmonella-Shigella (SS) agar to get pure culture and cultural characteristics.

Morphological characterization
The representative Salmonella isolates from SS agar were stained by Gram’s stain (Merchant and Packer, 1967). Motility test was performed by MIU (Motility, Indole, Urea) medium according to the procedure described by OIE (2000).

Biochemical test
Several biochemical tests such citrate utilization test (using Simmons citrate agar), triple sugar iron (TSI) agar slant reaction, Indole test, Methyl Red (MR) test, Voges-Proskauer (V-P) test, Dulcitol fermentation test and Ornithine test were performed according to the procedure described by Cowan (1967), Merchant and Packer (1967) and OIE (2000).

Antibiotic sensitivity test
In vitro antibiotic sensitivity test of isolated Salmonella was performed with the standardized commercial sensitivity discs manufactured by Oxoid limited (Basingstoke, Hampshire, England). Sensitivity to antibacterial agents (antibiotic) was studied on nutrient agar and Salmonella-Shigella agar plates with erythromycin (E) 15µg, sulphanmethoxazole/ trimethoprim (SxT) 25µg, ciprofloxacin (CIP) 5µg, nitrofurantoin (F) 300µg, amoxycillin (AML) 25µg, penecilin-G (P) 10 units and tetracycline (TE) 30µg. This test was performed according to the procedure described in OIE (2000).

RESULTS AND DISCUSSION

Seroprevalence study

The overall prevalence of salmonellosis was detected as 23.11% (Table 1). This finding is supported by Alam et al. (2003) and Sikder et al. (2005) who observed 23.8% and 23.46% prevalence, respectively. But Torzolo et al. (1977), Fruknar (1987), Ghosh (1988), Waltman and Horne (1993) and Yang et al. (1996) reported 9%, 13.9%, 7.5%, 10% and 15% prevalence of salmonellosis respectively which may be due to geographical variation. The prevalence was varied in terms of age (Table 1). It indicated that seroprevalence varied with the increase of age of the birds, which supports the findings of Truong and Tieuquang (2003), Sikder et al. (2005) and Islam et al. (2006).
Salmonellosis in layer chickens

Table 1. Seroprevalence of salmonellosis in layer birds

<table>
<thead>
<tr>
<th>Groups</th>
<th>Age (weeks)</th>
<th>No. of sera sample tested</th>
<th>Positive case</th>
<th>Seroprevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>~8</td>
<td>100</td>
<td>20</td>
<td>20.00</td>
</tr>
<tr>
<td>B</td>
<td>9-20</td>
<td>75</td>
<td>18</td>
<td>24.00</td>
</tr>
<tr>
<td>C</td>
<td>&gt;20</td>
<td>50</td>
<td>14</td>
<td>28.00</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>225</td>
<td>52</td>
<td>23.11</td>
</tr>
</tbody>
</table>

Isolation of causal agent

A total of 15 (7.5%) Salmonellae were isolated from 200 liver of dead birds of selected flocks of different groups and characterized by using specific biochemical tests and Gram’s staining technique (Table 2). The rate of isolation was slightly lower than Hossain et al. (2006) but markedly lower than Islam et al. (2006). This may be due to variation of severity. It was observed that the highest rate (12.98%) of Salmonella was isolated from group C followed by group A (5.71%) and group B (3.33%). This finding is supported by Islam et al. (2006), Lee et al. (2001) and Hoque et al. (1996).

Table 2. Isolation rate of Salmonella from different groups of birds

<table>
<thead>
<tr>
<th>Group of birds</th>
<th>No. of liver sample studied</th>
<th>No. of positive cases</th>
<th>Isolation rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>70</td>
<td>4</td>
<td>5.71</td>
</tr>
<tr>
<td>B</td>
<td>60</td>
<td>2</td>
<td>3.33</td>
</tr>
<tr>
<td>C</td>
<td>70</td>
<td>9</td>
<td>12.86</td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>15</td>
<td>7.50</td>
</tr>
</tbody>
</table>

Group A = ~8 weeks, Group B = 9-20 weeks, Group C = >20 weeks

It was observed that all isolates were gram negative, small rod shaped with single or pair shaped. All the isolates were indole negative and TSI agar slant positive. Out of 15 isolates 12 were positive on dulcitol and others were negative. On the other hand 3 were ornithine positive and other 12 were negative. It indicated that the 12 (80%) isolates were Salmonella gallinarum and 3 (20%) were Salmonella pullorum. The higher rate of Salmonella gallinarum than S. pullorum is supported by Hossain et al. (2006) who detected 62.5% S. gallinarum and 25% S. pullorum.

Antibiotic sensitivity of Salmonella isolates

The antibiogram study revealed that the isolates were sensitive to ciprofloxacin (80%), nitrofurantoin (100%), sulphamethoxazole/trimethoprim and amoxycillin (50%), tetracycline (60%) (Table 3). The isolates were found resistant to penicillin-G and erythromycin but nitrofurantoin was found highly effective (100%). These findings were strongly supported by Verma et al. (1993), Anjanappa et al. (1994) and Hui and Das (2001).

Table 3. Antibiotic sensitivity of the isolated Salmonellae

<table>
<thead>
<tr>
<th>Antibacterial agents</th>
<th>Sensitive*</th>
<th>Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Sulphamethoxazole/Trimethoprim</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Penicillin-G</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Amoxycillin</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

* ≥2 mm clear zone around the antibiotic disc indicated sensitive cases.

However, for useful application of the present research findings further studies should be conducted on serotyping of the isolated Salmonellae, isolation and identification of Salmonellae from different feed and environmental sample.
REFERENCES