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Research Article

The carrier rate of *Salmonella gallinarum* in free range chickens in Nasarawa state, Nigeria

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Abstract

The study determined the carrier rate of *S. gallinarum* in free range chickens in Nasarawa State, Nigeria. A total of 1,970 visceral organ samples, consisting of spleen, liver and cloacae were collected from the free chickens in 12 selected villages/towns across the three senatorial zones of Nasarawa State. They samples were cultured for isolation of *S. gallinarum* and the isolates were identified using standard biochemical and serological tests. The overall *S. gallinarum* occurence rate was 49 (2.5%) and chickens from Wamba and Keana had the highest rates of 5.0% each. The lowest bacterial prevalence of 0.9% was recorded for chickens from Akwanga. The variation in *S. gallinarum* bacterial prevalence among the individual study locations was not statistically significant ($p>0.05$). Chickens from Nasarawa West Senatorial Zone had an occurrence rate of 2.8%%, Nasarawa North 2.4% and Nasarawa West 2.2% ($p>0.05$). Bacterial yield from the spleen was 4.6%, which was significantly higher ($p<0.05$) when compared with the 3.1% yield from the liver and 0.6% from cloaca. respectively. The layer chickens had a bacterial isolation rate of 3.5%, followed by the pullets 2.3% and cockerel 1.7% ($p<0.05$). The highest ($p<0.05$) bacterial prevalence of 4.5% was found among chickens above 34 weeks of age and the least in chickens of age between 8-12 weeks. The isolation of *S. gallinarum* in these apparently healthy free-range chickens shows their carrier status for *S. gallinarum* and confirms infection of fowl typhoid in the free range chickens in Nasarawa State,Nigeria.

Keywords: Carrier rate, *S. gallinarum*, free range chickens, Nasarawa State, Nigeria.

Introduction

Salmonella gallinarum (*S. gallinarum*) is a non-motile Gram negative rod and the causative agent of fowl typhoid (Abdul, 2007; OIE Manual, 2008), a severe systemic disease of chickens and other galliforme birds (Shivaprasad, 2000).

Fowl typhoid is principally associated with an acute septicaemic illness of high morbidity and mortality in birds during the later growing period and in adult birds (Animal Health and Veterinary Laboratories Services AHVLA, 2012), but it may occur in young chickens where it is indistinguishable clinically from pullorum disease (OIE Manual, 2000).

Fowl typhoid has geographical distribution in Mexico, Central and South America, Africa, and Asia and the Indian subcontinent (OIE, 2005). In Nigeria, the disease endemic and is militating against successful operation of the poultry industry (Ibrahim *et al*., 2003). The disease has affected both government and

private farms and has also been found in birds in the chicken markets (Nwobu *et al.,* 1990). According to the Annual Animal Health Report on the Notification of the Absence or Presence of All Diseases in Nigeria (Federal Department of Livestock and Pest Control Services FDLPCS, 2010) and data on the diagnosis of animal diseases obtained from the Central Diagnostic Division of National Veterinary Research Institute, Vom, Nigeria, fowl typhoid ranks higher among the bacterial diseases of poultry, causing huge economic losses to commercial poultry farmers.

Currently, most of the reported isolations of *S. gallinarum* in Nigeria are associated with occurrence of fowl typhoid outbreaks in commercial poultry (Ibrahim *et al*, 2003; Mbuko *et al*, 2009; Agbaje *et al*, 2010). Report on isolation of this bacterium in apparently healthy free-range birds based on epizootiological surveys in Nasarawa State is not available. The role of this category of birds in the maintenance and spread of *S.gallinarum* is therefore obscured.

