VETERINARSKI ARHIV 76 (5), 391-401, 2006

Comparative studies of serum neuraminidase, free and erythrocytes surface sialic acid, packed cell volume and haemagglutination inhibition antibodies of chickens vaccinated with different Newcastle disease virus vaccines

Sunday Blessing Oladele[1]*, Andrew Jonathan Nok[2], Paul Abdu[3], Lawal Saidu[4], and King Akpofure Nelson Esievo[1]

[1]*Department of Pathology and Microbiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria*

[2]*Department of Biochemistry, Faculty of Science, Ahmadu Bello University, Zaria, Nigeria*

[3]*Department of Surgery and Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria*

[4]*Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria*

OLADELE, S. B., A. J. NOK, P. ABDU, L. SAIDU, K. A. N. ESIEVO: Comparative studies of serum neuraminidase, free and erythrocytes surface sialic acid, packed cell volume and haemagglutination inhibition antibodies of chickens vaccinated with different Newcastle disease virus vaccines. Vet. arhiv 76, 391-401, 2006.

ABSTRACT

Comparative studies of serum neuraminidase, free and erythrocytes surface sialic acid, packed cell volume and haemagglutination inhibition antibody titres were carried out on a total of seventy-five Shaver Brown chickens that were vaccinated with different Newcastle disease virus (NDV) vaccines. Trial birds were primed with NDV Hitchner B_1 vaccine prior to NDV La Sota and NDV Komarov vaccines administration. Twenty-five chickens received NDV La Sota vaccine; a further 25 were vaccinated with NDV Komarov vaccine, while 25 chickens served as control. The values of neuraminidase activity (NA), free serum sialic acid (FSSA), erythrocytes surface sialic acid (ESSA) concentration, packed cell volume (PCV) and haemagglutination inhibition (HI) antibody titres were determined sequentially for each chicken. The highest daily mean HI antibody titres of $\log_2$ 7.12 ± 1.20 and $\log_2$ 8.98 ± 1.73 were obtained from chickens vaccinated with NDV La Sota vaccine and NDV Komarov vaccine, respectively, by day 15 post-vaccination (pv). Neuraminidase activity and FSSA concentration increased gradually from days 1 to 5 pv in the two groups of chickens. In general, the period of increased NA and FSSA concentration coincided with the time of a decline in values of ESSA and PCV in the two vaccinated groups of

* Contact address:

Dr. Sunday Blessing Oladele, Department of Pathology and Microbiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria, Phone: +234 8023 672 720; E-mail: drbsunday@yahoo.com.

ISSN 0372-5480
Printed in Croatia

chickens. It was concluded that chickens which received NDV Komarov vaccine had higher daily mean values of NA, FSSA and HI antibody titres than their counterparts which received NDV La Sota vaccine.

Key words: chicken, neuraminidase, Newcastle disease virus, serum, sialic acid, vaccine

Introduction

Newcastle disease (ND) is one of the most important poultry diseases worldwide. The disease is known to cause serious economic losses to poultry industry due to its high morbidity and mortality rates (ALEXANDER, 1997; ALEXANDER et al., 1999).

Newcastle disease is controlled worldwide by routine vaccination (MEULEMANS, 1988; ALEXANDER et al., 1997). In Nigeria, NDV Hitchner B_1, NDV La Sota and NDV Komarov vaccines are routinely used to vaccinate chickens against ND. Newcastle disease virus LaSota vaccines are derived from lentogenic NDV strain which has low pathogenicity, but which produce an adequate immune response; while NDV Komarov vaccines are mesogenic NDV strain (ALLAN et al., 1978; MEULEMANS, 1988).

