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Journal Article

A ONE-TIME SCREENING OF NEWCASTLE DISEASE HI ANTIBODIES BEFORE SLAUGHTER AS AN INDICATOR OF VACCINE PERFORMANCE IN BROILER CHICKENS

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SUMMARY

Seven poultry farms participated in a study to determine the vaccine performance in broilers vaccinated with Newcastle disease (ND) vaccines following each of the farms own vaccination programmes and procedures. It is foreseen that knowledge of the antibody titres attained by the birds, will help poultry farmers or veterinarians to reflect on all routine procedures taken in their farms in ensuring that the vaccines delivered performed accordingly. The HI antibody titres of a batch of broilers before slaughter may provide these farmers a guide to assist them with future vaccination strategies for their farms. In this preliminary study, just before marketing, at the age of 27-37 days old, 15-20 broilers were submitted for post vaccination HI antibody determination and protection study. The HI-GMT of antibodies of broilers at approximately one month post–vaccination (pre-challenged) titres for five of the farms were below standard, ranging from as low as 1.83 to 4.70 and is associated with its low level of protection afforded i.e. as low as 38-76 %. For three farms, The HI-GMT antibodies were 115.36 and 78.8 and 6.8 and the protection afforded were 100%, 95% and 85% respectively. The lowest percentage of protection afforded were 38% and 44% in chickens vaccinated with killed vaccines at 3 days old. For the seven farms, at total of 122 broilers were screened and challenged, and out of these, 44 birds showed HI titres of <2. Out of these 44 birds, 41% however, survived the challenge.

Keywords: Chicken, Broiler, Newcastle disease, hemagglutination inhibition

INTRODUCTION

Newcastle disease (ND) is one of the major avian diseases worldwide. It is caused by Newcastle disease virus (NDV) or avian *Paramyxovirus* serotype 1 *(APMV-1)*. NDV causes a devastating disease in birds and remains one of the most important pathogens of poultry (Alexander, 2000). The disease is still a worldwide economic problem resulting in severe losses to the poultry industry. Virulent strains of Newcastle Disease (ND) are enzootic in Malaysia and despite vaccination, ND outbreaks still occurred throughout the country causing damage and serious losses to the farmers. Most of the domestic outbreaks occurred in areas with high density of poultry farms.

As ND is a continuing threat, farmers worldwide are vigilant of this disease and tremendous efforts to control and prevent ND are constantly being carried out through efficient vaccination programs and corresponding serological monitoring (Ricardo *et al.*, 2000). The testing of chickens for NDV antibodies to determine the potency of the vaccine is done at various intervals and in most cases at least 2 weeks after vaccination. Vaccination of chicks against NDV were performed as early as 1-3 days old and in some, vaccination is repeated at the age of 7-10 days, depending on the different vaccination programmes practiced and the types of vaccines used by each poultry farmer. Most of the time, humoral antibody vaccination response will not reached its optimum level after 2 weeks post-vaccination, therefore making it difficult to determine whether the vaccine and vaccination procedures were successful in stimulating the full potency or efficacy potentials.

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In this study, we investigated vaccine performance of broilers from seven commercial farms by demonstrating the NDV-HI antibody level attained, in a one-time screening just before marketing, i.e. as early as 27-37 days old, i.e. just before marketing. Knowledge of the antibody titres attained by the birds will help poultry farmers or veterinarians to reflect on all routine procedures taken in their farms in ensuring that the vaccines delivered performed accordingly, and may assist farmers with future vaccination strategies in their farms.

MATERIALS AND METHODS

Participation of farmers

Seven poultry enterprises located in Sitiawan, Perak, with poultry population of 15,000 to 54,000 participated in this study. The broiler birds from the seven farms were of the Cobb breed. Each of the enterprise practiced their own vaccination programmes against NDV and uses either single ND or various combinations (eg + infectious bronchitis vaccine), and types, i.e. either live or killed of the lentogenic or mesogenic vaccine strains. The routes of inoculation depended on the type of vaccines used and are according to the recommendations of the manufacturers. Just a few days before slaughter, i.e. at the age of between 27-37 days old, 15-20 of the broiler chickens from the vaccinated flock were submitted to VRI. VRI diagnostic numbers (077166-077172 and 077361-077367) were assigned for each of the broiler batch submitted from the seven farms.

