Influence Of The Use Of Homologous And Heterologous Newcastle Disease Virus Strains In The Haemagglutination Inhibition Assay On Sera Of SPF Chickens Vaccinated With Different Inactivated Newcastle Disease Vaccines

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ABSTRACT

In this study, the influence of different strains of Newcastle disease virus (NDV) (LaSota, Clone 30 and Hitchner B "HB1") was evaluated as homologous and heterologous antigens in the haemagglutination inhibition assay HI for the measurement of antibody titre after vaccination of chickens with different inactivated NDV strains. The mean value of HI test revealed that the use of LaSota strain as an antigen in HI assay after vaccination with inactivated LaSota vaccine gave significantly higher titre than Hitchner B1 or Clone 30 when used as antigen in HI assay. The result after vaccination with Clone 30 revealed that HI titres were significantly higher when Clone 30 was used as an antigen in HI assay in comparison to LaSota or HB1 when used as antigens in HI assay. In case of vaccination with Ulster strain the HI titres were higher when LaSota was used as antigen in comparison to Clone 30 on HB1 as an antigen in HI assay. It was found that vaccination with HB1 vaccine gave higher HI titre in HI assay on using HB1 as an antigen. The results of this study indicate that the NDV strains used in HI assay can have a considerable influence on the antibody titres that are detected, where the homologous antigens in HI assay revealed significantly higher titres comparing with those obtained by heterologous antigens.

INTRODUCTION

Potency of inactivated Newcastle disease virus (NDV) vaccines can be determined by measuring the protective dose 50% (PD_{50}) after administration of a number of different volumes of the vaccines. The measurement of serum antibody titres to determine the potency of inactivated ND vaccines is a reliable alternative to the measurement of PD_{50} of these vaccines *(1)*.

According to the European Pharmacopoeia, 1/50 dose of inactivated NDV vaccines should induce a mean serum titre of 4 (log_2) as measured by HI assay *(2)*.

However, no NDV strain is prescribed that should be used in HI assay, although differences in NDV strains have been well documented NDV strains can offer in the preferential site of replication *(3)* and their ability to cause haemagglutination and haemolysis of erythrocytes *(4)*. Sequence variability was demonstrated for both the fusion gene *(5)* and the haemagglutinin-neuraminidase gene *(6)*. These two genes encode the proteins that are recognized by neutralizing antibodies. This variability results in differences between the immunogenicity and antigenicity of strains, which is illustrated by the results of vaccination experiment *(7)* when determined antibody levels after vaccination. The question remains whether the virus strain used as antigen in the HI assay affects the antibody titres that are measured. So, this study was planned to determine the preferable strain used in HI assay.

MATERIAL AND METHODS

Newcastle disease virus (NDV) vaccines

The lentogenic strains of NDV (LaSota strain, Hitchner B1 "HB1", Ulster strain and $Clone_{30}$ derived LaSota) were purchased from local agency, Cairo, Egypt. They were used for vaccination of chickens at 21 days of age.

Newcastle disease vaccines

Newcastle vaccines from the following strain were used:

* The LaSota strain was obtained from Neuva Co. with HA 2^{10} and titre $10^{7.5}$ EID_{50}/ml.

* Clone strain was obtained from IZO Co. with HA 2^9 and titre 10 EID_{50}/ml.
* HB1 strain was obtained from Neuva Co. with HA 2^{10} and titre $10^{7.5}$ EID_{50}/ml.
* Ulster strain was obtained from Neuva Co. with $HA2^9$ titre $10^{7.5}$ EID_{50}/ml

Experimental birds

One day old chicks were obtained from Poultry Production Company, Egypt. They were floored reared, fed on commercial balanced poultry ration, and kept under good hygienic conditions. For each batch a group of birds (50 chickens each) was used. Each group of birds was coded according to the type of vaccine used (Lasota, $Clone_{30}$, HB_1 and ulster). Birds were vaccinated at 21 days old , and ten blood samples were obtained 3 weeks post vaccination from each group to determine HI test *(8)* .

Statistical analysis

Data obtained were statistically analyzed using analysis of variance (ANOVA) and comparing between groups were performed using least significant difference (LSD) at $P < 0.05$ *(9)* and computerized using SPSS.

RESULTS AND DISCUSSION

The occurrence of antigenic differences between Newcastle disease virus (NDV) strains has been demonstrated with the use of monoclonal antibodies *(10-12)*. Most antibodies against the HN glycoprotein of NDV block agglutination of the virus and attachment to target cells.

It is generally accepted that conventional serology can not discriminate between different NDV strains or isolates *(13)*, although some antigenic differences have been reported in early studies employing neutralization tests *(14)*. When a quantitative measurement has to be performed to determine antibody levels after vaccination, we discuss the effect of antigen used in HI assay on the antibody titres that are measured.

