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Effects of Vaccination with Lentogenic Vaccine and Challenge with Virulent Newcastle Disease Virus (NDV) on Egg Production in Commercial and SPF Chickens

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Abstract: Since 2002, control of Newcastle Disease (ND) in South Africa has become complicated following the introduction of lineage 5d/VIId Newcastle Disease Virus (NDV) strain (locally known as "goose paramyxovirus" - GPMV). Commercially available ND vaccines appeared less effective. In this study, commercial and SPF hens in lay were vaccinated with La Sota vaccine and challenged with GPMV isolate to assess the effect of both vaccination and challenge on egg production. This study also compared the efficacy of cloacal and ocular routes of vaccination against challenge, following reports that cloacal vaccination offered a better protection against egg production losses than the oro-nasal route of vaccination. Vaccinated birds were fully protected (100%) against challenge by La Sota vaccine, but not against infection and replication of the virus, as birds showed varying degrees of macropathology and confirms the ability of virulent ND strains to infect and replicate even in vaccinated birds. Results also showed no clear difference in the protection of the birds against challenge with GPMV by either the cloacal and ocular routes of vaccination. Mmarginal to severe egg production drop was observed in both commercial and SPF birds after vaccination and challenge experiments.

Key words: Newcastle disease, hen, vaccination, routes, production

INTRODUCTION

Newcastle Disease (ND) is a highly contagious and widespread disease of the avian species causing severe economic losses in domestic poultry, especially chickens (Alexander, 2001). This disease is still one of the most important disease in poultry production worldwide and remains a serious problem in spite of control measures, including vaccination which has been applied since the 1950s (Czeglédi *et al*., 2006). Its global impact is enormous and unsurpassed by any other poultry disease, although the recent epizootic of H5N1 avian influenza in some parts of the world seems to challenge this status (Alexander, 2003; Van Boven *et al*., 2008). ND remains a major barrier to international trade in poultry and poultry products and a great drain on the world economy (Alexander, 2003). The causative agent of the disease is Newcastle Disease Virus (NDV), also designated as Avian Paramyxovirus Serotype 1 (APMV-1) and belongs to the genus *Avulavirus* within family *Paramyxoviridae* (Mayo, 2002a, b). Since its first official report in poultry in Java, Indonesia in 1926 (Kraneveld, 1926) and Newcastle-upon-Tyne (from where the disease and the virus got its name) in 1927 (Doyle, 1927), ND has continued to re-emerge in both epidemic and endemic form throughout the world (Brown *et al*., 1999). ND was officially recorded to have entered South Africa through the port of Durban during 1944 (Kaschula *et al*., 1945). Since then, ND outbreaks in poultry have occurred sporadically in South Africa (Abolnik *et al*., 2004; Abolnik, 2007).

Poultry production is the most efficient and cost-effective way of increasing the availability of high-protein food (FAO, 1987), as eggs are known to provide the most perfectly balanced food containing all the essential amino acids, minerals and vitamins (Branckaert *et al*., 2000). However, the reproductive efforts of birds can be influenced by disease processes either by acting directly and altering the ability of the lining cells to perform their specialized functions or by generally compromising the health of the bird (Solomon, 2002). One of the diseases that affects and produces some form of pathology in the reproductive organs of affected birds, thus affecting the reproductive functions of chickens is ND (Biswal and Morrill, 1954; Rao *et al*., 2002). The net effect of ND is either a change in quality of the egg produced in terms of the shape and/or texture of the shell or a complete drop in the quantity produced. A marked effect on egg production is reported as one of the signs of ND and such effect could include partial to complete drop in production accompanied by production of smaller eggs, misshapen and rough-shelled eggs and shell-less to thin-shelled eggs containing watery albumen (McFerran

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and McCracken, 1988). The effect on egg production together with the mortalities caused by ND affects both the quality and quantity of dietary protein and significantly affects human health (Steneroden *et al*., 2004).