Protection studies

At VRI, the birds were tagged and sera collected to determine the post-vaccination or pre-challenged titre. The birds were challenged with a dose of $10^{4.3}$ EID_{50} of virulent (v) NDV per bird via the intranasal route. Birds were observed for clinical signs and death for up to 1 week after challenge, where sera was again collected from surviving birds at the end of the week. As controls, unvaccinated SPF chickens of the same age were also challenged intranasally, with the same vNDV, to prove its pathogenicity in chickens. All dead birds were autopsied and observed for NDV lesions in the intestines, cecal tonsils and other organs. Pooled organs of brain, kidney, trachea, lungs and intestines were collected and subjected to viral isolation. ND virus isolated was validated using the HA test as described previously (OIE, 2012).

The HI assay

Sera was obtained at pre and post challenge from all birds and tested by hemagglutination inhibition (HI) assay for HI antibody titers. The HI assay was performed using inactivated NDV antigen according to standard procedures with 4 HAU virus/antigen in 0.025 ml (OIE, 2012). Titers were calculated as the highest reciprocal serum dilution providing complete hemagglutination inhibition. Serum titers of 1:8 (3 log 2) or lower were considered negative for antibodies against NDV. The GMT of the HI antibody titres was calculated for each batch from each of the farm.

RESULTS

Seven poultry enterprises agreed to participate in the study. The poultry farms were all from Sitiawan, Perak as this area has one of the largest concentration of poultry farms. As observed, there were at least six vaccination types or protocols practiced by the seven poultry owners. Each of the protocols resulted in different outcomes of the vaccination in terms of the humoral HI antibodies elicited and the protection afforded. Referring to Table 1, 2 and 3, the GMT for the HI humoral antibodies for five of the farms were below standard, ranging from as low as 1.83 to 6.28 and this can be associated with its low level of protection afforded i.e as low as 38-76 %. The lowest percentage of protection afforded were 38% and 44% in chickens vaccinated with killed vaccines at 3 days old. The average antibody titre of the vaccinated flock may be satisfactory, however, there are still many birds that have poor antibody responses within the group. Three farms (Farms 4, 5 and 7) performed very well, where broilers were vaccinated with a killed followed by a live vaccine or by only the live vaccine i.e using La Sota strain.

Table 1: The types of vaccination practiced by the farmers, the age and HI-GMT at pre-challenged and the protection afforded after challenged with vNDV at $10^{4.3}$ EID_{50} per bird, for broilers from each farm.

Farm	*Commercial ND vaccine and its combination*	*Types of ND vaccine virus*	*Age at vaccination*	*Vaccination Route*	*HI-GMT titre at (pre-challenged age)*	*Percentage protection against challenge with vNDV*
1	ND +IB	Live	6 days old	Drinking water	2.9 (37 days)	76%
2	ND + IB	Killed	5 days old	Subcutaneous	1.83 (35 days)	38%
3	ND	Live (VG/GA strain)	1 day old at hatchery	Spray	4.70 (34 days)	62%
4	ND	Live (La Sota)	5 days old	Drinking water	115.36 (34 days)	100%
5	ND	Killed Live (La Sota)	3 days old 7 days old	Subcutaneous Drinking water	6.28 (27 days)	85%
6	ND + IB	Killed	3 days old	Subcutaneous	2.71 (30 days)	44%
7	ND	Live (ND F) Live (La Sota)	3 days old 10 days old	Intranasal/ocular Drinking water	78.80 (34 days)	95%

Table 2: The ND-HI titres at post vaccination i.e. before challenged and the outcomes after challenged depicted as dead (D) and HI titres for farm 1 – 7

