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HAEMAGGLUTINABILITY OF MAMMALIAN ERYTHROCYTES BY NEWCASTLE DISEASE VIRUS STRAINS ISOLATED FROM CENTRAL NIGERIA

Hemaglutinação dos eritrócitos de mamíferos por cepas de vírus da Doença de Newcastle isolados na Nigéria Central

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ABSTRACT

The haemagglutinability of mammalian erythrocytes by field and vaccine strains of the Newcastle disease virus was assessed. Variability in the pattern of agglutination of the various specie erythrocytes was observed. Whereas noticeable differences in the pattern of agglutination between field virus and vaccine strains were not apparent, differences between the velogenic and non-velogenic strains was observed. The possibility of developing haemagglutinability tests using mammalian erythrocytes for rapid strain differentiation in less developed laboratories is discussed.

Key words: heamagglutinability, Newcastle disease, vaccine virus, wild virus

RESUMO

A hemaglutinação dos eritrócitos de mamíferos por cepas de campo e vacinais do vírus da doença de Newcastle foi avaliada. Foi verificado variabilidade no modelo de aglutinação entre as várias espécies. Embora diferenças perceptíveis no padrão de aglutinação entre vírus de campo e cepas vacinais não tenham sido evidentes, diferenças entre as cepas velogénicas e não velogénicas foram observadas. A possibilidade de desenvolvimento de testes hemaglutinação utilizando eritrócitos de mamíferos para a rápida diferenciação de cepas em laboratórios menos desenvolvidos é aqui discutida.

Palavras-chave: Doença de Newcastle, Hemaglutinação, vírus selvagem, vírus vacinal

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INTRODUCTION

The Newcastle disease virus (NDV), like other viruses carrying haemagglutinins on the viral envelope is capable of causing agglutination of avian and some mammalian erythrocytes.

It has been observed that while erythrocytes of all amphibia, reptiles and birds are agglutinated to some degree by the NDV, some mammalian erythrocytes are in-agglutinable (Placid and Santucci, 1956). Human, mouse and guinea pigs are agglutinated by all strains of the NDV. Those of cattle, sheep, goats, swine and horses are agglutinated by some but not all strains (Winslow et al, 1950). The erythrocytes of certain individual cattle may be agglutinated by certain NDV strains, while the cells of other individual cattle are not (Hanson and Spalatin, 1978). The selective ability of mammalian RBCs as a tool for differentiation between natural and vaccinal immunity in a serological test has been reported (Adebayo, 2004).

The pattern of agglutination may also be related to the degree of virulence of some NDV strains. The more virulent strains appear to show less agglutinability of certain mammalian red blood cells than the less virulent ones (Haruna, *et al*, 1993). The agglutination of erythrocytes of different animal species could serve as a quick means of identifying and separating different viruses as well as different strains of one and the same virus (Tolba *et al,* 1960). Recently, 13 NDV isolates were obtained from wild birds captured from central Nigeria and characterized (Ibu et al, 2008a). In this study, the pattern of agglutination of erythrocytes from 10 species of mammals by twelve lentogenic, and one mesogenic field isolates of the NDV were assessed for the purpose of comparison with chicken erythrocytes in a standard Haemagglutination (HA) test (OIE, 2004). Also included in the study were four lentogenic vaccine strains, one mesogenic vaccine strain, one standard velogenic (Herts) strain and three local velogenic strains. The criteria for antigenic differences for the purpose of intra-strain differentiation are highlighted.

MATERIAL AND METHODS

Blood collection

Whole blood was collected from the following mammalian species: goat, sheep, horse, cattle, man, dog, pig, guinea pig, mouse, and rabbit. Blood from the chicken was taken as a positive control. Goat, sheep, horse, cattle and pig were bled from animals kept at the Livestock Investigation Department (LID) farm, of National Veterinary Research Institute, while guinea pig, mice and the rabbit blood were obtained from the Laboratory Animal Colony Unit of the Epidemiological Investigation Department of the Institute. The ruminants and the horse were bled via the jugular vein while the pig was bled through the ear vein. The guinea pig, mice and rabbit were bled from the heart. Blood was also taken from dogs kept at the Veterinary Clinic of the College of Animal Health and Production Technology via the cephalic vein. Chickens kept at the minimal disease unit of the Viral Research Department of the Institute were bled through the wing vein.

