African Journal of Microbiology Research Vol. 4(9), pp. 796-800, 4 May, 2010
Available online http://www.academicjournals.org/ajmr
ISSN 1996-0808 © 2010 Academic Journals

Full Length Research Paper

Observation on the occurrence and transmission pattern of *Salmonella gallinarum* in commercial poultry farms in Ogun State, South Western Nigeria

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Accepted 14 April, 2010

Reports of heavy mortality in three commercial layer poultry farms and two meat-type turkey farms in Ogun State were received separately between March and December, 2008 at the Veterinary Teaching Hospital (VTH), University of Agriculture, Abeokuta, Nigeria. We investigated the cause of mortality, isolated and serotyped the aetiological agent, and carried out antimicrobial susceptibility testing. All five isolates serotyped were *Salmonella* Gallinarum and showed identical patterns of resistance and susceptibility to all antimicrobials used. The occurrence of ciprofloxacin resistance in all five isolates is of importance since fluoroquinolone resistance has implications for both veterinary and human therapy and the abuse of such medications in poultry could result in the emergence of resistant zoonotic organisms.

Key words: Occurrence, transmission pattern, *Salmonella gallinarum* poultry farms.

INTRODUCTION

Poultry is an essential component of the Nigerian economy and a good source of high quality protein for the ever growing population of Nigeria. In livestock production, poultry occupies a prominent position in the provision of animal protein and this account for about 25% of local meat production in Nigeria. In Nigeria, apart from its contribution in feeding the growing population, it provides income for small-scale farmers. Despite the enormous potentials the poultry industry holds, it regularly suffers from major limitations including diseases, poor husbandry, low egg production, poor chick quality, poor and low performing breeds, poor weight gain/ feed conversion, feeding and management problems and lack of capital (Eekeren et al., 1995).

Fowl typhoid (FT) was first reported in England by Klein as 'infectious enteritidis' in 1888 and was named Fowl Typhoid (FT) in 1902 by Curtice in Rhode Island (Curtice, 1902). Although the disease has largely been eradicated from modern poultry in the developed countries of the world, it has increased in incidence in most developing countries of South America, Asia and Africa (Onunkwo, 1981; Bouzoubaa and Nagaraja, 1984). In Africa, the disease has been reported in a considerable number of countries like Nigeria (Sa'idu et al., 1994), Tanzania (Mtie and Msami, 1996), Uganda (Ojok, 1993), Zambia (Sharma et al., 1991), Libya (Hamid and Sharma, 1990) and Senegal (Arbelot et al., 1997).

FT disease is caused by the bacterium *Salmonella enterica* subsp. *enterica* serotype Gallinarum (Jordan and Pattison, 1992), a facultative anaerobic gram negative rod belonging to the family enterobacteriaceae. *S. enterica* subsp. *enterica* serovar Gallinarum is also composed of two distinct biovars under the serogroup D1, *Gallinarum* and *Pullorum*, both of which are host-adapted/specific for poultry (Shivaprashad, 2003). The organism grows optimally between 35 - 37°C, catabolises a variety of carbohydrates into acid and gas, utilizes citrate as the sole carbon source, produces H_2S and decarboxylates lysine and ornithine to cadaverine and

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putrescine, respectively. FT has a wide distribution around the world and transmission is either by horizontal or vertical route (Berchieri et al., 2001). Infected breeding flocks are mainly associated with vertical transmission of *Salmonellae* to their progeny (Zancan et al., 2000). Chickens typically get exposed to the disease at very young ages without any morbidity or mortality, but can harbour infection until they come into lay and can then produce infected egg and/or progeny (Wigley et al., 2001). Horizontal transmission can occur following ingestion of food or water already contaminated with faeces of clinically infected birds or carriers, cannibalism, presence of dead birds, wild birds; attendants (through hands, feet and clothes) as well as rodents and vehicles (Jordan and Pattison, 1992).

