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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*e. 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*e. In vitro antibiotic sensitivity test of isolated *Salmonella*e 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 antibiogram study revealed that the isolates were sensitive to ciprofloxacin (80%), nitrofurantoin (100%), sulphamethoxazole/ trimeoprim and amoxycillin (50%), tetracycline (60%) but resistant to penicillin-G and erythromycin. Further studies should be conducted on serotyping of the isolated *Salmonella*e, isolation and identification of *Salmonella*e 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*e 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 preformed according to the procedure described by OIE (2000) with crystal violet stained *Salmonella* antigen (Nobilis$^{(R)}$ 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*e were performed as per procedure described by OIE (2000), Merchant and Packer (1967) and Cowan (1985). *Salmonella*e 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*e 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-Proskaur (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, sulphamethoxazole/ 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), Prukner (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).

Table 1. Seroprevalence of salmonellosis in layer birds

Groups	Age (weeks)	No. of sera sample tested	Positive case	Seroprevalence (%)
A	~8	100	20	20.00
B	9-20	75	18	24.00
C	>20	50	14	28.00
Total		225	52	23.11

Isolation of causal agent

A total of 15 (7.5%) *Salmonella*e 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

Group of birds	No. of liver sample studied	No. of positive cases	Isolation rate (%)
A	70	4	5.71
B	60	2	3.33
C	70	9	12.86
Total	200	15	7.50

Group A = ~8 weeks, Group B = 9-20 weeks, Group A = >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 *Salmonella*e

Antibacterial agents	Sensitive*		Resistance	
	No.	%	No.	%
Ciprofloxacin	8	2	80	20
Nitrofurantoin	10	0	100	00
Tetracycline	6	4	60	40
Sulphamethoxazole/Trimethoprim	5	5	50	50
Erythromycin	0	10	00	100
Penicillin-G	0	10	00	100
Amoxycillin	5	5	50	50

* ≥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 *Salmonella*e, isolation and identification of *Salmonella*e from different feed and environmental sample.

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