A comparative study on the immunogenic and protective properties of the V4, Hitchener BI, and LaSota Newcastle disease vaccines in commercial chickens

K. G. ANING & J. N. BREWOO
Animal Research Institute, CSIR, P. O. Box AH 20, Achimota, Ghana

ABSTRACT

A commercial V4 vaccine was compared with the conventional Hitchener BI (HBI) and LaSota Newcastle disease (ND) vaccines for seroconversion and ability to protect commercial chicks against artificial challenge of ND virus. In the two experiments, groups of broiler and replacement pullet/cockerel chicks were vaccinated orally with HBI at 2 weeks and LaSota at 6 weeks or V4 at 2 and 6 weeks. In addition, another group of replacement chicks was given V4 at 2 weeks and LaSota at 6 weeks. Groups of unvaccinated controls were included in both experiments. At day-old and weekly intervals, semen samples were collected from all groups and tested for ND virus antibody titres, using the micro haemagglutination-inhibition test. Eight to 15 birds from the groups of birds were challenged with a velogenic ND virus strain at 5 and 9 weeks old. Due to interference of maternal antibodies, primary vaccination with HBI or V4 at 2 weeks generally resulted in low seroconversion, although in replacement chicks HBI caused an increase of geometric mean titre (GMT) of $\log_2 3.71 \pm 1.31$ at 2 weeks to 5.39 ± 2.10 at 3 weeks. Broiler chickens given LaSota at 6 weeks showed increasing titres from 1.30 ± 1.59 to a maximum of 3.40 ± 1.20 at 8 weeks, but fell rapidly to 1.86 ± 2.06 at 9 weeks. Replacement chicks given LaSota showed rising titres from 1.86 ± 2.1 to 6.94 ± 1.48 at 9 weeks. In contrast, the GMTs in V4-vaccinated birds rose from 1.67 ± 2.08 at 6 weeks to only 2.53 ± 2.44 at 8 weeks. Challenge tests at 5 weeks showed that replacement chicks vaccinated with HBI were better protected (45.7 % ± 27.00) than V4 - vaccinated chicks (27.14 % ± 28.60; $P<0.05$). Birds challenged at 9 weeks following re-vaccination with LaSota showed a significantly ($P<0.05$) higher survival rate (77.14 % ± 15.84) than those re-vaccinated with V4 (35.70 % ± 28.00) and unvaccinated control (41.63 % ± 30.37). Curiously, unvaccinated control chicks challenged at 5 weeks showed the highest survival rate of 60.00 % ± 20.95. Broiler birds challenged at 9 weeks after LaSota re-vaccination also showed

RÉSUMÉ

ANING, K. G. & BREWOO, J. N. : *Etude comparative sur les propriétés immunogéniques et protectrices des vaccins V4, Hitchener B1 et La Sota de la maladie de Newcastle dans les poulets commerciaux.* Un vaccin commercial V4 était comparé avec les vaccins conventionnels Hitchener B1 (HBI) et La Sota de la maladie de Newcastle (MN) pour la séro-conversion et la capacité de protéger les poussins commerciaux contre le défi artificiel du virus MN. Dans les deux expériences des groupes de poulet de chair et de jeunes poules/jeunes coqs de remplacement étaient vaccinés par voie orale avec HBI à 2 semaines et La Sota à 6 semaines ou V4 à 2 et 6 semaines à la fois. En plus, un autre groupe de poussins de remplacement étaient donnés V4 à 2 semaines et La Sota à 6 semaines. Des groupes de contrôles non-vaccinés étaient inclus dans les deux expériences. Par intervalles d'un jour ou d' une semaine des échantillons de sperme étaient prélevés de tous les groupes et mis à l' essai pour les titres anticorps de virus de MN utilisant l'essai de micro haemagglutination-inhibiiton. De 8 à 15 volailles des groupes de volailles étaient stimulés avec la souche du virus de MN vélogénique aux âges de 5 et 9 semaines. A cause de l'interférence des anticorps maternels, la vaccination primaire avec HBI ou V4 à 2 semaines aboutissait en générale à la séro-conversion basse, malgré le fait que dans les poussins de remplacement HBI provoquait une augmentation du titre de moyenne géométrique (TMG) de log 2 3.71 ± 1.31 à 2 semaines à 5.39 ± 2.10 à 3 semaines. Les poulets de chair donnés La Sota à 6 semaines montraient les titres augmentant de 1.30 ± 1.59 au maximum de 3.40 ± 1.20 à 8 semaines, mais tombaient rapidement de 1.86 ± 2.06 à 9 semaines. Les poussins de remplacement donnés La Sota montraient les titres levant de 1.86 ± 2.1 à 6.94 ± 1.48 à 9 semaines. Par contraste, les GMTs en volailles vaccinées avec V4 se le vaient de 1.67 ± 2.08 à 6 semaines à seulement 2.53 ± 2.44 à 8 semaines. Les essais de défi à 5 semaines montraient que les poussins de remplacement vaccinés avec HBI étaient mieux protégés (45.7 % ± 27.00) que les

