Assessment of antibody responses to Newcastle disease vaccination in Nigerian indigenous chicken lines selected for sheep red blood cell antigen

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This research was carried out to evaluate serum haemagglutination inhibition antibody titres to a Newcastle disease virus vaccine in two Nigerian indigenous chicken lines selected for sheep red blood cell antigens. One hundred Nigerian indigenous chickens were used. These chickens belonged to the fourth generation of flock that had been divergently distinguished into a high antibody titre chicken line (HATC) and a low antibody titre chicken line (LATC). Chicks were given a NDV vaccine at six weeks of age, while blood samples were collected from each chick at seven days post-administration. The antibody titres of chicks were determined through a haemagglutination inhibition test and the data collected were subjected to analysis of variance using SAS 9.2 version. It was found that sex had no significant effect on the antibody titre values ($p>0.05$), while the genetic line and sex by genetic line interaction had significant effects on the antibody titre values. Female chicks of LATC recorded a significantly lower ND antibody titre (4.21±0.47) compared to HATC females (8.04±2.02). Also, male chicks of LATC recorded a significantly lower ND antibody titre (4.26±1.20) compared to HATC males (7.25±0.48). Meanwhile, there was no significant difference between male and female chicks of LATC as well as between male and female chicks of HATC. Based on the differences observed in ND antibody titres between the genetic lines, emphasis should be placed on policies to develop HATC and LATC along ND resistance. Therefore, we recommend that when considering ND infection which exerts substantial losses to the poultry industry, HATC should be favoured over LATC.

Keywords: Nigerian indigenous chicken, Newcastle disease, antibody titre, sheep red blood cell antigen

Indigenous chickens in Africa are generally hardy, adaptive to rural environments, survive on little or no inputs and adjust to fluctuations in feed availability. Chickens largely dominate flock composition and make up about 98% (Gueye 2003) of the total poultry numbers (chickens, ducks and turkeys) kept in Africa. Nigerian indigenous chickens are found mostly in the tropical and sub-tropical rural areas representing a valuable resource for livestock development.

Newcastle disease (ND) is an important highly-contagious viral disease of poultry which is of major economic concern. It has world-wide distribution and affects a variety of avian species. ND is characterised by marked variation in morbidity, mortality, clinical signs and lesions (Alexander 1991); it causes severe respiratory distress, nervous disorders, decreased egg production, and haemorrhagic intestinal lesions. ND can be prevented and controlled by vaccination and/or by quarantine and slaughter of diseased flocks in confirmed outbreaks (Alexander 1991). Serological antibody levels to Newcastle disease virus have been commonly used for the evaluation of ND vaccination and are related to protection against virus challenges (Beard and Brugh 1975).

Cell-mediated immunity or passive immunity has been demonstrated to be the first immunological response following ND vaccination and has been proposed to play a key role in conferring protection to chickens (Cannon and Russel 1988). The cell-mediated immune response to ND vaccine has been demonstrated in vaccinated birds (Marino and Hanson 1987), but immune responses in birds are established by several antigenic substances. Sheep red blood cell (SRBC) antigen has been used to monitor immune responses in chickens. As reviewed by Osei-Amponsah et al. (2013), SRBC has been used as a potential antigen in the immune response because it is non-pathogenic and multi-determinant. Antibody levels are important dynamic parameters of immune response as they partially reflect the

potential of an animal to resist pathogenic infection (Henriksen et al. 2013). Johnson and Edgar (1982) suggested that resistance and susceptibility to ND is associated with inheritance, and ultimately, chicken lines resistant to ND could be established by genetic selection. However, Nigerian indigenous chickens have not been established as distinct lines on the basis of immune response as compared to their exotic counterparts (Ngongeh et al. 2017). Thus, we aimed to investigate whether the process of developing Nigerian indigenous chicken lines through genetic selection along antibody response to SRBC, will simultaneously lead to better response to Newcastle disease virus. We conjectured that, apart from the chicken genetic make-up, sex difference may also link with antibody response.

Material and method

Two chicken lines, high antibody titre (HATC) and low antibody titre (LATC), were provided by the Poultry Breeding Unit of the Department of Animal Breeding and Genetics, Federal University of Agriculture, Abeokuta, Ogun State.

