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The effect of different Newcastle disease live vaccines and vaccination schedules on the immune response and performance of broiler chickens serologically positive to Mycoplasmas

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This study was conducted to investigate the efficacy of the Newcastle disease (ND) live vaccines from different commercial sources used in different programs for vaccination of chicks having maternal antibodies against Mycoplasma infection. The immune response was estimated using HI and challenge tests. The effect on the chicken performance was estimated by the detection of the body weight gain. The role of vaccines in stimulating respiratory bacterial stress was pointed out by the lesion scores.

Birds vaccinated with live vaccines from source (2) showed higher HI titers than those vaccinated with vaccine from source (1) and birds received the 2nd vaccination from heterologous source showed lower titers than those received from homologous source.

Results of the challenge test indicated that birds vaccinated with live ND vaccines from one source and those vaccinated with Hitchner B1 at 33-days of age instead of La Sota showed 100% protection rate as compared with 95 % followed by vaccination with heterologous La Sota. Birds received Hitchner B1 of vaccine source (1) showed only 85% protection rate. All vaccinated chicken groups showed feed conversion rates lower than the non-vaccinated control one. Groups received Hitchner B_1 as a 3rd dose of the vaccine showed higher rates than those vaccinated with La Sota.

Results of the lesion score for chronic respiratory disease (CRD) in vaccinated groups with different regimes revealed that birds received ND vaccinal strains from source (1) having higher scores than those received ND vaccines from the 2nd source. Administration of Hitchner B1 vaccine at 33-days of age showed lower scores. Hitchner B1 can be recommended in vaccination of chickens derived from Mycoplasma infected hens.

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

ND vaccine production subjected to a continuous development to face the requirements of poultry men and that depending on the flock conditions, the aim of production, the prevalence of the latent infections as well as the epidemiological status of the disease.

On the other hand, considerable variations exist among the same strains produced by different manufacturers (Borland and Allan, 1980; Thronton *et al*., 1980). The demonstrated immunity was also different (Bunens *et al*., 1983). Furthermore, the field ND viruses are found to be different antiginically from the used vaccines (Panshsin *et al*., 2002). Eidson and Kleven, (1980) stated that ND vaccinal strains had the same pathogenic index differ in their immunogenicity based on geometric mean titers and challenge.

Live vaccines are different in their characters, mimic natural infection and induce circulating antibodies, secreted antibodies producing mucosal immunity and cell-mediated immunity (Allan *et al*., 1975).

An effective vaccination program must minimize the risk associated with the disease and

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maximize production efficiency in an economic and practical manner. Hitchner B1 (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; Gaunson *et al*., 2000). On the other hand, Amer *et al*., (1993) reported the immunosuppressive effect of Mycoplasma spp. in chickens vaccinated with Newcastle Hitchner B1 vaccine. In addition, Mycoplasma infections, could be aggravated by other bacteria and viruses to induce respiratory affections adversely affect chickens performance (MacOwan *et al*., 1982; Gross, 1990; Nakamura *et al*., 1994).

This work planed to study the efficacy of using of live Newcastle disease vaccines from different commercial sources in different vaccination programs on the immune response, the protection and the performance of broiler chicks serologically positive to Mycoplasmas.

Material and Methods

Chicks. Four hundreds and fifteen day old Cobb chicks were obtained from commercial farm at hatching. These chicks were floor reared and fed on commercial balanced ration with amprole plus and tylan premix as feed additive. 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, Holland. Titers of these vaccines were estimated to be $10^{9.28}$ and $10^{9.56}$ EID_{50} /vial 1000 dose; respectively.

2. Source (2). Liopest vaccines including Liopest B1 **(LB)** Lot No. N2/939 and Liopest La Sota **(LL)** Lot No V/02 produced by Iven laboratory, Maderd, Spain. They contained $10^{9.15}$ and $10^{9.42}$ EID_{50}/ vial 1000 dose respect-tively.