significantly higher survival rates (89.51 % ± 6.90) than those re-vaccinated with V4 (56.17 % ± 21.69) or unvaccinated birds (23.21 % ± 30.44). The results show that in commercial chickens the conventional vaccines are superior in their immunogenic and protective properties than the V4 vaccine.

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poussins vaccinés avec V4 (27.14 % ± 28.60 ; $P<0.05$). Les volailles stimulées à 9 semaines à la suite de la révaccination avec La Sota montraient une proportion (77.14 % ± 15.84) de survie considérablement ($P<0.05$) plus élevée que celles révaccinées avec V4 (35.70 % ± 28.00) et un contrôle non-vacciné (41.63 % ± 30.37). Curieusement, les poussins non-vaccinés de contrôle stimulé à 5 semaines montraient les proportions (60.00 % ± 20.95) de survie les plus élevées. Les poulets de chair stimulés à 9 semaines à la suite de la révaccination avec La Sota aussi montraient des proportions (89.51 % ± 6.90) considérablement plus élevées que ceux révaccinés avec V4 (56.17 % ± 21.69) ou les volailles non-vaccinées (23.21% ± 30.44). Les résultats montrent que dans les poulets commerciaux les vaccins conventionnels sont superieurs dans leurs propriétés immunogéniques et protectrices que le vaccin V4.

Introduction

The Hitchener BI (HBI) and LaSota vaccines are two tried and tested Newcastle disease (ND) vaccines used to control ND in commerical poultry worldwide. In Ghana, their use in a vaccination regime that has been adopted over two decades, following recommendations by Nutor (1973), has given satisfactory results in commercial birds. However, in free-roaming village chickens the use of these conventional vaccines is limited as cold-chain storage (usually unavailable in the rural setting) for the vaccines and confinement of the birds are prerequisites for their application (Samuel, 1987). Consequently, ND has become a very important obstacle in the growth of the national village chicken population as epidemics are frequent, especially in the dry, windy harmattan season (Bannor & Ogunsan, 1988; Spradbrow, 1993/94; Brewoo & Aning, unpublished data).

The V4 vaccine, like HBI and LaSota, is lentogenic. It is heat resistant and can withstand an ambient temperature of 32 °C for at least 24 h without loss of titre (Copland, 1992). It has been administered to birds through medication of various feeding stuff with good results (Ibrahim *et al.*, 1987). Prepared from an avirulent NDV strain isolated in Australia, V4 has been reported to protect chickens against virulent NDV and it spreads rapidly from bird to bird *via* the faecal-oral route (Westbury, 1981), thereby, protecting birds that had not been directly vaccinated.

On account of its high potential for use in Ghana, a batch of commercially available live, freeze-dried ND V4 vaccine was obtained from the manufacturers for comparative studies with HBI and LaSota conventional vaccines to ascertain its immunogenic and protective properties against a velogenic ND virus strain isolated in Ghana.

Materials and methods

Experimental animals

In the 1st experiment (Table 1), Anak broiler day-old chicks were brooded together in a Petersine electrical brooder till they were 2 weeks old. They were randomly assigned to three groups of 25 each and housed in separate deep litter pens.

