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Research Article
Immune Response of Nigerian Chicken Genotypes to Salmonella and Newcastle Vaccines

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Abstract

Background and Objective: Indigenous chickens as a source of meat and egg have made significant contributions to household food security throughout the developing world. The present study was aimed at evaluating the antibody titre of Nigerian chicken genotypes vaccinated with Salmonella and Newcastle vaccines. **Materials and Methods:** A total of 96 chickens were used for the study (24 normal feather, 24 frizzle feather, 24 naked neck, 24 exotic). Initial blood samples of the chicken were obtained from the wing vein and evaluated for Salmonella and Newcastle antibody titre using widal and haemagglutination inhibition test, respectively before vaccination. The birds were divided into two broad groups of 48 chickens each and treated with Salmonella and Newcastle vaccines, respectively. Blood samples were collected and analyzed at 3 and 5 days post vaccination. **Results:** Antibody titre was measured in the genotypes 3 days post vaccination with Salmonella vaccine as; 2.27 (frizzle feather), 2.14 (normal feather), 2.12 (naked neck) and 1.93 (exotic). The 3 days post vaccination titre with Newcastle vaccine were; 0.62 (Normal feather), 0.56 (frizzle feather), 0.51 (naked neck) and 0.09 (exotic). Responses to vaccination were significant ($p<0.05$) for antibody titre of the chicken vaccinated with Salmonella and Newcastle vaccines. The chickens showed significant responses to Salmonella vaccination and also, highest antibody titre was recorded in the birds 3 days post vaccination. **Conclusion:** It was concluded that frizzle and normal feather chicken genotypes showed more resistance to salmonella and Newcastle vaccines.

Key words: *Gallus domesticus*, Salmonella vaccination, indigenous chickens, exotic, newcastle vaccines, antibody titre, frizzle feather

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Indigenous chickens (*Gallus domesticus*) make substantial contribution to household food security throughout the developing world. They serve as a source of investment, generating economic benefits for many households. The market demands for local chicken are increasing[1,2] as they serve as affordable source of protein via their meat and egg consumption. It is reported that about 90% of poultry populations are chicken and they are the most important poultry species all over the world[3]. Prior to urbanization, indigenous chickens were basically raised by small household settings for consumption and for various socio-cultural practices. However, with human population expansion and increasing demands for affordable and accessible protein sources, domestication of chicken has been highly advocated. Thus, poultry production by families has become a common practice as a means of obtaining food security, income and gainful employment[4].

In Nigeria, with human population of over 180 million[5], the need for diversification of agricultural practices should not be overemphasized as there is a pressing demand for food supply to the growing populace. Protein supply is very important in ensuring healthy living.

Although there are many sources of protein, animal proteins are very reach in essential amino acid required to maintain the body system[6]. Interestingly, chicken offers affordable protein source in the poultry family. Their meat and egg are routine meal by many families in Nigeria. It is reported that indigenous chicken constitute 80% of the 120 million poultry types raised in Nigeria[7].

Despite the success recorded so far in the domestication of indigenous chicken, disease attack has been a challenging factor that has hampered poultry production in Nigeria. Salmonella and Newcastle are two common diseases that affect poultry production. Salmonella are gram negative enterobacteria which affect mammals and poultry birds especially domestic fowl around the world[8]. There are several serotypes which transmit pullorum disease, fowl typhoid and salmonellosis to poultry birds. Isolation incidence of salmonellosis in humans due to consumption of infected chicken eggs was reported[9]. Therefore, there is need for identification of proper poultry management practices to reduce economic losses and enhance public health. Similarly, Newcastle disease is a highly contagious viral infection of avian species especially poultry caused by Newcastle disease virus[10]. Infection of poultry with Newcastle disease (ND) has resulted in economic loss and essential transmission to human population as well as posing serious health challenge. Interests in vaccination of birds as means of controlling these challenging diseases have continued to rise[11-13].