B. La Sota virus. Laboratory La Sota strain was obtained from Veterinary Serum and Research Institute, Abassia, Cairo, Egypt and passed in SPF chicken embryo to be used as antigen for HI test.

C. Challenge virus. The local velogenic viscerotropic ND strain that was isolated from field outbreak 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 life with intermediate plus vaccine (E.228) (Intervet Co., Boxmeer, and Holland) against infectious bursal disease using eye drop route.

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

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

Haemagglutination inhibition (HI) test. The β-procedure of micromethodology according to Takatsy, (1956) was used. HI-titers 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 the 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 at 40-day-old sacrificed birds according to Awaad *et al*., (2003).

Challenge test. Experimental chickens were intra-nasally infected each with 0.2 ml of saline containing 10^6 EID_{50} of velogenic viscerotropic ND (VVND) virus. Symptoms, mortalities and post-mortem lesions 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 sera were individually collected at 1 and 7 days of age 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 until HI testing.

Mycoplasma antigen and antiserum *Mycoplasma gallisepticum (MG)* and *Mycoplasma synoviae (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 Institute, Dokki, Giza, Egypt.

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

Experimental design. The used chicks (415) were floor reared. At the first day of life, 25 chicks were weighed and sacrificed and their blood was collected for separation of sera. Those birds were subjected to bacteriological examination for detection of pathogenic bacterial infection.

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

At the 19th 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 2).

The consumed feed was recorded and the body weight of 25 bird/ group was detected weekly for 6 weeks to calculate the feed conversion rate at the 40th day of life.

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

At the 40th day of age, 10 birds / group were sacrificed and subjected to post-mortem examination for estimation of CRD lesion score. The remaining birds in all chicken groups (20 chickens / group) were challenged (each bird was taken 0.2 ml containing 10^6 EID_{50} VVND via intra-nasal route). The challenged birds were kept under daily observation for 10 days for clinical signs, mortalities, post-mortem lesions as well as virus reisolation from dead birds. The protection rate was calculated at the end of observation period.

Results

Bacteriological examination of experimental sacrificed birds showed negative results to bacterial pathogen. Testing of the collected sera at 1 and 7 days of age against stained Mycoplasma antigen 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).

Statistical analysis of HI results (Table 2, 3 Fig. 1) showed 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 were 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 were significantly higher than that of group 2 (5.57 ± 1.20) at P<0.05. Titers of group 8 (7.31 ± 2.25) was significantly higher than those of groups 9 (5.77± 1.87) and 10 (5.73 ± 1.61) at P< 0.05.

Generally, it was 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). On the other hand, bird received LB and /or LL vaccines at any time of vaccination showed relatively higher titers. Statistically, the results of the mean body weight at the 6th week of age (Table 4, 5) indicated that birds of non-vaccinated control group showed significantly higher mean body 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 was 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 addition, the obtained mean body weight values in groups 4, 7 and 8 were significantly higher than those of groups 1 (P<0.001) and 3 (P<0.01).

Generally, the results in Tables (4, 5) indicated that using of vaccines from the same source resulted in improved body weights than the using of vaccines from heterologous sources. LB and LL vaccines induced relatively higher values.

Feed conversion rates in Table (4) Fig. (3) showed that all vaccinated chicken groups showed lower 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 which were lower than those of groups 4, 6 and 8 (2.10, 2.12 and 2.16), respectively. Groups received La Sota ND strain vaccine at 33-days of age mostly showed lower rates than those received Hitchner B_1. Groups received NB and NL vaccines strains had lower rates than those

Table (1): Results of MG and MS plate agglutination test.

Age	No. of samples	MG		MS	
		No. of positive	%	No. of positive	%
1 day	25	9	36	11	44
7 days	25	7	28	15	60

MG=*Mycoplasma gallisepticum* **MS**=*Mycoplasma synoviae*

Table (2): Results of HI mean antibody titers in sera of chickens vaccinated against ND using different vaccination models. (n= 25).

