Occurrence, effect and control of pathogenic salmonella in some poultry farms in Abia state

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Full Length Research

Occurrence, effect and control of pathogenic *Salmonella* in some poultry farms in Abia State

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Accepted 13th November, 2011

Occurrence of pathogenic *Salmonella* species in the apparently healthy birds of organized poultry farms was investigated. The study was carried out from March to July 2010. A total of 600 samples of poultry were examined for *Salmonella* using standard bacteriological techniques. Out of the 600 samples, 4(0.66%) were positive for *Salmonella enteritidis*, 10(1.64%) were positive for *Salmonella pullorum* while 22(3.66%) were positive for *Salmonella gallinarum*. Antibiotic susceptibility testing of isolates revealed that some were highly sensitive to ceftriazone, ciprofloxin, cephalexin, gentamycin and chloramphencicol while some were resistant to co-trimoxazole, nalidixic acid, ampicillin, tetracycline and kanamycin. Disinfectant activity shows that quaternary ammonium compound reduced *Salmonella* load appreciably. Temperature did not affect the activity of the disinfectant. Haematological features of the isolates in orally infected healthy chickens showed that there was a decrease of red blood cells and haemoglobin level while the white blood cell increased. Regular screening of poultry farms for occurrence of pathogenic *Salmonella* is necessary to prevent outbreaks in the farms and possible human infections.

Key word: Poultry farm, *Salmonella*, antibiotic susceptibility, disinfectants

INTRODUCTION

*Salmonella* is one of the major bacterial agents that cause food born infection in human worldwide (Herikstand et al., 2002). The majority of salmonellosis outbreaks have been attributed to food such as eggs, chicken, beef and fish. There has been increase in turkey and chicken consumption (Buzby and Farah, 2006). Food animals are the reservoir for most domestically acquired human *Salmonella* infections and transmission from animal to human occurs through the food supply (Angulo et al., 2000). Direct contact with infected animals may also serve as a source of *Salmonella* infections (Tauxe et al; 2004., Benenson and Chin, 1995). The percentage of *Salmonella* positive birds and faecal samples on farms ranged from 5-100%. Eggs and egg containing foods are primary vehicles of *Salmonella* infection, having been implicated in 298 of the 371 known sources. *Salmonella* outbreak have been reported to the Centre for Disease Control and Prevention (CDC) from 1985 through 1999 (Patrick et al., 2004).

Typhoid fever caused by *Salmonella* organism continues to be a global health problem with an estimated 15 to 30 million cases occurring worldwide each year. The disease is common in developing countries especially Africa, Asian sub-continent, South and Central America (Miller and Peguela, 2000).

In several countries, feeds have been responsible for the infection of birds with multi drug resistance *Salmonella* (Kariuki et al., 2002). The widespread use of antimicrobial in animal resulted in drug resistant strain in human infection (Molbak et al., 1999). Emergence of *Salmonella* resistance antimicrobial strains is of world significance.

*Salmonella* in birds and humans have been of great concern to poultry farmers and health workers. Since *S. enteritidis* suddenly emerged, over the years it has caused both clinical disease on chicks and outbreak of food poisoning in humans. Once *Salmonella* infection occurs in birds, total elimination may be difficult.
Salmonella infection has been responsible for heavy losses by livestock farmers. Therefore the objectives of this study were to isolate and characterize Salmonella from the different farms, determine the antibiotics susceptibility or resistant profile of isolates as well as haematological and pathological effect of the test organism on healthy birds and screen for disinfectant sensitivity of Salmonella isolates.

MATERIALS AND METHODS
Sample collection
Six hundred (100 eggs, 100 litters and 400 faecal) samples were collected from some poultry farms in Abia State, Nigeria. The samples were collected between March and July 2010. The samples were labeled and kept in icepack and then transported to the veterinary microbiology laboratory of Michael Okpara University of Agriculture, Umudike within 4h of collection for analyses.