Variation existing within and among species has been very key in detecting disease resistance among breeds. There exist biodiversity that encompass the genetic variants within and among the local genotypes of chicken distributed around the world. These genetic variants have evolved due to domestication, selection and breeding[14]. Some of these variants are due to the presence of major morphological marker genes which increase the adaptability of these breeds to tropical climatic environments as seen in the feather size, feather pattern, body size and plumage colour[15].

The morphological distinction observed in local chicken may therefore, confer variation in their response to disease attacks. In this regards, selective breeding of resistance individuals may be adopted in poultry breeding to reduce the spread of diseases in poultry farms. Therefore, the present study was designed to observe the antibody titre of Nigerian chicken genotypes vaccinated with Salmonella and Newcastle vaccines.

MATERIALS AND METHODS

Study location and experimental materials: This study was carried out in the Animal House Unit, Department of Genetics and Biotechnology, University of Calabar, Nigeria. The different genotypes were obtained from two locations; Watt Market in Cross River State and Itam Market in Akwa Ibom State. The choice of these locations was hinged on the abundance of these genotypes in the areas. A total of 96 matured chickens were used for this research cutting across four genotypes (Normal feather, frizzle feather, naked neck and exotic). The Plate 1-4 shows the diagrams of frizzle feather, naked neck, normal feather and exotic chicken genotypes, respectively. Salmonella and Newcastle vaccines were purchased from Jojas Nigeria Limited in Calabar, Cross River State.

Management of experimental animals: The chickens were raised in standard cages with natural ventilation. The cages were properly cleaned and disinfected prior to the arrival of the experimental birds. They were allowed for 3 weeks to acclimatize and were fed with grower feed and water before commencement of vaccination. The cages were cleaned and the beddings changed on daily basis to ensure hygienic experimental conditions for the birds. Ethical care and handling of experimental animals were observed at all times and the study was approved by the University of Calabar ethical committee.

Plate 1: Frizzle feather chicken genotype

Plate 2: Naked neck chicken genotype

Plate 3: Normal feather chicken genotype

Plate 4: Exotic genotype

Vaccination procedure: The chickens were randomized into two broad groups of 48 birds each for salmonella and Newcastle vaccines, respectively. Each of the two groups had four sub-groups based on the 4 chicken genotypes (Fig. 1). After acclimatization, 1 mL of blood was collected via the wing vein of each bird using sterile needle and syringe to measure the initial antibody titre level before vaccination. One week after, the birds were vaccinated with 1 mL of salmonella and Newcastle vaccines, respectively through their wing veins. Blood samples were collected from all the birds into well labeled EDTA bottles at 3 and 5 days post-vaccination and taken to the laboratory for antibody titre measurement. Salmonella titre was determined using the widal test by tube agglutination method while Newcastle titre was determined using haemagglutination inhibition (HI) test[16].

Procedure for widal test: All the samples under salmonella vaccine were subjected to widal test by tube agglutination to assess salmonella antibody across the chicken genotypes. Four rows containing 6 test tubes were carefully prepared for the chicken genotypes. Double dilution of the test serum (1:10, 1:20, 1:40, 1:80, 1:160 and 1:320) were prepared in all the rows with each tube containing 0.4 mL of the diluted serum. About 50 µL of antigen O, H, AH and BH was added to each test tube, mixed thoroughly and incubated at 37°C for 24 h. Appropriate tubes were prepared for positive and negative controls as well. The pattern of agglutination was determine macroscopically using the magnifying lens and compared with the controls. Antibody titre was taken as the highest dilution of serum giving visible agglutination[17].