Although vaccination against ND has been practised for many years in Nigeria, frequent outbreaks of ND in vaccinated flocks are common occurrence, thus making ND a serious threat to the poultry industry (ADU et al., 1990, OLADELE et al., 2003). It is believed that frequent outbreaks of ND in vaccinated flocks are due to low immune status of birds, improper vaccine administration, and the use of non-viable vaccines (OKOYE et al., 2001). However, ADU et al. (1989) speculated that outbreaks of ND in vaccinated flocks could also be due to difference in pathogenicity of the strains of ND vaccine virus. It will, therefore, be of interest to know the levels of neuraminidase in the sera of chickens vaccinated with different types of NDV vaccines. This is because neuraminidase is one of the important components of haemagglutinin-neuraminidase protein of NDV (ALEXANDER et al., 1999), and it is known to play crucial role in the pathogenicity of diseases by enzymatic removal of sialic acids from carbohydrate-containing molecules, such as erythrocytes of chickens and other animal species (HERRLER et al., 1987; TRAVING and SCHAUER, 1998), thereby exposing the erythrocytes to destruction by macrophages (McNULTY et al., 1975; CORFIELD, 1992; LICHTENSTEIGER and VIMR, 2003).

OLADELE et al. (2002) found that chickens naturally infected with NDV had higher values of neuraminidase and free serum sialic acid than their apparently healthy counterparts. Also, it has been demonstrated in vitro that velogenic strain of NDV had higher neuraminidase content than lentogenic NDV strain (McNULTY et al., 1975). Since there is no information on in vivo studies of neuraminidase content of mesogenic velogenic, or lentogenic NDV strains, it is therefore imperative to determine whether differences in neuraminidase content also exist in vivo in the sera of chickens which have received different NDV vaccines.

The levels of NDV HI antibodies are commonly discussed in vaccinated chickens without emphasis on the levels of neuraminidase, free and erythrocytes surface sialic acid, and the effect of neuraminidase on erythrocytes of vaccinated chickens. Yet such information could provide an insight into the possible level of pathogenicity of NDV vaccines that are administered to chickens. According to available literature, this is the first report on the levels of neuraminidase, free and erythrocytes surface sialic acid of chickens vaccinated with NDV La Sota vaccine or NDV Komarov vaccine in Nigeria.

The objective of this study, therefore, was to determine the levels of NA, FSSA, ESSA, PCV and HI antibody titres of Shaver Brown chickens vaccinated with NDV La Sota vaccine or NDV Komarov vaccine.

Materials and methods

Chickens and management. A total of one-day-old 75 Shaver Brown pullet chicks were obtained. They were randomly selected into three groups of 25 chicks each from day one of age. The three groups of chicks were housed separately in an enclosed building protected from pathogens. Brooding and rearing were performed by deep litter systems. Water and feed were supplied *ad libitum*.

Newcastle disease virus vaccines. NDV Hitchner B_1, NDV La Sota and NDV Komarov vaccines were obtained from the National Veterinary Research Institute Vom, Plateau State, Nigeria.

Group one chickens received NDV Hitchner B_1 vaccine of $10^{7.0}$ embryo infective dose 50% end point $[EID]_{50}$ per chicken through intraocular (I/O) route at two-days-old. At three weeks, one vial of NDV La Sota vaccine of $10^{6.8}$ $[EID]_{50}$ per mL was reconstituted with two litres of chlorine-free water and each chicken was given 10 mL of the reconstituted vaccine per os, according to manufacturer's recommendation. The titre per chicken was $10^{6.5}[EID]_{50}$.

Group two chickens received NDV Hitchner B_1 vaccine of $10^{7.0}$ $[EID]_{50}$ per mL each through I/O route at two days old. At three weeks, each chicken was inoculated intramuscularly in the thigh muscle with 0.2 mL of NDV Komarov vaccines of $10^{6.5}$ $[EID]_{50}$ per mL, according to manufacturer's recommendation. The HI titre per chicken was $10^{5.0}$ $[EID]_{50}$.

Group three chickens comprised the control group. Chickens in this group did not receive any NDV vaccine during the experiment.

