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COMPARISON BETWEEN THE SEROLOGICAL RESPONSE IN VACCINATED CHICKS WITH DIFFERENT COMMERCIAL ND AND IBV VACCINES EITHER ALONE OR COMBINED VACCINES

(With One Table & 3 Fig.)

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(Received at 31/12/1994)

المقارنه بين مستوى الاجسام المناعيه الناتجه عن التحصين بلقاح النيوكاسل الثنائي مع الالتهاب الشعبي الوبائي أو نتيجة للتحصين بكل لقاح على حده

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تم تقسيم ١٦٠ كتكوت عمر يوم واحد الى أربع مجموعات وتحصين الاولى والثانيه بلقاح النيوكاسل أو الالتهاب الشعبى الحى كل على حده . وفى المجموعه الثالثه تم استخدام اللقاح الثنائى من النيوكاسل والالتهاب الشعبى بطريقة العين والانف أما المجموعه الرابعه تركت كضوابط .

بعد أسبوعين من التحصين (أما بلقاح النيوكاسل أو الالتهاب الشعبى كجرعه أولى) تم تقسيم المجموعات الى أ ، ب حيث تم تحصينهم بالجرعه المنشطه من النيوكاسل للمجموعه (أ) والالتهاب الشعبى للمجموعه (ب) تم اجراء اختبار التحدى لجميع المجاميع بعد أسبوعين من التحصين بالجرعه المنشطه بحقنهم بالعتره الضاربه للقاح النيوكاسل .

تم قياس مستوى الاجسام المناعيه والنسبه المئويه للصد باستخدام الاساليب السيرولوجيه المختلفه وأظهرت النتائج الاتيه :

تم قياس نسبة الاجسام المناعيه باستخدام أختبار التلازن الدموى وكانت النتائج (٥ ر٢ - ٥ ر٦) فى المجموعه الاولى - (٣ - ٩ ر٦) فى المجموعه الثانيه اما المجموعه الثالثه (٩ ر٢) للقاح الثنائى ووصل الى (٩ ر٣ - ٣) بعد الجرعه المنشطه سواء النيوكاسل أو الالتهاب الشعبى الحى كل على حده .

أظهرت النتائج باستخدام القوه المتعادله للسيرم ان مستوى المناعه يتراوح من (٦ - ٣ ر٧) ، (٤ ر٥ - ٥ ر٣) فى المجموعات تباعاً .

كان معدل النسبه المئويه للصد بعد اجراء اختبار تحدى المناعه (٨٩ ٪ - ٧٥ ٪) فى المجموعتين الاولى والثانيه المحصنين باللقاحين كل على حده ، (٥٨ ٪ - ٣٤ ٪) للمجموعه الثالثه المحصنه باللقاح الثنائى .

SUMMARY

One hundred and sixty one-day-old chicks were vaccinated in this study. They were divided into 4 groups; group I and II were vaccinated with single live attenuated vaccine either Newcastle disease vaccine or Infectious Bronchitis vaccine separately, while group III was vaccinated with a combined ND and IB vaccine. All birds were vaccinated via occulonasal route. The fourth group was kept as non vaccinated negative control. Two weeks post vaccination the 4 groups were subdivided into two subgroups A and B, twenty birds per each. Subgroup A was boostered with single ND vaccine in all 3 groups while subgroup B was boostered with single IB vaccine in all the three groups. Two weeks post second vaccination, all birds were challenged with virulent NDV. The level of antibodies and the protection percentage were determined using different serological methods. The results showed that:

1- The Arithmetic Mean (AM) HI titer in the first group (Group I) was 2.5 Log_2 2 weeks after boostering with either ND or IB in birds of subgroup A and subgroup B respectively.

2- In the second group (group II), the arithmetic mean (AM) HI titer was 3 Log_2 2 weeks post vaccination with IB vaccine. After boostering with either ND or IB, the (AM) HI titer was 5 Log_2 2 weeks post vaccination in birds of subgroup A and subgroup B respectively.

3- The third group (group III), the (AM) HI titer was 2.9 Log_2 2 weeks post vaccination with combined (ND+IB) vaccine. Two weeks post vaccination with the booster dose with ND or IB, the (AM) HI titer was 3.9 Log_2 and 3 Log_2 in birds of subgroup A and B respectively.