Group No.	Vaccination			Days of age						
	1st	2nd	3rd	0	7	14	19	26	33	40
1			NB							5.20 ± 1.66
2		NL	LB					3.56 ± 1.02	4.04 ± 1.14	5.57 ± 1.20
3	LB		NL			2.28 ± 1.48	3.60 ± 1.38			5.20 ± 1.20
4			LB							6.33 ± 1.32
5		LL	NB					4.16 ± 1.15	4.64 ± 1.09	5.25 ± 0.89
6			LL	2.48 ± 1.23	1.92 ± 1.19					7.21 ± 1.78
7			NB							6.00 ± 1.67
8		NL	LB					3.92 ± 1.22	4.80 ± .097	7.31 ± 2.25
9	NB		NL			1.48 ± 1.37	3.16 ± 1.12			5.77 ± 1.87
10			NB							5.73 ± 1.61
11		LL	LB					3.56 ± 1.02	5.72 ± 1.35	6.88 ± 1.01
12			LL							5.83 ± 1.47
13	Control negative					1.52 ± 1.23	0.96 ± 1.25	0.00	0.00	0.00

NB=Nobles Hitchner B_1. **NL**=Nobles La Sota. **LB**=Liopest B_1. **LL**=Liopest La Sota.

Table (3): Results of statistical analysis of HI-titres between groups at 40-days of age as seen in table (2).

Group No.	1	2	3	4	5	6	7	8	9	10	11	12	13
1						**		***			*		
2						*		*					
3						**		***			*		
4													
5						***		***			*		
6													
7													
8									*	*	*		
9													
10													
11													
12													
13													

Table (4): Results of weekly mean body weight of ND vaccinated chicken groups (n=25).

Group No.	Vaccination			Weeks of age							Conversion rate
	1st	2nd	3rd	0	1	2	3	4	5	6	
1			NB						836.8±88.6	1139±129.2	2.40
2		NL	LB				478.5±41.95	623.6±45.87	857±99.4	1191±110.8	2.29
3	LB		NL			255.65±29.94			856.4±2.7	1154±82.1	2.55
4			LB						901±58.1	1285±104.2	2.10
5		LL	NB				569.5±35.73	629.5±53.6	875±30.8	1061±35.6	2.37
6			LL	38.25±3.45	97.50±10.62				898 ± 97.9	1156.7±85.5	2.12
7			NB						942.5±90.6	1295±140.4	2.32
8		NL	LB				540±40.31	692±1.22	918.6±6.1	1281.6±114.3	2.16
9	NB		NL			256.18±20.55			898.3±96.4	1238±77.9	2.32
10			NB						911.2±97.9	1125±105.8	2.35
11		LL	LB				499±30.28	615.5±64.12	959.7±97.0	1226.5±95.1	2.28
12			LL						874.7±84.2	1253.5±119.1	2.23
13	Control negative					261±37.22	499.5±7.37	875.5±101.6	1311±102.3	1311±102.32	2.07

NB=Nobles Hitchner B1 . **NL**=Nobles La Sota. **LB**=Liopest B_1. **LL**=Liopest La Sota.

Table (5): Results of statistical analysis of body weight difference between groups at 40-days of age as seen in table (4).

Group No.	1	2	3	4	5	6	7	8	9	10	11	12	13
1				***			***	***				*	***
2					**								*
3				**			**	**					***
4					***	**				**			
5							***	***	***		***	***	***
6								**					***
7										***			
8										***			
9										*			
10												**	***
11													
12													
13													

*= Significant difference between vaccinated groups at *=p<0.05 **=p<0.01***=p<0.001

Table (6): Results of challenge test in ND vaccinated chickens (n=20).