In the 2nd experiment, four groups (50 each) of Starcross 579 pullet/cockerel chicks were housed in separate deep litter pens throughout the brooding period till the end of the experiment.

All birds used were wing-tagged for individual identification.

Feeding procedure

In both experiments, the birds were given appropriate diets and water *ad libitum* throughout the experiments. All groups of birds received similar vitamin supplements and prophylactic

TABLE 1

Vaccination Schedule of Broiler and Replacement Pullet/Cockerel Chicks with HBI, LaSota and NDV4HR Vaccines

Experiment 1 (broiler chicks)

Vaccination	Group of chicks A n = 25	B 25	C 25
HBI (2 weeks)	+	-	-
NDV4HR (2 weeks)	-	+	-
NDV4HR (6 weeks)	-	+	-
LaSota (6 weeks)	+	-	-

Experiment 2 (replacement chicks)

Vaccination	Group of chicks A n = 50	B 50	C 50	D 50
HBI (2 weeks)	+	-	-	-
NDV4HR (2 weeks)	-	+	+	-
NDV4HR (6 weeks)	-	+	-	-
LaSota (6 weeks)	+	-	+	-

antibiotic treatments.

Vaccines

The vaccines compared were as follows:

a) NDHR 'V4', manufactured by Malaysian Vaccines and Pharmaceuticals, SDN BHD, and presented in freeze-dried 1000-dose vials. According to the manufacturer's guide, it can be administered either intra-ocularly or in drinking water to all ages and classes of birds.
b) Newcastle disease vaccine B1 Type, Strain 1, manufactured by Vineland Laboratories, USA. It is recommended for use through either intra-nasal or intra-ocular routes or in drinking water as the initial vaccination or for re-vaccination.
c) Hipraviar-S, Newcastle disease LaSota strain, manufactured by Laboratories Hipra, SA, Spain. It is recommended for use through either intra-nasal or intra-ocular routes or in drinking water.

All birds were vaccinated against infectious bursal disease (IBD), using "Nobilis Gumboro D78" (Intervet, Boxmeer Holland) vaccine in drinking water at day 10, with re-vaccination at 23 days of age.

All vaccines were stored at 4 °C from the time they were received in Ghana, until they were used. Before administration, each 1000-dose vial was reconstituted in 10 ml of distilled water. A volume of 1 ml (100 doses) was further diluted in 19 ml of water and 0.2 ml of this dilution (1 dose) was delivered directly into the oral cavity of each bird by means of a tuberculin syringe.

Vaccination procedure

Table 1 shows the ND vaccination schedules and vaccine combinations used in the experiments. The adopted vaccination schedule against ND in Ghana was used. The first vaccination was done at 2 weeks and the booster dose (re-vaccination) given at 6 weeks. In both experiments, groups of unvaccinated control were included.

Serological tests

Blood samples were taken randomly from 10 birds in each group before vaccination and at weekly intervals subsequently, from birds also selected randomly, and left overnight. The serum samples were harvested and stored at 20 °C until serological testing. The microtitre haemagluttination-inhibition (HAI) test, described by Allan & Gough (1974) using round-bottom plates, was used to determine specific ND virus (NDV) antibody titres in serum samples.

The antigen used initially in the assay was commercial ND LaSota vaccine adjusted to give 4 haemogluttination (HA) units (Allan, Lancaster & Toth, 1978). Subsequently, a field isolate of NDV similarly adjusted to 4HA units, was used as antigen. This is a velogenic strain maintained at 20 °C in amnio-allantoic fluid. It was inactivated

in 1:1000 formalin at 37 °C before use (Allan *et al.*, 1978).

