ISOLATION AND ANTIBIOGRAM OF SALMONELLA SPECIES FROM WATER AND POULTRY FEED IN SELECTED COMMERCIAL FARMS IN ZARIA, NIGERIA

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ABSTRACT

Salmonella species remain a global issue in the poultry industry. Major sources of salmonella to poultry include contaminated feed, water and environmental reservoirs. This research was conducted to evaluate presence and antibiotic susceptibility of Salmonella species from poultry feed and water in selected poultry farms in Zaria. Ninety four (94) each of randomly selected feed and water samples were tested for the presence of Salmonella spp. Samples were collected from randomly selected feed sources using sterile polyethene bags while water samples from the primary sources, reservoirs and drinkers were collected using sterile universal bottles. Samples were cultured using selective isolation method with prior enrichment. Suspected isolates were identified and characterised using conventional biochemical methods. Eight each of water and feed samples were positive for Salmonella. All the Salmonella isolated from feed were from flocks kept on deep litter. Three Salmonella isolates were from commercial and five from self milled feeds on-farm. Isolated Salmonella organisms showed highest susceptibility to ciprofloxacin but resistant to commonly used antibiotics. Feed and water may serve as important means of Salmonella dissemination; they may also serve as critical control points for Salmonella in to poultry flocks.

Key word: Salmonella, poultry, feed, water, Zaria.

INTRODUCTION

The development of the poultry industry was described as the fastest means of bridging the protein deficiency gap in most developing nations. This has concomitantly required trade in hatching eggs, day old chicks; feed concentrates and additives from various controlled and uncontrolled local and international sources (Al-Nakhli et al., 1999; Apantaku, 2006). Poultry immediate micro-environment includes the housing, the feed they consume, climatic and management factors which are reported to have effects on the performance of the birds (Apantaku, 2006). From various studies conducted, poor performance, feeds and feeding, diseases and pests have been tagged major constraints to poultry production in Nigeria. Infections due to Salmonella spp. continue to cause global concern of morbidity and mortality in human and animals as well as significant economic losses. Salmonellosis occurs mainly by a faeco-oral route through the consumption of contaminated feed and water (WHO, 2010). Sources of Salmonella infections into poultry farms include contaminated feed and feed ingredients, water, equipments, personnel, rodents and hatchery related unhygienic activities (Ricardo and Brazil, 2012; Roth, 2012; Bryetembach, 2004; Al-Nakhli et al., 1999). Timely identification and serotyping of Salmonella
from clinical samples enables early outbreak detection while detection in contaminated feed and water prevents entry into food chain (WHO, 2006). Salmonellosis is endemic and a major threat to commercial poultry farming in Nigeria (Ogunleye et al., 2006; Ibe, 1998). Two major species of *Salmonella* are worldwide distributed which principally affect chickens and turkeys. *S. pullorum* is an acute systemic disease of chicks associated with high mortality rate. *S. gallinarum* is acute to chronic septicaemic disease of mature birds, also associated with mortality. Both are responsible for serious economic losses of death and decreased egg production (Breytenbach, 2004). Despite concerted efforts of test and slaughter to eradicate *salmonella* from poultry flocks, reports are still common in especially backyard poultry in advanced and developing countries (Breytenbach, 2004; Alnakhli, 1999). Rodents destructive roles on infrastructures, feed and feed ingredients are well known to farmers but their role as especially multi-drug resistant salmonella reservoir have been underestimated (Meerburg, 2007). Therefore, major challenges to tropical poultry production include quality feed, flock health and environmental control (Roth, 2012; Bale et al., 2002; Ibe, 1998). Hence effective Salmonella control on the farm must be primarily based on preventing *Salmonella* from entering and spreading in a farm (Ricardo and Brazil, 2012; Roth, 2012).

There is suppose to be zero tolerance to Salmonella infections in genetic stocks meant for breeding, hence positive flocks are culled and vaccination and treatment not permitted (Breytenbach, 2004). Antibiotic resistance by microorganisms especially *Salmonella* is a global issue (Jacob and Archer, 1991; Roth, 2012). Therefore, treatment of salmonellosis with antibiotics like sulphonamides, tetracyclines, aminoglycosides, or quinolones had only been successful in reducing mortality and temporarily improving clinical recovery (Saidu et al, 2010; Breytenbach, 2004; Okoli, 2004), as multi-drug resistant *salmonella* had developed in recent years to which no antibiotic appears to completely eliminate *Salmonella* infections in flocks (Breytenbach, 2004).

