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Survey for Serum Neuraminidase, Free and Erythrocyte Surface Sialic Acid from Chickens Vaccinated Against Newcastle Disease and the Unvaccinated Chickens Reared under Semi-Intensive Management System

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Abstract: The study was carried out to determine the levels of neuraminidase, erythrocyte surface and free serum sialic acid concentrations of chickens that were vaccinated against Newcastle Disease (ND) and unvaccinated chickens reared under semi-intensive management system in Zaria metropolis. A total of one hundred chickens, fifty each from vaccinated and unvaccinated flocks, randomly selected from ten backyard poultry farms were used for the study. Neuraminidase activity and concentrations of erythrocyte surface and free serum sialic acids were determined for each chicken, using thiobarbituric acid assay method. The values of Packed Cell Volume (PCV) and antibody titres to Newcastle Disease Virus (NDV) were also determined for each chicken by microhaematocrit and Haemagglutination Inhibition (HI) methods, respectively. The mean neuraminidase activity ($4.514 \pm 1.70 \mu\text{mol}/\text{min}$) and free serum sialic acid concentration ($0.075 \pm 0.03 \text{ mg}/\text{ml}$) obtained from the vaccinated chickens were significantly higher ($p < 0.001$) than the corresponding values of $1.870 \pm 0.16 \mu\text{mol}/\text{min}$ and $0.031 \pm 0.02 \text{ mg}/\text{ml}$ recorded for the unvaccinated chickens. Similarly, the mean HI antibody titres of $\log_2 5.66 \pm 3.56$ obtained from the vaccinated chickens was significantly higher ($p < 0.001$) than the values of $\log_2 0.980 \pm 0.42$ recorded for the unvaccinated chickens. On the other hand, there was no significant difference ($p > 0.05$) between the mean erythrocyte surface sialic acid concentration of $0.045 \pm 0.01 \text{ mg}/\text{ml}$ and $0.040 \pm 0.02 \text{ mg}/\text{ml}$ obtained from vaccinated and unvaccinated chickens, respectively. It was concluded that chickens that were vaccinated against ND had significantly higher levels of neuraminidase activity and free serum sialic acid concentrations than the unvaccinated chickens. Further studies are required to isolate and characterize neuraminidase of field strains of NDV present in the area of study. This could possibly give further insight into the role (s) of this enzyme during vaccination of poultry with NDV vaccines.

Key words: Neuraminidase, Newcastle disease virus, sialic acid

Introduction

In many countries of the world, Newcastle Disease (ND) constitutes one of the most important diseases of poultry (Gould *et al.*, 2003; Saidu *et al.*, 2006). It causes serious economic losses in poultry industry (Adu *et al.*, 1990; Maw *et al.*, 2003). The disease is currently being controlled by routine vaccination in many countries (Baba *et al.*, 1998; Okoye *et al.*, 2001; Czeglédi *et al.*, 2003).

Outbreaks of ND in vaccinated flocks have been reported in many parts of Nigeria (Adu *et al.*, 1990; Oladele *et al.*, 2005). It is often speculated that the frequent outbreaks of ND in vaccinated flocks are due to many factors, such as presence of antigenic differences between strains of NDV, low maternal antibodies, improper vaccine administration and the use of non-viable vaccines (Okoye *et al.*, 2001; Saidu *et al.*, 2006). However, Adu *et al.* (1989) reported that outbreaks of ND in vaccinated flocks often reported on the field may not only be due to differences in the antigenicity of the NDV wild field strains and vaccine strains, but could also be as a result

of differences in pathogenicity and virulence between different strains of NDV vaccines. This observation is worth investigating as neuraminidase which forms part of Haemagglutinin and Neuraminidase (HN) protein of NDV (McGinnes and Morrison, 1986) is known to play important role in the pathogenicity of many diseases (Corfield, 1992; Schauer *et al.*, 1995). Since sialic acids residues are cleaved by neuraminidase from cell surface or serum sialoglycoconjugates (Corfield and Schauer, 1982; Herrler *et al.*, 1987; Traving and Schauer, 1998) and consequently, resulting in disease processes, it is pertinent to determine the level of neuraminidase in the sera of chickens vaccinated against ND and compare the values with those obtained in the unvaccinated chickens. The results could give insight into the possible role of this enzyme with regard to its pathogenicity during vaccination of poultry with NDV vaccines on the field.