Challenge tests

In the first experiment, 15 birds each from Groups A and B and eight from Group C were challenged with a velogenic NDV strain at 9 weeks, that is, 3 weeks after the second ND vaccination (Allan *et al.*, 1978). In the 2nd experiment, 10 birds from each group were challenged at 5 weeks. Another 10 birds from each group were challenged at 9 weeks. The challenge virus used was isolated from a village chicken that died in a natural ND outbreak in the Achimota area. The virus was isolated by inoculating material from the trachea and lungs into the allantoic sac of NDV antibody-free, 9-day-old embryonating chicken eggs according to methods described by Nutor (1973). The allantoic fluid from these eggs containing about 10^9 EID_{50} per ml was diluted to give a HA titre of 1:256. The challenge dose of 0.1 ml of this dilution was estimated to contain about 10^6 EID_{50}. The challenge inoculum was given intramuscularly (Allan *et al.*, 1978; Biswas *et al.*, 1996). The challenged birds from all the groups were then housed together in a separate deep-litter pen and inspected daily for clinical signs of ND over a 7-day period. Birds that died after challenge were subjected to necropsy and suspected ND cases were confirmed by isolation in embryonated eggs and the virus was identified with a positive NDV antiserum (HAI test).

Statistical analysis

The survival rates of the challenged birds were analyzed for significant differences between the groups of birds (treatments) by using the Duncan's Multiple Range Test (Steel & Torrie, 1980). The model used considered the effects of type of vaccine and number of days after challenge on the cumulative death.

Results

Serological responses

The results of serological tests on serum samples of broiler birds (first experiment) showed that the geometric mean titres (GMTs) at 2 weeks (before the first ND vaccination) were high in all three groups (log_2 2.71-4.34). After vaccination with HBI and V4, the GMTs dropped slightly from 3.71 and 3.81 to 3.22 and 3.00, respectively (Groups A and B, Fig. 1). However, by 4 weeks of age when control birds (Group C, Fig 1) had lost NDV antibodies, the vaccinated birds still had GMTs of above 3.00.

The GMTs of broiler birds given LaSota rose

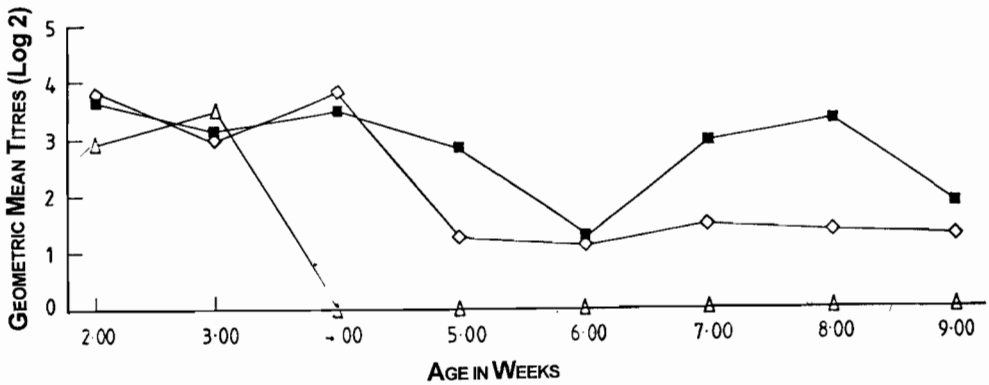

Fig. 1. Geometric mean titres (GMTs) of Newcastle disease (ND) virus antibodies in broiler chickens vaccinated with ND vaccines: HBI, LaSota and NDV4HR.
■ Vaccinated with HBI at 2 weeks and LaSota at 6 weeks ✧ Vaccinated with NDV4HR at 2 and 6 weeks Δ Unvaccinated control

from 1.30 ± 0.18 to 3.40 ± 1.14 at 8 weeks, but fell to 1.86 ± 2.06 at 9 weeks. In contrast, Group B broiler birds given V4 at 6 weeks showed GMTs of 1.81, 1.34 and 1.31 at 7, 8 and 9 weeks, respectively (Table 2, Fig. 1).

The results for replacement pullet/cockerel chicks (2nd experiment) also showed high GMTs at 2 weeks ($\log_2$ 3.57 -3.71) before first ND vaccination (Table 3, Fig 2). After vaccination with HBI (Group A), the mean GMT rose from 3.57 to 5.39 ± 2.38 at 3 weeks, while those given V4 (Groups B and C) or no vaccine (Group D) showed no rise in GMT (Fig. 2). At 6 weeks, all groups showed low titres (between 1.67 and 2.10). Those given LaSota (Groups A and C) showed high seroconversion from weeks 7 to 9 (3.67-7.39), while those given V4 as second vaccination (Group B) showed low mean GMTs (1.67-2.53), like the unvaccinated control Group D (Fig. 2), till 9 weeks when birds were challenged.