The free range poultry otherwise known as rural poultry or scavenging poultry in Nigeria is made up mostly of chickens, ducks, and guinea fowls, which are domesticated for the purpose of egg and meat production. These are managed under the extensive systems (Emikpe *et al*, 2005), and are usually not vaccinated (Adu *et al*, 1986). It is widely believed that they act as reservoirs of most important poultry diseases, including fowl typhoid (Bouzoubaa *et al*, 1992). Chickens (*Gallus gallus domesticus*) are the most important of the free range poultry species in terms of number and development (Oluyemi and Roberts, 1979). They are kept by over 90 % of rural households, especially women, as assets (Ajala *et al.,* 2007), providing an important source of high quality protein as well as a source of income for the families (Abubakar *et al*, 2008). In Nigeria, the local chickens form the bed-rock of the poultry industry, contributing over 80% of poultry products (National Agricultural Extension and Research Liaison Services NAERLS, 2000). They represent a significant part of the rural economy in particular and of the national economy as a whole (Ajala *et al.,* 2007). For example, in 1988 the rural poultry constituted 43% and 89% of the national egg and meat production respectively, with an annual output of 67,000 metric tons of meat and 82,000 metric tons of eggs (Adegeye *et al*, 1988). In the scavenging system, the birds are sheltered only at night. During the day, the birds are allowed to scavenge on insects, earthworms, residual grains, and green materials around the homestead. Occasionally, the birds are supplemented with rice bran, broken rice, cooked rice, millet, guinea corn and rice gruel in the morning or afternoon. This category of chickens is however faced with poor management, lack of external input for production, and poor disease control. Of these constraints, disease remains a major limiting factor in their production. The aim of this study was to determine the carrier rate of *S. gallinarum* in free range chickens in Nasarawa State, Nigeria.

Materials and Methods

Study Area

Nasarawa State is located in the North Central Nigeria otherwise known as the Middle-Belt region, with Lafia as its capital. The state is divided into 13 Local Government Areas (LGA) for administrative purposes, which are further grouped into three senatorial zones. Nasarawa State is bounded in the north by Kaduna State, in the west by the Federal Capital Territory, in the south by Kogi and Benue States and in the east by Taraba and Plateau States. The state has agriculture as the mainstay of its economy with an estimate poultry population of 863,647.7, which comprise 777,282.9 local breeds and 86,364.8 exotic breeds (FLDPCS, 2011). Located between latitude 8^{o} 24 17N and longitude 8^{o} 08 38E, Nasarawa State has a total area of 27, 117 Km^2 and population of 2, 040, 097 with density of 75/ km^2 (190/sq mi), according to 2005 Nigerian National Census. The state has a mean temperature of 32^{o}C (Hassan et al, 2013) and average rainfall of 104. 75cm.

Selection of Study Areas

Three villages and a local government headquarter were randomly selected from each senatorial zone of Nasarawa State. A total of 12 villages /towns were selected, which included Lafia, Keana, Agbashi and Asakkio from Nasarawa South Senatorial Zone; Akwanga, Andaha, Nasarawa Eggon and Wamba from Nasarawa North Senatorial Zone, and Keffi, Garaku, Gadabuke and Gunduma from Nasarawa West Senatorial Zone. The selected villages/towns are located in 10 (76.9%) out of the 13 LGAs of Nasarawa State.

Sampling Procedure

A stratified random sampling technique was employed in selecting the flocks. A total of 83 households were randomly selected from the selected villages and, all chickens in a selected household were considered as one flock (Mdegela *et al*, 2000). The number of flocks selected per area ranged from eight to ten and, from each of these households 10% of the total flock population was sampled (Mdegela *et al*, 2000; Peter *et al*, 2006). A total of 830 chickens were subsequently sampled. In the local government headquarters, samples were collected from chicken markets, and one chicken market was selected from each local government headquarters.

Sample Collection

Chickens of various types (pullets, cockerels and layers) and ages were sampled. Samples were obtained from the liver, spleen using sterile forceps and scapel bled, and about 10g was collected from each of the tissues. Sample from the cloacae was collected using sterile commercial swab sticks (Antech, UK) by inserting the swab stick into the cloaca of the bird for about 20 seconds, after which the swab stick was removed and immediately returned into the swab pack and labeled. Cloacal swab samples were placed in universal bottles containing 10ml Selenite F broth (Oxoid, Hamshire, England) (Mdegela *et al*., 2000), while tissue samples were kept in clearly labeled polythene bags. Samples were transported to the laboratory in cold boxes. A total of 1, 970 samples made up of 830 cloacal swabs and 1,140 tissues were obtained. The cloacal swabs were obtained from households during blood collection while the tissues, which comprised of 570 liver samples and 570 spleen samples, were collected from chickens in the markets. The tissue samples were made up of 530 from Lafia, 230 from Akwanga and 380 from Keffi markets. Of the total number of samples collected, 660 were from Nasarawa South, 640 from Nasarawa North and 670 from Nasarawa West Senatorial Zones.