These two chicken lines originated from Nigerian indigenous chickens, which went through four generations of selection since 2014 based on the response to sheep red blood cell (SRBC) at seven weeks of age. The antibody response to SRBC for all the first generation chickens was measured at seven weeks of age. The antibody response to SRBC of the second generation was measured and compared to that of the previous one, and chickens with lower or higher average antibody response were chosen for subsequent breeding. Afterward, the chicken lines were divergently selected for the fourth generation. Briefly, SRBC preparation and inoculation involved the collection of blood samples (5 ml) from West African Dwarf sheep (< 2 years old) via the jugular vein. The sheep was restricted and the jugular was palpated. The site of collection was cleaned with alcohol and, using a 5 ml syringe, the blood sample was obtained and emptied into a lithium heparinised tube to disallow clotting. The packed cell volume was determined using a haematospin machine (1400 rpm) and haematospin reader. The blood sample was centrifuged at 3000 rpm for 10 minutes at 4ºC. The supernatant was removed subsequently. The settled red blood cells were washed three times using phosphate buffered saline. One ml SRBC was intravenously administered to each chick via the jugular vein at six weeks of age. Blood was collected on the seventh day post-inoculation. The blood was collected in plain tubes and slanted 45º for the blood to clot and for serum separation. Serum was separated subsequently in the laboratory and kept at -20ºC in the refrigerator for haemagglutination inhibition (HI) assay. This was carried out by making serial dilutions of serum (50 µl) obtained from chicken blood samples and with a combination of PBS (50µl) and 1% SRBC (50 µl). Based on HI titre, chickens were classified as HAT and LAT chicken lines.

At the fourth generation, one hundred day-old unvaccinated chicks (50 per genetic line) were collected and raised up to six weeks of age before the start of the study, under intensive management using a deep litter system. Feed and water were provided *ad libitum* while a commercial Newcastle disease (ND) attenuated vaccine was given to the chicks at six weeks of age. Blood samples were collected from the chicks at seven weeks of age and serum was separated and stored at –20°C until use. Serum samples were analyzed by HI test using 1% chicken red blood cells (RBCs) according to the OIE (2015) procedures. The HI titre was determined as the highest dilution of serum sample that inhibited NDV agglutination of chicken RBCs. The antibody titre of each chick was calculated by geometric mean of the obtained HI titre.

Statistical Analysis

Data generated were subjected to analysis of variance (ANOVA) using SAS (version 9.2). The statistical model is given below:

$Y_{ijk} = \mu + T_i + S_j + (TS)_{ij} + e_{ijk}$

Y_{ijkl}= Trait of interest (HI titre $\log_2$)
μ = Overall mean for the parameter of interest
T_i= Effect of the i^{th} chicken lines (i= high antibody titre, low antibody titre)
S_j= Effect of the j^{th} sex (j= male and female)
$(TS)_{ij}$ = interaction effect of i^{th} (chicken lines) and jth (sex) factors
e_{ijkl}= Random residual error

Significant means were separated using Least Significant Difference procedure.

Results

The detection of Newcastle disease (ND) antibodies in chicken genetic lines was based on haemagglutination inhibition (HI) of the serum samples. An average negative control titre of 1.50 indicated absence of ND antibodies while the average positive titre of 6.85 showed the presence of ND antibodies. All chicks used in this study had antibodies present in their sera. Analysis of variance results showing effects of sex, genetic line and their interaction on the chicken antibody titre levels against an ND vaccine are presented in Table 1. Genetic line by sex interaction had an effect on the chicks' antibody titre levels ($p < 0.05$). Also, genetic line significantly influenced chicks' antibody titre levels ($p < 0.001$). However, effect of sex on antibody titre was not significantly different ($p>0.05$). The high antibody titre chicken line (HATC) had a higher antibody titre level (7.59 ± 0.45) compared to the low antibody titre chicken line (LATC) (4.72 ± 0.31) as shown in Table 2. Statistically, there was no significant difference between male and female chicks' response to the ND vaccine. Female chicks of LATC recorded a significantly lower ND antibody titre (4.21 ± 0.47) compared to HATC females (8.04 ± 2.02). Also, male chicks of LATC recorded a significant lower ND antibody titre (4.26 ± 1.20) compared to HATC males (7.25 ± 0.48). Meanwhile, there was no significant difference between male and female chicks of LATC as well as male and female chicks of HATC.

