

Preliminary Report on Neuraminidase, Erythrocyte Surface and Free Serum Sialic Acid Concentrations in the Serum of Healthy and Newcastle Disease Virus-Infected Chickens

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Key words

Chicken – Newcastle disease virus – Nigeria.

Summary

A survey was carried out in ten backyard poultry farms in Zaria metropolis to determine levels of neuraminidase, erythrocyte surface and free serum sialic acid concentrations in the serum of chickens. One hundred Shika brown layers, of which 50 were infected by the Newcastle disease virus (NDV) and 50 were apparently healthy, were used. Neuraminidase activity, erythrocyte surface and free serum sialic acid concentrations were determined for each chicken, using the thiobarbituric acid assay method. The packed cell volume (PCV) and antibody titers to Newcastle disease were also determined for each chicken by the microhematocrit and hemagglutination inhibition tests, respectively. The mean values of 8.079 ± 2.93 $\mu\text{mol}/\text{min}$ and 0.129 ± 0.04 mg/ml obtained for neuraminidase activity and free serum sialic acid concentration, respectively, in NDV-infected chickens were significantly higher ($P < 0.001$) than the corresponding mean values of 0.849 ± 0.61 $\mu\text{mol}/\text{min}$ and 0.041 ± 0.03 mg/ml obtained in apparently healthy chickens. On the other hand, the mean values of 0.009 ± 0.01 mg/ml and $21.96 \pm 6.89\%$ recorded for erythrocyte surface sialic acid concentration and PCV, respectively, in NDV-infected chickens were significantly lower ($P < 0.001$) than the corresponding mean values of 0.650 ± 0.04 mg/ml and $29.50 \pm 5.53\%$ recorded in their apparently healthy counterparts. In conclusion, the low mean values of PCV and erythrocyte surface sialic acid concentration obtained in NDV-infected chickens could result from the circulating NDV that produced neuraminidase *in vivo*, which, in turn, cleaved off the erythrocyte surface sialic acid, thus rendering the erythrocytes more prone to destruction by the reticuloendothelial system. Further studies are needed to isolate and characterize neuraminidases of NDV field strains in this local environment.

INTRODUCTION

The Newcastle disease (ND) constitutes one of the major disease problems of poultry in most parts of the world (4, 16). In Nigeria, ND has assumed greater importance, following the increase in the number of isolated ND virus (NDV) wild strains (11). Frequent

outbreaks of ND in both exotic and indigenous chickens resulted in serious economic losses in the poultry industry (1).

These frequent ND outbreaks have been associated with pathogenicity of NDV strains (2). This observation prompted the authors to determine the levels of serum neuraminidase, an enzyme known to play an important role in the pathogenicity of many diseases (8, 14). It has been established that viral neuraminidase assists viral spread by cleaving local sialyl residues, during budding of infectious particles that would have been trapped by hemagglutinin-host-sialyl interactions, thus playing an important role in the spread of viral infections (17). Neuraminidases are also known to cleave sialic acids that are believed to be present in appreciable quantities on the surface structures of erythrocytes in the chicken (15) and in other animal species (18, 19). These sialic acids are known to mask and protect the surface structure of erythrocytes (18).

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It was observed that neuraminidase cleaved surface sialic acid of erythrocytes in vivo in *Trypanosoma vivax* (12). If this mechanism is operative in ND infections in vivo, it might be accompanied by reduction of the life span of such desialylated erythrocytes, and their subsequent removal from circulation by the reticuloendothelial system (10), thus causing anemia in the infected chickens.

A number of investigations on ND in Nigeria focus on the epidemiology of the disease (3, 13), without emphasis on factors that could be responsible for the pathogenicity of the disease. From available literature, this is the first report on neuraminidase activity, erythrocyte surface and free serum sialic acid concentrations in the serum of chickens in Nigeria.

This study was therefore conducted to survey levels of neuraminidase, erythrocyte surface and free serum sialic acid concentrations in the serum of apparently healthy and NDV-infected chickens, reared in Zaria, Northern Guinea savannah zone of Nigeria.

■ MATERIALS AND METHODS

Animals and site

The survey was carried out in ten backyard poultry farms in Zaria metropolis (latitude 11° 10' N; longitude 07° 38' E), located in the Northern Guinea savannah zone of Nigeria. One hundred Shika brown layers, aged between three and eight months, and reared under a semi-intensive management system, were used. The Shika brown layer was developed at the National Animal Production and Research Institute, Shika, Ahmadu Bello University, Zaria, Nigeria, after many years of active breeding and selection, to produce chickens which will cope with harsh tropical environmental conditions.