4- The serum neutralization results showed that the neutralizing index (NI)) 2 weeks post second dose was 6, 6 in subgroup A and B in group I respectively and it was 2.3, 7.3 in subgroup (A and B) in group II respectively, while it was 5.4, 3.5 in subgroup (A and B) in group III respectively.

5- The protection percentage (%) after challenged with VVNDV (Velogenic Viscerotropic NDV) 2 weeks post second vaccination was 89% - 75% in subgroup

A and B in group I respectively and it was 75% in subgroup A in group II while it was 58%-34% in subgroup (A and B) in the group III respectively.

Keywords: Comparison, serological response, vaccinated chicks, ND, IBV Vaccines, alone or combined.

INTRODUCTION

Newcastle Disease (ND) and Infectious Bronchitis (IBV) are two of the major economically important viral respiratory diseases of chickens. IBV is a highly contagious disease of respiratory and urogenital tract of chickens. Young chicks develope respiratory disease whereas adult hens reduced egg production *(HOFSTAD, 1984)*. Despite the use of various vaccines and scheduling of vaccination programme still many losses due to mortality and reduced egg production in layer and air saculitis in broiler. Spray vaccines administrated in the hatchery have been used to improve protection, however serological profiling of broiler flocks after reveals poor immune response *(PARTADIREDJA et al., 1979)*. It has been shown that IBV strains can interfere with NDV growth in chickens, chicken embryos and cell culture *(BEARD, 1967)*. Previous work *(THORNTON and MASKETT, 1973)* has shown that the amount of challenge virus withstood by chicks given ND vaccine mixed with infectious bronchitis vaccine is less than in those given ND vaccine alone. During recent years, injectable oil emulsion inactivated vaccines against ND and IBV monovalent or bivalent have been extensively used *(GOUGH et al., 1981)*. The purpose of the present study was to investigate the effect of vaccination with ND and IBV, either alone or as a combined vaccine on the level of the immune response and the protection percentage in vaccinated chicks due to mixing two different viruses in combined vaccine.

MATERIAL AND METHODS

1) **Chicks:** One hundred and sixty 1-day-old chicks were used. They were obtained from the United Company for Poultry Production (UCPP).
2) **Vaccines:**
   a- Commercial living vaccine for NDV (Clone 30). It's titer was $10^{9.5}$ EID_{50}/ml.
   b- Commercial living vaccine for IBV (Strain H 120). It's titer was $10^{9.3}$ EID_{50}/ml.

3) **Virulent Challenge Virus:** Velogenic Viscerotropic NDV (VVNDV) strain, locally isolated *(SHEBLE and REDA, 1967)* with a titer of $10^{8.5}$ EID_{50}/ml.

4) **Chicken Eggs:** 9-10 day-old embryonated chicken eggs were used for virus titration. The eggs were supplied by UCPP.

Experimental Design:

One hundred and sixty birds 1-day-old chicks were divided into four groups:

Group I: Contained 40 birds were vaccinated with live attenuated single NDV vaccine (Clone 30) via occulonasal route, each bird received $Log10^5$ EID_{50}.

Group II: Contained 40 birds were vaccinated with live attenuated single IBV vaccine (Strain H 120) via oculaonasal route, each bird received $Log10^3$ EID_{50}.

Group II: Contained 40 birds were vaccinated with live attenuated single IBV vaccine (Strain H 120) via oculonasal route, each bird received $Log10^3$ EID_{50}.

Group III: Contained 40 birds were vaccinated with live attenuated two vaccines (Clone 30 + ND) via oculonasal route, each bird received 2 drops from both vaccines at the same time

Group IV: It contained 40 birds, were kept as positive control and non vaccinated negative control.

Two weeks post vaccination each group was divided into two subgroup A and B. All the birds in the subgroup A received the second dose (booster dose) of the vaccine with live NDV (Clone 30) via oculonasal at a dose of 0.03 ml while the birds in the subgroup B in all three groups were boostered with a single IB vaccine.

Two weeks post second booster dose all birds were challenged with VVNDV, blood samples were taken every weeks. The tested serum samples were inactivated at 56°C for 30 minutes and stored at - 20°C till the time of conducting the serological methods. PM and virus isolation from dead birds was performed for confirmation of the results.