Most countries where poultry is raised commercially and where the disease is endemic rely on vaccination to keep the disease under control (Alexander, 2001). However, there are several reports indicating that commercially available ND vaccines are not performing optimally against virulent NDV (Burridge *et al*., 1975; Kapczynski and King, 2005; Miller *et al*., 2007). The newly emerging virulent NDV strains of lineage 5d/VIId (locally called goose paramyxovirus - GPMV in South Africa) are of great concern and have been suggested to have the ability to overcome vaccination barriers (Panshin *et al*., 2002). In addition Kapczynski and King (2005) and Czeglédi *et al*. (2006) reported that currently available vaccines induced better protection against viruses that were isolated in past epizootics (1950s) than against viruses that are currently circulating in the poultry industry which comprises mostly of viruses of lineage 5d/VIId. In South Africa, protection offered by available commercial vaccines against lineage 5d/VIId strains has been suboptimal, as ND infection and disease are characterized by mortalities in broiler flocks and drop in egg production even in fully-vaccinated pullets (Bisschop, unpublished data). While the reasons and causes for the frequent "vaccine failures" in the field are not very clear, the efficacy and potency of most of the commercially available vaccines are being questioned. During velogenic field challenge in apparently well-vaccinated laying hens, there is seldom any significant increase in mortality associated with the challenge. Egg production is, however, frequently seriously depressed and often associated with abnormal egg-shell formation and white-shelled eggs (in brown egg layers). In order to limit these production losses, certain poultry producers in South Africa have resorted to cloacal application of live Newcastle disease vaccines before and during the laying period based on the assumption that superior immunity might be achieved in the oviduct through more direct application of the vaccine. Although the mechanism and theory behind the intracloacal vaccination is yet to be elucidated, the swabbing of the cloacae of 6 to 8-week old chickens with infective allantoic fluid have been used in the United State of America to assess tropism and distinguish between viscerotropic velogenic NDV and other strains and their virulence (Hanson, 1980).

Despite the depressing and pathologic effect of some ND vaccine and virulent ND strains on the reproductive system, only Biswal and Morrill (1954) looked at the pathology of the reproductive tract of laying pullets affected with ND while Rao *et al*. (2002) did an *in vitro* and *in vivo* evaluation of the virulence of Newcastle disease virus and vaccines for the chicken reproductive tract. The findings of this study will therefore add to the findings of Biswal and Morrill (1954) and Rao *et al*. (2002) especially on the effects of ND viruses and vaccines on the reproductive tract of chickens.

MATERIALS AND METHODS

Virus: The challenge virus used was a local velogenic Newcastle Disease Virus (NDV) strain. Its Mean Death Time (MDT) was determined to be 48 h and Intracerebral Pathogenicity Index (ICPI) was 1.85. Based on Polymerase Chain Reaction (PCR) and molecular sequencing, it was classified as highly pathogenic and of the genotype 5d/VIId (GPMV; GenBank Ref. # FJ985978). The virus challenge dose was $10^{7.0}$ EID_{50}/0.1ml/bird.

Experimental design: Eighty two (82) weeks old Specific Pathogen-Free (SPF) White Leghorn (n = 40) and 52 weeks old commercial Hyline Brown hens (n = 40) were procured from two reputable poultry establishments and assigned randomly into eight groups (groups 1-4 for SPF and groups 5-8 for commercial hens) of 10 hens per isolator. 12 extra SPF birds were also procured for the control experiment. The birds were allowed to acclimatize for two days after which they were vaccinated with NEW VAC-LS® Newcastle disease vaccine (La Sota strain, live virus - Forte Dodge®, Brazil; FD6033A; Batch No: 002/07) at the manufacturer's recommended dose. Birds in groups 1, 3, 5 and 7 were vaccinated via the cloacal route while birds in groups 2, 4, 6 and 8 were vaccinated via eyedrop. On day-12 post-vaccination, the remaining birds (five birds from each group have previously been removed and euthanized during vaccination experiment) were challenged via eyedrop with a lineage 5d/VIId strain of NDV (GPMV). Ten White Leghorn SPF birds were used as the positive control. The positive control birds were not vaccinated but challenged with the same GPMV at the same dose and by the same route. Two SPF birds were kept as negative control birds. They were neither vaccinated nor challenged.