Chicken	**HI titres challenged**													
	Farm 1 *13/17 (76%)		**Farm 2 6/16 (38%)**		**Farm 3 8/13 (62%)**		**Farm 4 20/20 (100%)**		Farm 5 *17/20 (85%)		Farm 6 7/16 (44%)		Farm 7 19/20 (95%)	
	Before	**After**	**Before**	**After**	**Before**	**After**	**Before**	**After**	**Before**	**After**	**Before**	**After**	**Before**	**After**
1	<2	D	8	8	256	256	32	64	4	1024	<2	D	256	1024
2	8	128	<2	D	<2	D	256	64	8	128	<2	D	8	D
3	<2	1024	16	128	<2	D	128	512	8	512	<2	D	128	2048
4	<2	2048	<2	D	128	32	256	512	<2	D	<2	8	128	512
5	8	32	<2	D	<2	D	256	256	8	1024	4	D	64	512
6	<2	1024	4	2048	4	1024	256	512	64	512	<2	2048	32	1024
7	<2	D	<2	<2	<2	2048	16	512	16	1024	16	128	256	2048
8	<2	1024	<2	D	<2	D	256	512	32	1024	16	D	256	1024
9	4	512	32	32	4	1024	64	32	<2	D	8	32	32	1024
10	64	128	<2	D	32	64	64	32	64	128	<2	D	32	64
11	16	D	<2	512	<2	D	8	16	16	512	<2	D	64	64
12	16	128	<2	D	32	64	128	128	<2	128	<2	512	128	512
13	<2	1024	<2	D	<2	512	64	64	<2	1024	32	D	32	256
14	<2	D	<2	D			256	1024	16	2048	<2	1024	64	256
15	16	1024	<2	D			32	128	4	1024	32	D	64	1024
16	<2	32	<2	D			16	256	32	1024	<2	512	128	1024
17	<2	2048					32	128	4	128			32	1024
18							128	256	<2	D			256	1024
19							64	128	16	512			128	512
20							128	64	<2	32			128	2048
HI GMT	**2.9**		**1.83**		**4.70**		**115.36**		**6.28**		**2.71**		**78.80**	

**a/b (%) = no. survived (with HI titre)/Total no. of broiler tested (% protection)*

Table 3: The no. and percentage of chickens with HI titre <2 for the seven farms that survived and died from the challenged with vNDV.

Total no chickens with HI titre <2	***n* = 44**	***Percentage***
Total no. of chickens that survived challenged	18	41% (survived)
Total no. of chickens that died after challenged	26	59% (died)

The HI - GMT of broilers at the pre-challenged titres was 115.36 and 78.8 and the protection afforded were 100% and 95% respectively. However, for broilers in Farm 5 with a GMT titre of 6.28, the vaccine seemed to provide reasonable protection of 85% against virulent ND virus challenged. This group of chicken was vaccinated with a killed followed by live La Sota vaccine strain via the subcutaneous and drinking water respectively. Compared to the vaccination regime using both live NDF and live La Sota strain via the intranasal/ocular and drinking water routes respectively, the vaccines seemed to invoke a much higher immune responses and afforded 95% protection to the vaccinated chickens. From the seven farms, 122 broilers were screened and challenged, and out of these 44 birds showed HI titres of < 2. Out of these 44 birds, 41% however, survived the challenge (Table 3).

DISCUSSION

For ND, the vaccines used for vaccination of large population of broiler chickens are usually the non-virulent live virus that is administered via spray or drinking water. Live virus vaccines provide acute antibody response, spreads systemically and invoke high cell mediated immune responses, thus providing greater and stronger protection to the chickens. However, the spray and drinking water methods of administering vaccines usually produce considerable variation in the individual immune responses, indicating potential variation in the levels of protection after vaccination (Senne *et al.*, 2004).

Immune responses are determined via the HI test, the most widely used conventional serological method for detection of NDV antibodies as it is quite specific and gives reproducible results (Ricardo *et al.*, 2000). Field results suggest that only birds with HI titers >16, usually after multiple vaccinations will survive a vNDV challenge and 66% of the flock with titres < 16 will not be protected and will succumbed to infection (Kapczynski and King, 2005). HI levels of 32 or higher have been typically shown to be protective against ND (Allan *et al.*, 1978).