Protection against virus challenge

Table 4 shows the results of virulent ND virus challenge at 9 weeks of broiler birds (first experiment) vaccinated with HBI or V4 at 2 weeks and re-vaccinated with LaSota or V4 at 6 weeks. Birds vaccinated with HBI and re-vaccinated with LaSota showed significantly ($P<0.05$) higher protection level (89.51 % ± 6.90) than those given two vaccinations of V4 (56.17 % ± 21.69). The unvaccinated birds showed a poor protection level of 23.21% ± 30.44.

Tables 5 and 6 show the challenge results for pullets/cockerels (second experiment). Birds challenged at 5 weeks after primary vaccination at 2 weeks with HBI showed a significantly higher

TABLE 2

HI Titres (Log_2) of Broiler Chickens Vaccinated with HBI, LaSota, and NDV4HR Vaccines

Group/age	*2 weeks*			*6 weeks*			*9 weeks*		
	$\bar{x}$	*SD*	*%>3.0*	$\bar{x}$	*SD*	*%>3.0*	$\bar{x}$	*SD*	*%>3.0*
A	2.71	1.61	92.9	1.30	1.59	20	1.86	2.06	40
B	3.81	1.18	100	1.15	1.68	7.1	1.31	1.74	22.2
C	4.34	1.19	100	0	0	0	0	0	0

$\bar{x}$ = Geometric mean titre
A = Vaccinated with HBI and LaSota
B = Vaccinated with only NDV4HR
C = Unvaccinated control

TABLE 3

HI Titres (Log_2) of Pullet/Cockerel Chickens Vaccinated with HBI, LaSota, and NDV4HR Vaccines

Group/age	*2 weeks*			*5 weeks*			*6 weeks*			*9 weeks*		
	$\bar{x}$	*SD*	*%>3.0*	$\bar{x}$	*SD*	*%>3.0*	$\bar{x}$	*SD*	*%>3.0*	$\bar{x}$	*SD*	*%>3.0*
A	3.71	1.31	94.7	2.33	2.04	57.9	1.86	2.21	38.9	6.94	1.48	100
B	3.57	1.49	90	1.99	1.86	50	1.67	2.08	36.6	2.53	2.44	55.6
C	3.57	1.49	90	1.99	1.86	50	1.96	1.91	55.6	7.39	1.30	100
D	3.57	1.42	95	1.95	1.80	42.9	2.10	2.05	57.1	1.74	1.56	22.2

$\bar{x}$= Geometric mean titre
A = Vaccinated with HBI and LaSota
B = Vaccinated with only NDV4HR
C = Vaccinated with NDV4HR
D = Unvaccinated control

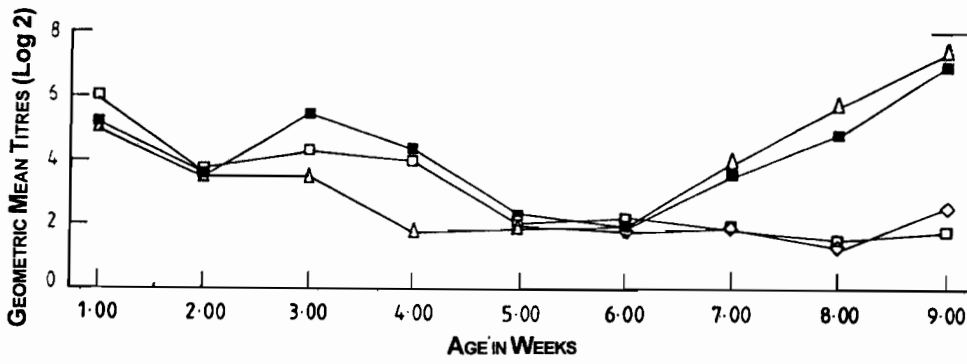

Fig. 2. Geometric mean titres (GMTs) of Newcastle disease:(ND) virus antibodies in pullet/cockerel chickens vaccinated with ND vaccines: HBI, LaSota and NDV4HR.
■ Vaccinated with HBI at 2 weeks and LaSota at 6 weeks ✧ Vaccinated with NDV4HR at 2 and 6 weeks
Δ Vaccinated with NDV4HR at 2 weeks and LaSota at 6 weeks ◻ Unvaccinated control