Serology: Both the SPF and the commercial birds (n = 10 each) were randomly bled on arrival to determine their Newcastle Disease (ND) antibody status. Following vaccination and challenge, randomly selected birds from each group were bled and euthanized. Details of the protocol have been reported more comprehensively in another paper (Bwala *et al*., unpublished report). Antibody titres to ND were determined using a commercial NDV ELISA Kit (Newcastle Disease Virus Antibody Test Kit - FlockChek*; IDEXX Laboratories Inc, Maine, USA). Results were presented as titre groups (range 1 to 18; equivalent to titre values of 397 to 32,000).

Clinical observation and statistics: Birds were monitored according to the Poultry Reference Centre's Standard Operating Procedure (PAS/PRC/035). Birds were observed daily both post-vaccination and post-challenge (2 times daily at 8-h interval) for clinical signs and egg production figures for each group were recorded. Birds were euthanized based on a designed protocol. Details of the protocol have been reported more comprehensively in another paper (Bwala *et al*., unpublished report). All euthanasia was done by asphyxiation with carbon dioxide (CO_2) according to the Poultry Reference Centre's Standard Operating Procedure (SOP 0104) which was previously approved by the Animal Use and Care Committee (AUCC) of the University of Pretoria. Data were imported into Microsoft® Excel Spreadsheets and subjected to simple statistical manipulations to produce percentages, bar charts and line graphs for interpretation.

Birds/samples were identified as follows:

- CV (1, 3, 5 and 7) = Cloacal vaccination (isolators 1, 3, 5 and 7)
- EV (2, 4, 6 and 8) = Eye (ocular) vaccination (isolators 2, 4, 6 and 8)
- Date of euthanasia were identified as: 2, 4, 6, 8 and 10 PV (post-vaccination) and 2, 4, 6, 8 and 10 PC (post-challenge),

Sample (S) numbers e.g. S1996 and the year of processing (2008, simply 08) were further given to processed tissues in addition to the earlier identifications of CV and EV, e.g. CV3 S1996-08; EV2 S2000-08 will mean cloacal vaccination in isolator 3, sample number 1996 processed in 2008 and eye vaccination in isolator 2, sample number 2000 processed in 2008 respectively.

RESULTS

Clinical signs and mortality: All vaccinated birds appeared healthy Post-Vaccination (PV). However, there was a transitory drop in egg production from 31 eggs (38.6%) before vaccination to 14 eggs (17.5%), a day post vaccination (day 1 PV). Post-vaccination and post-challenge egg productions are presented in Table 1. The PV egg production increased from 14 eggs (17.5%) on day 1 PV to 25 eggs (34.72%) on day 3 PV and 25 eggs (39.06%) on day 5 PV (Table 1). The transitory drop in production immediately after vaccination was observed in both the commercial and the SPF birds, but more marked in the SPF than the commercial birds.

Following challenge, all birds appeared clinically normal until day 3 PC, when two SPF birds in two of the groups vaccinated via the cloacal route (isolator 1 and 3) had ruffled feathers. One birds (isolator 3) died on day 4 PC from causes not associated with the trial, just before bleeding and was removed from the trial. All the remaining birds appeared healthy for the entire study period. There was no death from ND-related causes in all the groups. Egg production of the birds dropped from 18 eggs (45%) to 16 eggs (40%) on day 1 PC and increased again to 17 eggs (53.13%) on day 3 PC. Egg production finally plummeted to 1 egg (12.5%) on day 9 PC. Of the total 505 eggs produced by all the birds during the study, 106 eggs (20.99%), 279 eggs (55.25%) and 120 (23.76%) were produced by the SPF, the commercial and the control birds, respectively (Table 1). The positive control birds (unvaccinated but challenged with the same virus and dose and via the same route (eye drop) as the trial birds) started showing ND-related clinical signs on day 1 PC. Four of the birds passed greenish faeces on day 1 PC. Two hens were euthanized according to the research design while the remaining eight (100%) control birds appeared depressed, sleepy and anorexic at the last observation on day 2 PC. By the second observation on day 3 PC, one positive control bird was found dead. Five more of the positive control birds died on day 4 PC, one on day 5 PC and by the morning of day 6 PC, all the positive control birds were dead from viral challenge-associated causes. Pre-challenge egg production of 9 eggs (90%) dropped to 7 eggs (70%) and 5 eggs (50%) on days 1 and 2 PC, respectively (Table 1) and was 0% from day 5 PC. From day 2 PC to day 6 PC, when the last positive control bird died, 7 (18.42%) out of the total 38 eggs laid were either soft-shelled or shell-less (data not shown). The two negative control birds (neither vaccinated nor challenged) did not manifest any signs of disease throughout the trial period and were laying at 100% (2 eggs per day) until they were euthanized on day 10PV (Table 1). The negative control birds did not lay any soft-shelled or shell-less eggs.