From available literature, this is the first report on neuraminidase, erythrocyte surface and free serum sialic acid concentrations of chickens that were

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Table 1: Mean value of neuraminidase activity, free serum sialic acid, erythrocyte surface sialic acid, packed cell volume and haemagglutination inhibition antibody titre of vaccinated and unvaccinated chickens (Mean ± SD)

Chickens	Neuraminidase activity (µmol/min)	Free serum sialic acid (mg/ml)	Erythrocyte surface sialic acid (mg/ml)	Packed cell volume (%)	Haemagglutination inhibition antibody titre (log ₂)
Vaccinated (n = 50)	4.514 ± 1.70 ^a	0.075 ± 0.03 ^a	0.045 ± 0.01 ^a	25.30 ± 5.53 ^a	5.66 ± 3.56 ^a
Unvaccinated (n = 50)	1.870 ± 0.16 ^b	0.031 ± 0.02 ^b	0.040 ± 0.02 ^a	25.64 ± 5.61 ^a	0.980 ± 0.42 ^a

a,b = Data along the same column with different superscript alphabets are significantly different (p<0.001)

vaccinated against ND and those not vaccinated that were reared under semi-intensive management system in Nigeria. Therefore, the objective of this study was designed to determine the levels of neuraminidase, erythrocyte surface and free serum sialic acid concentrations in the chickens that were vaccinated against ND and unvaccinated chickens, reared under the semi-intensive management system in Zaria, Nigeria.

Materials and Methods

Survey was carried out in ten backyard poultry farms randomly selected in Zaria metropolis (latitude 11° 10'N; longitude 07° 38'E), located in the northern Guinea Savannah zone of Nigeria. A total of one hundred chickens, aged between three and eight months were used for the study. The chickens were reared under semi-intensive management system.

Two categories of farms were visited. The first category was farms where there was history of chickens vaccinated against ND with NDV Hitchner intra ocular (I/O) vaccine at day old. NDV LaSota vaccine at 3 weeks, NDV Komarov vaccine at 6 and 16 weeks, respectively and booster doses of NDV LaSota vaccine at 28 weeks. The dosage of each vaccine was administered as recommended by the manufacturer. The NDV I/O, NDV LaSota and NDV Komarov vaccines were obtained from National Veterinary Research Institute, Vom, Nigeria. The second category was farms where no history of vaccination of chickens against ND was carried out before the experiment.

Serum samples were prepared from blood samples taken from each vaccinated chicken between days 4 and 7 after the booster doses of NDV LaSota were given to the chickens, for the determination of HI antibodies to NDV, according to the method of Allan and Gough (1974) and neuraminidase activity and free serum sialic acid concentration by the methods of Aminoff (1961). Erythrocyte surface sialic acid concentration was determined first, by preparing haemoglobin-free erythrocyte ghosts from blood samples collected from each vaccinated chicken by the procedures of Dodge *et al.* (1963). The concentration of erythrocyte surface sialic acid cleaved from haemoglobin-free erythrocyte ghosts by neuraminidase was then assayed by the procedure of Aminoff (1961). The values of Packed Cell Volume (PCV) were determined by the method of Benjamin (1985). Blood and serum samples were also taken from

each unvaccinated chickens at the same time as their vaccinated counterparts, for the determination of all the above parameters.

All the data obtained were subjected to statistical analysis using Student's t-test and correlation analyses. The data were expressed as mean±standard deviation (mean±SD). Values of p<0.05 were considered significant.