RESULTS

Table (1) showed that in case of group I that vaccinated with Clone 30 alone the arithmetic mean HI titer was (2.5) 2 weeks post first dose while it reached upto (6.5) 2 weeks post second dose with ND and (5.4) 2 weeks post second dose with IB. The NI was 6 in both subgroup A and B, the protective percentage was 89% - 75% for subgroup A and B respectively.

In case of group II that vaccinated with IB (H 120) alone the arithmetic mean HI titer was (3.0) 2 weeks post first dose of vaccination, then it reached upto (5) 2 weeks post second

vaccination with ND and (6.9) 2 weeks post second vaccination with IB vaccine. The NI was 7.3 in both subgroup A and B, while the protection percentage after challenge with VVNDV was 75% in birds of subgroup A that boostered with ND vaccine. No challenge was done in subgroup B.

In group III that the birds were vaccinated with both NDV and IBV vaccine at the same time, the arithmetic HI titer was (2.9) 2 weeks post first dose of vaccination then it reached up to (3.9) HI titer 2 weeks post second dose of vaccination with ND vaccine (subgroup A) and (3.0) HI titer in 2 weeks post second dose of vaccination with IB vaccine (subgroup B). The NI was 5.4 - 3.5 in subgroup A and B respectively. The protection% was 58%-34% in birds of subgroup A and subgroup B respectively.

Fig. 1, 2 and 3 cleared the development of HI titer in chickens vaccinated with Clone 30 and IB vaccines either combined or separated in the three groups I, II and III.

DISCUSSION

Infectious Bronchitis Virus (IBV) causes serious economic losses in laying hens and young chicks worldwide *(HOFSTAD, 1984)*. Highly virulent strains of NDV affect both domestic and wild birds *(BEARD and HANSON, 1984)* and have been the major problem in the poultry industry throughout the world for many years. Avian respiratory diseases due to mixed infection by various organisms are very common and show more severe symptoms than diseases due to single infection *(MALIK and VERMA, 1969)*. Therefore, use of a mixed vaccine is preferable to that of a single agent vaccine for prevention of the common avian respiratory diseases.

Mixed vaccines have been become widely used. Most of these combination products are live attenuated vaccines. Mixed live virus vaccine may produce low antibody titers and the immunologically effective period may be shorter than that observed with live single virus vaccine. This inhibition in antibody response is a result of interference, a phenomenon demonstrated among various kinds of viruses *(BRATT and RUBIN, 1968, and CROWELL, 1966)*. During recent years, injectable oil - emulsion inactivated vaccines against NDV and IBV (monovalent or bivalent) have been extensively used *(GOUGH et al., 1977 and STONE et al., 1978)*. The purpose of the present study was to investigate possible deleterious effects on efficacy due to mixing two different viruses in combined vaccine between IBV and NDV. Our results indicated that the AMHI titer was 2.5 Log_2 2 weeks post vaccination with ND (Clone 30) in the first group,

after the second booster dose with ND in subgroup A the AMHI titer reached up to (6.5) Log2 2 weeks post second vaccination and the NI titer was 5 Log2. The Protection % after challenge with VVNDV was 89% (Table 1 and Fig. 1). While in case of subgroup B which took the second booster dose with IB vaccine the AMHI titer was 5.4 Log2 2 weeks post vaccination, the NI was (6) 2 weeks post vaccination and the protection % reached 75% post challenged with VVNDV (Table 1 and Fig. 1). In case of group two we started the vaccination with IB vaccine at 1 day old chicks and 2 weeks post first vaccination the subgroup A vaccinated with ND as a booster dose and showed AMHI titer (5) Log2 2 weeks post vaccination the NI titer was (7.3) Log2. While the protection % after challenged with VVNDV was 73%. In case of subgroup B in which the birds were vaccinated with IB vaccine as a boostered dose 2 weeks post first vaccination showed AMHI titer (5.9) Log2 and NI was (7.3) (Fig. 2 and Table 1).

HALVORSON et al. *(1991)* vaccinated broiler chicks against ND and IBV at 2 weeks of age as either primary or secondary vaccinations. The vaccine was administrated as a spray at 2 weeks of age to chicks that have received NDV alone, bronchitis alone, both vaccines in combination. They proved that the ND haemagglutination - inhibition response was significantly higher in chicks receiving a primary vaccination at 2 weeks of age than did chicks vaccinated at day 1 alone, chicks vaccinated day 1 and 2 weeks and unvaccinated control birds.