Serology (ELISA): Pre-vaccination serology (ELISA) confirmed the SPF status of all the White Leghorn SPF birds used in this study. All 10 of the SPF birds that were randomly selected and bled tested negative for NDV antibodies. The 10 commercial Hyline Brown hens had NDV antibody titres expressed as titre groups of between 2^5 and 2^{17} on arrival (data not shown). Post-vaccination titres of the SPF birds that were bled on each of the euthanasia days remained at zero (0) for the first six days PV, with the exception of EV2 S1965-08 that had a titre of $<2^1$ on day 6 PV. On day 8 PV, SPF birds CV1 S1987-08 and EV4 S1992-08 had an antibody titre of 2^1, while bird EV2 S1991-08 had a titre of 2^2. By day 10 PV, the euthanized SPF birds had titres of 2^5 (CV1 S1995-08); 2^7 (CV3 S1996-08) and 2^{12} (EV2 S2000-08 and EV4 S2001-08), signifying seroconversion to the vaccination. The post-vaccination and post-challenge NDV antibody titres for both the SPF and the commercial hens as detected by ELISA are presented in Fig. 1. The moving averages of all groups were calculated and displayed graphically (Fig. 1).

Table 1: Daily egg production of birds vaccinated with La Sota ND vaccine and challenged with velogenic NDV

			Control hens		
	SPF hens	COMM. hens	Challenged		Unchallenged
Days post-exposure	Eggs Laid (%) (No. of birds)	Eggs Laid (%) (No. of birds)	Eggs Laid (%) (No. of birds)	Mortality (%)	Eggs Laid (n) (%) (No. of birds)
Vaccination	**La Sota ND vac**	**La Sota ND vac**	**---------- Not Vaccinated ----------**		**Not vaccinated**
Day 0	27.5% (40)	50.0% (40)	70.0% (10)	0/10 (0%)	2 (100%) 2
Day 1 PV	15.0% (40)	20.0% (40)	60.0% (10)	0/10 (0%)	2 (100%) 2
Day 2 PV*	15.0% (40)	32.5% (40)	80.0% (10)	0/10 (0%)	2 (100%) 2
Day 3 PV	19.4% (36)	50.0% (36)	90.0% (10)	0/10 (0%)	2 (100%) 2
Day 4 PV*	19.4% (36)	61.1% (36)	80.0% (10)	0/10 (0%)	2 (100%) 2
Day 5 PV	18.8% (32)	59.4% (32)	90.0% (10)	0/10 (0%)	2 (100%) 2
Day 6 PV*	9.4% (32)	40.6% (32)	70.0% (10)	0/10 (0%)	2 (100%) 2
Day 7 PV	14.3% (28)	50.0% (28)	80.0% (10)	0/10 (0%)	2 (100%) 2
Day 8 PV*	14.3% (28)	39.3% (28)	90.0% (10)	0/10 (0%)	2 (100%) 2
Day 9 PV	8.3% (24)	50.0% (24)	90.0% (10)	0/10 (0%)	2 (100%) 2
Day 10 PV*	12.5% (24)	50.0% (24)	90.0% (10)	0/10 (0%)	2 (100%) 2
Day 11 PV	**---------- Challenged ----------**				**Unchallenged**
	20.0% (20)	70.0% (20)	70.0% (10)	0/10 (0%)	2 (100%) 2
Day 1 PC	20.0% (20)	60.0% (20)	50.0% (10)	0/10 (0%)	2 (100%) 2
Day 2 PC*	15.0% (20)	40.0% (20)	50.0% (8)	2[a]/10 (0%)	2 (100%) 2
Day 3 PC	31.3% (16)	100.0% (16)	42.9% (7)	1/8 (12.5%)	2 (100%) 2
Day 4 PC*	6.3% (16)	75.0% (16)	28.6% (7)	5/7 (71.4%)	2 (100%) 2
Day 5 PC	25.0% (12)	75.0% (12)	0.0% (3)	½ (50%)	2 (100%) 2
Day 6 PC*	33.3% (12)	58.3% (12)	0.0% (0)	1/1 (100%)	2 (100%) 2
Day 7 PC	37.5% (8)	75.0% (8)	0.0% (0)	0	2 (100%) 2
Day 8 PC*	25.0% (8)	75.0% (8)	0.0% (0)	0	2 (100%) 2
Day 9 PC	0.0% (4)	25.0% (4)	0.0% (0)	0	2 (100%) 2
Day 10 PC*	0.0% (4)	0.0% (4)	0.0% (0)	0	2 (100%) 2