Group No.	Vaccination			Challenge test	
	1st	2nd	3rd	No. of survivals	Protection rate %
1			NB	17	85*
2		NL	LB	19	95
3	LB		NL	18	90
4			LB	20	100*
5		LL	NB	19	95
6			LL	20	100*
7			NB	17	85*
8		NL	LB	20	100*
9	NB		NL	20	100*
10			NB	19	95
11		LL	LB	20	100*
12			LL	20	100*
13	Control negative			0	0

NB=Nobles Hitchner B_1. **NL**=Nobles La Sota. **LB**=Liopest B_1. **LL**=Liopest La Sota.
*=Significant difference between vaccinated groups at p<0.05.

Table (7): Mean lesion score of CRD in ND vaccinated chickens (n=10).

Group No.	Vaccination			Lesion score
	1st	2nd	3rd	Mean
1	LB	NL	NB	1.6
2			LB	1.4
3			NL	1.1
4			LB	0.9
5		LL	NB	0.9
6			LL	1.3
7	NB	NL	NB	1.2
8			LB	1.2
9			NL	1.9
10			NB	0.9
11		LL	LB	0.9
12			LL	1.5
13	Control negative			0.6

NB=Nobles Hitchner B1. **NL**=Nobles La Sota. **LB**=Liopest B1. **LL**=Liopest La Sota.