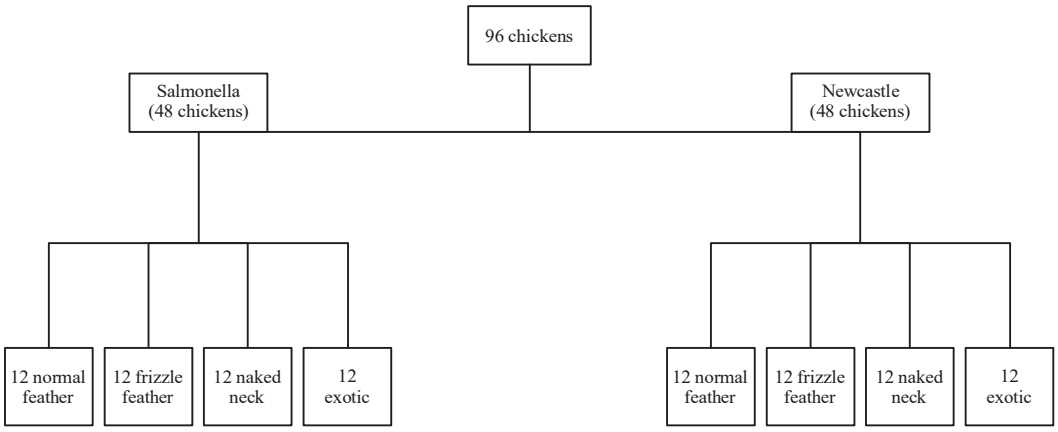

Fig. 1: Chart showing randomization of the chicken genotypes

Procedure for haemagglutination inhibition test: Haemagglutination inhibition (HI) test was carried out according to the procedure of OIE[16]. Two serial dilution of 25 µL serum was made with phosphate-buffered saline (PBS) in V-bottomed microtitre plates up to 10th well. About 25 µL of 4 haemagglutination units of Newcastle disease virus or antigen was added up to 11th well. The plates were kept at room temperature for more than 30 min to facilitate antigen-antibody reaction. Then 50 µL of 1% (v/v) chicken RBC suspension was added to each well. The 11th well containing the antigen and RBCs served as the positive control while the 12th well containing only RBCs served as the negative control. After gently mixing, the RBCs were allowed to settle at room temperature for 40 min and agglutination was assessed by tilting the plates. The samples showing peculiar central button shaped settling of RBCs were recorded as positive and maximum dilution of each sample causing agglutination inhibition was considered as the end point, which was used to estimate HI titire. The HI titre of each serum was expressed as reciprocal of the serum dilution.

Statistical analysis: Data obtained from the titre measurement were subjected to a one-way analysis of variance (ANOVA) using SPSS version 20.0. The Duncan multiple test was used to separate the significant means at $p<0.05$.

RESULTS

Response of chicken genotypes to salmonella vaccine: The result of the response of the chicken genotypes to salmonella vaccine at different days of exposure is presented in Table 1. The result revealed that there were significant differences ($p<0.05$) in the response of the chicken genotypes to salmonella vaccines as was measured by their antibody titre levels. The highest antibody titre was recorded by frizzle feather genotypes 3 days after vaccination (2.27) while the least was recorded from exotic genotype (1.93). There was no significant difference ($p>0.05$) in the antibody titre of normal feather and naked neck genotypes 5 days after vaccination while frizzle feather and exotic genotypes still gave the highest and the lowest antibody titre, respectively. As shown in Fig. 2, it was revealed that the response of chicken to salmonella vaccine was influenced by their genotypes ($p<0.05$). The pooled antibody titre measurement was highest in frizzle feather genotypes while the lowest from the exotic genotypes. However, there was no significant difference ($p>0.05$) in the pooled antibody titre level of normal feather and naked neck genotypes.

Response of chicken genotypes to Newcastle vaccine: Result of the response of chicken genotypes to Newcastle vaccine is shown in Table 2. It was shown that vaccination of the chicken genotypes with Newcastle vaccine had significant differences ($p<0.05$) in the antibody titre measured. The level of antibody titre was highest in all the genotypes 3 days after vaccination and was reduced at 5 days post vaccination. Normal feather chicken genotypes produced the highest antibody titre 3 days post vaccination (0.62) and 5 days post vaccination (0.59). The lowest was from the exotic genotype at 3 days (0.09) and 5 days (0.02) post vaccination. Figure 3 showed the pooled performance of all the genotypes of chicken exposed to Newcastle vaccine. Normal feather chicken was highest followed by frizzle feather, naked neck and exotic which were all statistically different ($p<0.05$).