PV = Post-Vaccination; PC = Post-Challenge; SPF = Specific Pathogen-Free hens; COMM = Commercial hens; * = Days that one hen each was euthanized/group and sera taken (i.e. one hen per treatment group, giving a total of 8; 4 SPF and 4 commercial hens a day), control birds were euthanized only on days 10PV and 2PC while all the remaining control birds died from challenge-related causes; n = number of eggs laid. [a]were birds taken and euthanized for sample collection and did not died from ND-related causes

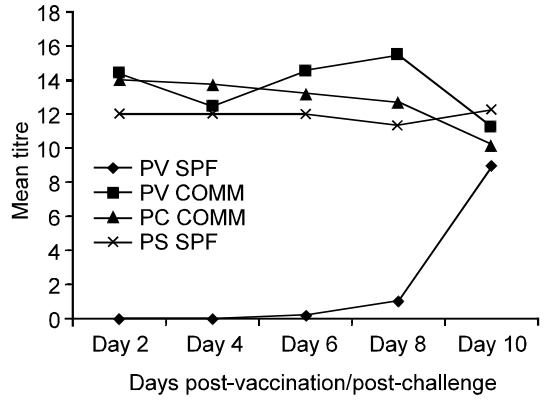

Fig. 1: ND antibody titre of both SPF and commercial hens vaccinated with La Sota vaccine and challenged with a virulent lineage 5d NDV (GPMV), as assayed by ELISA and expressed as titre groups. PV SPF = Post-Vaccinated SPF hens; PV COMM = Post-Vaccinated Commercial hens; PC SPF = Post-Challenge SPF; PC COMM = Post-Challenge Commercial hens

The average titres (Fig. 1) of the commercial birds (PV COMM) fluctuated between 2^{11} and 2^{16} while those of the SPF hens (PV SPF) picked up gradually from an average titre of $>2^{1}$ to the peak value of 2^{9}. The drop in the line graph for the PV COMM on day 4 PV was due to individual differences (birds CV5 S1953-08 and EV6 S1958-08 had antibody titres of 2^{9} and 2^{7}, respectively, while others had titres of up to 2^{17}). In addition, the drop in the graph (again for the PV COMM) seen on day 10 PV was due to the generally low antibody titres of birds euthanized on day 10 PV as compared to those euthanized on day 8 PV (data not shown). The mean titres of the PV SPF chickens picked up gradually from day 6 PV ($>2^{1}$) to day 8 PV (2^{1}), followed by a steep ascent to a mean peak titre of 2^{9} on day 10 PV (Fig. 1).

Post-challenge antibody titres of commercial birds (PC COMM) fluctuated between 2^{8} and 2^{18} throughout the 10 days period while those of the SPF hens were between 2^{3} and 2^{16} (data not shown). The post-challenge antibody titre graph (Fig. 1) revealed a gradual descent for the commercial birds (PC COMM) from 2^{14} to 2^{10}, although the commercial birds reached their peak antibody titre of

$2^{15.5}$ in response to vaccination on day 8 PV, thus the challenge with the virulent virus did not cause any substantial increase in antibody titre. The graph for the SPF birds (PC SPF) was almost a straight line descent with the average titre values fluctuating between 11 and 12 (Fig. 1). The drop in titre on day 8 PC was due to the low antibody titre (2^7) of bird CV3 S2174-08 (data not shown). This was probably due to the inability of bird CV3 S2174-08 to seroconvert optimally to the vaccination as well as to the challenge. Indeed, abundance of ND viral antigens were apparent with IHC staining in all the sections of oviduct of this particular bird (data have been reported in another paper), which support the suboptimal seroconversion theory in this case. Clearly this bird was not fully protected against virulent viral challenge, when compared with other members of the group. However, it did not develop or show visible clinical signs.