Two categories of farms were visited. In the first category, cases of ND had been reported and clinical signs of ND were observed. The chickens were then necropsized and ND lesions were observed in the affected chickens. In the second category, no reports of ND outbreaks had been reported and no clinical signs or postmortem lesions were found, when the chickens were necropsized. The survey was carried out in these farms irrespective of whether the chickens had been previously vaccinated against ND or not.

Methods

Serum samples were taken from each chicken to test for hemagglutination inhibition (HI) antibody titers to ND, according to the method of Allan and Gough (5). The HI test was performed to ensure that chickens that presented ND clinical signs and postmortem lesions

were really having antibodies to ND. Chickens with postmortem lesions that were not caused by ND were not used for the study.

Neuraminidase activity and free serum sialic acid concentrations in the serum samples of each chicken were determined (6). Erythrocyte surface sialic acid concentrations were determined by, first, preparing hemoglobin-free erythrocyte ghosts from blood samples collected from each chicken into test tubes containing acid citrate dextrose (9). The concentrations of erythrocyte surface sialic acid, cleaved from hemoglobin-free erythrocyte ghosts by neuraminidase, were then estimated (6). Blood samples collected from each chicken into a set of test tubes containing ethylene diamine tetra acetic acid were used to determine the packed cell volume (PCV) of each bird, according to the method of Benjamin (7).

All data obtained were subjected to statistical analysis using Student's t-test and correlation analysis. The data were expressed as means plus or minus standard deviations ($P < 0.05$ was considered significant).

■ RESULTS

The mean neuraminidase activity of 8.079 ± 2.93 $\mu\text{mol}/\text{min}$ obtained from NDV-infected chickens was significantly higher ($P < 0.001$) than that of 0.849 ± 0.61 $\mu\text{mol}/\text{min}$ recorded in apparently healthy chickens. Similarly, the mean free serum sialic acid concentration of 0.129 ± 0.04 mg/ml recorded in NDV-infected chickens was significantly higher ($P < 0.001$) than that of 0.041 ± 0.03 mg/ml recorded in apparently healthy chickens. On the other hand, the mean erythrocyte surface sialic acid concentration of 0.009 ± 0.01 mg/ml obtained from NDV-infected chickens was significantly lower ($P < 0.001$) than that of 0.650 ± 0.04 mg/ml recorded in apparently healthy chickens. The mean PCV value of $21.96 \pm 6.89\%$ obtained in NDV-infected chickens was significantly lower ($P < 0.001$) than that of $29.50 \pm 5.53\%$ recorded in apparently healthy chickens (Table I).

Correlation coefficients of neuraminidase activity with erythrocyte surface sialic acid concentration ($r = -0.447$, $P < 0.01$), and neuraminidase activity with PCV ($r = -0.698$, $P < 0.001$) were negative and highly significant in NDV-infected chickens (Table II).

There was a positive and significant relationship between neuraminidase activity and free serum sialic acid concentration ($r = 0.290$, $P < 0.05$), while the relationship between neuraminidase activity and erythrocyte surface sialic acid ($r = -0.461$, $P < 0.01$) was negative but significant in apparently healthy chickens (Table II).

Table I

Mean values of neuraminidase activity, free serum sialic acid, erythrocyte surface sialic acid, packed cell volume and hemagglutination inhibition antibody titers of Newcastle disease virus-infected and apparently healthy chickens (\pm SD)

	Neuraminidase activity ($\mu\text{mol}/\text{min}$)	Free serum sialic acid (mg/ml)	Erythrocyte surface sialic acid (mg/ml)	Packed cell volume (%)	Hemagglutination inhibition antibody titer (\log_2)
Newcastle disease infected chickens (n = 50)	$8.079 (\pm 2.93)^a$	$0.129 (\pm 0.04)^a$	$0.009 (\pm 0.01)^a$	$21.96 (\pm 6.89)^a$	$5.82 (\pm 2.35)^a$
Apparently healthy chickens (n = 50)	$0.849 (\pm 0.61)^b$	$0.041 (\pm 0.03)^b$	$0.650 (\pm 0.04)^b$	$29.50 (\pm 5.53)^b$	$0.940 (\pm 0.22)^b$

^{a, b} Data in the same column with different superscripts are significantly different ($P < 0.001$)

Table II

Correlation coefficients (r) of neuraminidase activity with free serum sialic acid, erythrocyte surface sialic acid, packed cell volume and hemagglutination inhibition antibody titers of Newcastle disease virus (NDV) infected chickens and apparently healthy chickens

	Neuraminidase activity			
	Free serum sialic acid	Erythrocyte surface sialic acid	Packed cell volume	Hemagglutination inhibition antibody titer
NDV-infected chickens	0.094 ^{NS}	-0.447**	-0.698***	0.604***
Apparently healthy chickens	0.290*	-0.461**	-0.560***	0.397*

^{NS} Not significant; * P < 0.05; ** P < 0.01; *** P < 0.001

■ DISCUSSION

It is believed that neuraminidases cleave the terminal sialic acid from sugar residues and glycoproteins (18, 19). Detection of neuraminidase in the serum, therefore, rests on the assay of free sialic acid split from the substrates (20).

