Beni-Suef Veterinary Medical Journal, (2006):16 (1): 18-26.

USE OF NEWCASTLE DISEASE VACCINES FROM DIFFERENT SOURSES IN VACCINATION OF CHICKENS HAVE MATERNAL ANTIBODIES AGAINT MYCOPLASMA

By
M. M. Amer and Wafaa A. Abd El-Ghany
Department of Poultry Diseases, Faculty of Veterinary Medicine, Cairo University

SAMMARY

This study was carried out to investigate the efficacy of the used Newcastle disease (ND) live vaccines from different commercial sources and in different programs for vaccination of chicks having maternal antibodies against *Mycoplasma*. The immune response was estimated using HI and challenge tests. Effect on chicken performance was estimated by detection of body weight gain. Role of vaccine in stimulation of respiratory bacterial stress was pointed by lesion scores. HI-test titers in birds vaccinated with Liopest vaccines showed higher titers than those vaccinated with Nobles. Birds received 2nd vaccine from heterologus source showed lower titers than those received homologus vaccine.
Results of challenge test proved that birds vaccinated with ND vaccines from one source and those vaccinated with Hitchner B_1 at 33 days of age instead of La Sota resulted in 100% protection as compared with 95 % following heterologus La Sota, While groups 1, 5 and 10 received Nobles Hitchner B_1 showed only 85%, 85% and 95%; respectively.
All vaccinated chicken groups showed lower conversion rates lower than the non vaccinated control. Groups received Hitchner B_1 as 3rd dose of the vaccine showed higher rates than those vaccinated with La Sota. Results of lesion score for CRD in vaccinated groups with different regimens showed that birds received traditional ND vaccinal strains having higher scores than those received ND vaccines from field isolates. Administration of Hichner B_1 vaccine at 33 days of age showed lower scores. Hichner B_1 can be recommended in vaccination of chickens derived from *Mycopasma* infected hens.

INTRODUCTION

Till now vaccination of chickens against Newcastle disease (ND) stills the only effective policy for prevention and control **(Saif, et al., 2003 and OIE, 2004)**. Since the recognition of the disease in Egypt veloginic viscerotropic strain of virus become endemic **(Lancaster and Alexander, 1975)**, and still reported to cause severe outbreaks with high losses in infected flocks. Till new there is no data about the antigenic variation among NDV isolates circulating in poultry reared in Egypt.

ND vaccine production and improvement have a continuous development to face requirement of poultry men depending on the flock conditions, the aim of production, the prevalence of latent infections as well as the epidemiological status of the disease.
In the other hand, considerable variation exists among the same strains produced by different biologics manufacturers **(Borland and Allan, 1980 and Thronton et al., 1980)** also, the demonstrated immunity was differ **(Bunens et al., 1983)**. Further more, the field ND viruses are found to be differ antiginically from the used vaccines

(Panshsin et al., 2002). **Edison and Kleven (1980)** stated that ND vaccinal strains having the same pathogenic index differ in their immunogenicity as compared by geometric mean titers and challenge.

Live vaccines are differ in their character, mimic natural infection and induce all three immune responses including circulating antibodies, secreted antibody producing mucosal immunity and cell mediated immunity **(Allan et al., 1975)**.

An effective vaccination program must minimize the risk associated with the disease and maximize production efficiency as economically and practically as possible.
Hitchner B_1 **(Hitchner and Johnson. 1948)** and La Sota **(Goldhaft, 1980)** strains are now the most widely used vaccines.
Recent work indicated an antigenic variation among the NDV strains **(Russell and Alexander, 1983)**.

Mycoplasmas may affect the cell-mediated immune system by inducing either suppression or stimulation of B and T lymphocytes and inducing cytokines **(Chabra and Goel. 1981; Reddy et al., 1998 and Gaunson et al., 2000)**. In the other hand, **Amer et al., (1993)** reported on the immunosuppressive effect of *Mycoplasma* sp. in chickens vaccinated with Newcastle Hitchner B_1 vaccine. Also, Mycopasma infections can be aggravated by other bacteria and viruses to induce respiratory affections affected chickens performance **(MacOwan et al., 1982; Gross, 1990 and Nakamura et al., 1994)**.