No HI antibodies were detected in the three groups of chickens at one-day-old, when the immune status of the chickens was assessed. As a result, chickens in groups one and two were administered NDV Hitchner B_1 vaccine though I/O route at two days old so that

the vaccine could elicit protective immune responses before the experiment commenced at three weeks of age.

The values of NA, FSSA, ESSA, PCV and HI were determined in the three groups of chickens three days (days - 3 to - 1, that is, pre-vaccination days) before the chickens in groups one and two were vaccinated with NDV La Sota vaccine and NDV Komarov vaccine, respectively, at three weeks of age. This was done to establish the values of these parameters before vaccinating the chickens with either NDV La Sota vaccine or NDV Komarov vaccine.

Blood sampling. Blood sampling was through wing venepuncture, using 23 gauge sterile hypodermic needles and syringes. About 0.5 mL of blood were collected on each day of the experiment from each chicken in the three groups.

Blood for haematological values were collected into labelled Bijou bottles, containing ethylene diamine tetra acetic acid (2mg/mL) as anticoagulant. Serum samples for biochemical and HI analyses were taken without anticoagulant. The serum samples were separated by centrifugation at 1,000 g for 10 min, and stored frozen in plastic vials until laboratory determinations were made.

Packed cell volume determination. PCV values were determined by the microhaematocrit method of BENJAMIN (1985). Blood samples containing ethylene diamine tetra acetic acid were aspirated into a set of plain capillary tubes. The tubes were sealed and then placed on Hittich centrifuge (Hawksley and Sons Limited, England), and spun at 5.000 g for 5 min. After five min, the tubes were removed and the PCV was read as a percentage directly from Graphic Reader (Hawksley and Sons Limited, England).

Serology. Newcastle disease virus HI antibody quantification was done using haemagglutination and HI procedures of BEARD (1989): Two-fold dilutions of 50 μL of the sera were made in phosphate buffered saline (pH 7.2), and four haemagglutination (4HA) units of La Sota virus as antigen in 50 μL were added. Thereafter, 50 μL of 1% erythrocytes suspension were added to each well of the microtitre plate and left for 45 min at room temperature (26-30 ^{0}C). The HI titre for each serum sample was determined at the highest dilution of serum causing complete inhibition of 4 HA units.

Assay for neuraminidase, free and erythrocytes surface sialic acid. NA, FSSA and ESSA values were determined using the procedures of REUTER and SCHAUER (1994), modified thus: An equal amount (10 μL) of the test serum and fetuin (substrate) were mixed for each test. The reaction mixture was incubated for one hour at 37 ^{o}C. Thereafter, the mixture was cooled to room temperature for two min. The quantity of N-acetylneuraminic acid liberated from fetuin after incubation was then assayed thus: About 250 μL of sodium periodate was added and the mixture was shaken and reincubated for 20 min in a water bath at 37 ^{o}C. Then, 100 μL of sodium arsenate was added. A brown colour immediately appeared, which disappeared after vigorous shaking. 100 μL of thiobarbituric acid was

then added. The mixture was also shaken and placed in boiling water for 10 min. A pink colour appeared after 10 min.

The reaction mixture was cooled by placing the tubes containing the mixture in running tap water. After cooling, 5.000 μL of acid-butanol was added to the mixture and vigorously shaken. The mixture was centrifuged at 1,000 g for 5 minutes. The supernatant was carefully transferred into cuvettes using Pasteur pipettes. The absorbance was read against blank on a spectrophotometer at 549 nm.

Statistical analysis. Data obtained were analysed using Student's *t*-test analysis. Values were expressed as mean ± SD. Values of ($P<0.05$) were considered significant. The analysed data were used to plot graphs.

Results and discussion

Chickens which received NDV La Sota vaccine or NDV Komarov vaccine at three weeks of age had reduced PCV values when compared with their control counterparts from days 1 to 11 pv. However, there was no significant difference ($P>0.05$) in PCV values between vaccinated and control chickens (Fig. 1).