Vaccination against ND is easy as all NDV are in one serotype and any NDV strain can be used as a vaccine which will prevent clinical disease and death from ND. Lentogenic Newcastle disease (ND) vaccines indicated for young chicks are mild in nature and hence safer for use. However, they usually do not evoke a strong and long lasting immunity. Although the average antibody titre of the vaccinated flock may be satisfactory, there are still many birds that have poor antibody responses within the flock. This is actually portrayed in the GMT value of the HI antibodies of the broilers of the seven farms, where at 27-37 days post-vaccination, The HI antibodies showing protective titres of ≥ 32 is only 4% (5/122). This is in agreement with many research that showed that lentogenic ND vaccinated flocks often do not achieve the required solid and uniform immune status following vaccination because a significant proportion of the population still remain susceptible. In the face of an outbreak, these flocks will experience some mortalities and a carrier status could be established in many of the infected birds (Senne *et al.*, 2004). In Malaysia, challenged with field virulent ND virus could occur in chicks as young as 3 to 4 weeks of age and in such cases, disease control by vaccination would be difficult because the lentogenic vaccines which are safe for young chicks are unable to provide a full and solid protection against field virulent viruses. These seven farms can be considered fortunate as they might not have been hit by field virulent NDV, at the time of the study. It was observed that in two of the farms (Farm4 and 7), administration of live NDV vaccines induced very good antibody responses resulting in significant protection of the vaccinated birds. For Farm 5, concurrent vaccination with killed oil emulsion and live NDV vaccine also afforded 85% protection.

In this study, challenged was done through a milder normal route of infection i.e via intranasal, so as to simulate natural field infection. In the vaccination of broilers, taking into consideration the short life span of 45-50 days of broiler chickens, quick and high antibody responses is most desirable to protect them against ND. Inactivated or killed vaccines are often administered to layers and breeders as they provide long lasting high antibody titers that can be passed also to the offspring (Al-Garib *et al.*, 2003). As killed vaccines are usually adjuvanted, they are given early in the life of the broiler chickens, due to withdrawal times problem between vaccination and slaughter. Furthermore, inactivated vaccines administered by the SQ route require individual administration, therefore, increasing also the cost of labour. Inactivated vaccines have been believed not to be able to induce a mucosal immunity, however, (Senne et al 2004) in a recent study, has demonstrated that both live and inactivated vaccines induced antibodies other than IgA not only in the serum but also in the tracheal and intestines (Chimeno Zoth *et al.*, 2008). It was observed that for all the vaccination programmes of five of the farms, there were substantial numbers of chickens with antibodies < 2 at post-vaccination or prechallenged titres, where 59% died after challenge. The other 41% survived the challenged with some, also demonstrating a four-fold or more seroconversion. This protective phenomenon could be contributed by CMI mediated T and B lymphocytes which had been implicated in the development of protection in chickens vaccinated against NDV (Cannon and Russell, 1986: Sharma 1999; Reynolds and Maraga, 2000). One of the reasons why the 59% has HI antibody titres < 2 is that they might have missed the vaccine or have not received the full dosage of the vaccine.

This is a preliminary study on only seven farms and we hoped that it will provide insights into new research or investigations on how to help farmers know that the vaccines that they are using, the vaccination programmes and the vaccination procedures implemented will protect their chickens until marketing age. Knowledge on the one-time HI antibody level at slaughter will help farmers in reviewing their whole complete vaccination programmes and vaccines used, so farmers can formulate future strategies

in vaccination to control ND. 'Good' or quality vaccines when administered correctly to healthy birds will help prevent death and disease. In cases of vaccine failure or poor vaccine take, farmers will need to reflect, identify reasons and review procedures pertaining to some of the important factors such as, storage, handling, dilution, vaccination routes and regimes and the types or strains of virus vaccines.

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