Cairo University
Faculty of Veterinary Medicine
Department of Virology

Preparation of saponin-treated vaccine from NDV genotype chinese 7d

A Thesis Presented By

Wahid Hussein Mahmoud El-Dabae

(B.V.Sc., Cairo University, 2004)
(M.V.Sc. Virology, Cairo University, 2010)

For the Degree of PhD in Veterinary Medical Science (Virology)

Under Supervision Of

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National Research Center

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Supervision Sheet

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ABSTRACT

In the present study, chemical attenuation was carried out for local NDV genotype VIId designated as NDV-F278-RLQP-CH-EG circulating in Egypt. The virus was propagated in 9-11 day SPF eggs via allantoic cavity and was serially passaged for 35 passages. Then, nitrous acid was used for attenuation and the treated virus was inoculated into SPF eggs. After incubation at 26°C for 4-6 days, the harvested allantoic fluid revealed negative hemagglutination even after five passages of such harvest. Assaying of the allantoic fluid harvested from fifth passage of treated virus by real time RT-PCR revealed positive result indicating that nitrous acid affects the HA property of the virus. Inoculation of treated virus in SPF chickens, mortalities developed along ten days observation period and HA property of the virus was recovered again following isolation in SPF eggs indicating failure of attenuation. Therefore, second cycle of treatment using saponin was applied on the propagated virus after 35 passages. Saponin was used to treat virus at different times 10,20,30,45 and 60 minutes then, inoculated into SPF eggs and incubated at 37°C for 3-5 days. The harvested allantoic fluid revealed positive hemagglutination. When treated virus incubated with saponin for 30 to 60 minutes inoculated in SPF chickens no clinical signs or mortalities were appeared. Challenge trial of different chicken groups with the virulent NDV genotype VIId NDV-B7-RLQP-CH-EG-12 revealed 100%, 90% and 90% protection at times 30, 45 and 60 minutes respectively; indicating that the reaction of virus with saponin for 30 minutes was the best to be used in vaccine preparation. Testing of tracheal swabs for virus shedding at 3, 5, 7 and 10 days post challenge revealed complete protection with no shedding of the challenged virus confirming the success of the prepared vaccine. Histopathological examination of the organs collected from vaccinated challenged chickens revealed nearly low to absence of lesion scores.
The study reports the success preparation of the saponin treated NDV vaccine from the circulating NDV genotype VIId in Egypt.

Keywords: NDV genotype VIId, chemical attenuation, saponin and chicken experiment.

بسم الله الرحمن الرحيم

(وَمَا أُوتِيتُمْ مِنْ الْعِلْمِ إِلاَّ قَلِيلاً)

صدق الله العظيم

سورة الإسراء 85

Dedication

To

My mother

My father

And

My sisters

Acknowledgment

First of all, my ultimate and heartiest thanks to our Lord God** (ALLAH) **the most Gracious, the Greatest and the most Beneficent.

*I heartily and cordially utter all the expressive and impressive words of indebtedness and thankfulness to my honorable supervisor **Prof. Dr. Hussein Aly Hussein** Professor of Virology, Faculty of Veterinary Medicine, Cairo University for his supervision, kind encouragement, and continuous interest.*

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1. INTRODUCTION

Newcastle disease (ND) is one of the most devastating diseases of poultry with worldwide distribution and remains a major threat to the poultry industry. It is caused by Newcastle disease virus (NDV), also known as avian paramyxovirus serotype 1 (APMV-1), and is classified as a List A notifiable disease by the World Animal Health Organization (Office International des Epizooties, OIE) because it is highly contagious and responsible for severe disease and high mortality in susceptible birds **(Alexander, 2004)**.

NDV is classified in genus Avulavirus in the family Paramyxoviridae and has broad host range being able to infect over 240 species of birds **(Umali et al., 2014a)**. Of them, gallinaceous birds such as chickens are highly susceptible to NDV **(Cappelle *et al.,* 2014)**.

NDV has a single stranded, negative sense RNA genome of 15,186 to 15,198 nucleotide in length **(Gogoi *et al.,* 2015a)**. The virus genome encodes structural proteins including nucleoprotein (N), phosphoprotein (P), matrix (M), fusion (F), hemagglutinin–neuraminidase (HN) and RNA-dependent RNA polymerase (L). The replication of the RNA genome is controlled by the rule of six, which requires the genome length to be multiple of six for the proper packaging of the RNA genome **(El Najjar *et al.,* 2014)**.

The first outbreaks to be recognized and termed ND occurred in poultry in 1926, in Java, Indonesia **(Kraneveld, 1926)** and in Newcastle-upon-Tyne, England **(Doyle, 1927)**. However, earlier reports of similar disease outbreaks in Central Europe before this date **(Halasz, 1912)**. In particular, **(Macpherson, 1956)** attributes the death of all the chickens in Scotland in 1896 as being due to ND. It is possible, that ND occurred in poultry before 1926, but its recognition as a specifically disease of viral etiology dates from the outbreaks during this year in Newcastle-upon-Tyne.

Although NDV has only one serotype but strains can differ considerably and cause a wide variation in virulence or disease for NDV isolates. NDV isolates are pathotyped into at least three groups based on their clinical presentations in chickens; the least virulent are lentogenic, moderately virulent strains are mesogenic and most virulent are velogenic **(Miller *et al.,* 2015a)**.

The cleavage site of F protein is main determinant of NDV virulence, as strains with an F protein cleavage site with at least 3 arginine or lysine residues between positions 112 and 116 and a phenylalanine residue at the position 117 are considered virulent **(Miller *et al.,* 2015a)**.

NDV isolates are grouped into two distinct genetic classes; class I and class II based on genome length and nucleotide sequence diversity **(Courtney *et al.,* 2013)**.

According to recent new genotype classification criteria proposed by **Diel *et al.,* (2012a)**, Class I viruses consist of single serotype while class II viruses consist of fifteen genotypes (I to XV) and four recently added genotypes (XVI-XVII-XVIII-XIX) these viruses were involved in five main global streams of infection in the history of ND panzootics in chickens and other bird species **(Snoeck *et al.,* 2013a and Dimitrov *et al.,* 2016)**. On the basis of the full sequence of the F protein gene. Class I NDV viruses with a genome size of 15,198 nucleotide long are frequently isolated from water fowl and live bird markets and most of them are avirulent **(Cai et al., 2011)**. Class II viruses are typically found in wild birds and poultry species. Most virulent NDV viruses belong to class II virus. The genotypes that are considered "early" (I to IV and IX) contain 15,186 nucleotides while the other genotypes that emerged "late" contain 15,192 nucleotides **(Diel *et al.,* 2012b)**.

Genotypes V, VI, and VII of virulent viruses are the predominant genotypes circulating worldwide. Of these, genotype VII is particularly