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The possible genetic influence on the susceptibility of exotic, fulani and yoruba ecotype indigenous chickens to experimental *Salmonella enteritidis*

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Abstract

This study was conducted to evaluate the possible genetic resistance of exotic and indigenous chicks to Salmonella enteritidis. A total of 72 9 weeks old chicks were for this study which include the Fulani ecotype (FSF), Yoruba ecotype (YSF), and the Exotic breed (Black Nera cockerel). Chicks were infected with 3.8 x104 CFU of S. enteritidis and were observed for 16 days. Evaluation of possible genetic resistance was based on clinical signs, mortality and differential leukocytes count of infected chicks.

Clinical signs were evident on day 3 with eighty three percent (83.3%) of the chicks showing severe clinical signs on day 8 post infection. The exotic breed had the most prolonged clinical signs with all the chicks showing severe clinical signs. Yoruba ecotype had the shortest timing for the clinical signs, with 62.5% of the Yoruba ecotype affected. 87.5% of the Fulani ecotype and 98% of the exotic breed were affected. No mortality was recorded with the chicks infected with S. enteritidis. Assessing the clinical signs observed from infected chicks revealed that Yoruba ecotype is the least susceptible. The result from the study also indicated that the exotic is more susceptible to Salmonella infection than the indigenous chicks. It also revealed that within the ecotypes in Nigeria, Fulani ecotype is more susceptible to Salmonella infection than the Yoruba ecotype. The differential susceptibility and the low mortality observed in Yoruba ecotype indicated probable genetic resistance to S. enteritidis

Key words: disease resistance pattern, Nigerian indigenous chickens ecotypes, Salmonella enteritidis

Introduction

The indigenous chicken have survived various diseases (Yongolo 1996) and various environmental stress (Otim 2005), which has led to the assumption that local chickens are naturally and generally resistant to diseases (Kulube 1990; Chrysostome et al 1995). However, Mdegela et al (2002), Okoye and Aba-Adulugba (1998) concluded that the resistance of an indigenous chicken to a diseases is disease-specific.

Fulani or savannah, Yoruba or forest (Olori 1992), Nsukka, Owerri and Awgu ecotypes (Hill and Modebe 1961) are some of the indigenous ecotypes chickens found in Nigeria. Variations in trait of each ecotype have been reported by Oluyemi et al (1982). However no report has been found on the resistance of these ecotypes to diseases.

High prevalence of diseases is among the major factors limiting high productivity of the local chickens in the tropics (Yongolo 1996; Alexander 2001; Otim 2005), among these diseases is that

caused by Salmonella enteritidis. One of the major changes in the epidemiology of non-typhoidal Salmonellosis in the second half of the 20th century was the emergence of Salmonella enteritidis as a major food borne disease (Velge et al., 2005). S. enteritidis is currently the second most common Salmonella serotype and is responsible for about 17% of all human Salmonellosis (Patrick et al 2004; CDC 2005; Cox et al 1996). The epidemic of S. enteritidis began in the late 1970s in the Northeast region of the United States and in certain areas of Europe (St. Louis et al., 1988).

Epidemiological investigations of sporadic cases and outbreaks of S. enteritidis infections have demonstrated that contaminated eggs and egg products are major risk factors (Coyle et al 1998; Mead et al 1999). Other sources such as poultry, meat (Kimura et al 2004), and raw almonds (CDC 2004) have been implicated as causes of S. enteritidis infection in humans. Since non-typhoidal Salmonellosis is currently being reported and research on in Nigeria (Ogunleye et al 2005; 2009; Ishola and Holt 2008), the need to evaluate the resistance of indigenous and exotic chicks to Salmonella enteritidis is expedient for the proper understanding of the probable role of indigenous chicken ecotypes in the epidemiology of salmonellosis in Nigeria.

Materials and Methods

A total of seventy-two (72) chicks of nine (9) weeks old of Exotic breed, Fulani and Yoruba ecotype were used for this experiment. The exotic breed was purchased from a local hatchery at day old and was reared for nine weeks; they were vaccinated against Newcastle disease on day one (1) and day twenty-one (21). They were given vitamins and amprolium (anticoccidal) at week six (6). The Fulani and Yoruba ecotypes were obtained from some Fulani and Yoruba Villages in Ilorin and Ibadan, Nigeria respectively at age nine (9) weeks. The chicks were vaccinated against Newcastle disease and were given prophylactic doses of Mebendazole (antihelmintic) and Amprolium (anticoccidal) in accordance with the manufacturer's recommendations. The chicks were challenged with *Salmonella enteritidis* and observation for clinical signs was carried out for the period of sixteen (16) days.