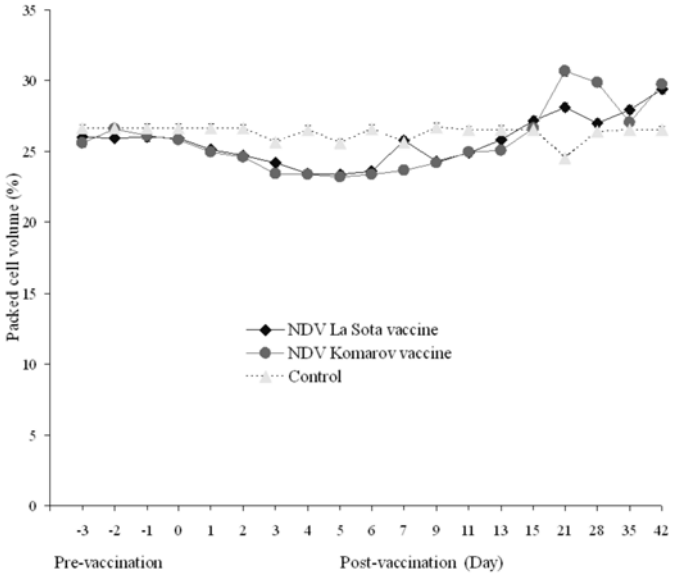

Fig. 1. Changes (mean ± SD) in packed cell volume of chickens vaccinated with NDV La Sota vaccine, NDV Komarov vaccine and control chickens

Chickens which received NDV La Sota vaccine or NDV Komarov vaccine had serological response to their respective vaccines following vaccination. The highest daily mean HI antibody titres of $\log_2$ 7.12 ± 1.20 and $\log_2$ 8.98 ± 1.73 were recorded for chickens which received NDV La Sota vaccine and NDV Komarov vaccine, respectively, by day 15 pv. No HI antibodies were detected in control chickens (Fig. 2).

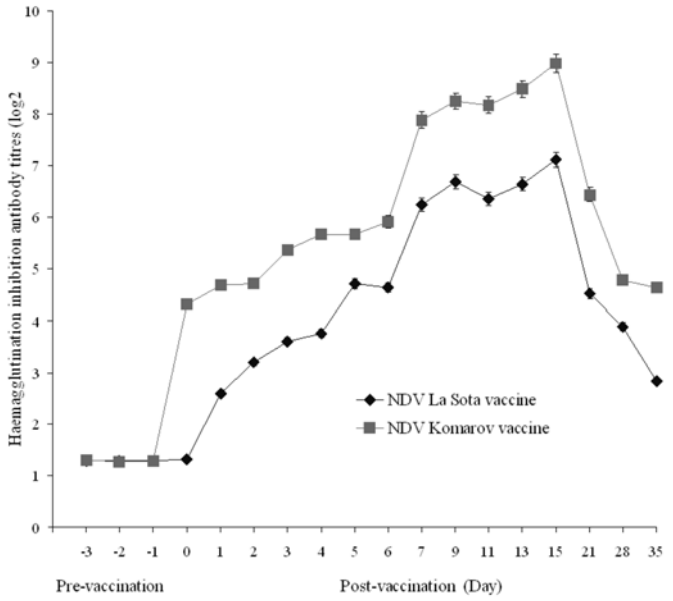

Fig. 2. Changes (mean ± SD) in serum haemagglutination inhibition antibody titres of chickens vaccinated with NDV La Sota vaccine and NDV Komarov vaccine

There was a gradual rise in daily mean NA from day 1 pv in the two vaccinated groups of chickens when compared with the control group. The highest daily mean NA values of 3.10 ± 0.40 μmol/min and 3.93 ± 0.55 μmol/min were recorded for chickens which received NDV La Sota vaccine and NDV Komarov vaccine, respectively, by day 5 pv. The daily mean NA values of the control chickens were low and relatively constant during the experimental period (Fig. 3).