Isolation of Bacteria from samples
In order to isolate bacteria from the egg samples, the content of 5 eggs was properly mixed in a sterile conical flask with a sterile rod. Then 10 ml of the content was suspended in sterile 90 ml of buffered peptone water (BPW) in conical flask. The sample was incubated at 37°C for 24h and after incubation, a loopful of the inoculum was streaked on xylose lysine deoxycholate (XLD) and incubated at 37°C for 24 hr. Thereafter, suspected single colony (white or yellow with black center) were isolated from XLD agar and streaked on Salmonella Shigella agar in order to obtain pure colony culture and incubated for 24hr. For the isolation of Salmonella from litter samples, a sterile spatula was used to introduce 5g of sample into 100ml of selenite-F broth in 200ml flask. The flask was mixed by rotating and thereafter incubated at 37°C for 24hr. Subcultures were made onto Salmonella Shigella agar plate. Suspected single colony was isolated and sub cultured repeated until pure isolate was obtained. Faecal samples (from the cloaca) were collected using sterile swabs sticks. The swab sample was inoculated into selenite-F and incubated at 37°C for 24hr and then plated on Salmonella Shigella agar (SSA) and incubated at 24hr. Isolates were biochemically characterized. All isolates were serologically confirmed using Salmonella anti-sera kit (Remel Europe Ltd. UK). Isolates were sub-cultured on Salmonella-Shigella agar (Difco Laboratories, USA) plates and after 18hr of incubation; serological evaluations were carried out by slide agglutination test.

Antibiotic susceptibility testing of isolates
Antibiotic susceptibility testing of all the Salmonella isolates were evaluated using disc diffusion method in accordance with the procedures of National Committee for clinical laboratory standards (NCCLS,2001). A sterile swab was dipped in a bacterial suspension (3.3 x 10^9/cfu/ml) and smeared on Mueller Hinton agar plate. The suspension was obtained by adding sterile physiological saline into a 24hr broth culture for turbidity equivalent to 3.3 x 10^9/cfu/ml of the McFarland standardization tube. Antibiotic disc was applied onto the inoculated plate using a sterile forcep. Agar plates were incubated at 37°C for 18hr. The zone of inhibition was determined and recorded in millimeters. Escherichia coli ATCC 25922 obtained from National Veterinary Research Institute, Vom, was used as control strain for susceptibility studies. The disc was made of ten different antibiotics namely cephalaxin (30mg), co-trimoxazole (25µ), tetracycline (30µ), ampicillin (25µg), ciprofloxacin (5µg), nalidixic acid (30µg), kanamycin (30µg), ceftriazone (30µg), gentamycin (10µg) and cephaloxin (25µg).

Oral infection of healthy chicken with Salmonella isolate
Firstly, the faecal samples of 14 healthy chickens of four weeks old were screened for the presence of Salmonella and those found to be negative were used. Out of this number, 7 chickens were orally infected with 0.2ml of the Salmonella suspension (3.3 x 10^8 cfu/ml) with the aid of catheter and monitored for 21 days (3 weeks). Seven chickens served as control. One ml blood sample was collected separately from the jugular vein of each of the chickens using 23 gauge needle at 1, 2, 3, 4, 7, 14 and 21 days after infection (Kokosharow, 1998). Blood samples were also collected from the control chickens accordingly for estimation of haemoglobin (Hb), red blood cell (RBC) and white blood cell (WBC).

Estimation of Hb, RBC and WBC of chicken
Haemoglobin estimation was determined by adding phosphate buffer saline to the 20 mark Sahlis apparatus followed by addition of 20µl blood sample with pipette. The tube was tapped intermittently while distilled water was added drop by drop until it assumed the consistency of the standard. The value was then read and recorded. The experiment was conducted thrice. Red blood cell and white blood cell were determined using Neubauer hemocytometer counting chamber (Usha,1988). The cover glass was firmly fixed to the counting chamber such that there was perfect bridging of the middle line. A drop of the RBC or WBC suspension was applied to the tip against the edge of the cover slip. Caution was taken to avoid the fluid from over-flowing into the channels. The suspension was prepared by adding 20µl of chicken blood to 4ml of red blood cell diluting fluid or 0.4ml white blood cell diluting fluid. The cells in the counting chamber were counted by using x 10 objective of the microscope. The WBC and RBC were differently counted and recorded.