Experimental Design

Completely randomized designed (CRD) was used. Twenty four (24) chicks from exotic breed, Fulani ecotype and Yoruba ecotype were allocated into four replicates consisting of six birds per replicate.

Inoculation of chicks with *Salmonella enteritidis*

Twenty four (24) chicks from each ecotypes were inoculated orally with a 1ml overnight tryptone soya broth culture of *Salmonella enteritidis* isolate (resistant to nalidixic acid), diluted to contain approximately to contain $3.8x10^4$ colony-forming units/ml. The chicks were given growers' marsh without antibiotics. They were observed twice daily for clinical signs and mortalities. Before the day of infection, approximately 2ml of whole blood was collected through the jugular vein of one chick each selected at random from each replicate and taken directly into sample bottles. On day 4, 8, 12 and 16, two blood samples were collected from each bird in each replicate for packed cell volume (PCV) determination and differential count.

Clinical Signs of Chicks Infected with *Salmonella enteritidis*

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 Christensen et al (1997) and Msoffe et al (2006), with (-) indicating no clinical signs; (+) indicates drowsiness, occasional closure of the eyes and drowsiness while (++) indicates closure of the eyes and reluctance to move.

Determination of PCV of Chicks Infected with *Salmonella enteritidis*

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.

Enumeration of Selected Leukocytes in Chicks Infected with *Salmonella enteritidis*

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 with Wrights stain (Drijver and Boon 1986). 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)

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.

$Yij = \mu + \alpha i + eij$

Where Yij = Individual observation assumed to be random elements

μ = Population means fixed, to be determined

αi = Treatment effect fixed, to be determined

eij = error associated with each record random and normally distributed.

Results

Clinical Signs of chicks infected with *Salmonella enteritidis*

Table 1 present the clinical signs for both the indigenous chickens and the exotic breed infected with *Salmonella enteritidis*. Clinical signs were observed in chickens on day 3 post inoculation with drowsiness and occasional closing of the eyes. From day 6-8, there was marked decrease in feed consumption. Clinical signs observed were more severe in the exotic breed and the least affected was the Yoruba ecotype. No clinical signs were observed beyond day 13 after infection and mortality was not recorded as all the birds that exhibited various clinical signs recovered on day 15.

Table 1: Clinical signs for chicks infected with *Salmonella enteritidis*

Infected chicks			
Days (p.i)	FSF	YSF	EB
3	+(2)	+(5)	+(3)
4	+(4)	+(7)	+(8)
5	+(5)	+(7)	+(9)
6	+(9) ++(4)	+(6) ++(2)	+(5)++(5)
7	+(4) ++(8)	+(2) ++(3)	+(9) ++(9)
8	+(2) ++(13)	+(1) ++(9)	+(3) ++(17)
9	+(8) +(1)	+(1)	++(1) +(8)
10	+(3)	+(2)	+(4)
11	+(1)	-	+(2)
12	+(1)	-	+(2)
13	-	-	+(2)
14	-	-	-
15	-	-	-
16	-	-	-
Number of Mortality	0	0	0
Number of Survivors	24	24	24

**P.I = post inoculation*

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

Means in the same row with different superscripts are significantly different ($P<0.05$) from each other.

(-): no clinical signs noticed

(+): Less severe clinical signs

(+ +): Very severe clinical signs

PCV and Leukocyte Enumeration for Chicks Infected with *Salmonella enteritidis*

PCV values for all the infected chicks fall within the normal range (>30%). Figure 1 shows the changes in the heterophilic population over 16 days of the experiment for both the indigenous (Yoruba and Fulani) chicks and exotic breed. The exotic breed had a high mean percentage distribution (Pd) on day 4 followed by a sharp decrease on day 8 and 12 with another slight increase on day 16. Yoruba ecotype however showed two peaks (day 4 and 16), this was followed by a decline on day 12. Generally, all the chicks had a peak on day 16 and decline on day 12. Comparing the means, significant difference was observed within the indigenous and between the exotic and the indigenous chicks on day 8, 12 and 16.