In Europe, transmission by the poultry red mite is a very important part of the epidemiology (Parmar and Davies, 2007). Although, a tentative diagnosis of FT can be carried out by considering flock history, clinical signs, mortality and lesions (Barrow et al., 1992), a definitive diagnosis requires isolation, genus identification and identification of serovar and biovar (*S. enterica* subsp. *enterica* serovar Gallinarum biovar Gallinarum). With the continuous expansion of poultry farming in Nigeria, fowl typhoid caused by *Salmonella* Gallinarum has also gained ground as a major disease of poultry. Salmonellosis involving *S.* Gallinarum is a bacterial disease that may cause heavy economic losses in poultry through mortality and reduced production (Khan et al., 1998). A recent report put the prevalence of fowl typhoid in sampled flocks in Kaduna state, northern Nigeria at 18.4% (Mbuko et al., 2009).

Prevention of Salmonella infection is important for the profitable expansion of poultry industry in Nigeria. Effective prevention and control measures cannot be undertaken unless the status of the disease and epidemiology are well elucidated. Therefore, the present study was undertaken to investigate the occurrence of FT by isolating, identifying and serotyping *S.* Gallinarum, as well as testing its susceptibility to widely used antimicrobials. This study also seeks to highlight uncertainties in the transmission pattern of the disease in the study location, which if not resolved might impede understanding of the epidemiology of the disease.

MATERIALS AND METHODS

Study area

The study was carried out in Ogun state, Nigeria. Ogun State is one of the thirty-six states in Nigeria. It lies within latitudes 6.2° and 8.0° north of the equator and longitudes 3.0° and 5.0° east. The state is bounded in the west by the Republic of Benin, on the South by Lagos state, on its east by Ondo state and on its north by Oyo and Osun states. It had in year 2003, human population of 3,728,098. Certain regions within the state have the highest concentration (density) of poultry population in Nigeria.

Sample collection

Reports of mortality in three commercial poultry layer farms and two meat-type turkey farms around Ogun State were made independently between March and December 2008. Similar outbreaks of unconfirmed aetiological agents were reported in other parts of the State within the same period. Sources of the birds could not be ascertained since several of the farms obtain their birds through unlicensed distributors. A typical history of somnolence, loss of appetite, droopy wings, weakness, palor, greenish–yellowish diarrhoea and sudden death without premonitory signs were reported by the farmers.

Visits were made to the various farms at different times of outbreaks and carcasses were collected. Carcass samples collected were transported to the laboratory in sealed containers on wet ice (4°C). Carcasses were opened aseptically in the post-mortem room and were investigated for lesions suggestive of sudden death in adult birds. Congested tissue samples (lung, liver, spleen, heart, ovaries and intestine) were harvested for bacteriological investigation(s).

Isolation and identification of *Salmonella* spp

Salmonella spp was isolated in accordance with standard methods (Waltman et al., 1998). Briefly described, whole samples of lung, liver, spleen, heart and ovaries were sectioned aseptically; parenchyma portion of internal organs (5 g) originating from the same farm were pooled together and aseptically inoculated into 50 ml of Rappaport-Vassiliades broth (Oxoid, Basingstoke, UK) for selective enrichment and incubated at 37°C for 24 h. Using a sterile wire loop, a loopful of each incubated broth culture was then inoculated onto Brilliant Green Agar (BGA) (Oxoid, Basingstoke, UK) supplemented with Sulphamandelate (Oxoid, Basingstoke, UK). The plates were examined for typical colonies of Salmonella, that is, pink colonies surrounded by a red medium (Doughlas et al., 1998). Catalase and Oxidase tests were performed according to previously described procedures (Shivaprasad, 2003). Motility test was performed using the hanging drop method (Barrow and Feltham, 2003).