This work was planed to study the efficacy of using Newcastle disease vaccines of different commercial sources, in different programs on chicken immune response, body weight as well as testing of models of vaccination in face of vertical Mycoplasma infection of broiler chicks.

MATERIAL AND METHODS

Chicks:

Four hundreds and fifteen Cubb chicks were obtained from commercial farm as hatched. These chicks were floor reared and feed commercial balanced ration with amprole plus and tylan premix as feed additives. The ration was given to the experimental birds *ad libitum.*

Newcastle disease (ND) viruses:

a. ***Vaccinal viruses****:*

1. Source 1: Nobles vaccines including Hitchner B_1 (NB) Lot No. 053176D and La Sota (NL) Lot No. 058166D produced by Intervet Co., Boxmeer, and Holland. Content of these vaccines were estimated to be $10^{9.28}$ and $10^{9.56}$ EIB_{50} /vial 1000 dose; respectively.
2. Source 2: Liopest vaccines including Liopest B_1 (LB1) Lot No. N2/939 and Liopest La Sota (LL) Lot No V/02 produced by Iven laboratory, Maderd Spain and containing $10^{9.15}$ and $10^{9.42}$ EID_{50} / vial 1000 dose; respectively.

b. ***La Sota virus***:

Laboratory La Sota strain was obtained from Animal Health Institute, Abassia and passed in SPF chicken embryo to be used as antigen for HI test antigen.

c. ***Challenge virus***:
The local velogenic viscerotrobic ND strain isolated and identified by **Sheble and Reda (1976)** was used for challenge test.

Infecious bursal disease vaccine:
All chicks were vaccinated at the 12^{th} day of live with intermediate plus vaccine against infectious bursal disease using eye drop route.

Detection of virus infectivity:
Both vaccinal and challenge ND strains were titrated in 9 day-old emberyonated SPF chicken eggs before their use according to **Anon (1971)**. Embryo infective dose $_{50}$ (EID_{50}) were calculated according to method of **Reed and Muench (1938)**.

Fertile eggs:
Fertile Specific Pathogen Free (SPF) eggs were obtained from Kom Oshem, Fayom and used for titration of the used vaccines, challenge virus, passage of HI antigen as well as virus reisolation from dead challenged birds.

Haemagglutination inhibition (HI) test:
The B-procedure of micromethodology according to **Takatsy (1956)** was used. HI-tiers were given titers reference numbers according to **Kaleta and Sigmonn (1971)** and the antibody titers were calculated as arithmetic mean of $\log_2$ end points.

Vaccination:
All used ND vaccines were applied allover this work using eye drop method by instillation of 0.05 ml containing a dose of 10^6 EID_{50}/ bird.

Lesion score:
Lesion score for chronic respiratory disease (CRD) were estimated in 40 days sacrificed birds according to **Awaad et al., (2003)**.

Challenge test:
Experimental chickens were intranasally infected each with 0.2 ml of saline containing 10^6 EID_{50} of velogenic viscerotropic ND virus. Symptoms, mortalities and post-mortem lesion were recorded during 10 days observation period post challenge. Samples for virus reisolation were taken from dead birds. All survived birds were sacrificed and subjected to post mortem examination for ND lesions.

Serum samples:
Twenty five clotted blood samples for serum were individually collected at 1 and 7days for detection of maternal HI antibodies against ND as well as at 14, 19, 26, 33 and 40 days of age to detect HI antibody in vaccinated and control groups. The sera were individually separated, labeled, heat inactivated and kept freeze till HI testing.

Mycoplasma antigen and antiserum:
Mycoplasma gallisepticum (MG) and *Mycoplasma synovaie (MS)* colored antigens were purchased from Intervet Co. and used for serum plate agglutination test. Chicken anti-*MG* and- *MS* sera were kindly gifted from Mycoplasma department, Animal Health Research Inst., Dokki, Giza, Egypt.

