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The comparative susceptibility of commercial and Nigerian indigenous chicken ecotypes to *Salmonella gallinarum* infection

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Abstract

This study was to evaluate the possible genetic resistance of exotic and indigenous chicks to *Salmonella gallinarum*. A total of 72 nine weeks-old chicks were used for the study. The Fulani ecotype (Fulani smooth feathers - FSF), Yoruba ecotype (Yoruba smooth feathers - YSF), and the Exotic breed (Nera Black) chicks were infected with a dose of *S. gallinarum* (8.3 x 10^6 CFU) and were observed for 16 days. Evaluation of resistance was based on clinical signs, mortality, pathology, leukocyte count, bacterial count from liver and spleen of infected chicks. The highest peak for clinical signs in *S. gallinarum* infected chicks coincides with highest mortalities recorded on day 11-12 dpi and bacterial count of both liver and spleen on day 8. The lymphocytes count declined on day 8 for all the experimental chicks except for the exotic breed. There was no significant difference between the bacterial counts of the different groups on day 8. In *S. gallinarum* infected chicks, 94.4% of all the chicks showed clinical signs after infection, the exotic breed showed a prolonged clinical signs while the Yoruba ecotype showed the least. 87.5%, 80.0% and 37.5% mortality were recorded in the exotic breed, Fulani and Yoruba ecotypes respectively. The study showed that the exotic chicken (Nera Black) was more susceptible to *Salmonella gallinarum* infection. It also indicated that within the ecotypes in Nigeria, Fulani ecotype was more susceptible to *Salmonella gallinarum* infection than the Yoruba ecotype. The lower clinical signs and mortality observed in Yoruba ecotype indicated a resistance of the ecotype to *S. gallinarum* infection.

Keywords: Ecotypes, Nigerian Indigenous chicken, *Salmonella gallinarum* infection.
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Introduction

An indigenous chicken is a pool of heterogeneous genetic material which differs in adult body size, weight and plumage and often found in an extensive system of poultry or scavenging freely (Ajayi, 2010). The indigenous chicken is a descendant of the species *Gallus gallus domesticus*. However, different strains and varieties have been identified and these differences are as a result of hereditary morphometric traits such as plumage, comb size or form, types of feather, amount of feather on the skin, size, eye colour etc. (Yakubu *et al*., 2009). Ecotype is the word used to describe chickens that are genetically adapted to a geographical location or environmental conditions (Yakubu *et al.,* 2009). They are often indigenous to a particular geographical location, kept mainly in an extensive system, scavenging free range and unimproved (Ajayi, 2010). These indigenous or local chickens constitute about 60 percent of the 120 million poultry birds found in Nigeria and more than 80 percent of these poultry population are found in the rural household (FAO, 2009, Oke, 2011). Fulani (savannah), Yoruba (forest), Nsukka, Owerri and Awgu ecotypes are some of the indigenous ecotypes of chickens found in Nigeria (Yakubu *et al*., 2009). Variations in trait of each ecotype have been reported by Oluyemi *et al*. (1982), however, there is paucity of information on the resistance of these ecotypes to diseases (Wales & Davies, 2011, Ogie *et al*., 2012)

High prevalence of diseases is among the major factors limiting high productivity of the indigenous chickens in the tropics (Yongolo, 1996), among these diseases are the fowl typhoid, which is caused by *Salmonella gallinarum.* Fowl typhoid is recognized worldwide as a septicaemic disease of social and economic significance which affects primarily

chickens and turkeys, although natural infections in ducks, pheasants, guinea fowls, ostriches, wood pigeons, swans, sparrows, peacocks and quail have been reported (Yongolo, 1996). 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). A recent report put the prevalence of fowl typhoid in sampled flocks in part of northern Nigeria at 18.4% (Mbuko *et al*., 2009). Although, *Salmonella gallinarum* infection is frequently considered as a problem of adult and grower chickens, chicks are also affected (Wales & Davies, 2011). *Salmonella gallinarum* is host specific and can cause morbidity of 10-100% in chickens and mortality of up to 100% in an immunocompromised flock (Wales & Davies, 2011). Previous study had evaluated the genetic influence on *Salmonella enteritidis* infection (Ogie *et al.,* 2012), this study in turn evaluates the susceptibility of exotic breed and Nigerian indigenous chickens to experimental *Salmonella gallinarum* infection.