Indole test was carried out by using Kovac's reagent according to previously described method (Cheesbrough, 2000). Presumptive Salmonella colonies were inoculated into triple sugar iron agar (TSI) by streaking colonies of the pure culture on the slant surface and stabbing the butt centrally. TSI tubes were then incubated for 24h at 37°C. Salmonella organism produced an alkaline slant (red) over an acid butt (yellow) reaction with hydrogen sulphide (H_2S) Production. *S.* Gallinarum does not form gas in TSI agar (Doughlas et al., 1998). Additional differentiation of Salmonella from other organisms was accomplished by biochemical analysis. Isolates were also tested for urease production, methyl red as well as citrate utilization. Isolates that were urea hydrolysis negative, methyl red positive, indole negative, citrate positive and non motile were considered to be *Salmonella* (Cox and Williams, 1976). Presumptive Salmonella colonies were then inoculated into nutrient agar slopes and shipped to Veterinary Laboratories Agency-Weybridge, UK for serotyping according to the Kauffmann-White Scheme (Grimont and Weill, 2007).

Antibiogram

Antimicrobial susceptibility testing was done at the Veterinary Laboratories Agency-Weybridge, UK, using a panel of 16 therapeutic antimicrobials in a disc diffusion method (Jones et al., 2002).

Table 1. Antimicrobial resistance pattern of 5 *S. gallinarum* Isolates.

Salmonella nomenclature	5010-09 Gallinarum	5011-09 Gallinarum	5012-09 Gallinarum	5013-09 Gallinarum	5014-09 Gallinarum
Antimicrobial	**Antimicrobial sensitivity pattern***				
Nalidixic acid	0R	0R	0R	0R	0R
Streptomycin	0R	0R	0R	0R	0R
Ciprofloxacin	10R	12R	11R	10R	16R
Tetracycline	27S	23S	22S	26S	25S
Neomycin	20S	21S	19S	19S	20S
Ampicillin	31S	31S	34S	31S	34S
Furazolidone	33S	31S	32S	34S	19S
Ceftazidime	36S	35S	39S	35S	38S
Sulphamethoxazole/trimethoprim	30S	31S	31S	30S	36S
Chloramphenicol	28S	29S	29S	28S	30S
Amikacin	29S	31S	29S	29S	31S
Amoxycillin/ clavulanic acid	29S	38S	30S	34S	36S
Gentamicin	30S	31S	33S	31S	32S
Sulphonamide compounds	31S	29S	28S	31S	27S
Cefotaxime	38S	46S	39S	41S	45S
Apramycin	24S	22S	26S	23S	24S

*R represents resistant; S represents sensitive; The numbers represent the measured zones of inhibition in millimetre; for example, 31S means the zone of inhibition is 31 mm on agar gel and the organism is resistant to the antimicrobial.

RESULTS

Of the five isolates cultured, 5 (100%) were positive for *Salmonella*. The 5 positive isolates were all *S. enterica* subsp. *enterica* serotype *gallinarum*. The isolates were labelled 5010-09 Gallinarum to 5014-09 Gallinarum in this work. One (20%) isolate came from the intestine while the 4 (80%) isolates were obtained from other internal organs (pooled visceral tissues).

All the five isolates of *S.* Gallinarum showed exactly the same antimicrobial sensitivity pattern. All five (100%) isolates tested against antimicrobial agents were resistant to nalidixic acid, streptomycin and ciprofloxacin (Table 1), and all the five (100%) were sensitive to tetracycline, neomycin, ampicillin, furazolidone, ceftazidime, sulphamethoxazole/trimetoprim, chloramphenicol, amikacin, amoxicillin/clavulanic acid, gentamycin, sulphonamide compounds, cefotaxime and apramycin (Table 1).