Table 1: Duration effect on the response of chicken genotypes to salmonella vaccine

	Genotypes			
Duration	Frizzle feather	Normal feather	Naked neck	Exotic
Pre vaccination (Mean log_e widal titre)	1.99±0.74[a]	1.89±0.85[b]	1.85±0.51[c]	1.18±0.00[d]
3 days post vaccination (Mean log_e widal titre)	2.27±1.18[a]	2.14±0.18[b]	2.12±0.35[c]	1.93±0.73[d]
5 days post vaccination (Mean log_e widal titre)	2.13±0.29[a]	1.98±0.93[b]	1.98±0.18[b]	1.85±0.76[c]

[abc]Means with different superscript on the same horizontal line are significantly different (p<0.05)

Table 2: Duration effect on the response of chicken genotypes to Newcastle vaccine

	Genotypes			
Duration	Frizzle feather	Normal feather	Naked neck	Exotic
Pre vaccination (Mean log_e HI titre)	0.30±0.13[a]	0.30±0.13[a]	0.25±0.01[b]	0.01±0.001[c]
3 days post vaccination (Mean log_e HI titre)	0.56±0.12[b]	0.62±0.16[a]	0.51±0.09[b]	0.09±0.03[c]
5 days post vaccination (Mean log_e HI titre)	0.46±0.12[b]	0.59±0.13[a]	0.37±0.11[c]	0.02±0.007[d]

[abc]means with different superscript on the same horizontal line are significantly different (p<0.05). HI: Haemagglutination inhibition

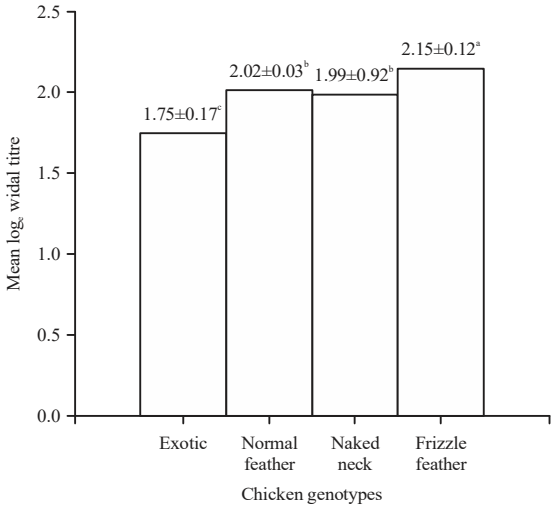

Fig. 2: Pooled performance of chicken genotypes to Salmonella vaccine

[abc]Means with different superscript are significantly different (p<0.05)

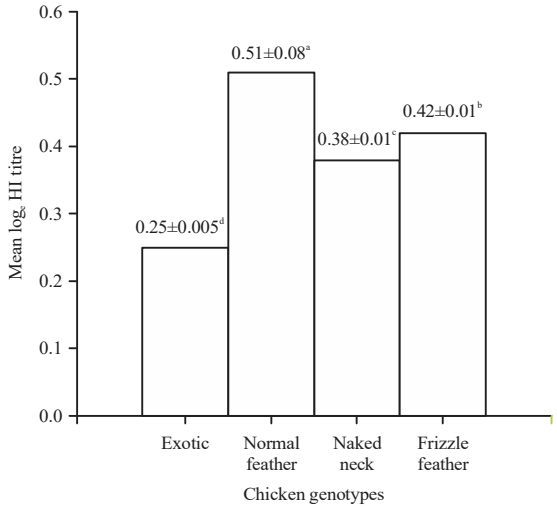

Fig. 3: Pooled performance of chicken genotypes to Newcastle vaccine

[abc]Means with different superscript are significantly different (p<0.05)