Isolation and Identification of *S. gallinarum*

The standard procedures described by Mdegela *et al* (2000); Murugkar *et al* (2005); OIE (2008) and HPA (2011) were used. The Selenite F broths containing cloacal swabs were incubated at 37^{o}C for 24 hr. Tissue samples originating from the same chicken were pooled in a sterile pestle and homogenized in nutrient broth. The homogenate was inoculated into Selenite F broths and incubated overnight at 37^{o}C for selective enrichment. Thereafter, subculture was made onto MacConkey agar (MCA)(Oxoid Ltd, UK, without salt), and smooth colourless colonies (non-lactose fermenters), suggestive of *Salmonella*, observed after 24 hour incubation at 37^{o}C were subcultured onto desoxycholate citrate agar (DCA) (Oxoid Ltd, UK) for purification. Dome-shaped colonies with black central spots observed after 24 hours incubation at 37^{o}C were suspected to be *Salmonella*. These were gram stained and morphologically studied (Merchant and Packer, 1967). Gram negative rods suggestive of *Salmonella* species were tested for motility. Isolates were identified as *Salmonella* using the following biochemical tests: oxidase test, urease test, citrate utilization test, glucose fermentation and dulcitol fermentation tests and triple sugar iron test for OIE (2008).

Serological Confirmation of *salmonellae* Isolates

Isolates were serologically confirmed as *S. gallinarum* and *S. pullorum* serotypes according to standard procedure OIE (2008), using the commercial *Salmonella* polyvalent 'O' (A-G) and group specific sera for *S. pullorum* and *S. gallinarum* serotypes ('O' 9 antiserum) (BD, United Sates of America) by the slide agglutination method. Pure colonies of the test organism from nutrient agar plate were used for the test. Before testing with sera, the test organism was tested with sterile saline to eliminate autoagglutinable strains. Material taken from a single colony of pure *Salmonella* culture using a sterile wire loop was transferred to a glass slide and mixed with a drop of sterile saline. The slide was rocked gently for 30-60 seconds and observed for agglutination with the aid of a magnifying glass. No clumping of bacteria into more or less distinct units was observed, which recognized the cultures as nonautoagglutinable. The nonautoagglutinable bacterial samples were tested as follows: Material from a single colony was dispersed in the drop of *Salmonella* Polyvalent 'O' antiserum to obtain a homogenous and turbid suspension. After gentle rocking for 30-60 seconds, the reaction was observed with the aid of magnifying glass for agglutination. Cultures that tested positive were tested further in the same manner with group-specific sera for *S. pullorum* and *S. gallinarum* serotypes ('O' 9 antiserum). Positive reaction was considered as *S.*

gallinarum and *S. pullorum* serogroup. They two serovars were differentiated using Glucose fermentation (with gas production) and dulcitol fermentation tests (Trabulsi and Edwards, 1962; OIE, 2008). Cultures of *S. gallinarum* were stored in NA slants at 4^{o}C.

Statistical analysis

The Chi-square statistical tests in Statistical Package for the Social Sciences (SPSS) software version 17.0 were used to compare the variations *S. gallinarum* carrier rates.

Results

Gram negative rods that were oxidase negative, urease negative and produced an alkaline reaction on a TSI slant and an acid reaction in the butt with gas bubbles and blackening were suspected to be *Salmonella*. Suspected *Salmonella* isolates that tested positive with polyvalent 'O' and antisera, and fermented dulcitol but did not ferment glucose were confirmed as *Salmonella gallinarum* (OIE, 2008).

The overall *S. gallinarum* isolation rate as shown in table 1 was 49 (2.5%), and chickens sampled from Keana and Wamba had the highest bacterial prevalence of 5.0% each, while the least prevalence rate of 0.8% was observed in chickens sampled from Akwanga. The differences in isolation rate from the entire study locations was not statistically significant ($p<0.05$).

