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Temporal Pattern of Elution of *Newcastle disease virus* (V4 and Komarov strains) Following Haemagglutination of Chicken and Guinea Pig Erythrocytes

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ABSTRACT

The temporal pattern of elution of V4 and Komarov strains of Newcastle disease virus (NDV) following haemagglutination of chicken and guinea pig red blood cells (RBCs) was investigated at different temperature conditions (4°C, 25°C, 37°C and 45°C). Chicken and guinea pig RBCs were haemagglutinated with V4 and Komarov strains of NDV and the haemagglutinated RBCs were centrifuged at 1,000 rpm for 5 minutes and the packed cells re-suspended in PBS, incubated at different temperatures and time intervals, centrifuged and the supernatants used to haemagglutinate fresh 2.5% suspension of chicken and guinea pig RBCs. The rate of elution of the two NDV strains increased with increase in incubation temperature against either RBC types. Significant elution pattern of the two virus strains were observed between the incubation temperatures of 45°C compared to 4°C and 25°C using chicken RBC and between 45°C and 4°C using guinea pig RBC. Significant differences (≥4 fold rise in HA titre) in elution patterns between the chicken and guinea pig RBCs was only exhibited by the NDV Komarov strain at 37°C and 45°C. Only the chicken RBC showed notable difference in elution pattern between the two viruses at 4°C. Maximum elution was recorded at 90-120 minutes of incubation, for both virus strains using the two RBCs and different incubation temperatures with the exception of NDV V4 at 45°C using chicken RBC. Both virus strains demonstrated least elution at 4°C and maximum elution at 45°C using either RBC type. The 4°C incubation temperature did not exhibit significant (≤4 fold rise in HA titre) elution by either virus strains against the different RBC types. In conclusion, the two NDV strains (Komarov and V4) were observed to elute from haemagglutinated chicken and guinea pig RBCs with increase in temperatures, with optimal elutions demonstrated at 45°C and for 90 – 120 minutes of incubation and least elution at 4°C throughout the period of incubation.

Keywords: Newcastle disease virus V4 and Komarov strains, haemagglutination and elution, chicken and guinea pig RBCs

INTRODUCTION

Newcastle disease virus (NDV), an avian paramyxovirus (APMV), is the causative agent of Newcastle disease (ND) (Uddin et al., 2017), an important acute, highly contagious, and economically significant infectious viral disease of poultry (Iram et al., 2013; Liu et al., 2023) that has the potential to cause large economic losses in the poultry industry (Chen et al., 2021) due to high morbidity and mortality, decreased growth rate, and reduced egg production. The NDV is an enveloped, single stranded, linear, negative sense RNA virus (Alexander, 2000; Daud *et al*., 2019; Awad, 2024). The NDV, also known as *Avian Orthoavulavirus-1* (AOAV-1), was recently classified into the Genus Avian *Orthoavulavirus*, family *Paramyxoviridae* (Abdelaziz *et al*., 2022). The virus genome encodes six virus coded structural proteins in the gene order of 3′-NP-P-M-F-HN-L-5′; that stands for nucleocapsid protein, phosphoprotein, matrix protein, fusion protein, hemagglutinin-neuraminidase, and large polymerase respectively (Daud *et al*., 2019; Abdelaziz *et al*., 2022).

The outer surface of the virus envelop has the HN protein, one of two immunogenic envelope glycoproteins, a multifunctional molecule that recognizes sialic acid-containing receptors on cell surfaces, promotes host cell fusion and penetration activity via the HN-F interaction and also acts as a neuraminidase during the release of viral progeny from the infected cell (Crennell *et al*., 2000; Connaris *et al*., 2002; Panda *et al*., 2004; Palermo *et al*., 2007). The NDV HN glycoprotein has the ability to agglutinate avian as well as mammalian red blood cells carrying HA receptors on their surface and reverses the agglutination (elution) (Kiani *et al.,* 2021). In addition to roles of HN in viral infectivity, it is critical to the protective immunity of vaccinated birds (Mao *et al*., 2022). While HA protein clumps specific erythrocytes, the neuraminidase is responsible for cutting off the bond between the virus HA protein and RBC receptors; a phenomenon referred to as elution (Spalatin *et al*., 1970). Pathogenicity of NDV isolates is a function of their haemagglutinin antigen (H-antigen) (Nagai *et al*., 1976; Collins *et al*., 1993; Peeters *et al*., 1999). It has been reported that the rate of elution of NDV could be used as a

genetic marker as well as a character for identifying NDV strains (Spalatin *et al*., 1970; Ezeibe and Ndip, 2005). This study was aimed at determining the influence of source of red blood cells (RBC), incubation time, temperature and elution time of NDV (V4 and Komarov) strains.