The post-challenge mean NDV antibody titres of both the SPF and commercial birds vaccinated by cloacal and eye-drop routes were compared (Fig. 2). Titres for the Cloacal-Vaccinated (CV COMM) and Eye-drop Vaccinated (EV COMM) commercial birds fluctuated between 10^{10} and 10^{15} for both groups while the titres for the EV SPF was between 10^{11} and 10^{16} and for CV SPF it was between 10^7 and 10^{13}. It may appear that Eye Vaccination (EV) provides better protection in both the commercial and SPF hens going by the higher titre values of birds vaccinated through this route. However, the differences seen were not significant as most of the fluctuations/variations observed could be attributed to individual bird variations within a group.

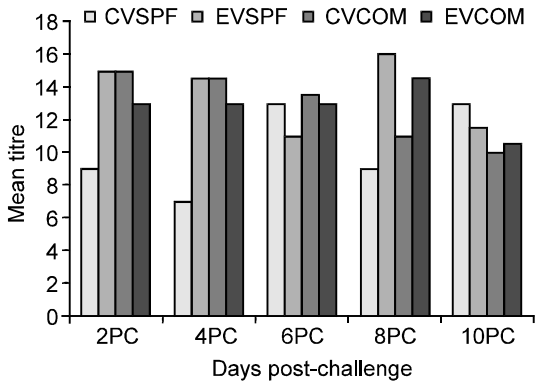

Fig. 2: Post-challenge mean titres of SPF and commercial hens vaccinated with ND La Sota vaccine by cloacal and eye-drop routes and challenged with virulent GPMV. PC = post-challenge; CVSPF = Cloacally-vaccinated SPF hens; EVSPF = Eyedrop vaccinated SPF hens; CVCOM = Cloacally-vaccinated commercial hens; EVCOM =Eyedrop vaccinated commercial hens

Gross pathology: Birds were necropsied immediately after they were euthanized (or after they died from challenge-related causes as in the case of the positive control birds). All birds euthanized on days 2, 4, 6, 8 and 10 Post-Vaccination (PV) had no grossly visible pathology. Bird CV1 S1946-08, one of the SPF hens euthanized on day 2 PV was found to have a metastatic uterine adenocarcinoma that had infiltrated almost all of the abdominal organs. Hens EV2 S1991-08, CV3 S1996-08 and EV4 S2001-08, all of which were SPF hens, had small, flaccid and inactive oviducts. These birds were probably not in active egg production. SPF hens, CV1 S2058-08, CV1 S2110-08, EV2 S2169-08 and EV2 S2177-08 euthanized post-challenge had small, flaccid and inactive oviducts indicating that they too were not in active lay. Birds CV3 S2111-08 and EV4 S2178-08 died from causes unrelated to the viral challenge and were therefore removed from the study. Bird EV8 S2180-08, euthanized on day 8 PC and birds CV5 S2199-08, CV7 S2200-08, EV2 S2201-08, EV6 S2203-08 and EV8 S2204-08, euthanized on day 10 PC, had necrohaemorrhagic foci in their caecal tonsils. Three of these hens (EV2 S2201-08, EV6 S2203-08 and EV8 S2204-08) were vaccinated by ocular route while birds CV5 S2199-08 and CV7 S2200-08 that were vaccinated through the cloacal route. In addition, all the birds with necrohaemorrhagic lesions in their caecal tonsils were commercial hens that had previous history of vaccination, except bird EV2 S2201-08, which was an SPF bird that had only one vaccination.