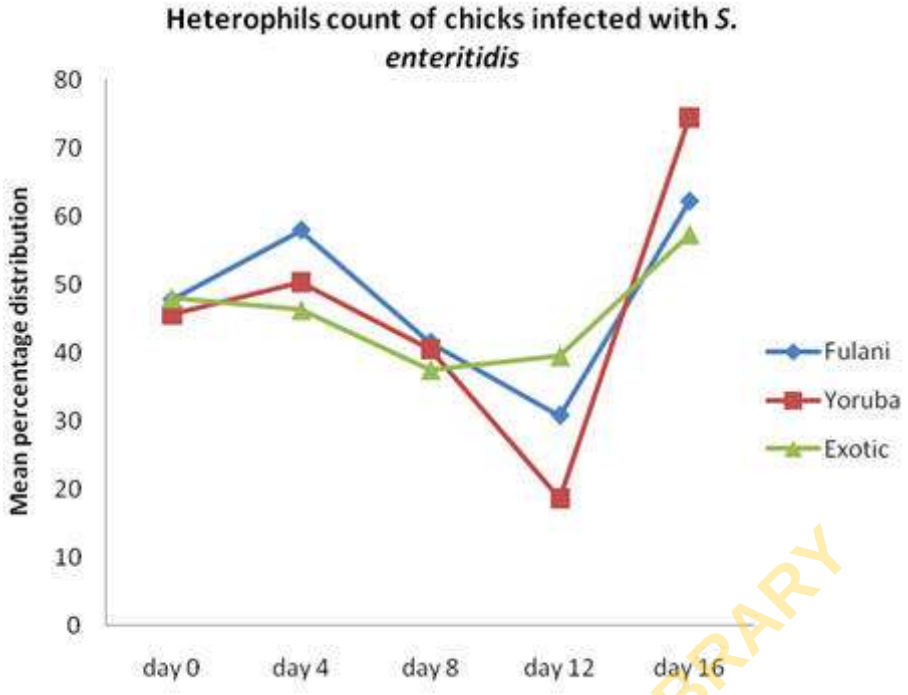

Figure 1: Mean Pd for heterophils in chicks infected with *S. enteritidis*

In figure 2, mean Pd for monocytes count for all infected the chicks showed a decrease on day 1-4 and an increase on day 8. There were significant difference ($p<0.05$) between all the mean values of the group of chickens infected with Salmonella enteritidis on day 8, 12 and 16. However Yoruba ecotype has highly significant Pd for monocytes when compared to other group of infected chicks.

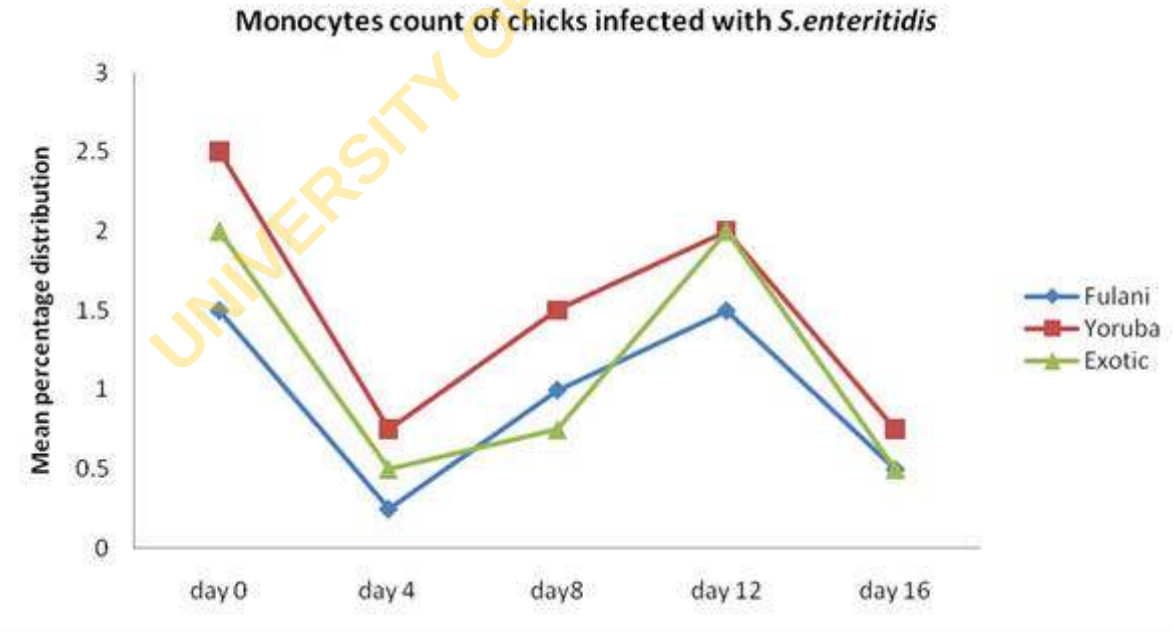

Figure 2: Mean Pd for monocytes count in chicks infected with *S.enteritidis*

The mean for the lymphocytic population for chicks infected with Salmonella enteritidis is presented in figure 3. Both the indigenous and the exotic chicks showed peaks on day 8 and 12 with a sharp decrease on day 8 and 16. All means are significantly different from each other.

