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Red blood cell elution time of strains of Newcastle disease virus

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Elution time of velogenic, mesogenic and lentogenic strains of Newcastle disease virus was determined. The differences in their elution time were also calculated. Four samples, each of a velogenic strain (VGF2), a mesogenic strain (Komarov) and a lentogenic strain (LaSota) were used for hemagglutination test with 0.6% chicken red blood cells. The time it took for wells of the end hemagglutination points (highest dilution that gave agglutination) to elute was recorded as elution time for each sample. The mean elution time of the three strains of Newcastle disease virus differed significantly ($p < 0.05$). The velogenic strain gave the highest mean elution time of 118 min, followed by the mesogenic strain with 59 min and the lentogenic strain with 25 min. Based on this result it appears that elution time could form a basis for rough characterization of isolates of Newcastle disease virus into the three major strains.

Key words: elution time, HA test, NDV

Introduction

Newcastle disease virus belongs to the family Paramyxoviridae [5], and the serotype paramyzovirus [6].

The disease produced often varies widely depending on the strain of the virus in affected chickens. So in diagnosis, it may be necessary to identify the strain of the virus involved in each outbreak.

Some biological activities of the Newcastle disease virus are characteristic of the different strains. These include hemagglutination of specific erythrocytes [2], neuraminidase activity, cell fusion and hemolysis [7]. Neuraminidase (mucopolysaccharide, N-acetyl neuraminyl hydrolase E. 3.2 : 1 : 18) is a component of the hemagglutinin-neuraminidase antigen. The neuraminidase is responsible for clipping off of the bond between red blood cell receptors and the viral hemagglutinins (elution) observed in hemagglutination of red blood cells by Newcastle disease virus.

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Hemagglutination test itself is very simple, cheap and quick technique [4]. It can be performed in the developing countries and in remote areas. The determination of elution time could be a very useful tool in the characterization of field isolates of Newcastle disease virus if the difference between the elution time of the different strains are found to be significant. Thus a study was carried out with the aim of developing a simple method of characterizing isolates of Newcastle disease virus involved in outbreaks of the disease which could be adopted in less sophisticated laboratories.

Materials and Methods

Four different samples of a velogenic strain (VGF2, NVRI Nigeria), four samples of a mesogenic strain (Kamarov) and four samples of a lentogenic strain (LaSota) were used to perform hemagglutination test with 0.6% chicken erythrocyte solution. The 0.6% erythrocyte solution was prepared according to the method described by Wosu [9] while the hemagglutination test was performed as described by Johnson [4]. Briefly, 0.05 ml of phosphate buffered saline (PBS) was put into each well in rows of 'U' bottomed microtitre plate. Equal volumes of the antigens were each serial diluted followed by the addition of 0.05 ml or the 0.6% chicken red blood cells to each well. Red blood cell controls consisting of wells containing only PBS and the RBC were include in the test. On each microtitre plate, samples of each of the three virus strains were used for hemagglutination test simultaneously at room temperature. The time taken by the red blood cells in the control to settle (when those in test wells failed to settle) was recorded as agglutination time. The interval between the agglutination time and the elution of the red blood cells in the highest dilution of the antigens, which gave a complete agglutination, was read as the elution time for each sample. For each set of samples, the test was repeated once and the average elution time recorded as the elution time for the sample. Then the mean elution time for the velogenic, mesogenic and lentogenic strain was calculated based on the elution time of the four sets of sample. The difference in the mean elution time of the three strains was tested by analysis of variance (ANOVA).

Table 1. Elution time of velogenic (VGF2), mseogenic (Komarov) and lentogenic (LaSota) strains of Newcastle disease virus

Samples	Elution time (min)*		
	VGF2	Komarov	LaSota
I	189	78	45
II	100	65	20
III	84	43	15
IV	99	50	20
Mean	118	59	25

*The elution time of Newcastle disease virus varied significantly between the three major strains ($p < 0.05$).

Results

The elution time for the Newcastle disease virus varied from 84 to 189 min for samples of the velogenic strain, 43 to 78 min for samples of the mesogenic strain and 20 to 45 min for the lentogenic strain (Table 1). The difference between the mean elution time of the three strains was statistically significant ($p < 0.05$).

Discussion

Among viral strains, the variation in elution time of samples of the same strain may be due to variation in room temperature since the tests were done on different days. However, for each set of samples tested, the velogenic strain consistently had the longest elution time, followed by the mesogenic strain. The lentogenic strain had the shortest elution time.

Viral elution is a function of the enzyme, neuraminidase, which is a component of the antigen, hemagglutinin-neuraminnidase complex [3]. Since elution time is inversely related to the neuraminidase activity, the result of this experiment suggests that elution time can be directly related to hemagglutinin activity for viral pathogenicity.

Stern *et al.* [8] reported that the pathogenicity of a paramyxovirus residues in the hemagglutinin glycoprotein. Since the hemagglutinin and neuraminiadase form a complex on the viral envelope, this result suggests that where the neuraminidase activity is decreased, (long elution time), the hemagglutinin activity (pathogenicity) is enhanced.

The hemagglutinin of the velogenic strain is stable even at 56°C for 15 min, whereas those of mesogenic strain and lentogenic strain are destroyed at that condition [3]. This demonstrates that hemagglutinin activity is highest in velogenic strain and lowest with lentogenic strain.

Our findings that the strains show high hemagglutinin activities possess low neuraminidase activity and that those with low hemagglutinin activities have high neuraminidase activity appears an important characteristic of the Newcastle disease virus. This characteristic could be useful in characterizing new isolates into the three major strains or pathotypes of Newcastle virus. It is however important that tests for new isolates be run concurrently with known samples of all the three strains in other to eliminate effects of other interfering factors such as environmental temperature.

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