In this study, the mean free serum sialic acid concentration obtained from NDV-infected chickens was significantly higher (P < 0.001) than that of apparently healthy chickens. This is in line with findings obtained by Esievo et al. (12) in cattle, as they observed higher levels of serum sialic acid concentration in cattle infected with *Trypanosoma vivax* than in their apparently healthy counterparts.

The significantly higher (P < 0.001) mean neuraminidase activity obtained from NDV-infected chickens compared to their apparently healthy counterparts is probably due to the presence of NDV in the serum of NDV-infected chickens. The higher levels of neuraminidase activity in NDV-infected chickens suggest that the virus produces neuraminidase in vivo. This is because neuraminidase activity is related to the number of parasites in the serum (12).

Sialic acids, the acylated neuraminic acid, occur in reasonable quantities on the erythrocyte surface structures of chickens (15) and other animal species, (18, 19). It is believed that sialic acids mask and protect the erythrocyte surface (18). The fact that the mean erythrocyte surface sialic acid concentration obtained from NDV-infected chickens was significantly lower (P < 0.001) than that obtained from the apparently healthy chickens suggests that the reduction in erythrocyte surface sialic acid of NDV-infected chickens is probably a mechanism of erythrocyte destruction, and this could be important in erythrocyte senescence (10, 19). This could probably explain the significantly lower (P < 0.001) mean PCV value obtained from NDV-infected chickens than from their apparently healthy counterparts. This is because it is known that neuraminidases cleave off sialic acid from erythrocytes (18, 19), thus rendering them prone to erythrophagocytosis (10, 12).

The negative and highly significant correlations between neuraminidase activity and PCV (r = -0.698, P < 0.001), and neuraminidase activity and erythrocyte surface sialic acid concentration (r = -0.447, P < 0.001) in NDV-infected chickens supported the fact that the presence of NDV might have caused significant reduction in the PCV value observed in NDV-infected chickens. Furthermore, the finding that reduced erythrocyte surface sialic acid concentration occurred concurrently with increased neuraminidase activity in the serum, preceded by reduced PCV values in NDV-infected chickens, was indirect evidence that NDV might produce neuraminidase in vivo.

The exact mechanism of the surface damage of NDV-infected erythrocytes was not investigated in this study, but the erythrocyte damage may have a physicochemical origin. The presence of certain levels of neuraminidase activity, erythrocyte surface and free serum sialic acid concentrations in the serum of apparently healthy chickens could be due to red blood cell hemolysis. This is because during the lifetime of the organism, sialic acid is known to be removed stepwise from surface of erythrocytes by the action of neuraminidases, and by spontaneous chemical hemolysis (18).

From the present results, it could be postulated that, as NDV concentration increased in circulation, it produced neuraminidase, which cleaved erythrocyte surface sialic acid, thus rendering erythrocytes more prone to hemolysis by the reticuloendothelial system.

In conclusion, the low mean values of PCV and erythrocyte surface sialic acid concentration observed in NDV-infected chickens could be due to the activities of circulating NDV that produced neuraminidase in vivo, which in turn cleaved off erythrocyte surface sialic acid, thus rendering the erythrocytes more prone to destruction by the reticuloendothelial system. Further studies are needed to isolate and characterize neuraminidases of NDV field strains in this local environment.

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Résumé

Oladele S.B., Abdu P., Nok A.J., Esievo K.A.N., Useh N.M. Rapport préliminaire sur les teneurs en neuraminidase et en acide sialique libre et de surface érythrocytaire dans les sérums de volailles indemnes et infectées par le virus de la maladie de Newcastle