MATERIALS AND METHODS

Antigen

The antigens used in this study were avirulent NDV4 and mesogenic Komarov ND vaccine strains obtained from the National Veterinary Research Institute (NVRI) Vom, Nigeria.

Preparation of red blood cell (RBC) suspensions

Four millilitres of blood was aseptically collected from chickens and guinea pigs through wing vein and intra-cardiac routes respectively, using a sterile 5ml syringe and 21G needle containing 1 ml of Alsever's solution (anticoagulant). Each blood sample was washed four times once with Alsever's solution and thrice with PBS (pH 7.2) by centrifugation at 1,500 rpm for 5 minutes in each wash. A 1% and 2.5% chicken and guinea pig RBCs were prepared in PBS (pH 7.2). The 1% RBC suspension was used for haemagglutination (HA) test and the 2.5% RBC suspension was used for the elution test.

Haemagglutination (HA) test

Haemagglutination tests were carried out with the two viruses (NDV V_4 and Komarov) to determine the optimum level of antigens to be used for the elution tests. The HA tests were carried out as described by Uddin *et al.* (2017). Briefly, 25 µl of PBS was dispensed into all the wells of a 96 well polystyrene V-shaped microtitre plate, and 25 µl of each antigen (V4 and Komarov) was dispensed in duplicate in the first wells of the plate. A two-fold serial dilution of either antigens (V4 and Komarov) was carried out by transferring 25 µl of each antigen from the first wells to the second to the last wells, the last 25 µl was discarded and the last wells were left as cell control. Finally, 25 µl of 1% RBC suspension was added to all the wells and the plates were incubated at room temperature for 45 minutes before the results were read. The last well that showed HA after which there was button formation was considered as the end-point titre carrying 1 HA unit of the individual virus.

Elution test

Elution test was carried out using a modification of the protocol described by Ramachandran *et al.* (1995). A 20 HAU of each of the two virus strains of NDV was prepared by dividing the HA titre of each virus by 20. A 2.5% RBC suspension in PBS was prepared from each of chicken and guinea pig RBCs. Fifty microlitres of each RBC was mixed with equal volume of 20HAU of each virus in 4 sets of 8 tubes labelled -45 minutes (time of the initial HA) to 180 minutes of 30 minutes' interval and incubated at different temperatures of 4°C, 25°C, 37°C and 45°C. The suspensions were then centrifuged at 1,000 rpm for 5 minutes to sediment the haemagglutinated RBCs, the supernatants were discarded and the packed RBCs re-suspended in 50 µl of fresh PBS and incubated at the different temperatures after which a tube each was removed at 30-minute intervals, centrifuged and the supernatant tested for haemagglutination activity against the two RBCs. Haemagglutination (HA) titres equal to or greater than 4-fold rise was considered significant.

RESULTS

The results of this study showed a significant elution of the NDV strains from the haemagglutinated RBCs with increase in temperature and length of time of incubation. The NDV V4 started to elute from the chicken RBC after 90 minutes of incubation for the 25°C, 37°C, and 45°C incubation temperatures; and the elution peaked at 90 minutes for 25°C, at 120 minutes for 37°C and at 150 minutes for 45°C. Significant elutions (≥4 fold rise in HA titre) were observed at 37°C and 45°C temperatures after 90 – 120 minutes of incubation (Figure 1). The NDV Komarov started eluting from chicken RBC from 90 minutes of incubation and peaked at 120 minutes for all the temperatures (Figure 2). A significant elution (≥4 fold rise in HA titre) of the Komarov strain was observed at 4ºC, 37ºC and 45ºC incubation temperatures after 120 minutes using chicken RBC (Table 1). The NDV V4 started to elute from the guinea pig RBC after 30 minutes of incubation at 45°C, at 60 minutes for 25°C and at 90 minutes for 37°C. The elution peaked at 90 minutes for all the incubation temperatures, except for the 4ºC (Figure 3). A significant elution (≥4 fold rise in HA titre) was observed only for the 45°C incubation at 90-120 minutes. The Komarov strain started eluting from the guinea pig RBC after 90 minutes of incubation for all the temperatures; and peaked at 90-120 minutes of incubation and the peak elution (≥4 fold rise in HA titre) was observed at 45°C after 120 minutes of incubation (Figure 4). Only the NDV Komarov showed significant elution (≥4 fold rise in HA titre) at 4°C incubation temperature using chicken RBC. There was no significant difference (≤4 fold rise in HA titre) in elution rate of NDV V4 observed between either chicken or guinea pig RBCs across all the incubation temperatures. However, a significant difference (≥4 fold rise in HA titre) in elution of NDV Komarov between the chicken and guinea pig RBCs was observed at 37°C and 45°C incubation temperatures (Table 1). There was no significant difference (≤4 fold rise in HA titre) in elution rate of NDV V4 or Komarov from either the chicken or guinea pig RBCs throughout the periods of the different incubation temperatures. The two virus strains exhibited significant difference in elution pattern using either RBC type, between the incubation temperatures of 45°C compared to 4°C and 25°C; also, between 37°C and 4°C only (Table 1).

