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International Journal of Current Research
Vol. 6, Issue, 04, pp.6302-6306, April, 2014

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

SEROTYPING OF CHICKEN SALMONELLAE IN JOS, PLATEAU STATE, NIGERIA

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ARTICLE INFO

Article History:
Received 19th January, 2013
Received in revised form
12th February, 2014
Accepted 06th March, 2014
Published online 23rd April, 2014

Key words:
Chicken salmonellosis,
Serotypes,
Chickens.

ABSTRACT

Serological characterization of chicken salmonellae isolated from four thousand, six hundred and sixteen (N= 4,616) samples from 1,319 chickens was done between 2006 and 2011 with the live bird market in Jos, Plateau state as the study site. Sample breakdown consisted of crop tissues (n=1,319), poultry droppings (n=1,319), gizzard tissue (n=1,319) and oviducts (n=659). Standard methods for bacterial cultivation and phenotypic identification were adopted prior to serological typing of the isolates. 28 (0.006%) of the total samples yielded *Salmonella* serotypes. 15 were from improved breed of chickens while 13 were from local breeds. Eighteen (64.3%) of the 28 *Salmonella* isolates were from layers while 10 (35.7%) were from cockerels. Samples from oviduct accounted for the most isolates (10) while the crop tissue accounted for the least (3) isolates. Serological analysis of the 28 isolates showed that 3(10.7%) each were Enteritidis, Typhimurium and Bargny. They were closely followed by St. Paul with 2 (7.14%). The remaining serotypes had one isolate each, with 3 of them typed to serogroup level only (one group D and 2 group B). Findings from this study indicate that chicken salmonellosis is prevalent in Jos. Also, the occurrence of highly invasive and rare serotypes is of great economic and public health significance. Concerted effort must therefore be made to improve on preventive and curative measures against chicken salmonellosis and other zoonoses.

INTRODUCTION

Salmonella belong to the family Enterobacteriaceae which are gram negative, non sporing and non capsulated rods. They are often motile usually by means of peritrichous flagella except for some serotypes notably Gallinarium – Pullorum that are non motile. They are aerobes and facultative anaerobes and grow readily on ordinary laboratory media. They are primarily intestinal parasites of vertebrates and are pathogenic for many species of animals including; chickens, pigs, cattle, fish, reptiles and humans. They cause the disease salmonellosis, which is a worldwide health problem; *Salmonella* infections are the second leading cause of bacterial food borne illness in the United States. Approximately 95% of cases of human salmonellosis are associated with the consumption of contaminated products such as meat, poultry, eggs, milk, seafood, and fresh produce. *Salmonella* can cause a number of different disease syndromes including gastroenteritis, bacteremia with focal infections , and typhoid fever, with the most common being gastroenteritis, which is often characterized by abdominal pain, nausea, vomiting, diarrhea, and headache with high mortality and morbidity in man and great economic loss in animals (Popoff and Le Minor, 2005). More than 95% of cases of *Salmonella* infection are foodborne, and salmonellosis accounts for more than 30% of deaths resulting from foodborne illnesses in the United States . Currently, there are over 2,500 identified serotypes of *Salmonella*. A smaller number of these serotypes are significantly associated with animal and human disease including Typhimurium, Enteritidis, Newport, Heidelberg, and Montevideo (Popoff and Le Minor, 2005; Old and Threlfall, 1998; Michael and Dirk, 2009).

Poultry, (particularly hens, ducks and turkeys) have been reported to be the most significant reservoir of food poisoning salmonellae (WHO, 2005). The organisms may also be found in their eggs and if slightly cooked or mixed with contaminated ones could lead to wide scale epidemics. *Salmonella* Enteritidis PT104 infections have been reported to result from consumption of hot cooked take away chickens in the United Kingdom (Cowden *et al*., 1989). *Salmonella* enteritidis associates more readily with intact chicken eggs (Guard, 2001; CDC/P, 2003A; CDC/P, 2003B). One of the reasons for this association may be its enhanced ability to survive in chicken egg albumen, an environment that is generally hostile to bacteria because it contains votransferin that chelates iron that is necessary for bacterial proliferation (Tranter and Board, 1992).The infection of laying hens with *Salmonella* Enteritidis is currently of great importance in the international pandemic of human infection (Rodrigue *et al*., 1990). Once inside the yolk, growth of *Salmonella* Enteritidis

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is rapid and an inoculation of only 5 cells reach 10^{11}cells/egg within 24hours at 20^{o}C (Rodrigue *et al*., 1990). The aim of this research is to ascertain the extent to which chicken salmonellosis is prevalent in live bird markets in Jos, Nigeria.