TABLE 4

Survival Rates of Broiler Chickens Vaccinated with HBI, LaSota, and NDV4HR Vaccines After Challenge at 9 Weeks with a Field Newcastle Disease Virus

	Days post inoculation									
Groups	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*No. survived No. inoculated*	*% mean level of protection*	*95 % CI*
A	100	100	93.3	93.3	80	80	80	12/15	89.51[a]	6.90
B	100	93.3	60	40	33.3	33.3	33.3	5/15	56.17[b]	21.69
C	100	62.5	0	0	0	0	0	0/8	23.21[c]	30.44

a, b, c groups with different superscript differ significantly ($P<0.05$)
A = Birds were vaccinated with HBI at 2 weeks and LaSota at 6 weeks
B = Birds were vaccinated with NDV4HR at 2 and 6 weeks
C = Served as unvaccinated control
% mean level of protection - Factor of % surviving and no. of days post-challenge they survived.

protection level (45.7 % ± 27.00) than those that received V4 (27.14 % ± 28.60). The mean level of protection of the unvaccinated control birds was significantly higher than the vaccinated groups (Table 5). The differences were statistically significant ($P<0.05$).

Table 6 shows that re-vaccination of pullets/cockerels with LaSota gave a significantly ($P<0.05$) higher protection (77.14 % ± 15.84) than with V4 (35.70 % ± 28.00) or in unvaccinated birds (41.63 % ± 30.37). Birds re-vaccinated with LaSota after primary vaccination with HBI (Group A) showed significantly higher survival rates ($P<0.05$) than those that received LaSota after V4 primary vaccination (Group C).

Discussion

The ND V4 vaccine has received high acclaim because of properties that make it suitable for use in rural village chickens (Ideris, Ibrahim & Spradbrow, 1990; Jagne *et al.*, 1991; Spradbrow, 1993/94). The V4 vaccine used in these

TABLE 5

Survival Rates of Pullet/Cockerel Chickens Vaccinated with HBI and NDV4HR Vaccines at 2 Weeks and Challenged at 5 Weeks of Age with a Field Newcastle Disease Virus

Groups	Days post inoculation 1	2	3	4	5	6	7	No. survived / No. inoculated	% mean level of protection	95 % CI
A	100	90	50	30	30	10	10	1/10	45.71[a]	27.00
B	100	60	20	10	0	0	0	0/10	27.14[b]	28.60
C	100	100	60	40	40	40	40	4/10	60.00[c]	20.95

a, b, c groups with different superscript differ significantly ($P<0.05$)
A = Birds vaccinated with HBI
B = Birds vaccinated with NDV4HR
C = Unvaccinated control
% mean level of protection - Factor of % surviving and no. of days post-challenge they survived.

TABLE 6

Survival Rates of Pullet/Cockerel Chickens Vaccinated with HBI, LaSota, and NDV4HR Vaccines and Challenged at 9 Weeks with a Field Newcastle Disease Virus

Groups	Days post inoculation 1	2	3	4	5	6	7	No. survived / No. inoculated	% mean level of protection	95 % CI
A	100	100	100	60	60	60	60	6/10	77.14[a]	15.84
B	100	80	20	20	10	10	10	1/10	35.71[b]	28.00
C	80	70	60	50	40	40	40	4/10	54.59[c]	11.99
D	100	90	60	10	10	10	10	1/10	41.43[bc]	30.37

a, b, c groups with different superscript differ significantly ($P<0.05$)
A = Birds were vaccinated with HBI at 2 weeks and LaSota at 6 weeks
B = Birds were vaccinated with NDV4HR at 2 and 6 weeks
C = Birds were vaccinated with NDV4HR at 2 weeks and LaSota at 6 weeks
D = Served as unvaccinated control
% mean level of protection - Factor of % surviving and no. of days post-challenge they survived.