Results and Discussion

The mean neuraminidase activity of 4.514±1.70 µmol/min obtained from chickens that were vaccinated against ND was significantly higher (p<0.001) than the value of 1.870±0.16 µmol/min obtained from the unvaccinated chickens. Similarly, the mean free serum sialic acid concentration of 0.075±0.03 mg/ml obtained from vaccinated chickens was significantly higher (p<0.001) than the mean value of 0.031±0.02 mg/ml obtained from their unvaccinated counterparts. On the other hand, there was no significant difference (p>0.05) between the mean erythrocyte surface sialic acid concentrations of 0.045±0.01 mg/ml and 0.040±0.02 mg/ml recorded for the vaccinated and unvaccinated chickens, respectively. The mean HI antibody titres of log₂ 5.66±3.56 obtained from the vaccinated chickens was significantly higher (p<0.001) than the mean HI antibody titres of log₂ 0.980±0.42 recorded for the unvaccinated chickens (Table 1).

The significantly higher (p<0.001) mean neuraminidase activity and free serum sialic acid concentrations in the vaccinated than the unvaccinated chickens show that the NDV vaccines used for vaccinating the chickens produced certain levels of neuraminidase *in vivo*, which in turn, cleaved off sialic acid from the erythrocytes surface. The relationship between neuraminidase activity and HI (r = 0.611, p<0.001); and neuraminidase activity and free serum sialic acid concentration (r = 0.062, p<0.05) in the vaccinated chickens were direct and positive (Table 2). A positive and direct relationship between neuraminidase activity and any of these parameters in the vaccinated chickens was not expected. This is because it is thought that with vaccination and high mean HI antibody titres; the activity of the virus would be reduced. This result, therefore, shows that it is possible that presumably protective high HI antibody titres could not prevent the NDV replication. This finding is in line with the results of Phillips (1973) and Alexander (1988) who observed that the level of ND

Table 2: Correlation coefficients (r) of neuraminidase activity with free serum sialic acid, erythrocyte surface sialic acid, packed cell volume and haemagglutination inhibition antibody titre of vaccinated chickens

	Free serum sialic acid	Erythrocyte surface sialic acid	Packed cell volume	Haemagglutination inhibition antibody titre
Neuraminidase activity	0.062*	-0.664***	-0.632***	0.611***

* = p<0.05, ** = p<0.01, *** = p<0.001

Table 3: Correlation coefficients (r) neuraminidase activity with free serum sialic acid, erythrocyte surface sialic acid, packed cell volume and haemagglutination inhibition antibody titre of unvaccinated chickens

	Free serum sialic acid	Erythrocyte surface sialic acid	Packed cell volume	Haemagglutination inhibition antibody titre
Neuraminidase activity	0.542**	-0.625***	-0.762***	0.397***

* = p<0.05, ** = p<0.01, *** = p<0.001

antibody which afford protection do not necessarily prevent NDV replication and fully vaccinated birds may still represent important sources of virus in the spread of ND.

The detection of significantly low levels of neuraminidase activity, erythrocyte surface and free serum sialic acid concentrations in the unvaccinated chickens (Table 1) and the direct and positive relationship between neuraminidase activity and free serum sialic acid (r = 0.542, p<0.01) in the unvaccinated chickens (Table 3) may be as a result of natural infection of these chickens by mild strain of field NDV. Also, it could be due to haemolysis of red blood cells. This is because during the lifetime of organism, sialic acids are known to be removed stepwise from surface of erythrocytes by spontaneous chemical haemolysis (Schauer *et al.*, 1995).

None of the chickens, including the unvaccinated showed any clinical signs of ND. Since the unvaccinated chickens had no previous history of vaccination against ND, therefore, it could be inferred that the significantly low ND antibodies detected in them (Table 1) were as a result of natural infection of these chickens by mild strain of field NDV. This is because all the chickens examined were between three and eight months old, thereby ruling out the presence of maternal antibodies. It is believed that under field condition where continuous challenge by NDV occur, the chicken's maternal antibodies are bound to be lost much rapidly than under experimentally controlled studies (Phillips, 1973). Therefore, the unvaccinated chickens could serve as reservoir of NDV to commercially reared poultry in our environment.

It was concluded that under field condition, chickens that were vaccinated against ND had significantly higher levels of neuraminidase activity and free serum sialic acid concentrations than the unvaccinated chickens. Further studies are required to isolate and characterize neuraminidase of field strains of NDV present in the area of study. This could possibly give further insight into the role (s) of this enzyme during vaccination of poultry with NDV vaccines.

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