MATERIALS AND METHODS

Sample Collection: Samples were collected and processed as earlier described by United State Department of Agriculture (12). Samples targeted were tissues of oviduct, gizzard and crop as well as intestinal contents. In all, 4,616 samples from 1,319 chickens. Crop tissue, gizzard and intestinal contents accounted for 1,319 samples each while oviduct tissues contributed to 659 of the samples. All samples were collected from live bird markets in Jos, Plateau State, Nigeria in a 5 year study between 2006 and 2011. Identified samples were aseptically collected into sterile universal plastic bottles and conveyed to the Medical Microbiology Laboratory of Jos University Teaching Hospital, Jos for processing. The breed and sex of each chicken was noted against each sample.

Sample Processing: In the laboratory, the oviducts, gizzards and intestines were cut open with a sterile scalpel blade and the contents scraped aseptically into the sample containers. The intestinal contents of the colon region were aseptically expressed and collected into the sample containers. Solid samples were emulsified in sterile normal saline before plating on to appropriate media (Baird-Parker, 1990).

Culture: A loop full of the emulsified sample was inoculated onto each of Deoxycholate Citrate Bile Salt Agar (DCA) and Xylose Lysine Decarboxylase Agar (XLDA). Also, 5mls of the sample was inoculated into 15mls of Selenite F (SF) broth for enrichment and incubated for 24±2hours aerobically at 37^{O}C. Cultures on selective media plates were examined for *Salmonella*-like characteristics as described by standard methods and depicted on Plate 1 below.

Plate 1. Colonial Appearance of *Salmonella* Typhimurium on Xylose Lysine Deoxycholate (XLD) Agar

Subcultures were made into urea agar and triple sugar iron (TSI) agar slope and incubated at 37^{o}C for 24hrs. Urease-negative samples were then subjected to full biochemical tests: including indole, triple sugar iron agar and citrate utilization test. Isolates with phenotypic characteristics suggestive of *Salmonellae* were subcultured onto nutrient agar slope for agglutination with *Salmonella* polyvalent O (A- 67) and Polyvalent H (specific and non specific) antisera (SIFIN Germany) by slide agglutination method. Isolates that agglutinated with poly O and poly H were then subjected to agglutination with group antisera, to determine the group antigens using the group specific antisera. After grouping with group specific antisera, isolates were then typed with monovalent sera. The O specific antigens were first determined followed by the H (both phases I&II antigens were determined) using the Kauffman White classification as a guide.

RESULTS

From the 4,616 samples collected from 1,319, 730 (53.3%) were from improved breed of poultry while 589 (44.7%) were from local breeds. 28 (0.06%) of the overall samples investigated yielded *Salmonella* serotypes with isolates from improved breed of chickens being slightly higher (15) than those from local breeds (13). Eighteen (64.3%) of the 28 *Salmonella* isolates were from layer birds while 10 (35.7%) were from cockerels (Table 1).

On the whole, 28 of the specimens yielded *Salmonella* isolates with oviduct specimens having the highest yield of 10(35.7%), followed by gizzard with 9(32.1%), droppings, 6(21.4%), while the least 3(10.7%) were recovered from crop specimens. 15 (53.4%) of the isolates were from improved breed chickens; with isolates from cockerels marginally more than those of the layers (8:7). However, isolates from the 13 local breed of chickens showed slightly higher isolation rate in layers than cockerels (8:5). These results are not statistically significant (P> 0.05). Hence, neither breed nor gender has any influence on the distribution of isolates (Table 2).

A breakdown of serotypes obtained from 15 isolates obtained from improved breeds of chicken included two each of Typhimurium and Bargny, one each of Enteritidis, koessen,Weston, Allerton, Everleigh,Brazaville, Tado, Phaliron, Hindimarsh, Tsevie and one,Non typable. Also, a breakdown of salmonella serotypes from the 13 isolates from the local breed of chicken showed that two each were of St Paul and Enteritidis, one each of Ngor, Typhimurium, Korbol, Bargny, Nigeria, Kambo,le and Norton while two were non typable. The most encountered serovars were Enteritidis, Typhimurium and Bargny. (Table 3).