In general, the daily mean FSSA concentration recorded per day in chickens which received NDV Komarov vaccine were higher than the corresponding values in the

group that received NDV La Sota vaccine from days 1 to 11 pv. Also, the values of FSSA obtained from these two vaccinated groups were higher than the values of FSSA obtained from the control group, which were relatively low throughout the experimental period (Fig. 4).

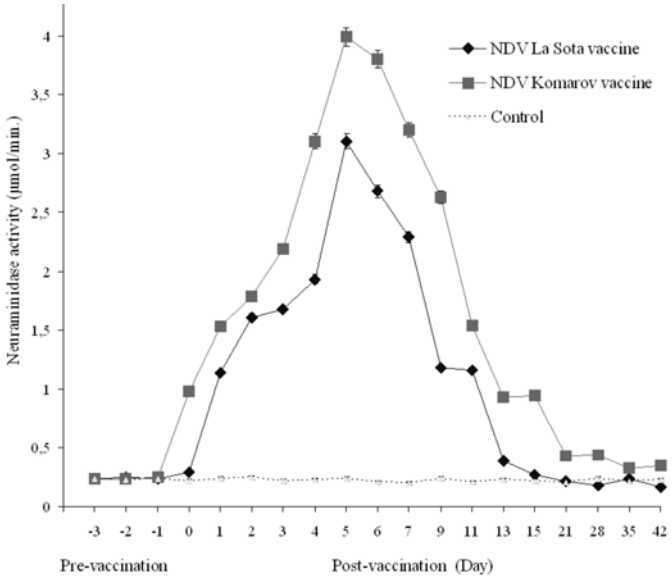

Fig. 3. Changes (mean ± SD) in neuraminidase activity of chickens vaccinated with NDV La Sota vaccine, NDV Komarov vaccine and control chickens

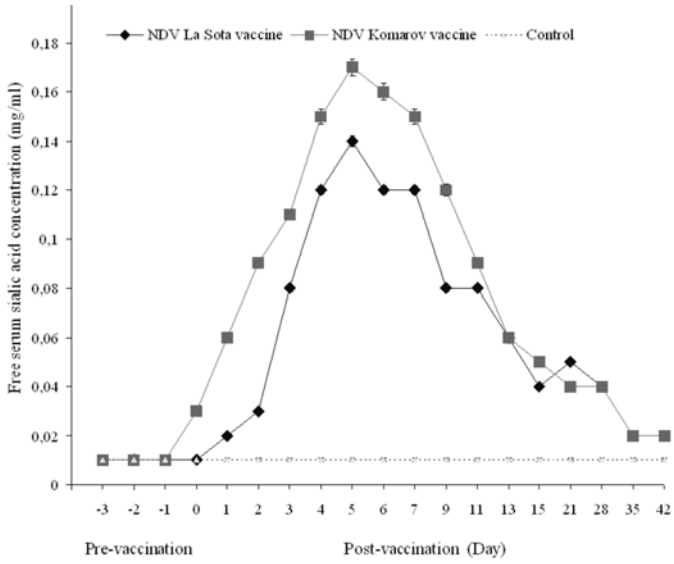

Fig. 4. Changes (mean ± SD) in free serum sialic acid concentration of chickens vaccinated with NDV La Sota vaccine, NDV Komarov vaccine and control chickens

The group of chickens which received NDV La Sota vaccine or NDV Komarov vaccine experienced a drop in their daily mean ESSA concentration following vaccination when compared with their control counterparts. Relatively high values of ESSA were recorded for control chickens throughout the experimental period (Fig. 5).