Statistical analysis:
The obtained results were statistically analyzed using ANOVA test at P< 0.001, 0.01 and 0.05.

Experiment:
The used chicks (415) were floor reared. At 1-day of life, 25 chicks were sacrificed with collection of their blood for separation of serum. These birds were subjected to bacteriological examination for detection of pathogenic bacterial infection.

At the 7^{th} day of life, the remaining chicks (390) were equally divided into 13 groups (1-13), 30 chicks each. Each group was kept in separate cage. Chicken groups 1-6 and 7-12 were vaccinated against ND using LB and NB vaccine; respectively. Chicks of group 13 were kept as non-vaccinated control. All chicken groups were vaccinated against infectious bursal disease via eye drop at the 12^{th} day of age.

At the 19^{th} day, birds of groups 1-3, 4-6, 7-9 and 10-12 were received NL, LL, NL and LL vaccines, respectively. Two weeks after (33 days of age), birds of groups 1, 5, 7 and 10, 2, 4, 8, and 11, 3 and 9; as well as 6 and 12 were revaccinated with NB, LB, NL as well as LL vaccines; respectively (Table 1).

The consumed feed and body weight / group were recorded to calculate the conversion rate at the 40^{th} day of life.

From each group, 25 clotted blood samples were randomly collected for serum at 7, 14, 19, 26, 33 and 40 days of age to determine HI antibodies against ND using HI test.

At the 40^{th} day of age, 10 birds / group were sacrificed and subjected to postmortem examination with calculation of CRD lesion score. Rests of birds in all chicken groups (20 chicks / group) were subjected to challenge test by given each bird 0.2 ml containing 10^6 VVND via intranasal rout. The challenged birds were kept under daily observation for 10 days for clinical signs, mortality, lesions as well as virus reisolation from dead birds. The protection rate was calculated at the end of observation period.

RESULTS

The obtained results are shown in tables (1-5) and Fig. (1-7).

The bacteriological examined sacrificed birds showed negative results to the detection of bacterial pathogens. While testing of collected sera at 0 and 7 days of age against stained *mycoplasma* sera using plate agglutination test proved the detection of 36% and 28% as well as 44% and 60% for *MG* as well as *MS*; respectively (Table 1).

Results of HI test in table (2) Fig. (1) proved the following:
Statistical analysis of HI results (table 2) proved that the obtained mean titers at 40 days of age in group 6 (7.21 ± 1.78), group 8 (7.31± 2.25) at P< 0.001 and group 11 (6.88± 1.01) at P<0.05 are significantly higher than those of groups 1 (5.20 ± 1.66), group 3 (5.20± 1.20), and group 5 (5.25± 0.89). HI mean titers in groups 6 and 8 are significantly higher than that of group 2 (5.57 ± 1.20) at P<0.05.

Titers of group 8 (7.31 ± 2.25) is significantly higher than those of groups 9 (5.777± 1.87) and 10 (5.73± 1.61) at P< 0.05.
Generally, it is observed that chicken group received LB and LL vaccine showed higher HI mean titers than those received NB and NL vaccines (group 6 and 9). In the other hand bird serviced LB and /or LL vaccines at any time of vaccination showed relatively higher titers.

Post-mortem lesions of died challenged birds showed the typical lesions of VVNDV infection in control, while intestinal lesions were more obvious in vaccinated group 1, and 7 than those of 3 and 5.

Results of challenge test (Table 6 Fig. 2) proved statistically that birds of groups 1 and 7 those showed significantly lower protection rates (85%) than the rate of 100% in groups 4, 6, 8, 9, 11, and 12. Birds received all ND vaccines from (group 6 and 9) source showed 100% protection. In the other side administration of LB vaccine instead of homologues La Sota at 33 days of age (Groups, 4 and 8) resulted in 100% protection as compared with 95% and 100% following heterologus La Sota (group 3 and 8). While groups 1, 5 and 10 received NB vaccine showed only 85%, 85% and 95%; respectively.