PARTADIREDJA et al. *(1979)* showed that aerosol NDV vaccine at day 1 in chicks from parents vaccinated with killed NDV vaccine resulted in a lower or variable HI response.

KELLEHER and LEMAR (1990) reported high *mortality* after NDV challenge of broiler vaccinated in the field or the laboratory. After analyzing vaccine titers, they conducted that in their study low post vaccinal enzyme - linked immunosorbent assay (ELISA) results and poor protection were the result of sub-optimal NDV vaccine dosages as established by *WINTERFIELD and SEADALE (1957).*

HALVORSON et al. *(1991)* showed that a secondary vaccination for IBV (Ark99) at 2 weeks of age resulted in significantly higher titers than vaccination alone at day 1, vaccination alone at 2 weeks and no vaccination. Our results showed that the protection % was 89% in chicks of group I and subgroup A in which they received ND as primary and secondary dose while it reached 75% in chicks subgroup B in which they received IB as a secondary vaccination. While in the group III in which the birds vaccinated with the mixed combined vaccine (IB + ND) the birds in subgroup A in which they received ND

vaccine as a second dose their protection % was 57% while chicks in subgroup B in which the chicks received IB vaccine as a secondary dose, the protection % was 34%.

Our reslts is consistent with those of *RAGGI and LEE (1964)* as they found that IB virus interferes with ND virus as judged by the absence of significant HI antibodies by susceptibity to ND challenge test *RAGGI,(1963)* has recently shown that IB virus is extremely small and also capable of tremendous invasiveness (*RAGGI, 1963).* It is therefore theorized that the capacity of IB virus to rapidly invade the cells and become a part of metabolic patterns of a cell may prevent ND multiplication in the same cells resulting in the absence of significant HI antibody titer and susceptibility to an ND challenge in the majority of the birds

The same author proved that marked interference occurred when the two viruses were given simultaneouly under field conditions. In the absence of IBV virus a maximal multipication of ND virus would occurred which in all probapility would have resulted in resistance. They found that, in all field trials, the majority of birds given the IB and ND combination were found to be susceptible to ND challenge coupled with the results in obtained in the laboratory trials.

RAGGI and PIGNATTELLI (1975) reported that (Mass and Conn) types of IBV were identified by interference in embryonating chicken eggs (ECE) with the production of haemagglutinin by the B_1 isolant of NDV. This interference test appears to be specific because the above interference was eliminated by adding type - specific anti - IBV serum to the IBV-NDV system , however , interference was not detectable when fowl pox virus and infectious Laryngeotracheitis virus (ILT) were substituted for IBV.

Our results indicated that in group III in which chicks were vaccinated at 1 day old with mixed combined vaccine of IBV and NBV as first dose then subgroup A received NDV as a second dose vaccination 2 weeks post 1 st vaccination with combined vaccine while subgroup B received IBV as a second dose of vaccination 2 weeks post frist vaccinatation with both vaccine. The AM haemagglution inhibition was 2.9 Log_2 2 week post vaccination with combined vaccine it reached to 3.9 Log_2 HI in case of subgroup A and (3) HI titer in case of subgroup B. Also the NI was (5.4) and (3.5) 2 weeks post vaccination of second dose in subgroup A and B respectively (Table 1 and Fig 3). The protection % dropped till 58% and 34% after challenge with VVNDV 2 weeks post vaccination in subgroup A and B respectively.

KOICHI and YOSHIKAZU (1973) proved that mixed live - virus vaccine may produe low antibody titers and the immunologically effective period may be shorter than observed with live single virus vaccine. The author prepared a new mixed vaccine composed of inacitvated NDV , and HG, protection levels of antibodies were evident 3 weeks after vaccination , it is recognized that interference occurs when different live viruses are mixed in a vaccine (*SOSAK et a1., 1969*) .This is one of the factors responsible for reduction in the efficacy of a combined live vaccine . On the other hand interference is not expected when vaccine consisted of different kinds of inactivated viruses.

From the previous results we recommended that polyvalent vaccines perform better when made up of combined monovalent vaccines rather than with mixed antigens that are then emulsified. The ND (HI) response was significantly higher in chicks receiving ND vaccines as a primary vaccine. In contrast the bronchitis HI response was significantly higher in chicks receiving bronchitis vaccine as a secondary vaccination. Also from the previous results the combining IB and ND vaccines is discouraged.