Interestingly, 6 of the serotypes encountered were of the invasive type. Incidentally, they composed of 3 serotypes each of Enteritidis and Typhimurium. Two of the 3 Enteritidis were from gizzard samples of locally bred chickens, while the third was from the crop of an improved breed. Two of the 3 Typhimurium were from the oviduct samples of improved breeds while the third was from the oviduct of a local breed chicken. There was no *Salmonella* Gallinarum-Pullorum isolated from chickens in this study (Table 4).

Table 1. Number of Salmonella Isolates from Chickens of both Breed and Sex in Jos

Chicken Breed	Chicken Sex Number (%) Tested / NO (o/o) Positive					
		Cockerel			Layers	
	Total No (%) Tested	No (%) Positive	NO (%) Tested	NO (%) Positive	NO (%) Tested	NO (%) Positive
Improved Breed	730(55.3)	15(53.6))	230(33.8	8(61.5)	500(78.2)	7(46.7)
Local Breed	589(44.7)	13(46.4)	450(66.2)	5(38.5)	139 (21.8)	8(53.3)
Total	1319	28(2.12)	680(51.6)	13(2.0)	639(48.4)	15(2.3)

Table 2. Distribution of the Number of *Salmonella* Isolates from Chickens in Jos According to Specimen Types, Chicken Breed and Gender

	Chicken Types Local breed		Improved breed		
	No (%)				
Types of specimens	Gender	Gender			Total No(%) of isolates
	Layer	Cockerel	Layer	Cockerel	
Oviduct	4(50)	0(0)	6(85.7)	0(0)	10(35.7)
Crop	1(12.5)	1(20)	0(0)	1(12.5)	3(10.7)
Gizzard	2(25)	2(40)	0(0)	5(62.5)	9(32.1)
Droppings	1(12.5)	2(40)	1(14.3)	2(25)	6(21.4)
Total	8(28.6)	5(17.9)	7(25.0)	8(28.6)	28(100%)

(P>0.05)

Table 3. Distribution of the No of Salmonella Serotypes Isolated From Chicken Specimens in Jos

Salmonella Serotypes	CHICKEN BREED									
	IMPROVED					LOCAL				
	SPECIMENS NO /(%)ISOLATES									
	Crops	Droppings	Gizzards	Oviducts	Total	Crops	Droppings	Gizzards	Oviducts	Total
Koessen		1(33.3)	0(0)	0(0)	1.(16.7)	0(0)	0(0)	0(0)	0(0)	0(0)
Weston	0(0)	0(0)	0(0)	1.(16.7)	1.(16.7)	0(0)	0(0)	0(0)	0(0)	0(0)
Ngor	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(33.3)	0(0)	0(0)	1(7.7)
Allerton	0(0)	0(0)	1(20.0)	0(0)	1.(16.7)	0(0)	0(0)	0(0)	0(0)	0(0)
Everleigh	0(0)	0(0)	0(0)	1(16.7)	1.(16.7)	0(0)	0(0)	0(0)	0(0)	0(0)
Enteritidis	1(100)	0(0)	0(0)	0(0)	1.(16.7)	0(0)	0(0)	2(50)	0(0)	2(15.4)
Typhimurium	0(0)	0(0)	0(0)	2(33.3)	2(13.3)	0(0)	0(0)	0(0)	1(25)	1(7.7)
Korbol	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(33.3)	0(0)	0(0)	1(7.7)
Bargny	0(0)	0(0)	0(0)	2(33.3)	2(13.3)	0(0)	1(33.3)	0(0)	0(0)	1(7.7)
Brazaville	0(0)	0(0)	1(20.0)	0(0)	1(16.7)	0(0)	0(0)	0(0)	0(0)	0(0)
Tado	0(0)	0(0)	1(20.0)	0(0)	1(16.7)	0(0)	0(0)	0(0)	0(0)	0(0)
Nigeria	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(25)	1(7.7)
Kambole	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(25)	1(7.7)
Phaliron	0(0)	0(0)	1(20.0)	0(0)	1.(16.7)	0(0)	0(0)	0(0)	0(0)	0(0)
Norton	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(25)	1(7.7)
Hindmash	0(0)	1(33.3)	0(0)	0(0)	1.(16.7)	0(0)	0(0)	0(0)	0(0)	0(0)
St. Paul	0(0)	0(0)	0(0)	0(0)	0(0)	1(50)	0(0)	1(25)	0(0)	2(15.4)
Tsevie	0(0)	1(33.3)	0(0)	0(0)	1.(16.7)	0(0)	0(0)	0(0)	0(0)	0(0)
NT	0(0)	0(0)	1(20.0)	0(0)	1.(16.7)	1(50)	0(0)	1(25)	0(0)	2(15.4)
Total	1(6.7)	3(20)	5(33.3)	6(40)	15(100)	5(15.4)	3(23.1)	4(30.8)	4(30.8)	13(100)

KEY NT : Non Typable (Typed only to group level.