S. gallinarum isolation rate from free-range chickens in relation to senatorial zone of Nasarawa State presented in figure 1 showed that Nasarawa West Senatorial Zone had the highest bacteria isolation rate of 2.8%, followed by Nasarawa North 2.4% while Nasarawa South had the least rate of 2.2%. The variations in bacterial prevalence was found to be statistically insignificant ($p<0.05$).

The spleen tissue yielded 4.6% *S. gallinarum* isolates, which was the highest when compared with the liver which yielded 3.1% isolates and the cloacae 0.6% (Figure 2). Distribution of *S. gallinarum* isolates in relation to type of chickens is presented in table 2. The egg-laying chickens produced 3.5% bacterial isolates, followed by the pullets 2.3% and cockerels 1.7%.

In relation to age, there was higher bacterial prevalence among the older chickens than the younger once. Thus chickens above 34 week of age had 4.5% prevalence, followed by the 28-34 week-old birds 2.8% and 21-27 week-old birds 2.5%, while 8-12 old chickens had the least *S. gallinarum* carrier rate of 1.1%.

Discussion

Salmonella gallinarum is one of the avian-adapted serovars and causes a systemic disease of fowl typhoid in chickens (Shivaprasad, 2000; shivaprasad, 2003). Currently in Nigeria, reported isolations of *S. gallinarum* are associated with occurrence of disease outbreaks in commercial chickens (Orji *et al*, 2005; Agbaje *et al*, 2010; Fasure *et al*, 2012). In the present investigation, *S. gallinarum* was isolated from apparently healthy free range chickens sampled from Nasarawa State, Nigeria. As these chickens are usually not restricted in movement they may have contacted the bacteria through contact with infected wild birds, rodents and flies, which are reservoirs of *S. gallinarum* and may act as important mechanical spreaders of the organism (Field Manual of Wildlife Diseases, 2001; OIE, 2005; Valiente et al, 2009). This finding is in agreement with those of Hoop and Albicker – Rippinger (1997), Msoffe *et al* (2002) and Bouzoubaa *et al*, (1992) who also reported isolation of *S. gallinarum* from free-range chickens. Chickens are the primary host for *S. gallinarum* and have been reported to be reservoirs of the organism (Bouzoubaa *et al*, 1992). Futhermore, Calnek *et al* (1997) and Jordan & Pattison (1996) reported that *S. gallinarum* is among the important bacterial pathogens of free range chickens.

S. gallinarum was isolated from 49(2.5%) of the 1,970 tissue samples cultured, with the liver and spleen having the highest bacterial yield when compared with yield from the cloacae. This isolation rate might have been higher if more than one plating media was used per sample, as recommended by Wray *et al* (1996). The isolation rate is low and similar to the level reported by Hoop and Albicker – Rippinger (1997), and it is evident that *S. gallinarum* infection in scavenging local chickens is normally low (Mdegela *et al*, 2000) compared to the relatively higher rates often reported in the commercial chickens, which are usually reared intensively. The environmental condition of commercial poultry rearing favours the

The higher bacterial yield observed from the liver and spleen is supported by the finding of Ibrahim *et al* (2003) who consistently isolated *S. gallinarum* from liver, spleen and bile in Zaria, Nigeria. Also, OIE (2008) reported that the liver and spleen are the most preferred tissues for recovery of *S. gallinarum* from carrier birds. This is further buttressed by the findings of Silva *et al* (1981); Barrow *et al* (1994) and Wingley *et al* (2005) who reported on the pathology of *S. gallinarum* in the liver and spleen of infected bird, and bacterial persistence for several weeks at these sites. This shows that the liver and spleen are important tissues for pathological study of fowl typhoid. The study shows a low bacterial yield in the cloacae samples. This is similar to the rates reported by Peter *et al* (2006) and Mdegela *et al* (2000). This has been reported by Smith and Tucker (1980) to be due to the fact that generally, *S. gallinarum* infected chickens are not efficient excreters, unlike birds infected with motile *Salmonellae*, that cause enteric rather than system infections. Contrary to the finding of the present study however, Mdegela *et al* (2000) did not isolate *S. gallinarum* from scavenging local chickens in Tanzania. The author suggested that such birds were probably not in active phase of the disease when they were sampled. This probably accounted for the non isolation of the bacteria from some of the sampled chickens in the present study.