Blood film differential count
A smear of the blood sample was made on a clean glass
slide, dried and then stained with Leishman stain. The smear was then viewed with × 100 objective lens of a microscope for differential count.

**Disinfectant susceptibility testing**

The susceptibility of the isolates to the disinfectants was determined by the modified technique of dilution as described by Association of Official Analytical Chemists (AOAC, 1984) and Robinson (1988) for evaluation of disinfectant efficacy. The dilution ratio was 1:100 litre phenols, 0.8:100 litre gluteraldehyde and 0.5:100 litre quaternary ammonium compound. A control of the test organism was in a test tube containing distilled water without disinfectants. In each experiment, 0.5ml of the test organism suspension (10⁸ cfu/ml) was placed into 4.5ml of each of the three diluted disinfectants in 10ml tubes. The tubes were later incubated at 37°C for 48hrs and examined for turbidity.

**Effect of disinfectant against Salmonella after 12 week storage at various temperatures**

The reconstituted disinfectant of various ratio was stored at temperatures of 4°C, 20°C, 32°C and 42°C respectively. The Salmonella isolate was subjected to treatment at 4weeks, 6weeks, 10weeks, and 12 weeks (Robinson, 1988).

**Statistical analysis**

The data obtained was means of three parallel determinations and the statistical significance was assessed by analysis of variance (ANOVA, P=0.05).

**RESULTS**

The various Salmonella isolates indicated that Salmonella gallinarum was 22 (3.66%), Salmonella enteritidis was 4(0.66%) and Salmonella pullorum was 10 (1.64%) as shown in table 1. The result of the antibiotic susceptibility test showed that the isolates were more sensitive to chloramphenicol, cephalaxin, ciprofloxacin, gentamycin, and cephalaxin. There was high resistance for tetracycline, nalidixic acid, ampicillin and kanamycin (Table 2). Cephalaxin, chlorophenicol, ciprofloxacin and ceftriaxon recorded 100% sensitivity followed by gentamycin (88%). Ampicillin and nalixidic acid were the most resistance to the Salmonella with 77% and 83% resistance respectively. Out of the 10 antibiotics investigated, 16% showed intermediate sensitivity. Table 3 and 4 shows the distribution of red blood cell, white blood cell, haemoglobin level and lymphocytes in both control and infected chickens. The 7 day post infection showed the highest amount of white blood cells while the lowest was in day 21. The red blood cell and haemoglobin showed marked progressive decrease as low as 1.66 and 5.86 respectively. Decrease in the lymphocytes was also observed. Analysis of variance showed no significant difference at p<0.05. Disinfectant effect on Salmonella demonstrated that quaternary ammonium compound was better than phenol and gluteraldehyde at various concentrations as shown in figure 1. Effect of disinfectant against Salmonella after 12

### Table 1. Occurrence of *Salmonella* in different poultry farms

<table>
<thead>
<tr>
<th>Location</th>
<th>Name of farms</th>
<th>No of samples collected</th>
<th>Samples positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aba South L.G.A</td>
<td>Elijah's farm</td>
<td>80</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Monday's farm</td>
<td>50</td>
<td>3</td>
</tr>
<tr>
<td>Ossisioma L.G.A</td>
<td>Mgboema's farm</td>
<td>80</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Chijicke's farm</td>
<td>70</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Ebere's farm</td>
<td>50</td>
<td>1</td>
</tr>
<tr>
<td>Ikwuano L.G.A</td>
<td>Patrick's farm</td>
<td>80</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Ogbonna's farm</td>
<td>70</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Okezie's farm</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>9 farms</td>
<td>600</td>
<td>36</td>
</tr>
</tbody>
</table>