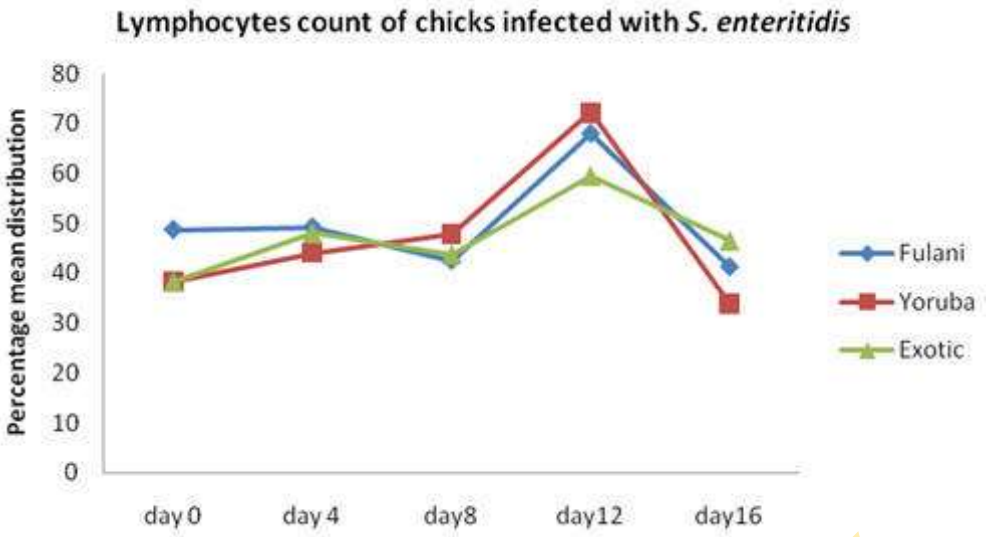

Figure 3: Mean Pd for lymphocyte count in *S. enteritidis* infected chicks

Discussion

This present study describes the clinicopathologic effect of *Salmonella enteritidis* in the indigenous and the exotic breed of chickens in Nigeria as demonstrated by the clinical and haematological features. This finding supports previous observation that showed that exotic and indigenous breeds are susceptible to Salmonella enteritidis (Ishola and Holt 2008). Clinical signs in chicks infected with *S. enteritidis* were most severe and lasted longer among the exotic breed than the ecotypes while the Yoruba ecotype had lesser clinical signs than the Fulani ecotype. This observation suggests a probable genetic influence. Earlier work by Bumstead and Barrow (1993) had earlier reported genetic variation in susceptibility to some *Salmonella enterica* serovars in in-bred white leghorn lines and this may also account for the difference in the chicken used. It is therefore reasonable to assume that the differences observed reflect the differences in the genetic background of the experimental chicks. The mechanism associated with this difference needed to be further elucidated. Although various investigations in to the humoral response of chickens in Nigeria to various agents revealed that indigenous chicken has a better humoral response than exotic breed (Ohore et al 2007; Oladele et al 2007).

It was observed that clinical signs progressively increased from mild to severe and peaked at day 8 (Table 1) however, no clinical signs exceed day 13. This is contrary to the report of Msoffe et al (2006) who reported that no clinical signs after day 9. The probable reason for this difference may be the different strain of pathogen and the types of experimental chicks used. In this study it was 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 also observed that exotic breeds shows more severe clinical signs than the indigenous. It was observed that Yoruba ecotype suffered less severe clinical signs and recovered earlier than the exotic breed. This probably is due to better immune responses observed in these indigenous chicks as reported by Aire and Ojo (1974) that indigenous chicks have better immune responses than the exotic chicks. However, the Fulani ecotype was more susceptible to Salmonella than the Yoruba ecotype. This observation confirms the report of Tiamiyu (1999) that Fulani is more susceptible to bacterial infection.

In *S.enteritidis* infected chicks, it was noticed that despite the level of clinical signs (62.5 -98%) observed by all the infected chicks, no mortality was recorded. Moreover, all the chicks did not show any clinical signs after day 13. The reason for the absence of mortality in the infected chicks may be attributed to the strain of S. enteritidis used in the study. This is supported by the report by

Msoffe et al (2001) that reported that chickens exhibit different rate of immune response to different strain of the same pathogen.

This study also assessed the haematological changes of the indigenous and the exotic breed to these pathogens in order to determine the most susceptible ecotype to Salmonella enteritidis infection. The result from the heterophils, lymphocytes and monocytes dynamics in S. enteritidis revealed significant difference between indigenous chicks and exotic breed, in terms of peak values ($P < 0.05$).