The thirteen field virus isolates were compared with four lentogenic vaccine strains, one mesogenic vaccine strain and four standard velogenic strains. At

least, one millilitre of blood was taken from each animal using appropriate gauge of needle and syringe containing acid citrate dextrose (ACD), (1 part ACD to 4 parts blood) and mixed. The blood was washed three times through the process of centrifugation at 900 rpm in phosphate buffered saline (PBS, pH 7.2). A 20% red blood cell (RBC) suspension was made as a stock. Just before use, a 1% RBC concentration was made in PBS as a working dilution.

Haemagglutination test

A standard HA test was carried out on all the virus strains according to standard methods (OIE, 2004). Briefly, twenty five microlitre (25µl) of Phosphate buffered saline (PBS) was dispensed into each well of a plastic V-bottomed microtitre plate. The same volume of the infective allantoic fluid harvest was placed in the first well and two - fold dilutions of the virus was made in PBS. Another 25 µl of PBS was then dispensed into each well bringing the diluted virus to 50 µl per well.

Just before use, 1% suspension of fresh RBC was made from the 10% stock and 25 µl was dispensed into each well.

The mixture was tapped gently and the RBC allowed to settle for about 30-40 min at room temperature. Fifty percent suspension of NDV Lasota in glycerin was used as positive control antigen. Red blood cells alone in PBS was used as negative control.

When the RBCs in the negative control wells were sufficiently settled to form a distinct botton, the test result was read by tilting the plate and observing the presence or absence of tear drop streaming of RBC.

Only those wells in which RBCs streamed at the same rate as the RBC in control wells were considered HA Positive.

RESULTS

The results of the HA test using erythrocytes from 10 species of domesticated mammals (goats, sheep, horse, cattle, man, dog, pigs, guinea fowl, mouse, rabbit) by the 13 field NDV isolates, 5 vaccine strains (Lasota, intraocular, V4, I2, K) and 4 known velogenic strains (Herts, Kudu, VGF1, and NCD) are presented (Table 1).

All the virus strains haemagglutinated caprine Red Blood Cells (RBCS) with varying titres except four strains (Bn2, Herts, Kudu, VGF1) which failed to agglutinate the goat RBCS. Similarly, sheep RBC was agglutinated by all NDV strains used except three (Pl029, Herts, Kudu). Five virus strains (Bn2, Bn8, Herts, Kudu, NCD) failed to agglutinate bovine and equine RBCS while VGF1 agglutinated the former but not the latter. All the other virus strains agglutinated RBC from these two species.

On the other hand, the human erythrocytes were agglutinated by all the virus strains used in the experiment except the NDV4 vaccine strain. The Canine RBC was agglutinated by only 5 field isolates but not by any of the vaccine strains, Herts and Kudu. Of the 22 virus strains used for the test, ten, (Pl032, Bn2, Bn8, Bnll, Jz2, Jz6, NDV(L), NDV4, NDVI2, Herts) agglutinated porcine RBCS while the rest did not.

Also, all the virus strains except Herts agglutinated guinea pig and mouse erythrocytes. Eight strains (Pl016, Bn2, Bn7, Jz2, Jz6, Jz13, NDV (L) and NDV(I2) agglutinated RBC from the rabbit.