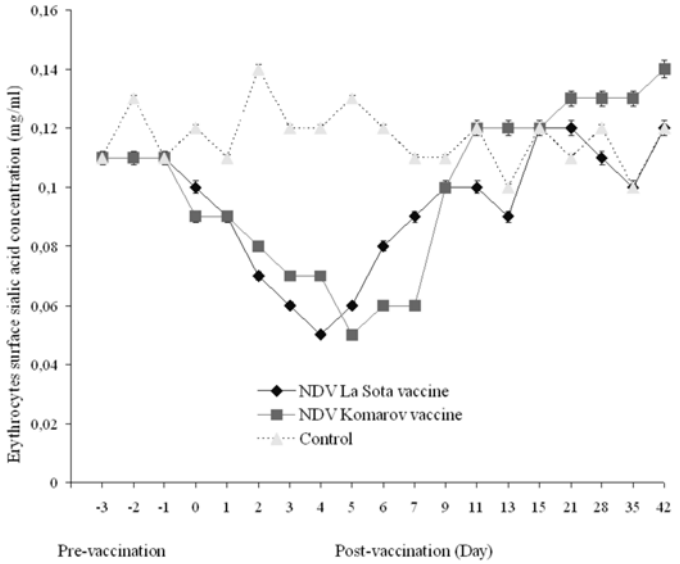

Fig. 5. Changes (mean ± SD) in erythrocytes surface sialic acid concentration of chickens vaccinated with NDV La Sota vaccine, NDV Komarov vaccine and control chickens

The two groups of vaccinated chickens had an elevated daily mean NA in their sera above their control counterparts, beginning from day one pv. The concentration of FSSA recorded for the two vaccinated groups of chickens were elevated at a period which coincided with the time of an increase in NA. Similarly, the period of increased NA also coincided with the time of high HI antibody titres to NDV in the sera of the two groups of vaccinated chickens. These findings support the results of MAAS et al. (2000), that in NDV vaccinations, quantified haemagglutinin-neuraminidase protein of NDV is an indicator of serological response after vaccination.

In general, NA and FSSA values recorded for the group of chickens which received NDV Komarov vaccine were higher than the corresponding values obtained from their counterparts which received NDV La Sota vaccine. This result supports, at least in part, the findings of McNULTY et al. (1975) who found that the neuraminidase content of velogenic NDV strain was higher than the lentogenic NDV strain in vitro.

The two groups of vaccinated chickens had relatively lower daily mean ESSA concentration when compared with their control counterparts until day 11 pv. This period also coincided with the time of a slight decrease in PCV values of the vaccinated groups when compared with the PCV values of control chickens. However, the values of PCV in the three groups of chickens during this period were within the normal range value of 24.39 ± 0.33 - 34.33 ± 0.25% established for chickens reared in Nigeria (OYEWALE, 1987; OLADELE and AYO, 1999; OLADELE et al., 2000).

The fact that there was a slight decrease in the values of PCV, occurring concurrently with an increase in NA, FSSA and HI antibody titres to NDV in both vaccinated groups of chickens, indicates that the vaccine viruses used in this experiment also produced neuraminidase in vivo, which cleaved off erythrocytes surface sialic acid, increasing FSSA concentration in the plasma, and the removal of desialylated erythrocytes by macrophages (DUROCHER et al., 1975; OLADELE et al., 2002). Although the level of involvement of NDV vaccines' neuraminidase in cleaving ESSA was not determined in this study, it is likely to depend on, among other things, the pathogenicity and/or virulence of the viruses from which NDV vaccines were produced. This is evidenced in this study by the higher values of NA and FSSA in chickens vaccinated with NDV Komarov vaccine (known to be derived from mesogenic NDV strain) than NDV La Sota vaccine which was derived from lentogenic NDV strain (MEULEMANS, 1988).

It was concluded that chickens which received NDV Komarov vaccine had higher daily mean values of NA, FSSA and HI antibody titre than their counterparts which received NDV La Sota vaccine.

References

ADU, F. D., A. OYEJIDE, O. TOMORI (1989): Antigenic relationships among the clones of Nigerian field and vaccine strains of Newcastle disease virus. Zar. Vet. 4, 11-17.