Materials and methods

Experimental chickens

The experimental design was similar to the earlier described (Ogie *et al*., 2012) where a total of 72 nine-weeks-old chicks were used. Twenty four chicks each of Exotic breed, Fulani and Yoruba ecotype were used. The Nera Black cockerels were purchased from day old and screened using ELISA technique as described by Ohore *et al*. (2002). They were reared for nine weeks; they were vaccinated against Newcastle disease on day one and day twenty-one and were given vitamins and Amprolium (anticoccidial) at week six. Fulani and Yoruba ecotypes were obtained from farmers residing in Fulani and Yoruba villages in Ilorin and Ibadan, Nigeria, respectively at 9 weeks of age. On arrival, the chicks were rested for 72 hours to recover from the stress of transportation. Growers' mash and water were later fed to the chicks *ad-libitum*. The chicks were vaccinated against Newcastle disease and were given prophylactic doses of Mebendazole antihelmintic and Amprolium (anticoccidial) before the start of the experiment in accordance with the manufacturer's recommendations.

Inoculation of Salmonella gallinarum into experimental chicks

Twenty four chicks from each ecotypes were inoculated orally with 1ml of overnight culture containing $8.3x10^6$ colony-forming units/ml virulent *Salmonella gallinarum* obtained from the field and donated by Prof Adetosoye of the Department of Veterinary Microbiology and Parasitology, University of Ibadan was grown in Lauryl L broth (Difco™) incubated at 37^oC. The chicks were made to rest for eleven days before the inoculation with *Salmonella gallinarum*. The chicks were observed for sixteen days. The chicks were given growers' mash without antibiotics and were observed twice daily for clinical signs and mortalities. Before the day of infection, approximately 2 ml of whole blood was collected into capillary tubes coated with heparin by brachial venipuncture from three randomly selected chicks from each groups on days 4 and 8 post inoculation, two blood samples were collected from each bird for packed cell volume (PCV) determination and for differential count.

Clinical signs and mortality in sSalmonella gallinarum Infected Chicks

All the chicks were observed twice daily for clinical signs and mortality up to 16 days post infection. The presentation of clinical signs was the modification of the system used by Ogie *et al.,* (2012), with (-) indicating no clinical signs; (+) indicates drowsiness, occasional closure of the eyes and drowsiness while (++) indicates closure of the eyes and reluctance to move.

Bacteriological examination

Post mortem examination was done on at least 2 dead or euthanized chicks from each group on day 4, 8, 12 and 16 days post challenge. The livers and spleens of these chicks were aseptically removed for determination of viable bacterial cell counts using standard methods (Chiu *et al.,* 2010). Bacteriological samples were prepared by weighing 1 gram of each macerated sample into sterile cooled 9mls of peptone broth. Ten (10) fold serial dilutions were made by transferring 1ml of sample from the peptone broth, this continued until serial dilution of all sample were completed. All chicks that were sacrificed were subjected to a thorough post-mortem examination as described by Fowler (1996).

One (1) ml of prepared bacteriological samples from dilution $x10^{-3}$ and $x10^{-6}$ respectively, were inoculated into sterile petri plates and about 20-25 ml of sterile, cooled to 40^0C Eosin methylene blue agar (Holt, Harris & Teague) was poured into the inoculated sterile petri plates, swirl thoroughly and allowed to cool and gel after which it was transferred to the incubator at 37^0C for 24-48 hours. Bacterial growth

was observed on each plate and colony counted, calculated and represented in colony forming units per milligram (cfu/ml).

Determination of PCV to Salmonella gallinarum
The PCV was determined by centrifugation of the stabilized blood in a micro-haematocrit centrifuge (Hawksley and Sons Ltd, lancing, UK) then read on a haematocrit reader.