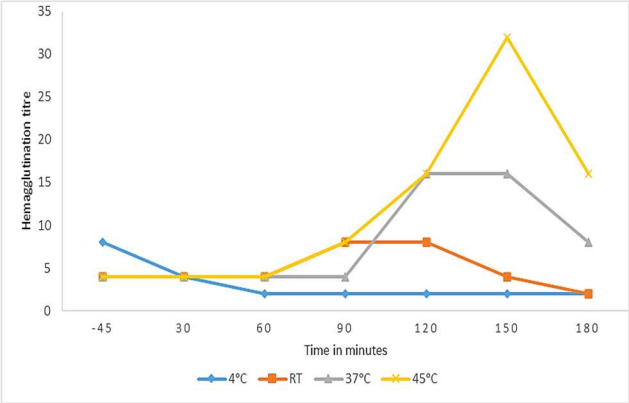

Figure 1: Temporal elution pattern of NDV V4 from chicken red blood cells at different temperature.

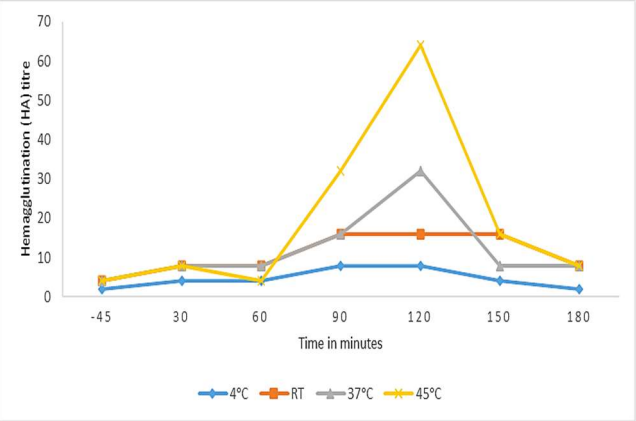

Figure 2: Temporal elution pattern of NDV Komarov from chicken red blood cells at different temperatures.

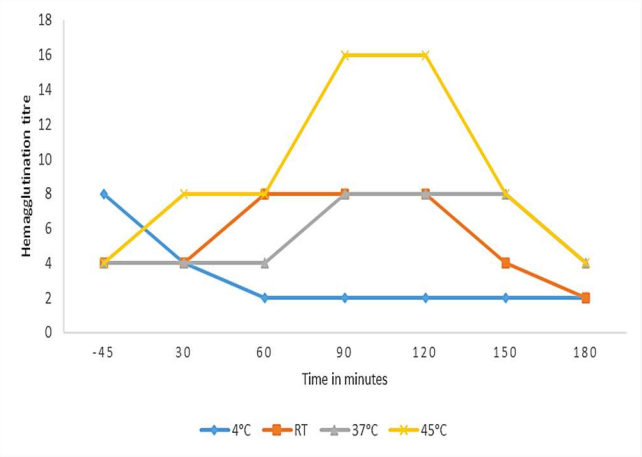

Figure 3: Temporal elution pattern of NDV V4 from guinea pig red blood cells at different temperatures.