The macropathology observed in the challenged-control birds (positive control) was variable, depending on the duration of the infection. Birds euthanized on day 2 PC (CX 1A 2PC and CX 1B 2PC) has no gross lesions. Bird CX 1A 3PC, that died on day 3 PC, had pin-point haemorrhages on the serosal surface of the spleen, slight haemorrhage in the lumen of the proventriculus, congested ovarian follicles and a segmentally congested duodenum filled with greenish watery content. Five birds died on day 4 PC, four of which had matted vents stained with greenish or whitish faecal material. Macroscopic lesions included congested tracheas, kidneys and lungs, pericardial haemorrhages as well as necrohaemorrhagic lesions in the caecal tonsils. Only one bird had haemorrhagic lesions in the proventriculus. In addition, all the positive control birds that died on day 4 PC showed marked degeneration of the ovarian follicles, characterized by resorption of the yolk, ill-defined external follicle outlines and congested follicles, with some having yolk material lying freely in the abdominal cavity. The bird that died on day 5 PC had a diffusely congested trachea, pin-point white spots throughout the spleen, a congested heart and haemorrhagic lesions in the proventriculus and ceacal tonsils. The duodenum of this bird was haemorrhagic and the ovarian follicles were also severely haemorrhagic and degenerated. Similar lesions were

seen in the last bird that died on day 6 PC. In addition, the spleen in this bird was markedly enlarged and diffusely haemorrhagic.

DISCUSSION

Newcastle disease is a global disease of enormous economic importance. The virus is capable of infecting many avian species with a marked effect on the poultry industry, principally due to mortality but also due to the effects on the quality and quantity of meat and eggs produced by affected birds. In this study, trial birds were protected from clinical disease and deaths from NDV challenge when vaccinated with the field dose recommended by the manufacturers (which is usually 10^6 EID_{50}/bird). After vaccination with ND La Sota vaccine, both immunologically naïve SPF and the commercial laying hens showed no clinical reaction to vaccination. However, the temporary drop in egg production witnessed post-vaccination indicate that vaccination with the La Sota vaccine had a transitory depressing effect on egg production. This drop in egg production can be attributed to some sort of reaction to the vaccine that had effects on FSH/LH secretion (hormonal control) possibly through the stress-corticosterone pathway of the oviduct (Blalock, 1987; Dunn *et al*., 1989; Chowdhury and Yoshimura, 2002; Johnson and Gous, 2006; reviewed in Borghetti *et al*., 2009). La Sota vaccine have also been reported to have a high "stress factor" which may produce adverse effects (Mészáros, 1983; Allan and Borland, 1979) that could cause a temporary drop in production in vaccinated birds. In addition, the SPF hens were also much older (82 weeks) than the commercial hen (52 weeks) at receipt and therefore did not lay at peak performance. This may be responsible for the production gaps noticed between the commercial and SPF chickens.

The absence of post-challenge clinical signs in the vaccinated trial birds could be attributed to the protection offered by the vaccine administered to the birds, as most of the birds had high ND antibody titres sequel to vaccination and prior to challenge, indication of seroconversion. This 100% protection from clinical disease seen in the vaccinated layer hens, shows that both commercial and immunologically naïve SPF hens can be protected from ND-related clinical disease when vaccinated with La Sota ND vaccine, either by the cloacal or ocular route, as none of the birds that were challenged after vaccination via either route developed clinical signs or died. The vaccination-related protection of this study was particularly true for the immunologically naïve SPF birds that were never vaccinated against ND before this trial. The La Sota vaccination for the commercial hens on the other hand only served as a booster vaccination, since they had history of previous vaccinations.

Generally, La Sota vaccines were reported to confer greater protection than other lentogenic ND vaccines such as Ulster 2C, B1 and F (reviewed in Thornton *et al*., 1980; Rehmani, 1996). The protection result emanating from the present study concurs with previous ND vaccine trials (Parede and Young, 1990; Kapczynski and King, 2005; Miller *et al*., 2007; Perozo *et al*., 2008), all of which demonstrated that the proper application of ND vaccines can protect birds against clinical signs and mortality from ND challenge. In addition, the present study also confirmed that a single application of ND vaccine like La Sota can confer protection against clinical ND, since none of the immunologically naïve SPF birds manifested clinical signs or died from the challenge. This agrees with Rehmani (1996), who reported that a single application of La Sota vaccine at 12 days of age was sufficient to offer reasonable protection until the chickens were 7 weeks old. The present study also showed that birds exposed to repeated vaccination are better protected against challenge, as was shown by the commercial birds that had higher antibody titre values as compared to their SPF counterparts. The results also concurs Parede and Young (1990), who reported that in birds with high antibody titres (immune birds), clinical signs are either mild or absent and there may not be any mortality after challenge with virulent field strains.