### Table 2. Serotypes of *Salmonella* isolates in different samples

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Litte</th>
<th>Egg</th>
<th>Cloaca</th>
<th>Total</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.gallinarum</td>
<td>6</td>
<td>0</td>
<td>16</td>
<td>22</td>
<td>3.66%</td>
</tr>
<tr>
<td>S.enteritidis</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>4</td>
<td>0.66%</td>
</tr>
<tr>
<td>S.pullorum</td>
<td>2</td>
<td>0</td>
<td>8</td>
<td>10</td>
<td>1.64%</td>
</tr>
</tbody>
</table>
Table 3. Antibiotic susceptibility pattern of *Salmonella* isolates.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Amp</th>
<th>Ceph</th>
<th>Chlo</th>
<th>Nal</th>
<th>Tetr</th>
<th>Cotri</th>
<th>Ceprof</th>
<th>Gen</th>
<th>Cerf</th>
<th>Kan</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of isolates</td>
<td>36</td>
<td>36</td>
<td>36</td>
<td>36</td>
<td>36</td>
<td>36</td>
<td>36</td>
<td>36</td>
<td>36</td>
<td>36</td>
</tr>
<tr>
<td>No. sensitive</td>
<td>6</td>
<td>36</td>
<td>36</td>
<td>6</td>
<td>10</td>
<td>10</td>
<td>36</td>
<td>36</td>
<td>36</td>
<td>36</td>
</tr>
<tr>
<td>% sensitive</td>
<td>16</td>
<td>100</td>
<td>100</td>
<td>16</td>
<td>31</td>
<td>31</td>
<td>100</td>
<td>88</td>
<td>100</td>
<td>77</td>
</tr>
<tr>
<td>No. resistant</td>
<td>14</td>
<td>-</td>
<td>-</td>
<td>15</td>
<td>10</td>
<td>13</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>% resistant</td>
<td>77</td>
<td>-</td>
<td>-</td>
<td>83</td>
<td>65</td>
<td>73</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>11</td>
</tr>
<tr>
<td>No. intermediate</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>% intermediate</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>16</td>
<td>-</td>
<td>11</td>
<td>-</td>
<td>-</td>
<td>11</td>
</tr>
</tbody>
</table>

Key: Amp: Ampicillin; Ceph: Cephalaxin; Chl: Chloramphenicol; Nal: Nalidixic acid; Tetr: Tetracycline Cotri: Cotrimoxazole; Ceprof: Ciprofloxacin; Gen: Gentamycin; Cerf: Ceftriazone; Kan: Kanamycin. Zone of inhibition = 4 mm sensitive Zone of inhibition = 2 mm resistant Zone of inhibition = 3 mm intermediate

Table 4: Red and white blood cells of chickens orally infected with *S. gallinarum*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>7</th>
<th>14</th>
<th>21</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC</td>
<td>2.18±0.60</td>
<td>2.22±0.25</td>
<td>2.4±1.0</td>
<td>2.86±1.18</td>
<td>3.24±1.16</td>
<td>5.42±0.10</td>
<td>2.12±0.42</td>
<td>1.86±0.14</td>
</tr>
<tr>
<td>RBC</td>
<td>3.64±0.11</td>
<td>3.42±0.24</td>
<td>2.88±22</td>
<td>2.74±20</td>
<td>2.22±18</td>
<td>1.66±18</td>
<td>2.04±14</td>
<td>1.66±0.16</td>
</tr>
<tr>
<td>Hb</td>
<td>9.52±0.42</td>
<td>9.32±0.32</td>
<td>8.98±54</td>
<td>8.26±58</td>
<td>6.28±60</td>
<td>5.86±56</td>
<td>5.80±54</td>
<td>5.86±0.56</td>
</tr>
<tr>
<td>Bandnuclei</td>
<td>2.86</td>
<td>3.16</td>
<td>4.80</td>
<td>4.82</td>
<td>7.12</td>
<td>8.20</td>
<td>9.32</td>
<td>6.82</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>73.10</td>
<td>66.40</td>
<td>58.84</td>
<td>56.40</td>
<td>54.10</td>
<td>56.00</td>
<td>55.00</td>
<td>70.00</td>
</tr>
</tbody>
</table>