The higher *S. gallinarum* isolation rates from chickens in Keana, Assakio and Wamba shows that chickens from these areas were more infected. This may be due to proximity of these areas to the state capital, where commercial poultry population was higher. Most commercial poultry workers held from households where local birds are reared. It is probable that under poor biosecurity condition in commercial poultry such workers may contribute to spreading the organism to the free range birds in their households.

It was observed in this study that *S. gallinarum* was isolated from chickens of the various age categories. This is not out of place since studies have shown that chickens of all ages are susceptible to *S. gallinarum* infection (Wekhe, 1992; OIE, 2005). However, it has been reported that fowl typhoid is more often observed in the later growing period and in mature stock (OIE, 2008). This report corroborates the findings of the present investigation which shows that the older birds had higher bacterial prevalence than the younger ones. The isolation of *S. gallinarum* in these apparently healthy free-range chickens shows the carrier status of these birds for *S. gallinarum* and confirms infection of fowl typhoid in the free range chickens in Nasarawa State, Nigeria. This is a threat to the development of poultry industry in Nasarawa State taking into consideration that the free range chickens are not usually confined hence could contribute to further spread of the infection in the environments. Infected carrier birds are most important in the transmission of *S gallinarum*, and will invariably pass the organism to their progeny through egg transmission. Once infection is established in a breeding flock, the infection spreads within flock by horizontal means through feeding on contaminated materials or by inhalation of *Salmonella* by birds in close contact when pecking at contaminated surface of infected birds (Field Manual of Wildlife Diseases, 2001).

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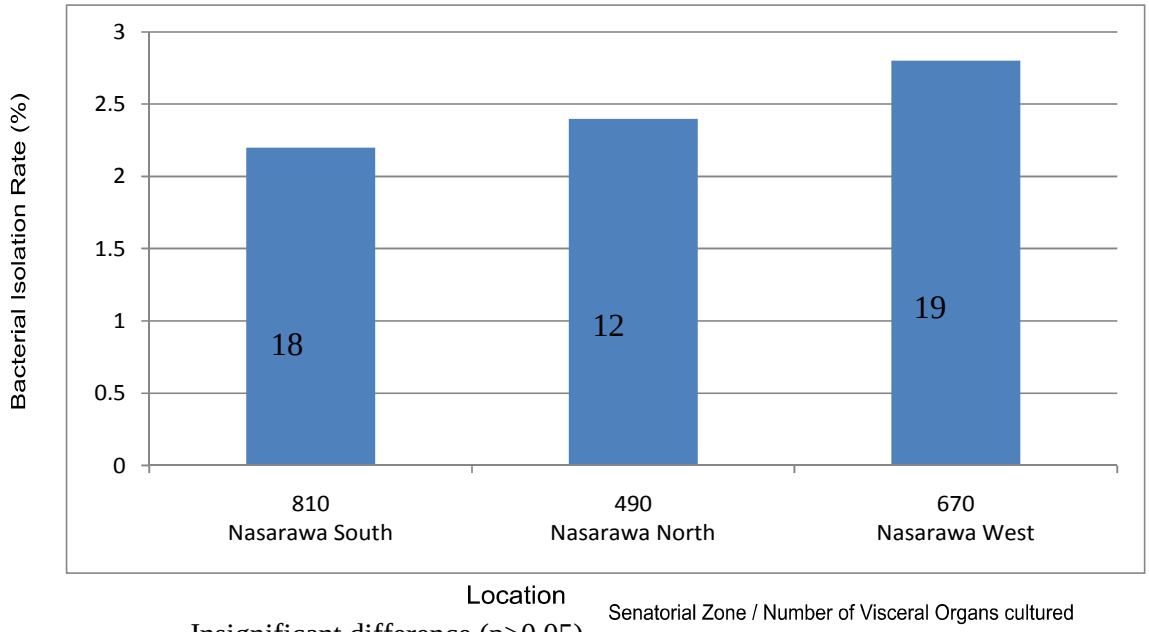
Table 1: Distribution of *S gallinarum* Isolates from Free Range Chickens in Nasarawa State, Nigeria, in Relation to Study Location