In Table 1, HI titres obtained using LaSota antigen were significantly higher than titres obtained using either Hitchner B1 or clone derived LaSota antigens, where HI titres reached 7.06 log2 in case of LaSota antigen. In case of clone 30 and HB1 antigens HI titres were 6.606 log2 and 5.66 log2 respectively. The potency of inactivated vaccines containing LaSota will be significantly overestimated when LaSota is used in the HI assay *(15)*.

Table 2 showed significant higher titres obtained after clone vaccination as measured in a HI assay using Clone antigen than LaSota or HB1 antigens where the mean HI were 7.02 log2 in case of Clone antigen, while in case of LaSota antigen and HB1 antigens reached 6.77 log_2 and 5.75 Log_2, respectively. On contrary it has been showed previously that after clone 30 vaccination the resulting HI titres were significantly higher when LaSota antigen was used *(15)*.

Table 3 revealed that higher HI titres after Ulster vaccination when clone 30 antigen used where mean HI titre was 6.3 log2, while in case of LaSota and HB1 antigens the mean HI titres were 4.53 log2 and 4.9 log2, respectively.

From above mentioned results, it could be concluded that the implications, the influence of NDV strain in HI assay on the antibody titres that are measured are evident, where the obtained results demonstrated the importance of the use differences between the immunogenicity and antigenicity of NDV strains and spot the light on the importance of the use homologous antigens in HI assay to obtained accurate results .

Table 1. The mean of NDV haemagglutination inhibition titre (HI) in sera of chickens vaccinated with inactivated NDV LaSota strain using different antigens

Batch No.	Haemagglutination Inhibition (HI) titre (Log_2 / ml)		
	LaSota antigen	HB1 antigen	Clone antigen
1	6.8	6.5	6.2
2	5.6	6.7	5.1
3	7.7	7.6	6.8
4	7.5	6.8	6.0
5	7.7	6.8	6.3
6	7.5	6.8	5.0
7	6.8	5.7	3.8
8	6.6	6.2	4.1
9	6.2	5.3	2.3
10	6.4	5.9	5.3
11	7.4	6.8	6.3
12	7.6	7.6	7.0
13	7.7	7.6	6.8
14	6.8	5.5	5.2
15	8.0	7.8	6.2
Mean	7.06	6.606	5.66

Table 2. The mean of NDV haemagglutination inhibition titre (HI) in sera of chickens vaccinated with inactivated NDV Clone LaSota derived strain using different antigens

Batch No.	Haemagglutination Inhibition (HI) titre (Log_2 / ml)		
	LaSota antigen	Clone antigen	HB1 antigen
1	6.0	6.3	5.1
2	5.2	5.8	3.7
3	6.7	7.2	5.7
4	6.5	7.5	5.2
5	6.2	7.8	5.5
6	7.0	6.3	5.3
7	6.8	6.7	5.2
8	7.7	7.9	6.9
9	7.2	7.6	6.6
10	6.9	7.0	6.3
11	7.8	5.5	6.7
12	7.5	6.8	6.0
13	6.2	7.5	5.7
14	6.8	7.0	6.4
15	6.8	7.0	5.5
16	7.2	7.8	5.8
17	6.6	7.6	6.2
Mean	6.77	7.02	5.75

Table 3. The mean of NDV haemagglutination inhibition titre (HI) in sera of chickens vaccinated with ulster and HB_1 inactivated NDV strains using different antigens

Batch No.	Type of vaccine	Haemagglutination Inhibition (HI) titre (Log_2 / ml)		
		HB1 antigen	Clone antigen	LaSota antigen
1	Ulster	3.5	3.8	5.7
2	Ulster	5.0	5.3	6.5
3	Ulster	5.1	5.6	6.7
Mean		4.53	4.9	6.3
4	HB1	6.4	4.9	5.1
5	HB1	7.1	6.3	6.4
Mean		6.75	5.6	5.75