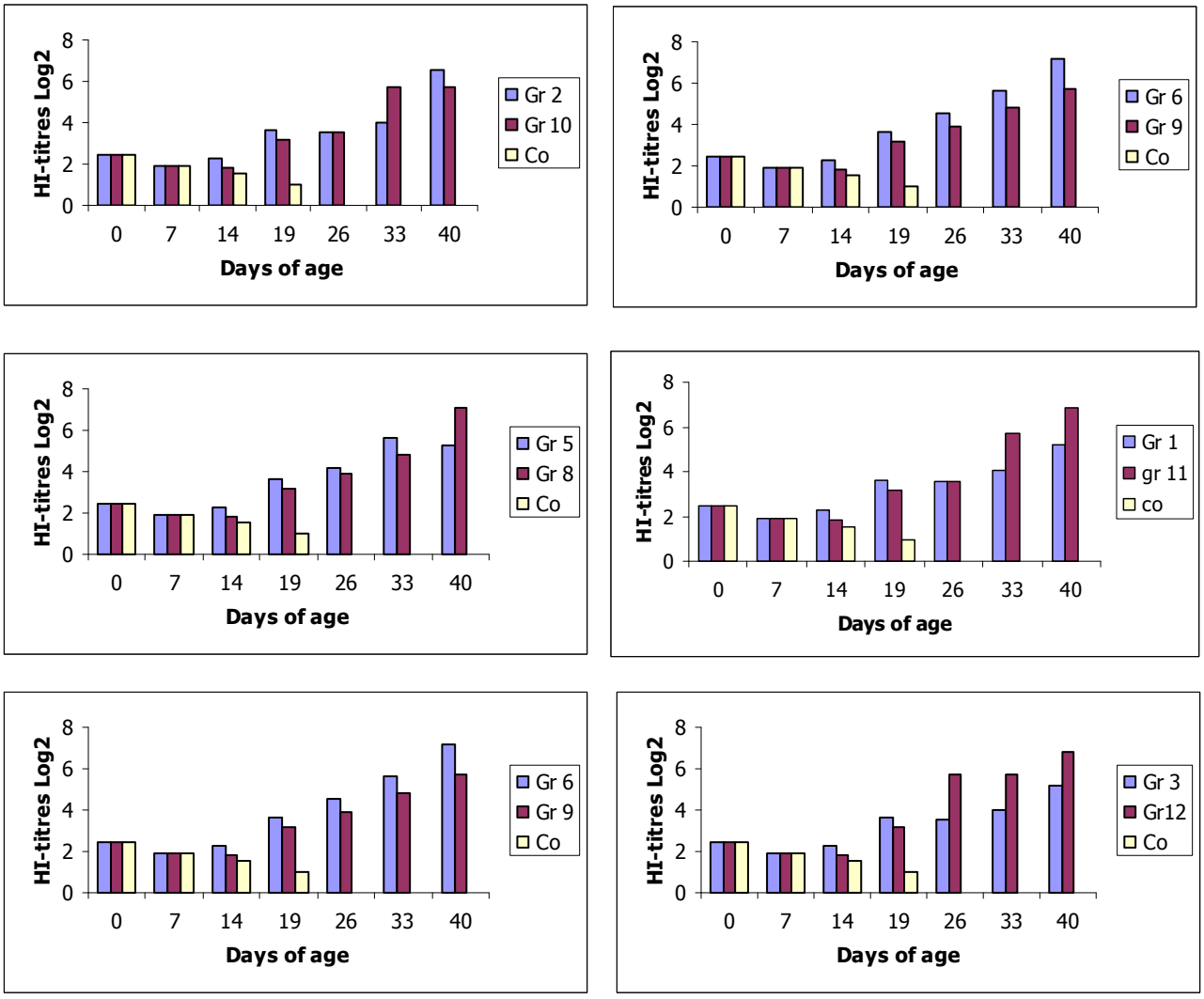

Fig. (1): Level of HI antibody log $_2$ titers in sera of chickens vaccinated against ND.

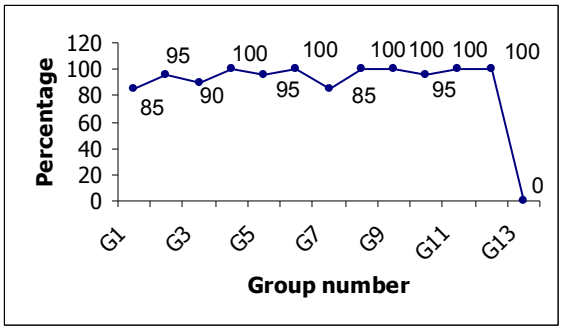

Fig. (2): Protection rate of challenged ND vaccinated chickens.

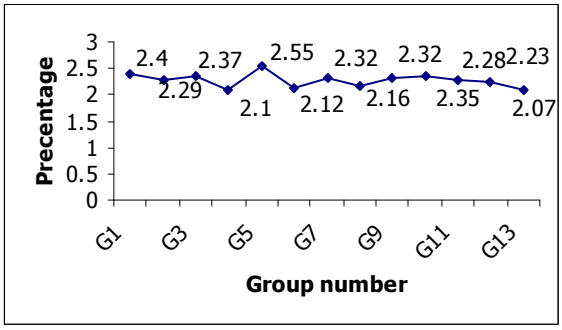

Fig. (3): Conversion rate of 40-days old ND vaccinated

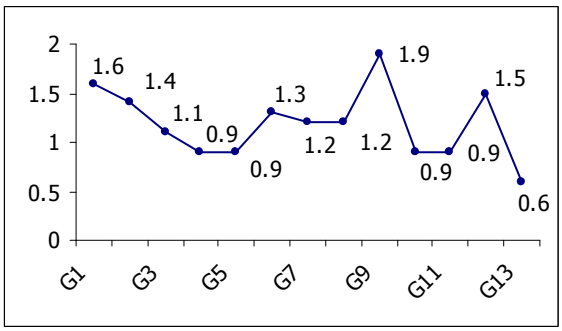

Fig. (4) : CRD lesion score in 40-days old ND vaccinated chickens.

received LB and LL vaccines. Results of challenge test (Table 6, Fig. 2) revealed statistically that the protection rate was only 85% for each of groups 1 and 7 which were significantly lower than groups 4, 6, 8, 9, 11 and 12 (100%). Birds received all ND vaccines from the same source (group 6, 9 and 12) showed 100% protection. In the other side, administration of LB vaccine instead of homologous La Sota at 33-days of age (groups 4 and 8) resulted in 100% protection rate as compared with 90% following heterologous La Sota (group 3). While groups 1, 7 and 10, which received, NB vaccine showed protection rates only 85%, 85% and 95%, respectively.