So far the most important single source of infection for fowl typhoid and pullorum disease remain infected birds. The bacteria are transmitted from generations via eggs or by direct contact or mechanical transfer by people, equipment, feed or water. Recovered birds become carriers perpetuating the disease especially in multi-aged layer farms so that the disease is continuously maintained with susceptible batches of pullets (Breytenbach, 2004). Most human cases of salmonellosis are food borne but infections can be acquired from farm environments and animal related settings as clinically infected animals exhibit a higher prevalence of shedding the organism into the environment over a long period of time. *Salmonella* has repeatedly been isolated from mice, rats and pet rabbits indicating their possible reservoir roles (Hoelzer et al., 2011). Since *Salmonella* reservoirs have continued to huddle efforts in salmonella elimination from poultry flocks (Ogunleye et al., 2006), and hazard analysis and critical control points programs adapted in most advanced nations had been difficult or even impossible to implement in most developing nations (Uwaezuoke et al., 2000), salmonellosis has become endemic and unsuccessfully treated in many developing countries. The better understanding of poultry and human *Salmonella* sources and patterns of distribution will enable significant improvement in their control strategies.

**Materials and Methods.**

The study was conducted in Zaria, Kaduna state, Nigeria with agro-climatic conditions typical of savanna vegetation located between longitude 11°07N and latitude 7°44E. The poultry industry in this area like in other parts of the country is very fast developing but dominated by sector III (FAO classification) poultry farms (Brandenburg, 2008). Important water sources to poultry farms include wells, public boreholes and pipe borne, and harvested rain water.

**Sampling procedure**

A total of 94 feed samples were collected (5 each from commercial feed outlets, toll-milling stands and self-milled feeds and 79 feed samples cutting across the 3 sources but collected from feeders in poultry houses) between the months of December 2010 to July 2011. The commercial feed brands were vital (VF) Hybrid (HF), Livestock (LF), Rebson (RF) and PLS (PF) feeds which included grower, layer, finisher, starter and chick mash. 10 grams of feed was collected midway into the sterile polythene bags, water collected using sterile universal bottles. 100 ml of water was collected directly from primary source (bore hole, well and tap) and from secondary sources (reservoirs and drinkers) and transported to the laboratory within 2 hours of collection.

**Isolation and Identification of Salmonella**

To each 10g feed type was added 90 ml of sterile one broth salmonella for enrichment in a stomacher and thoroughly mixed for 1 min. The homogenates was then poured into sterile conical flask and incubated at 37 °C for 24 h. A loopful of the thoroughly shaken homogenates was streaked on XLD plate to ensure isolated colonies which were then incubated 37°C for 24 h. Colonies appearing pinkish with or without black centers on XLD
were picked and inoculated into Triple Sugar Iron (TSI) agar and Urea agar. Colonies that gave reactions suggestive of Salmonella i.e. alkaline/acid with or without gas and hydrogen sulhide on the TSI, urease negative were kept at 4°C on Nutrient agar (NA) slants until further characterization.

Biochemical Characterization of Isolates

This was done based on standard techniques in which all isolates that gave reactions typical of Salmonella were considered to belong to the genus Salmonella. The reactions typical of Salmonella were indole negative, methyl red positive, Voges-Proskauer negative, citrate positive, motile in motility medium, produce H2S, nitrate positive, lysine decarboxylase positive, oxidase negative, ferments glucose, manitol, ducitol, and maltose but fail to ferment lactose, sucrose, adonitol and raffinose.