Statistically, results of body weight at the 6^{th} week of age (Tables 4 and 5) proved that birds of non vaccinated control group showed significantly higher mean bogy weight (1311± 102.32) than those of vaccinated groups 1 (1139 ± 129.2), group 2 (1191± 110.8), group 3 (1154 ± 82.1), group 6 (1156± 85.5) and group 10 (1125± 105.8) at P<0.001.
Body weight in group 10 is significantly lower than those of group 7 (1295± 140), group 8 (1281± 114.3) at P< 0.001 as well as lower than that of group 9 (1238 ± 77.9) at P<0.05. In the other hand the obtained body weight values in groups 4, 7 and 8 are significantly higher than those of groups 1 (P<0.001) and 3 (P<0.01).

Generally, the results in table (3) and Fig. (3) are indicating that the use of vaccines from the same source resulted in improved weights than usage of vaccines from heterologous sources. LB and LL vaccines induced relatively higher values.
Conversion rates in table (4) fig (3) showed that all vaccinated chicken groups showed lower conversion rate than the control non vaccinated one. Birds of groups 1 2, 3 , 5 ,7 ,9 ,10 ,11 and 12 had rates of 2.40, 2.29, 2.55, 2.37, 2.32, 2.32, 2.35, 2.28 and 2. 23; respectively lower than those of groups 4, 6 and 8 where it was 2.10,2.12 and 2.16; respectively. Groups received La Sota ND strain vaccine at 33 days mostly showed lower rates than those received Hitchner. Groups received NB and NL vaccines strains having lower rates than those received LB and LL vaccines.

Results of lesion score for CRD in vaccinated groups with different regimes (Table 7 Fig. 4) showed that birds received ND vaccinal strains source 1 having higher scores than those received vaccines from source 2. Administration of LB vaccine at 33 days of age shower lower scores.

Vaccination of chicken groups by ocular LB vaccine at 33 days of age resulted in good immunity as measured by higher HI titers and protection rates as well as higher performance than those received NB vaccine. Birds received LL vaccine 10 days

following homologues vaccine induced higher immunity and performance than those received NL vaccine.

DISSCUSION

Newcastle disease live vaccines from lentogenic strains had been adopted to use in the disease prevention, since its 1st use till now. In our field there are vaccines from different sources. The usage of such vaccines resulted in different results their potency in prevention of Newcastle disease. In this study we used ND vaccines from 2 sources, in vaccination of broiler chicks positive to Mycoplasma.
The obtained mean titers at 40 days of age in group 6 and 8 those given all vaccines from one source (7.21 ± 1.78 and (7.31± 2.25) are significantly higher than those of groups 1 (5.20 ± 1.66), group 3 (5.20± 1.20) , and group 5 (5.25± 0.89) those received vaccines from different sources. Titers in groups 6 and 8 are also significantly higher than that of group 2 (5.57 ± 1.20) at $P<0.05$. This result indicated that the usage of ND live vaccines from one source is better.

Titers of group 8 (7.31 ± 2.25) is significantly higher than those of groups 9 (5.777± 1.87) and 10 (5.73± 1.61) at $P< 0.05$.
Generally, it is observed that chicken group received vaccine from the 2nd source showed higher titers than those received 1st source vaccines (groups 6 and 9).
In the other hand bird serviced Hitchner and /or La Sota vaccines of source 2 at any time of vaccination showed relatively higher titers. Similar results had been reported by **Amer et al. (l993).**

Results of challenge test showed that birds of groups 1 and 7: those given 2nd and 3rd vaccines from source 1: showed significantly lower protection rates (85%) than 100% in groups 4, 6, 8, 9, 11, and 12; those received vaccines from source 2. In this way **Rananvare et al. (2001)** concluded that some vaccines were less potent than others in comparison with four commercial La Sota vaccines from different manufactures.