Post-mortem lesions of dead challenged birds were typical lesions of VVNDV infection, while the intestinal lesions were more obvious in vaccinated group 1 and 7 than those of 3 and 5. Results of the mean lesion score for CRD in vaccinated groups with different regimes (Table 7, Fig. 4) showed that vaccination with ND vaccinal strains source (1) inducing higher scores than vaccination with vaccines from source (2). Administration of LB vaccine at 33-days of age induced lower scores.

Vaccination of chicken groups with LB vaccine by ocular route at 33-days of age (boostered to 2 vaccinations) resulted in better immunity, better protection rates as well as higher performance than those received NB vaccine. Birds received LL vaccine 19-days of age following homologous vaccine revealed higher immunity and performance than those received NL vaccine.

Discussion

Newcastle disease live vaccines from lentogenic strains have been adopted to be used in the disease prevention since their first use until now. In our field there are vaccines from different sources, such vaccines are differ in their potency for prevention of the disease. In this study, we used ND vaccines from two sources for vaccination of broiler chicks serologically positive to Mycoplasmas.

The obtained mean HI titers at 40-days of age in group 6 and 8 that received vaccines from one source were (7.21 ± 1.78) and (7.31 ± 2.25), respectively. They were significantly higher than those of groups 1 (5.20 ± 1.66), group 3 (5.20 ± 1.20) and group 5 (5.25 ± 0.89) that received vaccines from different sources. Titers in groups 6 and 8 were 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) was significantly higher than those of groups 9 (5.77 ± 1.87) and 10 (5.73 ± 1.61) at $P< 0.05$.

General speaking, chicken groups received vaccine from the 2nd source showed higher titers than those received vaccines from the 1st source (groups 6 and 9). In addition, birds that were given Hitchner B1 and /or La Sota vaccines of source (2) at any time of vaccination age showed relatively higher titers. Similar results had been reported (Amer *et al*., l993; Min *et al*., 2002).

Results of the mean body weight at the 6th week of age pointed out that birds of non-vaccinated control group showed significantly ($P<0.001$) higher mean body weight (1311 ± 102.32) than those of vaccinated groups 1, 3, 6 and 10. The mean body weight in-group 10 was

significantly lower than those of group 7 (1295 ± 140.4), group 8 (1281 ± 114.3) at P< 0.001 and lower than that of group 9 (1238 ± 77.9) at P<0.05. In addition, the obtained body weight values in groups 4, 7 and 8 were 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 among groups received different vaccines.

In general, the usage of vaccines from homologous source induced improving in body weights than the usage of vaccines from the heterologous sources.

In addition, control non-vaccinated group showed higher feed conversion rate than all vaccinated chicken groups. Groups given La Sota ND strain vaccine at 33-days of age mostly showed lower rates than those received Hitchner.

Results of the challenge test indicated that birds of groups 1 and 7 that were given 2nd and 3rd vaccination from source (1) showed significantly lower protection rates (85%) for each than those of groups 4, 6, 8, 9, 11 and 12 that were given vaccines from source (2). In this way, Bananvare *et al*., (2001) concluded that, some vaccines were less potent than others up on comparing four commercial La Sota vaccines from different manufactures.

In the other side administration of Hitchner B_1 vaccine of source (2) instead of homologous La Sota at 33-days of age (groups 4 and 8) induced 100% protection percentage as compared with 90% following heterologus La Sota (group 3). Those results could be explained by findings of Thornton *et al*., (1980) who detected variation in protection among vaccinated chickens with vaccines made from the same strains, depending on their source. Also, Borland and Allan, (1980) found differences in the immunization capacities, potency levels and respiratory distress of 18 ND vaccines from different sources. La Sota vaccines were more varied, more heterogenous and more immunogenic than HB_1 vaccines. The relation between HI titers and challenge test had been discussed (Min *et al*., 2002).