ADU, F. D., O. TOMORI, A. OYEJIDE (1990): Susceptibility of chickens vaccinated with three live Newcastle disease vaccines to challenge by velogenic strains. Prev. Vet. Med. 10, 131-135.

ALEXANDER, D. J. (1997): Newcastle disease and other avian paramyxoviridae infections. In: Diseases of Poultry (Calnek, B. W., H. J. Barnes, C. W. Beard, L. R. McDougald, Y. M. Saif, Eds.), Iowa State University Press, Ames, United States of America, pp. 541-569.

ALEXANDER, D. J., J. BANKS, M. S. COLLINS, R. J. MANVELL, K. M. FROST, E. C. SPEIDEL, E. W. ALDOUS (1999): Antigenic and genetic characterization of Newcastle disease viruses isolated from outbreaks in domestic fowl and turkeys in Great Britain during 1997. Vet. Rec. 145, 417-421.

ALLAN, W. H., J. E. LANCASTER, B. TOTH (1978): Newcastle disease vaccines-their production and use. FAO Animal Production and Health Series No. 10, Rome, Italy.

BEARD, C. W. (1989): Serological procedures. In: A Laboratory Manual for Isolation and Identification of Avian Pathogens (Purchase, H. G., L. H. Ard, C. H. Domermuth, J. E. Pearson,

Eds.), American Association of Avian Pathologists, Kenneth Square, PA, United States of America, pp. 192-200.

BENJAMIN, M. M. (1985): Outline of Veterinary Pathology, 3rd Edition, Kalyani Publishers, New Delhi, India, pp. 365.

CORFIELD, T. (1992): Bacterial sialidases-Roles in pathogenicity and nutrition. Glycobiol. 2, 509-521.

DUROCHER, J. R., R. C. PAYNE, M. E. CONRAD (1975): Role of sialic acid in erythrocyte survival. Blood. 45, 10-20.

HERRLER, G., G. REUTER, R. ROTH, H. D. KLENT, R. SCHAUER (1987): N-acetyl-9-O-acetylneuraminic acid, the receptor determinant for influenza C virus, is a differentiation marker on chicken erythrocytes. Biol. Chem. Hoppe-Sey. 368, 451-454.

LICHTENSTEIGER, C. A., E. R. VIMR (2003): Purification and renaturation of membrane neuraminidase from Haemophilus parasuis. Vet. Microbiol. 93, 79-87.

MAAS, R. A., M. P. DE-WINTER, S. VENEMA, H. L. OEI, I. J. CLAASSEN (2000): Antigen quantification as in vitro alternative for potency testing of inactivated viral poultry vaccines. Vet. Qual. 22, 223-227.

MCNULTY, M. S., E. J. GOWANS, M. J. HOUSTON, G. FRASER (1975): Neuraminidase content of strains of Newcastle disease virus, which differ in virulence. J. Gen. Virol. 27, 399-402.

MEULEMANS, G. (1988): Control by vaccination. In: Newcastle disease, Kluwer Academic Publishers, Boston, M.A., pp. 318-332.

OKOYE, J. O. A., M. C. IFE, C. N. IKEJIAKU, V. C. AKOWUNDU, D. B. ADELABU, R. C. OKWOR, L. J. E. ORAJAKA, G. O. N. ECHEONWU (2001): Effect of post infection vaccination on mortality associated with velogenic Newcastle disease. Proc. of the 38th Ann. Conf. Nig. Vet. Med. Assoc. pp. 69-71.

OLADELE, S. B., J. O. AYO (1999): Comparative studies on haematocrit, haemoglobin and total protein values of apparently healthy and clinically sick indigenous chickens in Zaria. Bull. Anim. Hlth Prod. Afr. 47, 163-165.