In the other side administration of Hitchner B1 vaccine of source 2 instead of homologues La Sota at 33 days of age (Groups, 4 and 8) resulted in 100% protection as compared with 95 and 100% following heterologus La Sota (groups 3 and 8). These results can be explained by **Thornton et al. (1980)** who detected a variation in protection in vaccinated chickens with prouducts made from the same strains, depending on their source also, **Borland and Allan (1980)** reported difference in immunizing capacity, potency levels and respiratory stress of 18 ND vaccines from different sources, and the La Sota vaccines were more varied, more heterogenous and more immunogenic than B1 vaccines. The relation between HI titers and challenge test had been discussed by **Wu Qing Min et al. (2002).**

Results of body weight at the 6th week of age proved that birds of non vaccinated control group showed significantly higher mean bogy weight (1311± 102.32) than those of vaccinated groups 1-3, 6 and 10 at $P<0.001$.
Body weight in group 10 is significantly lower than those of group 7 (1295± 140), group 8 (1281± 114.3) at $P< 0.001$ as well as lower than that of group 9 (1238 ± 77.9) at $P<0.05$. In the other hand the obtained body weight values in groups 4, 7 and 8 are significantly higher than those of groups 1 ($P<0.001$) and 3 ($P<0.01$). **Bunens et al.**

(1983) found no significant difference in weight gain and feed conversion between groups received different vaccines.

Generally, the results indicating that the usage of vaccines from the same source resulted in improving of weights than the usage of vaccines from the heterologous sources.

All vaccinated chicken groups showed lower conversion rate than the control non vaccinated one. Groups received La Sota ND strain vaccine at 33 days mostly showed lower rates than those received Hitchner.

CRD lesion score in vaccinated groups with different regimes showed that birds received source 1 vaccines having higher scores than those received vaccines from the other source. Administration of LB vaccine at 33 days of age shower lower scores than La Sota. The result which pointed out the role of vaccine in stimulation of CRD **(MacOwan et al., 1982; Gross, 1990 and Nakamura et al., 1994)**

Results of this work can be explained by the difference of potency and immunogenicity or antigenic relation between vaccinal and challenge strain as stated by **Schloer et al. (1975), Eidson and Kleven, (1980) and Russel and Alexander, (1983).**

We can concluded that ND vaccines as preventive measure must be carefully chosen according to the disease history of birds. Not all vaccines in the field are equal in their potency. Hitcher B1 vaccines can be used as 3rd vaccine at 33 days in birds under Mycoplasma stress.

REFERANCES

Allan, W. H.; Lancaster, J. E. and Toth, B. (1975): Newcastle disease vaccines, production and use. FAO, Rome, Itally.

Amer, M . M. ; Khilfa, D.G.; Metwalli, A. S. E. and El-Kady, M. F. (1993): Immune responce against Hichner B_1 vaccine in chickens infected with Mycoplasma. Beni-Suef, Vet. Med. Res., III(1): 108-123.

Anon, (1971): Methods for examining poultry biologics and for identifying and quantifying avian pathogens. National Academy of science-Washington, D. C.

Awaad, M. H. H; Sahar, A. Zoulfakar; El-Shazely, M.O. ; Manal, A. Afify and Osman, L.H. (2003): Effect of "Pediococcus acidilactic" on zootechnical Performance and E. coli infection in broiler chickens. Vet. Med. J. Giza., 51 (2): 273-281.

Bananvare, P. S.; Kulkarni, D.D. and Gujar, M. B. (2001): Quality assessment of La Sota strain vaccines. Ind. J. of Comp. Microbol. Immunol. Inf. Dis., 22 (2): 154-155.

Borland, L. J. and Allan, W. H. (1980): Laboratory tests for comparing live lentognic Newcastle disease vaccines. Av. Pathol., 9 (1): 45-59.

Bunens,B.; Spanoghe, L.; Devos, A.; Vlaene, N. (1983): Commercial Newcastle disease vaccines: II: Vaccination of SPF layer chicks and ordinary broilers with five vaccines derived from La Sota and clone 30 virus strains. Valaams Diergeneeskundig Tijdschrift., 52 (4): 237-254.