Table 1: Analysis of variance showing effects of genetic line, sex and genetic line by sex interaction on the antibody titres against Newcastle disease of chicks at 7 weeks of age

Source	Df	Mean Squares
Genetic line	1	10.874***
Sex	1	0.372
Genetic line by sex interaction	1	3.525*
Error	97	

***$p < 0.001$ *$p < 0.05$, Df - degree of freedom

Table 2: Effects of genetic line and sex on the antibody titres against Newcastle disease of chicks at 7 weeks of age

Parameters	Subclass	HI titre ± SE
Genetic line	High antibody titre chicken line	7.59 ± 0.45^a
	Low antibody titre chicken line	4.72 ± 0.31^b
Sex	Male	6.45 ± 0.81
	Female	7.13 ± 0.62

HI: haemagglutination inhibition, SE: standard error.
Values with different superscripts within parameters differ significantly (*$p < 0.05$) within columns

Table 3: Effect of genetic line by sex interaction on the antibody titres against Newcastle disease of chicks at 7 weeks of age

Genetic line/Sex	Male	Female
High antibody titre chicken line	7.25 ± 0.48^{a}	8.04 ± 2.02^{a}
Low antibody titre chicken line	4.26 ± 1.20^{b}	4.21 ± 0.47^{b}

Values with different superscripts differ significantly ($^{*}p < 0.05$) within columns

Discussion

The level of circulating antibodies against ND in chickens could be obtained via the HI test (Boakye et al. 2016). Although, chicken antibody titres were not significantly influenced by sex, our results revealed that antibody titres in female divergently-selected chicks of the Nigerian indigenous chickens were comparatively higher than those of the male counterpart. This is in line with reports of Boakye et al. (2016) who concluded that the mean antibody titre of male chickens was lower than those of female chickens but was not statistically significant.

Based on OIE (2017) reports, HI antibody titre is considered as positive if there is an inhibition at 1:16 dilution (2^4) or more via 4HA units as working antigen. Positive antibody titre observed in this study may be considered as diagnostic evidence of ND. Rezaeianzadeh et al. (2011) reported positive serology on unvaccinated chickens as a signal that the chickens have circulation of ND virus but there was no occurrence of active viral infection based on RT-PCR detection. Also, Jibril et al. (2014) reported positive serology as an indication of sub-clinical infection in chickens.

However, the large difference in antibody titres observed between genetic lines in this study may be attributed to variation in response to ND antigen if the chicks were exposed to the ND virus. This implies that HATC will produce more antibodies compared to LATC in combating virulent ND virus if the two genetic lines are exposed to the virus instead of the vaccine used in this study. Meanwhile, Luc et al. (1992) ascribed the large range of antibody titres in chickens to natural infection which is recognized to yield greater antibody titres than vaccination. Our result on genetic line effect on antibody titres is in contrast to the observation of Hossain (2010), who reported that there was no difference between genetic groups in the antibody response against Newcastle disease virus and antibody titres ranging from 10.033 ± 0.056 to 11.045 ± 0.049 in two strains of chicken.

The results of genetic line by sex interaction demonstrated that sex of the chicks did not influence antibody degradation, while genetic line as a factor was superior relative to the sex factor. This suggests that genetic line plays a significant part in managing the time required for the deterioration of the ND antibody titres. Leandro et al. (2011) also reported variation in antibody titre in commercial broiler breeder lines. Finally, our results showed clearly that the amount of antibodies against ND will be high in the HATC compared to LATC.

Conclusion

This study showed that there was antibody titre variation in response to ND vaccine in the fourth generation of two selected genetic chicken lines. Therefore it was established that the divergently selected genetic chicken lines will retain their immune titre in actual challenge with Newcastle disease. Hence, HATC should be used for breeding programmes as they will be less susceptible to the virulence of the Newcastle disease virus. The results from this study did not confirm any differential performance

between male and female chickens in response to Newcastle disease vaccine.

Compliance with ethical standards

The manuscript does not contain clinical studies or patient data.

Statement of animal rights

All the protocols for this research were approved by the Animal Care and Use Committee of the Federal University of Agriculture, Abeokuta, Nigeria.

Conflict of interest

The authors declare that they have no conflict of interest.

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