DISCUSSION

The isolation of *S.* Gallinarum from clinical cases in five different farms (3 chickens and 2 turkeys) scattered around Ogun State over a period of time suggests a high incidence of *S.* Gallinarum in the state. Although not all of the sources of the birds are known since some of the farmers source their birds from unlicensed distributors whose sources of birds are most often unknown. Ogun state is one of the highest poultry farming states in Nigeria. However, the high incidence of fowl typhoid is not unconnected with the role of infected hatching eggs as well as contamination spread by attendants, feed dealers, farm-gate buyers and visitors who move from farm to farm disseminating the pathogens through their foot-wear, hands and clothing. While there is paucity of published information to support the existence of

vertical transmission of S. Gallinarum in Ogun State, Nigeria, not much is also available in literature to substantiate horizontal transmission of *S.* Gallinarum in Ogun State poultry farms. However, Mohammed et al. (2009) has earlier suggested the existence of horizontal transmission of *Salmonella* Kentucky and *Salmonella* Hadar at some poultry hatcheries in Plateau State, Nigeria; more work need to be done to determine the transmission pattern of *S.* Gallinarum. Infected birds (reactors and/or carriers), contaminated poultry industry (processing plant and feed mill) and poultry feed, water or litter have been known to transmit and/or perpetuate *S.* Gallinarum (Shivaprashad, 2003) as may be the case in Ogun State. Similarly, contaminated crates and trucks may be involved in the transmission of this disease (Christensen et al., 1994). Wild birds, rodents, flies and especially red mites (Valiente et al., 2009) may also be important mechanical spreaders of the organisms, although this is yet to be proved in Nigeria (Shivaprashad, 2003).

Antimicrobial susceptibility pattern of the five *S.* Gallinarum isolates in this study emanating from different poultry farms in different locations of Ogun State in two different species (Chickens and turkeys) presented exactly the same antimicrobial susceptibility pattern. The use of antibiogram in the conventional typing of *Salmonella* based on the expression of phenotypic traits is well documented (Wachsmulth, 1986), and in this study, suggests the possibility of all five isolates originating from a common source and a widespread dissemination of the bacterium.

In this study, resistance to nalidixic acid and ciprofloxacin was common in all five isolates, an indication that the Salmonella serotype isolated has accomplished the first step in quinolone resistance development (single-point gyrase gene). The tendency may be increasing resistance to fluoroquinolones. This agrees with earlier findings on the resistance of Salmonella including *S.* Gallinarum, found predominantly in poultry as been resistant to quinolones (Threlfall et al., 1997; Piddock, 2002). Quinolones as a member of the large family of antimicrobials have been widely used due to the advantage of oral administration and potency. Quinolones have been known to be highly successful in the treatment of salmonellosis, including infections caused by multi-resistant Salmonella serotypes (Barness et al., 1990; Reina et al., 1993). The resistance observed with ciprofloxacin should attract great interest in view of the fact that ciprofloxacin is a drug of choice in the treatment of invasive salmonellosis both in animals and humans, even though *S.* Gallinarum is not a zoonotic pathogen as it is host-adapted to avian species. Unfortunately, ciprofloxacin is licensed for use both in animals and humans in Nigeria. Being a member of the large family of quinolones, resistance to ciprofloxacin can confer resistance to all other members of the family since quinolones have the same mechanism of action. Streptomycin resistance was also commonly observed among all five *S.* Gallinarum isolates and has been frequently reported from other previous studies (Bokanyi et al., 1990; Lee et al., 1993).

Although tetracycline has been one of the most commonly used antibiotics for livestock and companion animals in Nigeria, its use in poultry has diminished perhaps, due to availability of some more potent and result oriented drugs especially members of the family quinolones. In this study, all five *S.* Gallinarum were sensitive to tetracycline, suggesting possible disuse and/or moderate usage. Several studies have discovered resistance pattern of streptomycin associated with tetracycline (Bokanyi et al., 1990; Manie et al., 1998). However, in this study, all five isolates showed resistance to streptomycin and sensitivity to tetracycline. This study has revealed the need for wider studies to be conducted in order to find out the predominant method(s) of transmission of *S.* Gallinarum in Ogun State and Nigeria. This will help in better understanding the epidemiology of the disease and hence, reduce morbidity and mortality arising from this devastating but preventable disease of poultry. The study has also confirmed the dangerous trend of resistance to quinolones in Salmonella from poultry thereby exposing human populations to the threat of acquiring resistant pathogens.

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