Chabra, P. C. and Goel. M. C. (1981): Immunological response of chickens to

Mycoplasma gallisepticum infection. Avian Dis., 25: 279-93.
Gaunson, J. E. ; Philip, C. J. ; Whithear, K. G. and Browning. G. F. (2000): Lymphocytic infiltration in the chicken trachea in response to Mycoplasma gallisepticum infection. Microbiology Reading. 146: 1223-1229.
Goldhaft, T. M. (1980): Historical note on the origin of the La Sota strain of Newcastle disease virus. Av. Dis., 24: 297-301.
Gross, W. B. (1990): Factors affecting the development of respiratory disease complex in chickens. Av. Dis., 34: 607-610.
Eidson, C. S. and Kleven, S. H. (1980): Vaccination of chickens with a clone-selected La Sota strain of Newcastle disease virus. Poult. Sci., 59 (5): 979-984.
Hitchner, S. B. and Johnson. E. P (1948): A virus of low virulence for immunizing fowls against Newcastle disease (avian pneumoencephalitis). Vet Med., 43:525-530.
Kaleta, E. F. and Sigmonn, O. (1971): Comparative studies on the demonstration of hemagglutinating inhibiting and neutralizing antibodies after vaccination against Newcastle disease. Arch. Geflugelk., 35:79.
Lancaster, J. and Alexander, D. J. (1975): A Newcastle disease virus and spread. A review of some of the literature. Canada Depart. Of Agri., Monograph No 11.
MacOwan, K. J.; Randall, C. J.; Jones, H. G. R. and Brand. T. F. (1982): Association of Mycoplasma synoviae with respiratory disease of broilers. Av. Pathol., 11: 235-244.
Nakamura, K.; Ueda, H.; Tanimura, T. and Noguchi, K. (1994): Effect of mixed live vaccine (Newcastle disease and infectious bronchitis) and Mycoplasma gallisepticum on the chicken respiratory tract and on Escherichia coli infection. J. comp Pathol., 111: 33-42.
OIE (2004): Manual of diagnostic tests and vaccines for terrestrial animals. 5th Ed. Office International des Epizootics, Paris, France.
Panshsin, A.; Shihmanter, E.; Weisman, Y.; Orveli, C. and Lipkind, M. (2002): Antigenic heterogenicity amongst the field isolated Newcastle disease virus (NDV) in relation to vaccine strain Part NII: Studies on viruses isolated from domestic birds in Israel. Comp. immunol. Microbiol. Inf. Dis., 25 (3): 173-185.
Reddy, S. K. ; Pratik, S.; Amer, S.; Newman, J. A ; Singh, P. and Silim. A. (1998): Lymphoproliferative responses of specific pathogen-free chickens to Mycoplasma gallisepticum strain PG31. Av. Pathol., 27: 277-283.
Reed, L. J. and Muench, H. (1938): A simple method of estimating fifty percent end point. Amer. J. Hyg., 27: 493-497.
Russell, P. H. and Alexander, D. J. (1983): Antigenic variation of Newcastle disease virus strains detected by monoclonal antibodies. Arch. of Virol., 75 (94): 243-253.
Saif, Y. M.; Barnes, H. J.; Fadly, A. M.; Glisson, J. R..; McDougald, L.R. and Swayne, D. E. (2003): Diseases of Poultry, 11th Ed., Iowa State Press, A Blackwell Publishing Co.
Schloer, G.; Spaltin, J. and Hanson, R. P. (1975): Newcastle disease virus antigens and strain variation. Am. J. Vet. Res., 36 (4): 505-508.
Sheble, A. and Reda, I. M. (1976): *Cited by Khaphagy, A. K.(1977)* Thesis M. V. Sc., Facult. Vet. Med., Cairo Univ.
Takatsy, G. Y. (1956): The use of spiral loops in serological methods. Acta. Microbiol. Hung.,3: 191.
Thornton, D. H.; Hopkina , I. G. and Hebert, C. N. (1980): Potency of live Newcastle disease vaccines. Av. Pathol., 9 (3): 457-464.
Wu Qing Min, Pei Jian Wu, Wang Kal Dong, Zhang Tian Shui (2002):

Beni-Suef Veterinary Medical Journal, (2006):16 (1): 18-26.

Immunopotency test on some attenuated vaccines against Newcastle disease chickens. Chinese. J. Vet. Med., 28 (5): 12-13.