Enumerations of selected leukocytes in chicks infected with Salmonella gallinarum
Some of the stabilized blood was used to make thin microscopic blood film for leukocyte enumeration. The thin blood films made were air-dried and fixed on absolute methanol for 30 sec and stained by Wrights staining method. The films were observed under light microscope (X1000) and the cells (heterophils, lymphocytes and monocytes) were enumerated according to their morphology (200 cells were counted on each slide). The cells were counted and the result are expressed as the percentage distribution (Pd)

Pathology of the liver and spleen of Salmonella gallinarum infected chicks
The livers and spleens from infected birds were examined grossly on day 4, 8, 12 and 16 days post challenge while tissues from these organs were fixed in 10% formal saline and processed routinely for histopathological examination.

Statistical model
All data collected from the experiment were subjected to one way analysis of variance using statistical analysis software (SAS) (2001) and New Duncan multiple range test of the same software.
$Y_{ij} = \mu + \alpha_i + e_{ij}$
Where Y_{ij} = Individual observation assumed to be random elements
μ = Population means fixed, to be determined
α_i = Treatment effect fixed, to be determined
e_{ij} = error associated with each record random and normally distributed.

Results
Clinical signs of chicks infected with Salmonella gallinarum
Table 1 presents the clinical signs for indigenous chickens and exotic breed infected with *Salmonella gallinarum*. Clinical signs were evident in chicken from day 4 after inoculation and it included drowsiness and occasional closure of the eyes. From day 5-7 the clinical sign were more severe with marked decrease in feed intake and reluctant to move. Clinical signs were most severe in exotic breed and least in Yoruba ecotype based on the scoring system used. No clinical signs were observed beyond days 14 post challenge.

Mortality in chicks infected with Salmonella gallinarum
Table 2 presents the mortality pattern observed in the indigenous ecotypes and exotic chicks infected with *Salmonella gallinarum*. A total of 20 chicks from Fulani ecotype, 9 chicks from Yoruba ecotype and 21 chicks from the Exotic breed died. The highest percentage mortality was observed in the exotic breed with 87.5% while Yoruba recorded the lowest with 37.5% percentage mortality. The highest number of mortalities was recorded on day 8 post challenge in Fulani ecotype and the exotic breed.

PCV and leukocyte enumeration for chicks infected with Salmonella gallinarum
Packed cell volume (PCV) for all the infected chicks were within the normal range (>30%). Figure 1 shows the changes in the mean lymphocytic population over the period of 8 days post challenge for all the infected chicks. The indigenous chicks showed peaks on day 4 with a decline on day 8. While the exotic breed shows peak only on day 4. The mean Pd for heterophilic population was presented in figure 2. The entire infected chicks showed peak on day 4, Yoruba ecotype being the lowest. Figure 3 showed the change in the monocyte population over the period of the experiment. The exotic breed showed a decrease from day 4 to 8, while the Fulani and Yoruba ecotypes showed peak only on day 8 post challenge.

Pathology and bacterial count
Necropsy of the sacrificed chicks infected with *Salmonella gallinarum*, showed a marked enlargement and multifocal necrosis of the liver and spleen at day 4 post challenge. The histopathological examination also revealed marked multifocal hepatic necrosis and splenic follicular hyperplasia. These pathological features appeared in both the indigenous and exotic chicks that were sacrificed with lesions more in Yoruba than Fulani ecotype and the exotic breeds. After day 4 post challenge, there were no notable gross pathological changes in the internal organs of the sacrificed chicks. The mean viable bacterial cell counts (log_{10}) in the liver and spleen varied within the indigenous and between

the indigenous and the exotic. The highest mean values were recorded on day 8 pc (post challenge) from both liver and spleen. The counts from the spleen were consistently lower compared with those from the liver. The bacterial count from the livers of Yoruba, Fulani and the exotic breeds on day 8 are 7.45×10^3, 7.43×10^3 and 7.45×10^3 respectively. This same pattern was observed in the spleen of the infected chicks. These were presented in figure 4 and 5. Comparing the means on day 8, there were no significant differences between the bacteria counts of the liver and spleen from the infected groups.