Evaluation of the *in vitro* susceptibility of the isolates to antimicrobial agents

All the biochemically confirmed *Salmonella* isolates were tested for anti-microbial susceptibility to 8 antimicrobial agents with the following disc contents: Chloramphenicol, CH (30 µg), gentamycin, GN (10 µg), norfloxacin, NO (10 µg), ciprofloxacin, CP (10 µg), tetracycline TE (30 µg), amoxicillin clavulanate, AU (30 µg), ampicillin, AM (30 µg), nalidixic acid, NA and nitrofurantoin, NF (30 µg), by the disc diffusion method described by Bauer, Kirby, and Turck (1966) and based on recommendations of CLSI (2006). The outcome of the susceptibility testing was qualitatively recorded as sensitive or resistant.

Statistical analysis

All data collected were analysed for incidence of isolation rate and their antimicrobial susceptibility profile using simple descriptive statistics.

Results and Discussion

Microbial analysis

Salmonella is an enteric pathogen that is shed predominantly in faeces making faecal pollution the main source of feed and water contamination (Roth, 2012; Breytenbach, 2004; Wray et al., 1999). Of the 188 feed and water samples processed, 51 (27%) *Salmonella* suspects were obtained, 34 (18%) from feed samples and 17 (9%) were from water samples. Isolates were subjected to further biochemical tests. All the biochemical rate was seen in houses that used the pelletized type reactions were noted and each suspect was classified based on its biochemical reaction. Of the 51 suspects, 8 (4.3%) (4 each from feed and water) isolates were confirmed to be *Salmonella* from farms B, C, D, M. Samples of commercial poultry feeds gotten from retailer shops were not positive for *Salmonella*. 4(11.76%) isolates showed typical of *Salmonella* appearance from the 34 suspected feed samples. The 17 suspected water samples yielded 4(23.53%) isolates that showed typical *Salmonella* appearance. The remaining suspects were however unclassified.

*In vitro* susceptibilities of the *Salmonella* isolates to 8 antimicrobial agents

All the 8 positive *Salmonella* subjected to disc diffusion method showed high sensitivity to ciprofloxacin as indicated by the greatest diameter of the zone of inhibition followed by Gentamycin. However, Norfloxacin, Tetracycline, Amoxicillin, Ampicillin, Nitrofurantoin, and Chloramphenicol, were all found to be resistant (plate 1). The result of the *salmonella* anti-microbial resistance profile in this study has two major concerns; first the isolates are multi-drug resistant implying commonly used, cheap and readily available antibiotics in the study area will not be effective against salmonellosis of both poultry and probably humans, secondly, norfloxacin resistance is of concern because it belongs to the fluro-quinolones which constitute drug of choice of human and poultry salmonellosis. This may be responsible for salmonellosis that is refractory to treatment in both human and poultry. Since the isolates were not characterised and since zoonotic salmonellae (*S. enteritidis* and *S. typhimurium*) have been recovered in many organic livestock farms and poultry products (Meerburg and Kijlstra, 2007; Al-Nakhli, 1999; Henzler and Optitz, 1992), this study is of public health significance.

Environmental and farm practices influencing the isolation rates of *salmonella*

The influence of routine farm management practices on the occurrence of *Salmonella* was determined using the information obtained from structured questionnaires, and each factor obtained was correlated with the incidence of *Salmonella* isolates

All the 8 isolates were found in houses that raised birds on deep litter system (Table 1). Flock sizes of between 250-500 birds had the highest isolation rate of 4%, while flock sizes of less than 250 and greater than 500 had isolation rates of 2% each. 5% isolation rate was found in houses that used mash type of feed while 3% isolation feed (Table 2). *Salmonella* is an enteric pathogen that is
Plate II: Sensitivity test result CIP (ciprofloxacin), N (neomycin), GN (Gentamicin), AM (Ampicillin), ORF (Orfloxacin), C (Chloramphenicol), AX (Amoxicillin), TE (Tetracycline)