Locations	No.of samples Tested	isolation rate	
		S. gallinarum Isolated	(%)
Lafia	530	7	1.3
Keana	80	4	5.0
Agbashi	100	3	3.0
Assakio	100	4	4.0
Akwanga	230	2	0.9
Andaha	90	3	3.3
Wamba	80	4	5.0
N/Eggon	90	3	3.3
Keffi	380	9	2.4
Garaku	100	3	3.0
Gadabuke	100	3	3.0
Gunduma	90	4	4.4
Total	**1970**	**49**	**2.5**

Insignificant difference (P>0.05)



Insignificant difference (p>0.05)

Figure 1: Distribution of *S gallinarum* Isolates from Free Range Chickens in Nasarawa State, Nigeria, in Relation to Senatorial Zones

Table 2: Distribution of *S. gallinarum* Isolates from Free Range Chickens in Nasarawa State, Nigeria, in Relation to Type of Chicken

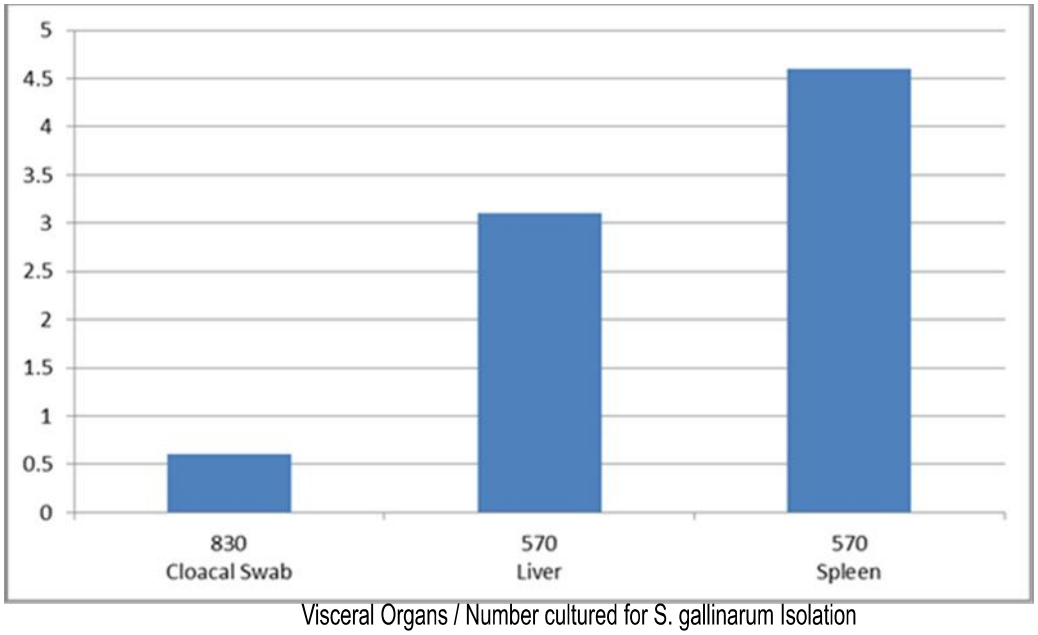
Type of chicken	No. of Samples Tested	No. of *S. gallinarum* Isolated	Carrier Rate (%)
Pullet	503	13	2.3
Layer	601	21	3.5
Cockerel	866	15	1.7
Total	**1,970**	**49**	**2.5**

Significant difference ($p<0.05$)

Table 3: Distribution of *S. gallinarum* Isolates from Free Range Chickens in Nasarawa State, Nigeria, in Relation to Age.

Age of Chicken (weeks)	No. of Samples Tested	No. of *S. gallinarum* Isolated	Carrier Rate (%)
8-12	455	7	1.1
13-16	397	9	2.2
17-19	238	5	2.1
21-27	355	9	2.5
28-34	280	8	2.8
>34	245	11	4.5
Total	**1,970**	**49**	**2.5**

Significant difference ($p<0.05$)



Significant difference ($p<0.05$)

Figure 2: *S. gallinarum* Isolates from Visceral Organs of Free Range Chickens from Nasarawa State, Nigeria

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