In this experiment, the unvaccinated control birds were not protected as all died from the challenge within six day after challenge. This is in consonance with the OIE requirements for such challenge trials. The unvaccinated control birds manifested clinical signs, and had mortalities and lesions that were consistent with that of velogenic NDV infection in non-immunized birds as reported by Biswal and Morrill (1954), McFerran and McCracken (1988), Parede and Young (1990) and Hamid *et al*. (1991). Torticollis was however not observed but there were marked degeneration of the follicles as reported by Biswal and Morrill (1954). The unvaccinated but challenged control hens that manifested morphological changes in the oviduct lay soft-shelled and shell-less eggs; this concurs with the reported findings of Biswal and Morrill (1954) and McFerran and McCracken (1988). Also dramatic falls in egg production have been reported as a consistent feature of infection with all pathotypes of ND (Biswal and Morrill, 1954; Al-Garib *et al*., 2003) as the degeneration of follicles leads to arrest of ovulation and subsequent oviposition (Biswal and Morrill, 1954). Reduced feed and egg production efficiency and/or temporary damage of the shell-producing mechanism of infected fowl are reported as a constant feature of Newcastle disease (Riddell, 1996).

In general, the protection achieved in this study did not prevent the challenge virus from infecting and replicating in the host tissues and organs as varied degree of gross pathology were encountered even in euthanized and necropsied apparently healthy challenged birds at the termination of the trial. This also agrees with the report that vaccination of poultry against ND can only

protect birds from the more serious consequence of virulent NDV infection (severe clinical signs and mortality) but not infection and replication of the virulent strains of the virus (Parede and Young, 1990; Kapczynski and King, 2005; Miller *et al*., 2007). Necrohaemorrhagic lesions seen in the caecal tonsils of some of the challenged birds were similar to macropathology reported by Parede and Young (1990) and Hamid *et al*. (1991) in high-antibody titre/immune birds. The demonstration of gross lesions in the challenged birds that had clinical signs and the assertion that vaccination protects against clinical signs and death and not infection and replication of the viruses in the host tissues, were corroborated by the demonstration of viral antigens in the various sections of the oviduct of vaccinated birds in this study (data have been reported in another paper). This probably explains the presence of macroscopic lesions in the "healthy challenged birds" that were euthanized at the end of the trial period. The inability of vaccines to fully protect against viral replication and possibly shedding of viruses (though shedding assessment was not done in this study) especially in natural infections in field situations according to Alexander (2001) presents a bigger problem as it may mask the possible introduction and spread of virulent virus which becomes endemic, but only becomes apparent when immunity level is down.

Though reports have shown that vaccination routes can influence the level of protection offered by the same vaccine against challenge (Kojnok *et al*., 1977; Rehmani, 1996), this study showed no clear difference in the protection of the oviduct between the two application routes. This therefore disproves the perceptions by some farmers that cloacal vaccination might provide "better" protection against infection and decreased egg production than ocular vaccinations.

Generally, stresses (e.g. diseases) have been suggested to have the ability to prevent the secretion of Luteinizing Hormone (LH) (Chowdhury and Yoshimura, 2002; Johnson and Gous, 2006) which is required for ovulation to occur. The neuroendocrine response to disease and stress induced by viral challenge has been reported to lead to the activation of the hypothalamic-pituitary-adrenal axis and the hypothalamic-pituitary-gonadal system with the subsequent peripheral secretion of cortisol and corticosterone that affects the metabolism and availability of calcium ions required for use in egg calcification (Blalock, 1987; Eiler, 2004; reviewed in Borghetti *et al*., 2009) with the resultant production of poor quality and malformed eggs. This could be another possible cause for the production of eggs with poor shell quality, in addition to structural damage that may be caused by the infecting ND viruses in the shell gland and the albumen-secreting section (magnum) of the oviduct of infected birds.

Conclusion: The fact that ND viruses can infect well-vaccinated flocks and replicate within the tissues represents a continuing threat to the poultry industry. The development of improved vaccines against the emerging lineages of ND that can more effectively reduce the replication of virulent virus during infection will be essential for the long term control of this disease.

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