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Application of Heterologous Erythrocyte Indicator Systems in the Differentiation of Vaccinal and Natural Newcastle Disease Induced Antibodies

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Abstract: The potentials of heterologous red blood cells (RB) indicator systems for differential assay of vaccinal and field (natural) ND antibodies were investigated. In a preliminary experiment on the Haemagglutination (HA) and Haemagglutination Inhibition (HI) standardization tests, results showed that 0.5 and 1% of goat and guinea-pig red blood cells (RBC) suspensions gave similar results with tests conducted with standard or control 0.5% chicken RBC as indicator systems. The HA time for the standardized tests were 50 and 52 minutes with the goat and guinea-pig RBC indicator systems while the corresponding Elution times (Elt) were 86 and 120 minutes respectively. In a field trial using the heterologous indicator systems on the HI test of sera obtained from conventional (field) poultry flocks with or without history of ND, the HI titres with the guinea-pig indicator systems was selectively and consistently higher than titres with the standard chicken RBC by $5\log_2$ in flocks with confirmed ND history. However, the titres for flocks without ND outbreak were generally similar with the three indicators thus showing the ability of the guinea-pig RBC indicator for the selective detection of high titre in ND infected flocks. The ability of these indicators as tools for differential detection of vaccinal ND antibodies in routinely vaccinated chickens was also investigated. The results showed that the ND vaccinal antibodies detected by the three indicators were similar or identical and ranged from $2\log_2$ in one group to $9\log_2$ in another. This strengthened the findings that the selective ability of the guinea pig RBC was only on antibodies due to ND natural outbreaks thus fulfilling the demand for a serological test that could differentiate between vaccinal and natural ND outbreak (field) induced antibodies.

Key words: Newcastle disease, antibodies, erythrocyte indicator systems

Introduction

Newcastle Disease (ND) is an infectious, highly contagious, viral disease of poultry and a wide range of non-poultry avian hosts characterized by variable clinical and pathological manifestations with variable morbidity and mortality.

Despite the production and improvement in ND vaccines and vaccination techniques, the disease has persisted as a major cause of mortalities and production losses in poultry. It is in the light of this that an holistic approach to improved diagnostic surveillance and alternative preventive measures should be intensified to determine the immune status of the flocks or efficacy of vaccinations so as to integrate antibody status with vaccination strategies. Thus, it has become increasingly necessary to develop technology for differentiating between vaccinal and disease induced antibodies as proposed by Bell (1990).

Sero-diagnostic methods in ND such as the Haemagglutination Inhibition (HI) test, Enzyme Linked Immunosorbent Assay (ELISA) and Serum Neutralization Test (SNT) cannot differentiate between vaccinal and disease induced antibodies when conducted under the existing format. Existing systems of HI test, based on paired serum samples, are not only incapable of differentiating between vaccinal and field viral antibodies but extremely slow as they demand one to a few weeks of waiting period. There is therefore an important requirement for achieving this differentiation assay between antibodies to vaccinal virus and field virus.

The Haemagglutination (HA) and HI tests that provide a rapid and useful diagnostic method for ND require the use of erythrocytes as indicators from disease-free or specific pathogen free (SPF) chickens. Unfortunately, SPF facility is non-existent in most developing countries due to very expensive and highly demanding nature of its operations. This inadequacy has posed serious constraints on experimentation and routine diagnostic works on ND in many places around the world. As a result, alternative indicator systems (erythrocytes) other than from chickens had to be sought in lieu of erythrocytes from SPF chickens.

It has been found that RBC from mammalian species such as rabbits, rats, guinea-pig, sheep, goat and cow are agglutinated by ND virus (Chu, 1948; Winslow, *et al*., 1950). The fact that these mammals are not natural hosts (not naturally infected) with ND in addition to their availability and easy management make them good candidates or substitutes for SPF chickens in the supply of indicator systems for HI titrations.

Recently, from the trial experiments conducted, it was

observed that some erythrocyte indicator systems differed in their sensitivity to the detection of ND antibody. Consequently, this investigation was conducted to standardize some mammalian erythrocytes as alternative indicator systems for ND HA and HI tests and determine the differential sensitivity of such erythrocytes to vaccinal and field NDV induced antibodies.

Materials and Methods

Blood Donors

Chickens: Red blood cells were obtained from two adult ND antibody-free unvaccinated cockerels .

Goats: Jugular venipuncture technique was adopted in withdrawing whole blood from two West African Dwarf goats housed at the Teaching and Research Farm.

Guinea Pig (*Cavia porcelus*): Direct cardiac puncture was employed in collecting blood from the experimental guinea-pigs.

Washing and Standardization of RBC Suspensions: The citrated blood samples obtained from the ND-antibody free cocks were washed using fresh normal saline prepared just few minutes before centrifuging the blood. Centrifugation was done several times until the supernatant was clear. This same procedure was employed in washing the guinea-pig and goat erythrocytes.