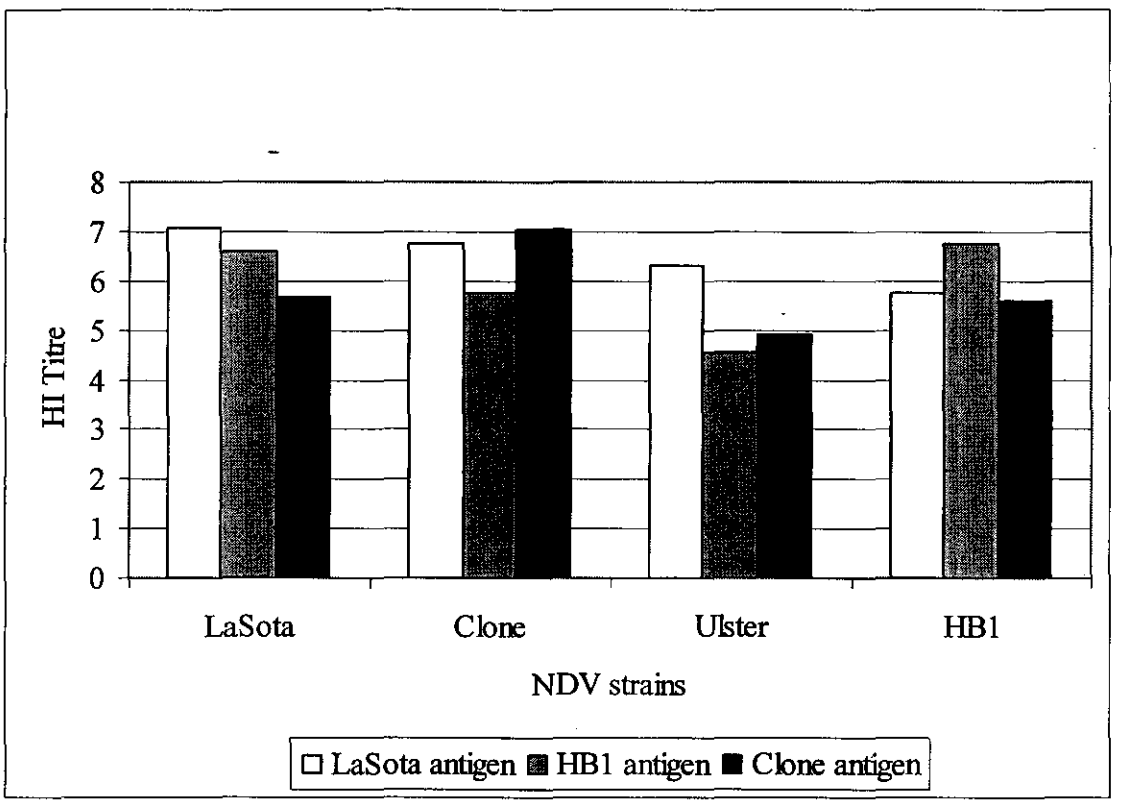

Fig. 1. The mean of NDV haemaggulation inhibition titre (HI) in sera of chicken vaccinated with inactivated NDV strains using different antigens

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الملخص العربى

تأثير استخدام العترات المماثلة والمختلفة لفيروس مرض النيوكاسل فى قياس الأجسام المناعية المانعة للتلازن الدموى فى أمصال الدجاج المحصن بمختلف لقاحات مرض النيوكاسل المثبطة

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تم فى هذه الدراسة تقييم تأثير استخدام العترات المختلفة من فيروس النيوكاسل مثل اللاسوتا أو كولون ٣٠ أو هتشنر ب١ كأنتيجينات متجانسة أو غير متجانسة عند تحصين الدجاج بالعترات المختلفة للقاح النيوكاسل المثبط فى اختبار مانع التلازن الدموى وذلك عند قياس المستوى المعيارى للأجسام المناعية.

وقد أوضحت النتائج أن استخدام عترة النيوكاسل (اللاسوتا) كأنتيجين مماثل أعطت أعلى قياس فى المستوى العيارى للاجسام المناعية وذلك مقارنة باستخدام عترة كولون ٣٠، هتشنر ب١، كانتيجينات غير مماثلة فى الدجاج المحصن بلقاح اللاسوتا المثبط.

كما وجد أن عند التحصين بلقاح كولون ٣٠ أن الاجسام المناعية كانت أعلى عند استخدام كولون ٣٠ كانتيجين فى اختبار مانع التلازن الدموى بعد التحصين مقارنة باستخدام هتشنر ب١ او اللاسوتا كانتيجينات غير مماثلة فى الاختبار.

أماعن تحصين الدجاج بلقاح السترا فقد كان المستوى المعيارى للاجسام المناعية أعلى عند استخدام اللاسوتا كانتيجين.

وبالنسبة للدجاج المحصن بلقاح هتشنر ب١ فقد أوضحت النتائج أن استخدام هتشنر ب١ كانتيجين أعطى أفضل النتائج فى اختبار مانع التلازن الدموى.

ومما سبق يمكن استنتاج أن استخدام العترات المختلفة للنيوكاسل كانتيجينات عند إجراء مانع التلازن الدموى لها تأثير واضح عند قياس المستوى المعيارى للاجسام المناعية حيث أن أستخدام العترات المتجانسة أوضحت أعلى نتائج عند إجراء اختبار مانع التلازن الدموى مقارنة بالعترات الغير متجانسة.