Values were calculated from seven individual chickens and expressed as means ± SEM.

Figure 1: Effect of disinfectant on Salmonella at various concentrations

DISCUSSION
The occurrence of *Salmonella* in poultry farms was investigated. *Salmonella* remains the main food borne bacterial disease in human (Barrow, 2000). Many of the world outbreaks are related to food containing poultry products (Rodrigue et al., 1990; Cox, 1995). In this study, out of 600 samples screened for *Salmonella* 36 were...
positive for *Salmonella*. Twenty-two (3.66%) isolates were obtained from environmental or litters, 10 (1.64%) from cloaca samples while 4 (0.66%) were from egg samples. Similar result was obtained by Lee *et al.*, (2001). The species of *Salmonella* isolated included *S. pullorum*, *S. gallinarum* and *S. enteritidis*. The antibiotic sensitivity test carried out showed that most of the isolates were sensitive to ceftriazone, ciprofloxacin, gentamycin, cephalexin and chlorphenicol. Nalidixic acid, ampicillin, tetracycline, kanamycin and co-trimoxazole showed resistance. This agrees with study by Van *et al.*, (2005). The sensitivity of cephalexin, chlorphenicol, ciprofloxacin, and cerfrizone was 100% against *Salmonella*, while gentamycin had 88% sensitivity against *Salmonella*. The most resistant of the antibiotics under consideration is nalidixic acid which had 83% resistance.

The chickens infected with the isolate showed that WBC was significantly different from that of the uninfected controls. There was a progressive decrease in the red blood cell as well as haemoglobin from day 1 to 7 of post infection. This could probably be attributed to the virulence activity of the *Salmonella* which destroys the red blood cell and the haemoglobin. However the increase in WBC could be due to the response of the chicken to the infectious agent. The decrease observed in the white blood cell from day 14 to day 21 is suggestive of the inability of the white blood cell to overcome the infective *Salmonella*. Similarly, there was a marginal decrease in the lymphocytes.

Disinfectant on *Salmonella* demonstrated that quaternary ammonium compound was more effective than phenol and gluteraldehyde at various concentrations. Similar finding was reported by Anderson *et al.*, (1997). Possible explanation for the difference in action could be the ingredients of each of the disinfectants. The major goal of disinfectant usage is to reduce the risk of *Salmonella* load in farms and other microbes. However, inefficiency of the disinfecting process may contribute to the maintenance of *Salmonella* at farms (Davies and Wray, 1995). A great number of disinfectants are used in poultry industry. Quaternary ammonium compound may be more effective in the management of poultry farms against *Salmonella* when used in the right concentration. Disinfectants stored at 4°C, 20°C, 32°C and 42°C for 12 weeks did not reduce the efficacy of the disinfectant as earlier reported by Dvorak (2005). The right concentration of disinfectant, the right quantity of water, application method and correct dilution should be fully considered (Prince *et al.*, 1991).

This study showed the occurrence of pathogenic
Salmonella species in poultry farms. The drugs of choice in elimination or reductions of this organism are chlorphenicol, gentamycin, cephalixin, ciprofloxacin and ceftriazone. Quaternary ammonium compound is more efficient in reducing microbial load. Therefore proper disinfection of poultry farms, appropriate antibiotic management and adequate cooking of chicken, their eggs and egg products for human consumption to avoid being infected are highly recommended.

REFERENCES