The erythrocyte concentration (packed cell volume) of the carefully washed blood samples were determined using the micro-haematocrit method. The values obtained were used in preparing the 1.5, 1% and 0.5 RBC suspensions to determine HA time, Elution time and the optimal concentration of RBC from guinea–pig and goat for HA and HI tests and to standardize the test format such that the titres obtained will compare with the standard 0.5% chicken RBC results for HA and HI titres.

The HA Antigen Titre Determination: NDV La Sota (Lento genic strain) was used based on trial results earlier obtained as this strain gave more uniform titres than any other strain. A 200 dose vial produced from the National Veterinary Institute (NVRI), Vom, Nigeria was constituted with 4ml normal saline. This dilution factor was used in the HA titre determination.

HA Titration Procedure: The micro-haemagglutination technique was used with the aim of determining the HA titre and thus calculating the 4HA units (4HAU) required for the determination of HI. The method employed followed standard micro-titration procedure as described by Anonymous (1971) using two-fold serial dilutions of antigen in 50ul normal saline contained in U-bottomed polystyrene micro-titration plates with 50ul micropipette. With two rows of diluted antigen for each of the two tests and the control indicators, a 50ul volume of the respective erythrocyte suspension was applied to each well using a dropping pipette.

HI Titration: The beta (β-) micro-haemagglutination inhibition (HI) technique (constant antigen, varying serum) was employed; (Beach, 1948). Serum samples were obtained from experimentally vaccinated chickens and commercial flocks categorized into confirmed (C), doubtful (D) and negative (N) history of ND on the field. The serum samples were well labeled and stored at –20^{o}C till use.

The HI test followed similar two-fold serial dilutions of each serum against a constant 4HA units of antigen in 50ul volumes using the appropriate pipettes as described under HA. Two rows of the serial dilution of each serum was made in triplicates ear-marked for the chicken (Ch), goat (Gt) and guinea-pig (Gp) erythrocytes.

Experimental Chickens: One hundred (100) day old Isa-Brown cockerels were purchased from a local hatchery and randomly divided into three groups A, B and C. The chicks were reared in wooden cages with floors and sides laced with half-inch wire mesh. Feed and water were served *ad-libitum* throughout the period of the experiment while vaccines and drugs were administered according to the manufacturers' recommendations.

The chicks were reared for five weeks before the commencement of sero-monitoring (using the control and the two test indicator systems) which was done at fortnightly interval thereafter.

Results

HA Titration: The erythrocytes from chicken and the mammalian species were agglutinated by ND virus. The HA titre of the NDV La-Sota with the 0.5% chicken erythrocytes (Ch.E) was 8log$_2$, with HA and Elt times of about 30 and 120 minutes respectively.

Standardization of Goat and Guinea-pig Erythrocyte (Test) Indicator Systems: The HA titres, HA time and Elution (Elt) time are presented in Table 1.Using NDV LaSota as antigen, one of the three optional concentrations of goat RBC (0.5% RBC suspension) gave a titre that was identical or similar to the titre from the control (chicken) RBC. Similarly, the 1% guinea-pig RBC concentration gave HA titre that was identical to that from the control RBC with 256 HA titre, 35 HA time and 125mins of Elt.

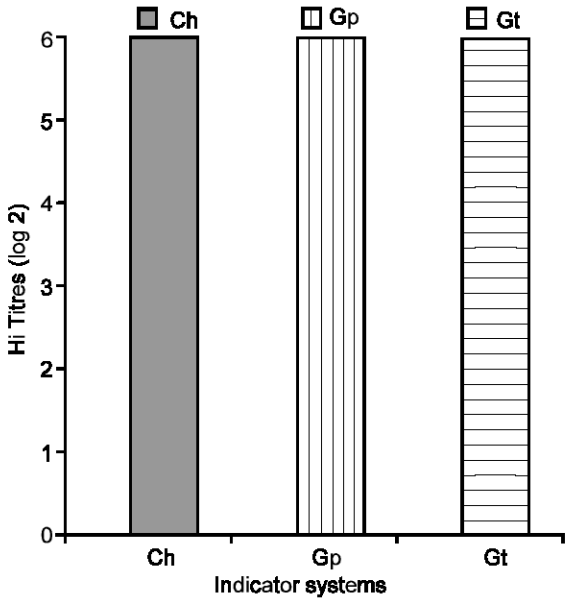
HI Titrations

Experimental Chickens: The responses of the chickens to the vaccines administered are presented in Fig. 1. While there was an appreciable response in the HI titre following the administration of the booster dose of ND vaccine, the three indicators did not differ in their

Table 1: Haemagglutination Titres and Times With Chicken Test Indicators

RBC DONOR	Chicken	Goat			Guinea-pig		
% RBC	0.5	0.5	1.0	1.5	0.5	1.0	1.5
HA Titre (log_2)	8	8	7	N	(11)*	8	6
HA Time (min)	30	50	80	120#	42	35	25
Elusion (min)	120	85	100	NA	120	105	45

N – No Agglutination. ( )* = No clear end point. # = last observation. NA = Not Applicable.