Table 1: The clinical signs for indigenous chickens and exotic breed infected with Salmonella

	Infected chicks		
Days (p.i)	FSF	YSF	EB
3	-	-	-
4	+(2)	+(2)	+(4)
5	+(1)++(5)	++(1)	+(2)++(4)
6	+(5)++(1)	++(5)	+(5)++(3)
7	+(4)++(4)	++(4) +(2)	+(3)++(5)
8	+(5)++(12)	++(6)+(1)	+(3)++(13)
9	+(4)	+(1)	+(3)++(2)
10		+(1)++(3)	
11		+(1)	+(4)++(1)
12			+(1)
13			+(2)
14			
15			

*P.I = post inoculation

*FSF = Fulani smooth feathers, YSF = Yoruba Smooth feathers and EB = Exotic Breed

(-): no clinical signs noticed

(+): Less severe clinical signs

(+ +): Severe clinical signs

Table 2: The mortality pattern observed in the indigenous ecotypes and exotic chicks infected with *Salmonella gallinarum*

Days (p.i)	FSF	YSF	EB
3	-	-	-
4	-	-	-
5	-	-	-
6	-	-	2
7	3	4	
8	13	2	11
9	4	1	1
10		2	
11			5
12			2
13			
14			
15			
Total mortality	20	9	21
% Mortality	80.0%	37.5%	87.5%

*P.I = post inoculation

*FSF = Fulani smooth feathers, YSF = Yoruba Smooth feathers and EB = Exotic Breed