Table 4. Invasive *Salmonella* Serotypes Isolated from Chickens in Jos Distributed According to the Chicken Breed and Specimen Types

	Chicken Types									
Serogroup	Improved breed					Local breed				
	Crop	Droppings	Gizzard	Oviduct	Total No (%)	Crop	Droppings	Gizzard	Oviduct	Total No (%)
Paratyphi A	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Paratyphi B	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Paratyphi C	0(0)	0(0)	0(0)	0(0)	(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Typhi	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Enteriditis	1(100)	0(0)	0(0)	0(0)	1(33.3)	0(0)	0(0)	2(100)	0(0)	2(66.6)
Typhimurium	0(0)	0(0)	0(0)	2(100)	2(66.6)	0(0)	0(0)	0(0)	1(0)	1(33.3)
Total	1(33.3)	0(0)	0(0)	2(66.7)	3(100)	0(0)	0(0)	2(66.7)	1(33.3)	3(100)

DISCUSSION

The presence of Salmonellae in the poultry industry in Plateau state as confirmed from this study corroborates current research findings indicating the prevalence of the infection in this locality. Okwori *et al.* (2013) and Agada *et al.* (2014) have recently reported the infections among poultry, poultry workers and poultry products in Jos, Plateau state. This indeed is a huge concern considering the public health importance of the disease which has been reported as a major public health challenge globally (Akinyemi *et al*., 2007). Salmonellosis alone has been reported to be responsible for over 50% of food poisoning the world over with poultry and poultry products the leading vehicles of transmission (CDC, 1996, Bryan and Doyle, 1995). It therefore suggests that the residents of Jos, plateau state are at risk of salmonellosis from their poultry industry. Also, the socio-economic impact of this development on poultry farmers is not encouraging. Reports have it that salmonellosis can lead to 100% morbidity and mortality leading to huge economic waste. Farmers with salmonella-infected flock are at risk of the zoonosis apart from spending more on treatment with the resulting poultry products being no match in market value with salmonella-free flock (Wasyl *et al*., 2005). The fact that demographic data of gender, age and breed with respect to the infection as found in this study did not show any statistical significance is a sure call for poultry farmers to take preventive and control measures against the infection more serious since it does not discriminate on the basis of these parameters. This if applied will definite reduce the rate of chicken salmonellosis (Jordan and Pattison, 1999). The 0.06% prevalence of the infection in chicken encountered in this study is however lower than 5% prevalence recently recorded amongst inmates of the zoological garden in Jos also in Plateau state (Oladapo *et al*., 2013). This is interesting because some animal species other than birds found in the zoo have been reported to be asymptomatic carriers of the infection (Palmgreen *et al*., 2006). The conclusion can therefore be that these asymptomatic carriers in the Jos zoo which is situated at the heart of the town are responsible for the infection of the poultry industry. It is also lower compared with 9.0% prevalence reported by Muhammed *et al*. (2010) as responsible for chick mortality in hatcheries within Jos and10.9% obtained by Agada *et al.* (2014) among poultry workers and poultry products within the same study area. The seemingly low prevalence rate in this study is likely attributed to the subjects we restricted our research to. The research targeted chicken sold at live bird markets which by all standard should possess an assembly of healthy and marketable poultry products as most farmers or buyers select or screen healthy and robust looking birds from farms for display in the market to enhance their patronage and profitability. As such diseased birds are reduced to the minimum although the few that get to the market have been reported to constitute a great public health challenge in the market place (Chollom *et al*., 2013).

The presence of various serotypes of salmonella encountered in this study further confirms the multitude of salmonella serotypes in the study area as reported previous by some authors (Muhammed *et al*., 2010; Okwori *et al.* (2013) and Agada *et al*., 2014). Also, the presence of non-typable serotypes reveal the possibility of the emergence of new strains and therefore call for further studies using molecular and epidemiological tools to precisely identify and characterize them. In conclusion, the outcome of this study reveals that chicken salmonellosis could be transmitted from live bird markets as such marketers, customers and the public are at risk of the infection in Jos if nothing is done to disrupt the infection transmission ecology.

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