CRD mean lesion scores in groups vaccinated with source (1) vaccines were higher than birds received vaccines from the other source. Administration of LB vaccine at 33-days of age shower lower scores than La Sota. Results that pointed out the role of vaccine in stimulation of CRD were reported (MacOwan *et al*., 1982; Gross, 1990; Nakamura *et al*., 1994).

Results of this work could be referred to the difference in the potency and in the immunogenicity or the antigenic relation between the vaccinal and the challenge strain. Similar explanation was stated (Schloer *et al*., 1975; Eidson and Kleven, 1980; Russel and Alexander, 1983).

We can conclude that when ND vaccines are used as preventive measure, they must be carefully chosen according to the disease history of the birds. Not all vaccines in the field are equal in their potency. Hitcher B_1 vaccines can be used as a 3rd vaccination dose at 33-days of age in birds under Mycoplasma stress.

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تأثير بعض لقاحات النيوكاسل الحية و برامج التحصين المختلفة على الإستجابة المناعية و الكفاءة الإنتاجية لدجاج تسمين إيجابى سيرولوجياً لعدوي الميكوبلازما

أجريت هذه الدراسة لإستبيان كفاءة لقاحات النيوكاسل الحية المنتجة من شركات مختلفة عند إستعمالها فى برامج مختلفة فى تحصين لقاحات النيوكاسل الحية فى دجاج التسمين الإيجابى مصلياً لكلٍ من الميكوبلازما جاليسيبتيكم و الميكوبلازما سينوفى ، تم إستبيان الإستجابة المناعية للقاحات النيوكاسل المستخدمة بإختبار مانع تلازن الدم و إختبار التحدى، كما قيمت الكفاءات التحويلية للطيور بتعيين متوسط الأوزان المكتسبة و كذلك تم إختبار مدى قدرة اللقاحات المستخدمة على إثارة البكتيريا التنفسية فى حساب معدل الآفات التشريحية. أوضحت الدراسة أن الطيور اللتى حصنت باللقاح من المصدر الثانى أعطت أجساماً مناعية مانعة اللتلازن أعلى من تلك الناتجة من إستخدام اللقاح من المصدر الأول. وأن الطيور اللتى تلقت التحصين الثانى من مصدر مخالف أعطت أجساماً أقل من اللتى حصنت بلقاح من نفس المصدر. نتائج إختبار التحدى أظهرت أن الدجاج المحصن ضد النيوكاسل بلقاحات من نفس المصدر و تلك اللتى تلقت لقاح من عترة هتشنر ب١ عند عمر ٣٣ يوم بدلاً من لاسوتا أعطيا نسب حماية ١٠٠% إذا ما قورن ذلك بـ ٩٥% فى المصادر المختلفة وكذلك اللاسوتا عند عمر ٣٣ يوم. كما أن المجموعات اللتى حصنت بلقاح هتشنر ب١ من المصدر الأول أعطت نسبة صد أقل (٨٥%). الطيور المحصنة أعطت معدلات تحويل أقل من المجموعات القياسية بصفة عامة، حيث أن المجموعات اللتى أعطيت لقاح عترة هتشنر ب١ كجرعة ثالثة أعطت معدلات تحويل أعلى من تلك المستخدم فى تحصينها لقاح من عترة لاسوتا. كما أن النتائج المحسوبة لمعدل التغير النسبى المرضى للمرض التنفسى المزمن فى الطيور المحصنة بالمعاملات المختلفة أظهرت أن الطيور المحصنة من المصدر الأول كانت أعلى منها فى اللقاحات من المصدر الثانى. أوضحت الدراسة أن إستخدام لقاح النيوكاسل المحضر من عترة هتشنر ب١ فى الدجاج عند عمر ٣٣ يوم كان أقل إحداثاً للتغيرات المرضية المحسوبة للمرض التنفسى المزمن مما يعطى الإنطباع بأهمية إستخدامه فى الطيور المصابة رأسياً بالميكوبلازما إذا ماكان هناك ضرورة للتحصين ضد النيوكاسل عند هذا العمر.