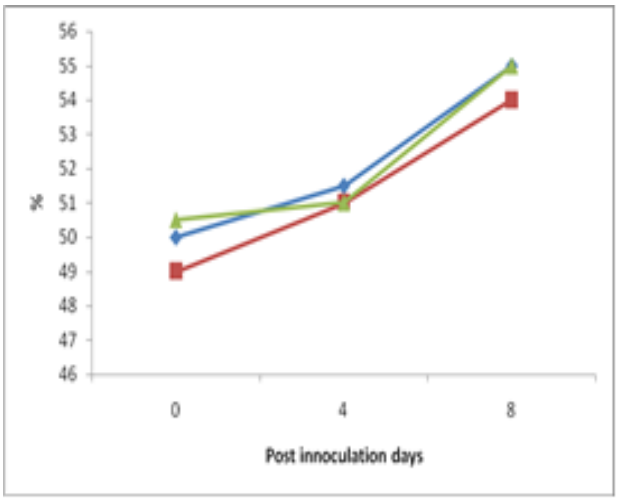

Figure 1: Lymphocytes count of chicks infected with S. *gallinarum*

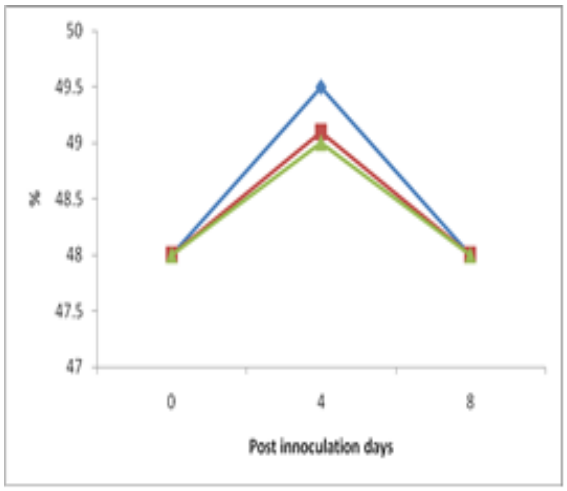

Figure 2: Heterophils count of chicks infected with S. *gallinarum*

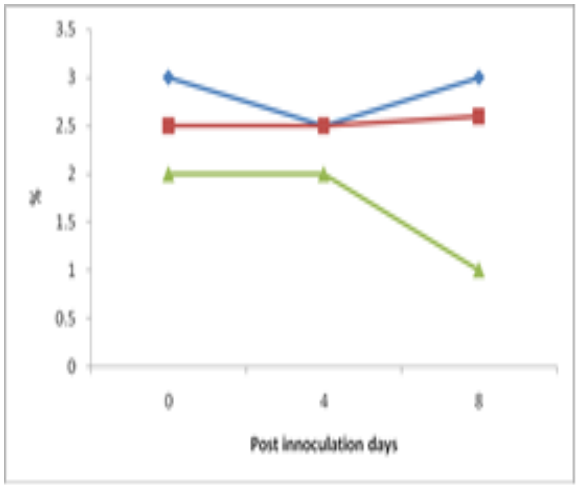

Figure 3: Monocytes count of chicks infected with S. *gallinarum*

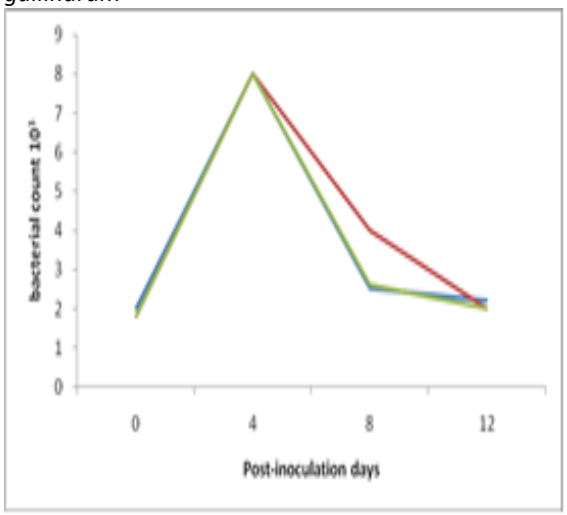

Figure 4:Bacterial count in the liver of chicks infected with S. *gallinarum*

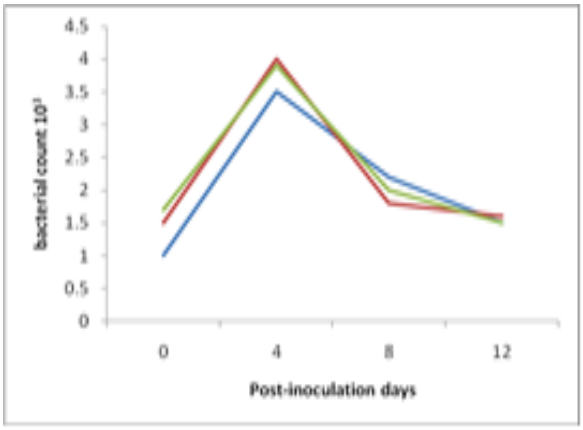

Figure 5: Bacterial count in the spleen of chicks infected with S. *gallinarum*

Discussion

This present study showed the effect of inoculation of *Salmonella gallinarum* in both the indigenous and the exotic breed of chickens in Nigeria as demonstrated by the clinical signs, mortality, pathology, leukocyte count, bacterial count from liver and spleen of infected chicks. This finding supports previous observations that exotic and indigenous breeds are susceptible to *Salmonella gallinarum* (Alvarez *et al.,* 2003). Clinical sign in chicks infected with *S. gallinarum* was most severe and lasted longer in the exotic breed. In this study, the same strain and concentration of *Salmonella gallinarum* were inoculated into all the experimental birds. Earlier work by Bumstead & Barrow (1993) reported genetic variation in susceptibility to some *Salmonella enterica* serovars in in-bred white leghorn lines due to general mechanism resistance experimental chicks used. In this study it was also observed that severe clinical signs were more pronounced in the exotic breed than the indigenous chicks, this support the observation of Mdegela *et al.* (2002), who observed that exotic breeds show more severe clinical signs than the indigenous. It was also observed that the peak of viable bacteria counts coincide with the period with severe clinical signs and mortality in the infected chicks especially in Fulani ecotype and exotic breed. This is in agreement with earlier observation of Mdegela *et al.* (2002), who reported a correlation between the viable bacteria counts and severity of clinical signs. It was also observed that Yoruba ecotype suffered less severe clinical signs than the exotic breed. This is probably due to better immune responses observed in this indigenous chicken which had been reported to have better immune responses than the exotic chicks (Aire & Ojo, 1974). Within the Nigerian indigenous chicken, the Fulani ecotype was more susceptible to *Salmonella* compared to the Yoruba ecotype which further corroborated our recent observation (Sola-Ojo & Ayorinde, 2011) that the Fulani ecotype is more susceptible (Ogie *et al.,* 2012).