In the chicks infected with *Salmonella enteritidi*s, monocytes fell from day 1 to 4 and peaked at day 12 followed by a decline on day 16. This is in agreement with observation made by Msoffe et al (2006) who reported a fall on day 3, peak on day 6 and final fall on day 14. The sharp decline for monocytes count from day 1 to 4 after infection may have been attributed to the cytotoxic effects of Salmonella that leads to apoptosis of the leukocytes (Monack et al 1996). The fall of the Pd for monocytes count coincided with the increase in Pd for both heterophils and lymphocytes to other cells known to influence resistance to Salmonella infections (Stabler et al 1994; Kogut et al 1994; Kogut et al 1998; Harmon, 1998).

It was observed that the Pd for heterophils count fell on day 12 after inoculation for S. enteritidis infected chicks. It follows that the fall of the heterophils count on day 12 signifies the increase of the bacteria activities and decrease in the number of heterophils in the system of the chicks. Nevertheless, there was clearance of bacteria as indicated by the low number of bacterial counts and the increase in heterophils count on day 16. This observation is in agreement with the pattern observed by Msoffe et al (2006), but while Msoffe et al (2006) reported a fall in the heterophils count on day 10, this current study reports a fall on day 12. The difference in the day of the heterophils fall could be attributed to the differences in the bacteria used and the genotypic differences in the experimental chicks. Comparing the means of the heterophils count, between the genetic groups on day 12 and day 16, there were significant differences ($p<0.05$). This infer that the heterophils commenced the bacterial clearing after day 12 post inoculation and with the Yoruba and Fulani ecotypes having the highest heterophils count on day 16, this is an indication of level of resistance to Salmonella enteritidis. This suggestion is in agreement with Aire and Ojo (1974), who reported that Nigerian indigenous chicks have better immune responses than the exotic breeds.

In the Pd for lymphocytes counts in chicks infected with S. enteritidis, it was observed that exotic breed, Fulani and Yoruba ecotypes peaked on day 12. However, there were early peak by the Fulani ecotype and exotic breed. This report is in agreement with the report of Msoffe et al (2006) who reported early peaks in the lymphocytes count of the commercial and indigenous chicks. They further reported that chicks with early peaks in lymphocytes count may be susceptible to diseases. This early report supports the current study as Fulani ecotype and Exotic breed were observed to have early peaks, hence were more susceptible to Salmonella infection. Comparing the means of the lymphocytes count between the genetic groups on day 12, there was significant difference ($p<0.05$). The highest lymphocytes count was observed in Yoruba ecotype with 72.25 ± 2.02, followed by Fulani ecotype with 68 ± 0.82 and exotic breed with 59.5 ± 0.65. A high lymphocytes count may be an indication of resistance to the Salmonella infection. This observation is supported by Harmid and Sharma (1990) who reported that Salmonella is more invasive in the exotic chicks than the indigenous chicks.

It has been long proposed that the Nigerian indigenous chickens could be more resistant to diseases than the exotic breeds due to their hardy nature (Aire and Ojo, 1974). This current investigation further suggests that the exotic breeds are more susceptible to diseases than the indigenous chickens. (Okoye and Aba Adulugba 1998; Mdegela et al 2002). From the report obtained from the current study, the exotic breeds are more susceptible to Salmonella enteritidis than the indigenous chickens. It was also observed that among the Indigenous chickens, the Fulani ecotype is more susceptible to Salmonella enteritidis than the Yoruba ecotype. The susceptibility of the Fulani ecotype could be as a result of its origin. Ogundipe, (1990) was of the opinion that Fulani ecotype is

from a cross between the Rhode Island Red and the indigenous fowl used previously in cockerel exchange programmes. Rhode Island Red has been observed to be a very susceptible chicken to bacterial infection (Tiamiyu 1999).

Conclusion and Recommendations

- It can therefore be summed that, the indigenous chickens are more resistance to *Salmonella enteritidis* than the exotic breed. Also within the indigenous ecotypes the Yoruba ecotype is more resistance than the Fulani ecotype. Based on these results, it is suggested that the observed differences between indigenous ecotypes and exotic breed are genetic and should be utilized in selective breeding both within and between ecotypes as suggested by Msoffe et al., (2006).

- Further studies on disease resistance may provide insight on the most resistant varieties within this Yoruba ecotype. It would be interesting to establish the link between high disease resistance potential in indigenous chicken ecotypes and varieties that include Naked neck, frizzled feathers, smooth feathers and dwarf (Sonaiya and Olori 1989) with productivity parameters such as body weight, egg weight and growth rate.

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