DISCUSSION

In the study, administration of the vaccines to chicken genotypes showed significant variation in their immune response as measured by their antibody titre. In general, the local genotypes were higher in their antibody titre response than the exotic counterpart. This may suggest that the local breeds of chicken are more resistance to salmonella and Newcastle diseases over the exotic breeds which corroborates earlier findings that the local chicken genotypes are well adapted to adverse environmental conditions than the exotic breeds[18,19]. It is possible that the heterogenous genetic nature of these local breeds accounted to the high antibody titre response to the vaccines. Administration of Newcastle vaccine increases the humoral immune response of chicken[20]. Therefore, its administration to the chickens may have caused humoral increase in the chickens leading to increase in the antibody titre measured across the breeds which were consistently higher than the pre-vaccination titre measurement in all the chicken genotypes.

Administration of salmonella vaccine yielded higher antibody titre in the frizzle feather genotypes followed by normal feather, naked neck and exotic. On the other hand, Newcastle vaccination of the chickens yielded higher antibody titre response in the normal feather followed by frizzle feather, naked neck and exotic. These findings supported the earlier submissions that frizzle feather genotype performed better in their morphological characteristics than naked neck[21]. Also, it has been opined that the amount and diversity of antibodies correlates to the strength of the body's immune system[22].

Therefore, it can be said that the high amount of antibody detected in the frizzle and normal feather chicken genotypes in this study contributed to their survivability and abundance in the local environments compared to the naked neck genotypes.

Interest in the amount of Newcastle virus shed into the environment by vaccinated birds has arisen as a potential indicator of vaccine efficiency[11]. In this study, the chicken genotypes showed higher and significant salmonella and Newcastle antibody titre 3 days post-vaccination and decreased across all the genotypes 5 days post-vaccination. This suggests that the efficacy of vaccines in poultry decrease with time. This aligns with earlier report that the efficacy of vaccines decline with time[23]. This is also in line with the results from previous study which observed that antibody titire after vaccination was relatively high in treated birds compared to controls and the level of antibody initially observed reduced with time as weeks passed on Muir *et al.*[24]. Similarly, the amount antibody detected in the serum 3 days post vaccination is consistence with that of Loa *et al.*[25] and Kremer *et al.*[26], who observed that the amount of antigen that was present in the system was highly elevated 3 days post vaccination indicating a strong response to vaccination. It is important for poultry farmers to adopt regular vaccination of chickens and other poultry for improvement of performance and enhancement of economic values. Antibody neutralizes virus particles by binding and preventing attachment of virus to the host cells[27]. Antibody titre measurement of the chicken genotypes used in this study indicated their ability to withstand salmonella and Newcastle viruses especially in the frizzle and normal feather genotypes. As reported, approximately 30% of IgY and 1% of IgM and IgA antibodies present in hen's plasma is passively transferred to offspring and if the level of the antibody is high enough, it can provide protection until the levels fall below a protection level[28]. It is interesting to opine that local chicken genotypes may be directly challenged with these viruses and selected for breeding with the aim of improving disease resistance. From the present findings, routine vaccination should be considered cardinal in all poultry management practices as a way to enhance performance and high yield especially among the local breeds of chicken.

CONCLUSION

From the results obtained, it can be concluded that frizzle and normal feather chicken genotypes are more resistance to salmonella and Newcastle vaccines than naked neck genotypes and on a general note, the indigenous chicken genotypes are more resistance to salmonella and Newcastle vaccines over the exotic counterparts.

SIGNIFICANCE STATEMENT

This study discovered that frizzle feather and normal feather chicken genotypes are more resistance to salmonella and Newcastle vaccine, respectively than the naked neck genotype. Considering the increasing demands for meat from poultry, the findings here in will be useful to poultry breeders both in selective breeding and management of poultry farms for the overall genetic improvement of local chicken genotypes.

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