OLADELE, S. B., P. ABDU, K. A. N. ESIEVO, A. J. NOK, N. M. USEH (2003): Prevalence of Newcastle disease virus antibodies in chickens reared in Zaria. Proc. of the 28th Ann. Conf. Nig. Soc. for Anim. Prod. 28, 5-7.

OLADELE, S. B., P. ABDU, A. J. NOK, K. A. N. ESIEVO, N. M. USEH (2002): Preliminary report on neuraminidase, erythrocyte surface and free serum sialic acid concentrations in the serum of healthy and Newcastle disease virus-infected chicken. Rev. d' Élev. Méd. Vét. Pays Trop. 55, 265-268.

OLADELE, S. B., K. A. N. ESIEVO, J. O. AYO, S. O. OGUNDIPE (2000): Effects of season and sex on packed cell volume, haemoglobin and total protein of indigenous chickens in Zaria, Nigeria. J. Med. All. Sci. 3, 174-178.

OYEWALE, J. O. (1987): Haematological studies on apparently healthy Nigerian domestic chickens (Gallus domesticus). Bull. Anim. Hlth Prod. Afr. 35, 108-112.

REUTER, G., R. SCHAUER (1994): Determination of sialic acids. Meth. Enzymol. 230, 168-199.

TRAVING, C., R. SCHAUER (1998): Structure, function and metabolism of sialic acids. Cell. Mol. Life Sci. 54, 1330-1349.

Received: 28 February 2005
Accepted: 8 September 2006

OLADELE, S. B., A. J. NOK, P. ABDU, L. SAIDU, K. A. N. ESIEVO: Poredbena istraživanja aktivnosti serumske neuraminidaze, slobodne i na površinu eritrocita vezane sijalinske kiseline, hematokrita i protutijela inhibicije hemaglutinacije u pilića cijepljenih različitim cjepivima protiv newcastleske bolesti. Vet. arhiv 76, 391-401, 2006.

SAŽETAK

Poredbena istraživanja aktivnosti serumske neuraminidaze, slobodne i na površinu eritrocita vezane sijalinske kiseline, hematokrita i titra protutijela inhibicije hemaglutinacije (IHA) provedena su na 75 pilića pasmine Shaver Brown cijepljenih različitim cjepivima protiv newcastleske bolesti. Pokusni pilići bili su najprije cijepljeni cjepivom proizvedenim od soja Hitchner B1, a zatim cjepivima proizvedenim od soja La Sota i soja Komarov. Dvadeset i pet pilića bilo je potom cijepljeno sojem La Sota, drugih 25 sojem Komarov, dok je sljedećih 25 poslužilo kao kontrola. Vrijednost aktivnosti serumske neuraminidaze, slobodne i na površinu eritrocita vezane sijalinske kiseline, hematokrita i titra protutijela IHA bile su postupno određene za svako pile. Najveća dnevna srednja vrijednost titra protutijela IHA $\log_2$ 7,12 ± 1,20 i $\log_2$ 8,98 ±1,73 dobivene su za piliće cijepljene sojem La Sota i sojem Komarov petnaestog dana nakon cijepljenja. Neuraminidazna aktivnost i koncentracija slobodne serumske sijalinske kiseline povećavale su se postupno od prvoga do petoga dana nakon cijepljenja u dvije skupine pilića. Općenito se razdoblje povećane aktivnosti neuraminidaze i koncentracije slobodne serumske sijalinske kiseline podudaralo s vremenom smanjene vrijednosti sijalinske kiseline vezane na eritrocite i hematokrita u dvije cijepljene skupine pilića. Zaključeno je da su pilići koji su bili cijepljeni sojem Komarov imali veću dnevnu srednju vrijednost aktivnosti neuraminidaze, koncentracije slobodne serumske sijalinske kiseline i titra protutijela IHA nego skupina cijepljena sojem La Sota.

Ključne riječi: pilići, neuraminidaza, virus newcastleske bolesti, serum, sijalinska kiselina, cjepivo