The test and control indicator systems showed similar or identical HI titres with no differential sensitivity to the vaccination induced antibody.

Ch Chicken erythrocyte indicator system
Gp Guinea-pig erythrocyte indicator system
Gt Goat erythrocyte indicator system

Fig. 1: HI Titres of the test sera from vaccinated flock

sensitivity to the indicators used. In other words, there was no differential sensitivity in the HI tests to vaccinal antibodies

Commercial Flocks: A total of 320 commercial flocks were sampled for sera in Oyo, Ogun and Ondo states of Nigeria. Although each flock had history of primary ND vaccination with Hitchner B1 strain from the Hatchery suppliers, only ninety of them had received the secondary (booster) dose at the time of visit. From retrospective case studies based on flock histories and data, one hundred and fifty flocks which had confirmed cases of ND were placed in category C, one hundred flocks which had history of low mortality and no recent ND outbreak were put in category N while the other seventy flocks with bacterial induced mortalities and

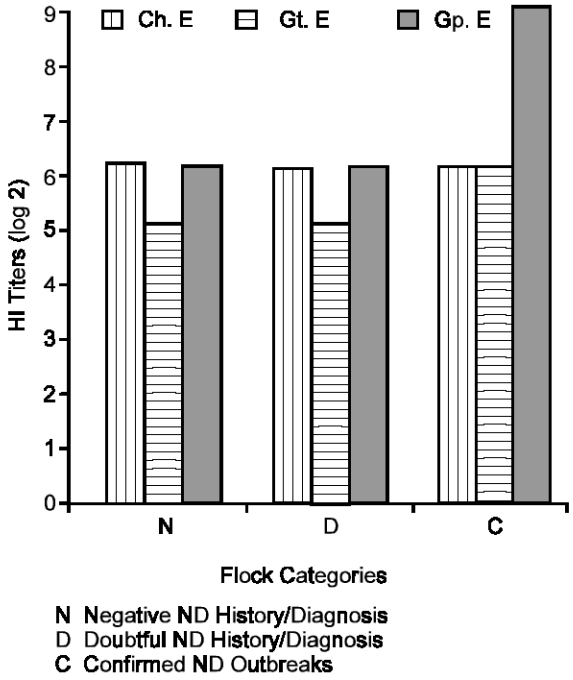


Fig. 2: Differential sensitivity of Guinea-pig Erythrocytes to the Antibody from ND Outbreaks

inadequate diagnostic tools and doubtful ND history were in category D. Serum samples obtained from these farms were subjected to HI titrations using the control (Ch.E) and the two test erythrocyte indicator systems (Gp.E and Gt.E). The results are as shown in Fig. 2.

Discussion

The accuracy of the two test (heterologous) indicator systems as well as the consistency of the results obtained and their application are similar and in many cases identical to those obtained with the conventional standard Ch. E RBC indicator. The results showed that the 0.5% Gt E and 1% Gp indicators were as good as the control Ch.E indicator in the HA tests in terms of the HA time, Elt HA titres which are the essential analytical parameters for HA test. These standardized mammalian erythrocyte systems therefore offer potential or ad-hoc tools which can be applicable in diagnostic centres generally and developmental rural poultry disease diagnostic studies as well. Although the pair of titres obtained under the Ch.E and Gp.E indicators might be a

useful guide to the detection of field NDV in vaccinated stocks, a two-fold or more difference in the pair of HI titres under the standardized guinea-pig and chicken erythrocyte indicator systems would be indicative of field ND exposure as in category C flocks in this study. HI titres from the N and D categories do not comply with these diagnostic criteria. Therefore, the HI results of the mammalian erythrocytes especially the Gp.E indicator in differentiating between the vaccinal and field ND induced antibodies could be useful in complementing other diagnostic procedures.

A previous report by Alexander *et al.* (1984) indeed showed that Gp.E produced considerably higher titre than Ch.E with sera from Infectious Bronchitis (IB) infected flocks. The roles of ND virus pathotype, the time intervals between outbreak and sampling as well as experimental (as opposed to natural infections) are worthy of investigation especially in view of their epidemiologic implications as highlighted by Hanson (1988).

The over 700 million rural poultry in Africa and Asia, which apparently subsist in ND endemic range systems are generally ND sero-positive partly due to sub-clinical ND. Therefore, there is a demand for test capable of discriminating between mild or vaccine strains and field strains of ND virus which account for over 65% of the mortalities in these stocks (Fatunmbi and Adene, 1979; Bell and Moulodi, 1991; Bell, 1990; Asadullah, 1992). With the increasing global awareness of the need for the development of rural agricultural resources of Africa and Asia (Tribe, 1994) and the introduction of Australian V4 thermostable ND vaccine, there would be requirements for the sero-surveillance of wild type NDV. Existing serologic tests are of little value as previously noted by Spradbrow (1992). As previously explained, the test system reported here could serve as a useful preliminary format to the more complex bio-molecular virus cleavage techniques These heterologous RBC donors are naturally free of the ND and many other diseases of poultry, easy to manage and readily available.

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