Table 1 - Haemagglutinability of mammalian erythrocytes by the NDV field isolates and vaccine strains

S/No	NDV	Goat	Sheep	Horse	Cattle	Man	Dog	Pig	G/Pig	Mouse	Rabbit	Chicken
1.	Pl016	32	64	128	16	32	2	0	16	1024	1024	512
2.	Pl029	64	0	128	512	512	0	0	256	128	0	4096
3.	Pl032	2048	1024	1024	1024	512	16	4	64	256	0	1024
4.	Pl038	512	512	256	256	128	0	0	32	256	0	512
5.	Bn 2	0	64	0	0	4	0	8	128	128	512	128
6.	Bn 7	128	2048	256	2	4096	0	0	32	128	2048	4096
7.	Bn 8	16	8	0	0	256	512	0	64	1028	0	256
8.	Bn 11	32	128	32	128	512	2	0	256	256	0	1024
9.	Jz2	128	128	32	128	64	0	4	32	128	512	512
10.	Jz4	256	128	1024	2048	4096	0	128	64	128	0	2048
11.	Jz6	256	1024	2048	512	1024	0	2	64	16	512	512
12.	Jz13	128	256	256	128	64	2048	0	64	32	2048	512
13.	KD	128	256	64	128	256	0	0	128	64	0	4096
14.	NDV(L)	32	128	128	512	128	0	8	64	1024	128	256
15.	NDV(i/o)	16	128	256	1024	128	0	4	512	2048	0	512
16.	NDV4	16	4	128	256	0	0	8	256	2048	0	256
17.	NDVI2	128	32	8	64	64	0	128	64	16	128	512
18.	NDV(K)	32	512	32	16	512	0	0	64	256	0	128
19.	Herts	0	0	0	0	8	0	2	0	0	0	128
20	Kudu	0	0	0	0	0	0	0	32	256	0	512
21	VGF1	0	4	0	64	32	16	0	8	64	64	512
22	NCD	4	8	0	0	4	32	0	32	32	0	128

With the exception of the pig, isolate Pl016 agglutinated erythrocytes from all the mammalian species with varying titres while isolate Pl029 agglutinated 9 mammalian RBC except that of the sheep, dog, pig and rabbit. Whereas isolate Pl032 failed to agglutinate only the rabbit RBC, isolate Pl038 agglutinated all others except those of the dog, pig and the rabbit.

Isolate Bn2 did not agglutinate RBC from 4 mammalian species (goats, horse, cattle and pig) while isolate Bn7 agglutinated RBC from all but two mammalian species (dog and pig). Erythrocytes from four species were not agglutinated by isolate Bn8 (horse, cattle, pig, and rabbit). Also isolate Bn11 did not agglutinate erythrocytes from two species (Pig and rabbit).

Whereas isolate Jz13 failed to agglutinate RBC from the pig, isolate KD4 agglutinated erythrocytes from all but three mammalian species (dog, pig, and rabbit).

All the 5 vaccines used failed to agglutinate canine erythrocytes. While NDV (L) and NDVI2 agglutinated RBC

from the rest of the mammalian species tested NDV (I/O) and NDV4 did not agglutinate RBC from the rabbit. Similarly, NDV (K) failed to agglutinate RBC from the rabbit and the pig.

Table 2 - Proportion of viral HA titres using mammalian RBCs to the HA titres using chicken RBCs