In terms of mortality, the exotic breed and Fulani ecotype had higher mortality than Yoruba ecotype. The higher mortalities recorded could be a function of their susceptibility to *Salmonella gallinarum*. This observation is in agreement with earlier report (Adeleke *et al.,* 2011), that *Salmonella gallinarum* is more invasive in the exotic chicks than the indigenous. The higher susceptibility of the Fulani ecotype to *S. gallinarum* could also be as a result of its origin as reported by Ogundipe, (1990) that Fulani ecotype is a cross between the Rhode Island Red and applied to various serotypes of *Salmonella.* It is evident that the local chickens used in this study are different ecotypes which represent different genetic groups. It is therefore reasonable to believe that the differences observed reflect the differences in the genetic background of the experimental chicks. This suggests that the indigenous chickens differ in disease resistance to *Salmonella gallinarum* infection.

It was observed that clinical signs progressively increased from mild to severe and peaked at day 8 pc (Table 1 and 2). However, no clinical signs exceeded day 13 pc in infected chicks. This is contrary to the earlier report of Msoffe *et al*. (2006) who reported that no clinical signs exceeded day 9. The probable reason for this difference may be the different strain of pathogen and the types of the indigenous. However, Rhode Island Red had been reported to be a very susceptible chicken to some bacterial infection (Sonaiya, 1992). The peak mortality in this study was recorded on day 8 which coincides with the peak of clinical signs and the viable bacterial count. This is in agreement with the report of Ogie *et al.* (2012), who reported a strong correlation between viable bacterial count and the severity of clinical signs observed.

The overall mean counts for the liver and spleen also peaked on day 8 pc which agreed with Mdegela *et al*. (2000, 2002), who reported peak bacterial cell counts from the liver and spleen of the indigenous chickens on day 9 after infection while other studies revealed 6 -10 days (Monack *et al.,* 1996, Msoffe *et al.*, 2006). The differences between the current observation and those of other workers may be due to the genetic differences between the strains of bacteria used, concentration of the pathogen as well as the chicks used in the study.

The result of the heterophils, lymphocytes and monocytes dynamics in *S. gallinarum* infection revealed significant difference between indigenous chicks and exotic breed, in terms of peak values ($P < 0.05$). In the infected chicks, the monocytes count for the Fulani ecotype dropped on day 4 and later increased on day 8. The sharp decline in monocytes count from day 1 to 4 after infection may have been attributed to the cytotoxic effects of *Salmonella* in Fulani ecotype (Monack *et al.,* 1996). However, there was no decrease in the monocytes count in the exotic breed and Yoruba ecotype on day 4, rather the counts maintained a steady rate from day 1 to 4. The result obtained is in contrast with report of Msoffe *et al*. (2006), who reported a decline on day 3 and a peak on 6 in chicks infected with *S.*

gallinarum. The differences could also be due to the genotype of chicks used. The decrease in the Percentage distribution (Pd) for monocytes count coincided with the increase in Pd for both heterophils and lymphocytes which are cells known to influence the susceptibility to *Salmonella* infections (Redmond *et al*., 2011). It was observed that the bacterial counts of the internal organs rose to the peak on day 8 after the infection. This observation is in agreement with the pattern observed by Msoffe *et al.* (2006) which further corroborate the reports of Aire & Ojo (1974), that Nigerian indigenous chicks have better immune responses than the exotic breeds. In the Pd for lymphocytes count in chicks infected with *S. gallinarum*, only one peak was observed by the exotic on day 8. This report is contrary to the report of Msoffe *et al.* (2006) who reported multiple peaks. The probable reasons for a single peak in the lymphocyte count of chicks infected with *Salmonella gallinarum* could also be differences in the genotype of the experimental chickens. The gross pathological changes also showed the same pattern of the susceptibility with Yoruba ecotype being more resistant with least changes but the lesion was not observed from day 4 pc.

In Conclusion, this study further confirms the earlier observations that the exotic breeds are more susceptible to *Salmonella gallinarum* than the indigenous chickens. It has also shown that among the indigenous chickens, the Fulani ecotype is more susceptible to *Salmonella gallinarum* than the Yoruba ecotype.

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