Une étude a été réalisée dans dix petits élevages de volailles dans l'agglomération de Zaria pour déterminer les teneurs en neuraminidase et en acide sialique libre et de surface érythrocytaire dans les sérums de volailles. Cent pondeuses brunes Shika ont été utilisées, parmi lesquelles 50 étaient infectées par le virus de la maladie de Newcastle (NDV) et 50 étaient apparemment indemnes. Les teneurs en neuraminidase et en acide sialique libre et de surface érythrocytaire ont été déterminées pour chaque poulet par la méthode de l'acide thiobarbiturique. L'hématocrite et les titres des anticorps anti-maladie de Newcastle ont aussi été déterminés pour chaque poulet, respectivement par la technique de microhématocrite et par la réaction d'inhibition de l'hémagglutination. Les valeurs moyennes de $8,079 \pm 2,93 \mu\text{mol}/\text{min}$ et $0,129 \pm 0,04 \text{ mg}/\text{ml}$, obtenues respectivement pour les teneurs en neuraminidase et en acide sialique libre du sérum chez les volailles infectées par le NDV, ont été significativement plus élevées ($P < 0,001$) que les valeurs moyennes correspondantes de $0,849 \pm 0,61 \mu\text{mol}/\text{min}$ et $0,041 \pm 0,03 \text{ mg}/\text{ml}$, obtenues chez les poulets apparemment indemnes. Inversement, les valeurs moyennes de $0,009 \pm 0,01 \text{ mg}/\text{ml}$ et $21,96 \pm 6,89 \text{ p. } 100$, relevées respectivement pour la teneur en acide sialique de la surface érythrocytaire et pour l'hématocrite chez les poulets infectés par le NDV, ont été significativement plus faibles ($P < 0,001$) que les valeurs moyennes correspondantes de $0,650 \pm 0,04 \text{ mg}/\text{ml}$ et $29,50 \pm 5,53 \text{ p. } 100$, relevées chez les poulets apparemment indemnes. En conclusion, les faibles valeurs moyennes de l'hématocrite et de la teneur en acide sialique de la surface érythrocytaire chez les poulets infectés par le NDV seraient dues à la circulation de ce virus qui sécrète la neuraminidase in vivo, laquelle à son tour sépare l'acide sialique de la surface érythrocytaire, exposant les hématies à la destruction par le système de défense réticulo-endothélial. D'autres études seront nécessaires pour isoler et caractériser les souches de NDV sur le terrain dans les conditions locales présentes.

Mots-clés : Poulet – Virus de la maladie de Newcastle – Nigeria.

Resumen

Oladele S.B., Abdu P., Nok A.J., Esievo K.A.N., Useh N.M. Reporte preliminar sobre neuraminidasas, superficie eritrocitaria y concentraciones de ácido siálico libre de suero en sueros de pollos saludables e infectados con el virus de la enfermedad de Newcastle

Se llevó a cabo un estudio en diez fincas con pollos de jardín en los suburbios de Zaria, con el fin de determinar los niveles de neuraminidasa, la superficie eritrocitaria y las concentraciones en suero de ácido siálico libre de suero, en ponedoras marrón Shika. Se utilizaron cien ponedoras marrón Shika, de las cuales se infectaron 50 con el virus de la enfermedad de Newcastle (NDV) y 50 permanecieron aparentemente sanas. Se determinó la actividad de la neuraminidasa, la superficie eritrocitaria y las concentraciones en suero de ácido siálico libre de suero en cada pollo mediante el método de titulación de ácido tiobarbitúrico. El volumen eritrocitario y los títulos de anticuerpos para cada ave, mediante los tests de microhematocrito e inhibición de la micro aglutinación, respectivamente. Se obtuvieron los valores promedio de $8,079 \pm 2,93 \mu\text{mol}/\text{min}$ y $0,129 \pm 0,04 \text{ mg}/\text{ml}$, respectivamente para la actividad de la neuraminidasa y la concentración en suero de ácido siálico libre de suero en pollos infectados con NDV, los cuales fueron significativamente ($p < 0,001$) más altos que los valores de $0,849 \pm 0,61 \mu\text{mol}/\text{min}$ y $0,041 \pm 0,03 \text{ mg}/\text{ml}$ de los animales aparentemente sanos. Por otro lado, los valores respectivos, registrados para la concentración de ácido siálico en la superficie del eritrocito y para el volumen eritrocitario, de $0,009 \pm 0,01 \text{ mg}/\text{ml}$ y $21,96 \pm 6,89\%$ en los pollos infectados con NDV fueron significativamente inferiores ($p < 0,001$) que los valores promedio correspondientes de $0,650 \pm 0,04 \text{ mg}/\text{ml}$ y $29,50 \pm 5,53\%$, registrados en aquellos aparentemente sanos. En conclusión, los valores bajos de volumen eritrocitario y de la concentración de ácido siálico en la superficie del eritrocito, obtenidos en los pollos infectados con NDV podría resultar de los NDV circulantes que produjo la neuraminidasa in vivo, la cual, a su vez disminuyó el ácido siálico en la superficie del eritrocito, facilitando la destrucción de los eritrocitos por el sistema retículo endotelial. Se necesitan estudios más profundos para aislar y caracterizar las neuraminidasas de las cepas de campo de NDV en este ambiente local.

Palabras clave: Pollo – Virus de la enfermedad de Newcastle – Nigeria.