experiments has, however, also been manufactured and presented for commercial use in 1000-dose freeze-dried ampoules and recommended for chickens of all classes and ages. In these comparative studies with HBI and LaSota, therefore, it was decided to evaluate the commercial NDHR "V4" within the prevailing vaccination protocol in the country. Routinely, chickens receive the primary ND vaccine in the drinking water at 2 weeks and are re-vaccinated at 6 weeks, also orally. Pullets receive a second booster at 16 weeks *via* inactivated ND vaccine given intramuscularly.

The afore-mentioned results show that before the primary vaccinations, the HAI titres of chicks, presumably derived from maternal antibody sources, were high and usually protective, as GMT levels of $\log_2$ 3.00 and above are considered protective (Allan & Gough, 1974; Spradbrow *et al.*, 1978). The high initial titres no doubt depressed seroconversion when chicks were vaccinated at 2 weeks with HBI or V4. High maternal antibody levels in chicks interfere with antibody production in these birds when they are

vaccinated (Nutor, 1973).

This phenomenon notwithstanding, replacement chicks that received HBI at 2 weeks showed a mean GMT increase from $\log_2 3.71$ to 5.39 a week later, although those given V4 showed no increase. Some live vaccine types are able to overcome the maternal antibody levels to elicit antibody production by the host immune system (Rehmani, 1996). At 5 weeks, while the mean GMT of broiler chicks vaccinated with HBI was above $\log_2 3.00$ and therefore protective, that of birds which received V4 was lower than the protective level (Fig.1). These results confirm the observations of Biswas *et al.* (1996) that HBI is superior to V4 in eliciting antibody production.

The LaSota vaccine elicited high GMTs in broiler and replacement chicks. In broilers, the mean titre of birds rose from $\log_2 1.30$ to 3.20 at 8 weeks, but fell rapidly to 1.86 at 9 weeks. In pullet/cockerel chicks, the GMT rose steadily to 6.94 at 9 weeks, showing no fall as in broilers. Box (1985) observed that antibody decay begins earlier and falls at a more rapid rate in broilers than in layer birds. In contrast, V4 elicited little antibody production when used for re-vaccination in broilers and pullets/cockerels. Interestingly, LaSota produced slightly increased antibody titres in V4-primed than in HBI-primed chicks.

V4 is less immunogenic than conventional vaccines, but gives satisfactory protection against experimental and field virus challenge (Spradbrow, 1993/1994; Biswas *et al.*, 1996). In these experiments, the survival rate of birds vaccinated with V4 at 2 weeks was only 27.14 per cent as against 45.71 per cent in birds vaccinated with HBI, when challenged. The difference is significant ($P<0.05$). Interestingly, unvaccinated controls showed a survival rate of 60.00 per cent, indicating the strong influence of circulating maternal antibodies in interfering with antibody production. Allan *et al.* (1978) have recommended that chicks with high levels of maternal antibody be given the first vaccination at 3 weeks, as only 60 per cent of such chicks will actively respond to vaccination at an earlier age. The results presented here have also shown that LaSota gave better protection to broiler and pullet/cockerel birds than V4. However, at the ages of virus challenge (9 weeks), unvaccinated control birds were significantly ($P<0.05$) more susceptible, as there were no circulating ND antibodies.

The results presented here generally agree with the findings of Biswas *et al.* (1996) in the superior immunogenic and protective qualities of conventional vaccines over the V4. However, the performance of this make and batch of V4 vaccine was lower than expected. For example, the V4 vaccine used by Biswas *et al.* (1996) gave 86 per cent protection in 19-week-old indigenous chickens vaccinated at 7 weeks as against 96 per cent protection shown in birds given conventional vaccines. Further tests with other batches of the vaccine, and perhaps other V4 vaccines are indicated, considering the potential of the V4 for use in village chickens, especially in the rural areas of the country.

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