Table 1: Distribution of salmonella isolates based on poultry management system

<table>
<thead>
<tr>
<th>Management system</th>
<th>Salmonella positive</th>
<th>Salmonella negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deep litter</td>
<td>8</td>
<td>86</td>
<td>94</td>
</tr>
<tr>
<td>Battery cage</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Flock size</th>
<th>Salmonella positive</th>
<th>Salmonella negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;250</td>
<td>2(2.13%)</td>
<td>17(18.09%)</td>
<td>19(20.21%)</td>
</tr>
<tr>
<td>250-500</td>
<td>4(4.26%)</td>
<td>44(46.81%)</td>
<td>48(51.06%)</td>
</tr>
<tr>
<td>&gt; 500</td>
<td>2(2.13%)</td>
<td>25(26.60%)</td>
<td>27(28.72%)</td>
</tr>
</tbody>
</table>

shed predominantly in faeces making faecal pollution the main source of feed and water contamination (Roth, 2012; Breytenbach, 2004; Wray et al., 1999). Therefore, the deep litter system of poultry management becomes a leader in the sustenance and transmission of salmonella. Higher incidence of salmonella in mash type feed seen in this study agrees with its low incidence in heat treated pelletized type (Breytenbach, 2004). Notwithstanding, heat treatment may not always protect feed against recontamination during transportation and storage (Roth, 2012). From this study it may deduced that bacterial contamination of feed occurred since all the salmonella isolates were from on-farm feeds and none from commercial or toll mill feeds at their various outlets. Further, it has been documented that animal-derived protein and oil seed meal are the major sources among feed materials through which Salmonella may be introduced to industrial compound feed and feed mills (Roth, 2011).

Based on source of poultry drinking water, 3% Salmonella rate of isolation was found in houses using borehole water, 4% isolates were found in houses using well water and 2% isolates were found in houses using pipe-borne water (Table 3). 5% salmonella isolation rate
was for poultry houses that reserved water before use, while 3% isolation rate was recorded in houses that did not reserve water before use. Houses that never treated poultry drinking water before use had the highest isolation rate of 7%, while only 1% isolate was found in houses that regularly treated poultry drinking water before use. It is alarming to observe all sources of drinking water to poultry which were also same sources to humans to contain multidrug resistant salmonella. It is an established fact that antimicrobial resistant bacteria or antimicrobial resistance genes can be transmitted via feed or water (Roth, 2012). In this study, the highest incidence in well water may not be unconnected to unrestricted access by common environmental, livestock and human contaminants. This is further supported by higher incidence in sources of water reserved before use in poultry farms, while in water routinely treated before use had very low salmonella incidence. In fact Salmonella can persist and grow in water given the right conditions and that the diversity and concentration of Salmonella increases as temperature rises. Therefore, a better approach to Salmonella control in farms will also involve the microbiological test of water especially if the source of water is a well or river (Roth, 2012). Salmonella isolation rate of 6% was recorded in poultry farms that never used protective clothing; however, 2% isolation rate was seen in poultry farms that used protective clothing (Table 4). Houses where foot bath was not being used gave 7% Salmonella isolation rate but only 1% isolation rate was seen in houses that foot bath was functional (Table 4). Furthermore, the uses of protective clothing and functional footbaths on-farms, and the fencing of farm premises gave lower incidences of salmonella when studied. It is on record that contamination of the farm environment can be a source of Salmonella infection and that improving farm personnel hygiene like washing of hands, cleaning of overalls and disinfection of boots before entering farms and the control of visitors had decreased Salmonella prevalence (Roth, 2012).

Isolation rate of 7% was established in poultry farms that had rodents in their farm premises while farms free of rodents had 1% isolation rate (Table 4). Poultry farms that were fenced had 2% salmonella isolation rate in comparison to 6% salmonella isolation rate from farms that were not fenced. Little or no attention has been given to farm management practices and rodent control in poultry farms. It is obvious from this study that farm management practices ranging from choice of production system, stocking density, routine hygienic practices and rodent control had significant influences on salmonella persistence on farms.
Organic animal production systems offer ideal environment for rodents to thrive. Rodents for decades have been known for their role as reservoir of salmonella organisms (zoonotic salmonella inclusive) that can contaminate feed, water and environment and transmit organisms to poultry (Henzler and Optitz, 1992; Meernburg and Kijlstra, 2007). All vertebrates including pests are susceptible to the Salmonella infection and therefore rodents have often been implicated as potential sources of Salmonella (Roth, 2012).

CONCLUSION

Timely identification of Salmonella from clinical samples, contaminated feed or water prevents Salmonella entry into food chain. There is therefore the need to institute salmonella monitoring in poultry farms to reduce incidence of poultry and human salmonellosis.

REFERENCES