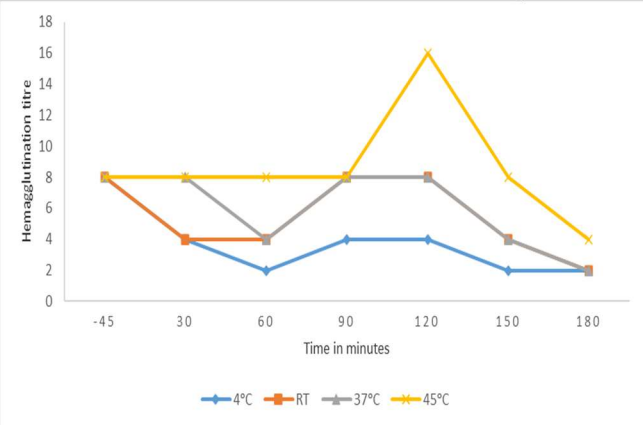

Figure 4: Temporal elution pattern of NDV Komarov from guinea pig red blood cells at different temperatures.

DISCUSSION

The results of this study revealed that NDV (V4 and Komarov) agglutinated chicken and guinea pig RBCs, when centrifuged and suspended in PBS, and incubated at different temperatures their supernatants haemagglutinated fresh chicken RBCs. This HA titres recorded with the supernatants were clear indications of elution of the two virus strains. This shows that agglutinated RBCs were carrying the NDV HA-antigen bound to the N-acetylneuraminic acid (sialic acid) receptors until such a time when neuraminidase (NA) (sialidase) in the HA-sialic acid configuration cuts off the bond between the virus and the RBCs. While the previously haemagglutinated RBCs loss their NDV HA receptors sites, the eluted NDV HA antigen still retains its haemagglutination potentials. This is similar to what was reported by Ramachandran *et al.* (1995) using *Peste des petits ruminants virus*.

Table 1: Highest haemagglutination titres of NDV strains (V4 and Komorav) eluted from chicken and guinea pig erythrocytes incubated at different temperature conditions

NDV strain	RBC type	Incubation temperatures and HA titres			
		4°C	25°C	37°C	45°C
V4	Chicken	1:2	1:8	1:16	1:32
	Guinea pig	1:2	1:8	1:8	1:16
Komarov	Chicken	1:8	1:16	1:32	1:64
	Guinea pig	1:4	1:8	1:8	1:16

The results of the temporal pattern of elution demonstrated with NDV V4 and Komarov strains against chicken RBCs showed higher HA titres at 37°C and 45°C, but only at 45°C against the guinea pig RBCs. This is consistent with the fact that enzyme activity is highest at 37°C. The enzyme neuraminidase destroys the haemagglutinin receptors on the host cells and the virus elutes from the cells due to the destruction of the neuraminic acid in the receptors on the cells by the virus neuraminidase enzyme (Mohanty and Dutta, 1981). Maximum elution was reported to occur at higher temperatures with avian influenza viruses (Hussain *et al*., 2010). Other workers have observed considerable variations in elution rates from fast elution to absence of elution among avian paramyxoviruses (Shihmanter *et al.*, 1995). The difference in the elution pattern of the two NDV strains could possibly be due to the difference in the conformation and composition of the neuraminidase molecules of the two virus strains (Yuan *et al*., 2011). Wiley and Skehel (1991), had observed some differences in the sequence homology among the neuraminidase molecules of related strains in the Influenza virus group. Maximum elution by both virus strains in this study were observed after 120-150 minutes and 90-120 minutes of incubation using chicken and guinea pig RBCs respectively. The observation does not agree with the study by Ezeibe *et al*. (2014) that reported erythrocyte elution time of Lentogenic and Mesogenic NDV strains to be only few minutes (Ezeibe *et al*., 2014). This difference could be due to the fact that they only looked at the button formation by the haemagglutinated RBCs rather than testing the supernatant for HA activity as carried out in the current study. The NDV strains used in this study did not demonstrate significant variation in the time of incubation required for elution to take place and the species of RBCs used. In conclusion, the two NDV strains (Komarov and V4) have shown to elute from haemagglutinated chicken and guinea pig RBCs with increase in temperatures and at 90 – 150 minutes, with optimal elution demonstrated at 45°C and least at 4°C. It is therefore recommended that the

elution pattern of all the NDV strains be further looked at with the aim of adopting the haemagglutination and elution patterns as biomarker for identifying and characterizing NDV strains and possibly using the guinea pig RBCs for HA and HI tests.

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Conflict of Interest

The authors have no conflict of interest to declare.

Author's Contribution

Both authors equally contributed to the work.

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