S/No	Virus Isolates	Goat	Sheep	Horse	Cattle	Man	Dog	Pig	G/Pig	Mouse	Rabbit	Chicken
1	Pl016	0.063	0.13	0.25	0.031	0.063	0.0039	0	0.031	2	2	1
2	Pl029	0.02	0.02	0.03	0.13	0.13	0	0	0.06	0.03	0	1
3	Pl032	2	2	1	1	0.5	0.016	0.0039	0.06	0.25	0	1
4	Pl038	1	1	0.5	0.5	0.25	0	0	0.063	0.5	0	1
5	BN2	0	0	0	0	0.03	0	0.063	1	1	4	1
6	BN7	0.031	0.031	0.063	0.0048	1	0	0	0.0078	0.031	0.5	1
7	BN8	0.063	0.063	0	0	1	2	0	0.25	4	0	1
8	BN11	0.031	0.031	0.031	0.13	0.5	0.002	0	0.25	0.25	0	1
9	Jz2	0.25	0.25	0.063	0.25	0.13	0	0.0078	0.063	0.25	1	1
10	Jz4	0	0.13	0.50	1	2	0	0.06	0.03	0.06	0	1
11	Jz6	0.5	0.5	4	1	2	0	0.0039	0.131	0.031	1	1
12	Jz13	0.25	0.25	0.5	0.25	0.13	4	0	0.131	0.063	4	
13	KD4	0.03	0.03	0.016	0.03	0.63	0	0	0.03	0.16	0	1
14	NDVL	0.13	0.13	0.5	2	0.5	0	0.03	0.25	4	0.5	1
15	NDV1/10	0.03	0.03	0.5	2	0.25	0	0.0078	1	4	0	1
16	NDV4	0.063	0.063	0.5	1	0	0	0.031	1	8	0	1
17	NDV12	0.25	0.25	0.02	0.13	0.13	0	0.25	0.13	0.03	0.25	
18	NDVK	0.25	0.25	0.25	0.13	2	0	0	0.5	2	0	1
19	Herts	0	0	0	0	0.063	0	0.016	0	0	0	1

The virulent virus strain (NDV Herts) only weakly agglutinated erythrocytes from two of the 10 mammalian species tested (man and pig). All the thirteen field virus strains, the 5 vaccine strains, and the velogenic virus strains, agglutinated the control chicken erythrocytes (Table 1). The proportion of HA titres using mammalian erythrocytes to the chicken RBCs is shown in Table 2.

DISCUSSION

The variability in the pattern of agglutination of certain mammalian erythrocytes by the field virus, vaccine strains, and the challenge strains, is demonstrated herewith. The pattern of variability as observed could be of theoretical and practical significance. Although twelve out of thirteen field virus isolates were classified as lentogenic by in-vivo tests as well as by molecular studies (Ibu *et al*, 2008a), some intra-strain differences are still apparent in terms of their haemagglutinability patterns.

A marked variability in the inhibitory capacity between the heterologous antigen and antisera of the isolates used in this study was reported earlier (Ibu, et al, 2008b).

Whereas, some isolates (Pl032, Jz4, Jz6, Jz13) haemagglutinated erythrocytes from mammalian species with relatively high titres, other virus strains (Bn2 & Bn8) only sparingly agglutinated the same RBCS. Also, there appeared

to be no obvious differences in the pattern of haemagglutinability between the field viruses and vaccine strains. This is in contrast with the 4 velogenic strains which sparingly haemagglutinated the mammalian erythrocytes.

The RBCs from man, guinea pig and mouse were agglutinated by all field virus strains while that of cattle, sheep, goats and horses were agglutinated by most but not all the NDV virus isolates tested. This observation is similar to that by previous workers (Winslow et al., 1950). On the other hand, swine erythrocytes were found by the same authors to be inagglutinable. In the present study, RBCS from the pig, dog and rabbit were not agglutinated by most of the field viruses, vaccine strains and the two out of the four velogenic strains used.

The lack of agglutinability of most mammalian erythrocytes by the velogenic strains appears to agree with the findings of Haruna et al, (1993). These workers observed a direct linkage between the agglutinability of mammalian RBCS and virulence of ND viruses. A similar observation was made earlier (Tolba, et al, 1960). The latter authors concluded that the agglutination of erythrocytes of different animal species could serve as a quick means of identifying and separating different viruses as well as different strains of one and the same virus.

From the present study, it appears that an NDV isolate which does not agglutinate fresh RBCs from the horse but agglutinates mouse and guineapig erythrocytes is of the velogenic strain. Consequently, any isolate which agglutinates RBCs of all three animals (horse, mouse, guineapig) is either of a mesogenic or lentogenic strain.

It is therefore possible to employ haemagglutinability tests using mammalian erythrocytes as a tool for strain differentiation. Such test procedures will be particularly useful in less developed laboratories with minimal diagnostic facilities.

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