REFERENCES

Beard, C.W. *(1967):* Infectious bronchitis virus interference with Newcastle Disease virus in monolayers of chicken kidney cells Avian Dis , 11: 399-406.

Beard, C.W. and Hanson, R.P. (1984): Newcastle Disease In: Diseases of Poultry , 8 th ed .M.S. Hofstad; H.J. Barnes, B.W. Calnek; W, M.Reid and H.W. Yoder, Jr, Eds. Iowa State Univ. Ames Iowa pp.452-470

Bratt, M.A. and Rubin, H. (1968): Specific interference among strains of Newcastle Disease Virus. II. Comparison of interference by active and inactive virus. Virology , 35:381- 394.

Crowell, R.L.(1966): Specific cell surface alteration by enterovirus as reflected by viral - attachment interference. J. Bacteriol., 91: 198-204

Gough, R.E.; Allan, W.H. and Nedelciu, D.(1977): Immune response to monovalent and bivalent Newcastle Disease and Infectious Bronchitis inactivated vaccines.Avian Path .,6:131-142

Gough, R.E.; Wyeth, P.J. and Bracewell, C.D. (1981): Immune response of breeding chicken chicken to trivalent oil emulsion vaccines :Response to infectious bronchitis . Vet Rec .,108:99-101

Halvorson, D.A.; Shaw, D.; Sivanandan; Barbour, E.K.; Masheshkum, S.; Newman, J.A. and Newman, L. (1991): Serological response in broiler chicks to different commercial Newcastle Disesase and Infectious Bronchitis vaccine Avian Dis., 35: 978-981.

Hofstad, M.S. (1984): Avian Infectious Bronchitis. In: Diseases of Poultry 8 th ed. M.S. Hofstad H.J. Barnes, B.W .Calnek, W.M. Reid and H.W. Yoder, Jr ,eds. Iowa State Univ .Ames Iowa PP. 429-443.

Kelleher, C.J. and Lemar, M.S. (1990): Newcastle Disease Virus vaccination of broiler. Proc. 25 th National Meeting on Poultry Health and Condemnations , Delmarva Poultry Industry , Inc., Occan City , Md . PP 77-79. October 1990.

Koichi, O. and Yoshikazu, I. (1973): Preparation and Immunological Reponse to a New Mixed Vaccine Composed of Inactivated IB and Inactivated Haemophilus gallinarum. Avian Dis ., Vol. 18, NO. (3), (1973).

Malik, B.S. and Verma, K.C. (1969): Coexistence of antibody against respiratory diseases, Infectious Laryngotracheitis and Infectious Bronchitis on poultry farms of Uttar. Pradesh, Andra Pradesh and Madras. Avian Dis., 13: 158-162

Partadiredja, M.; C.S. Eidson and Kleven, S.H. (1979): A comparison of immune response of broiler chickens to different mthods of vaccination against Newcastle Disease. Avian Dis., 23: 622-632.

Raggi, L.G. (1963): Unusual characteristics of Infectious Broncchitis Virus. Presented at the AVMA annual meeting New York, N.Y.

Raggi, L.G. and Lee, G.G. (1963): Infectious Bronchitis virus interference with the growth of Newcastie Disease Virus. I. study of interference in chicken embryo. Avian Dis., 7: 106-122.

Raggi and Pignattelli (1975): Identification of IB virus by interference with the B-1 isolant of Newcastle Disease Virus. Waxing and waning of interference. Avian Dis., 19: 334-342 (1975).

Sasaki, N; Hirahara, T. and Izumida, A. (1969): Application to comparative of vaccination against Newcastle Disease (ND), Avian Infectious Bronchitis (IB) and Avian infectious Iaryngo-tracheitis (ILT). I. Some observations on several combination vaccines and occasionally with vaccination. Japan. J. Vet. Sci., 31(Suppl.) :18 (Japanese).

Sheple, A. and Reda, I. (1967): Cited by Khaphagy, A.K. (1972), M.V.Sc. Thesis, Fac. Vet. Med., Cairo University.

Stone, H.D.; Brugh, M.; Hopkins, S.R.; Yoder, H.W. and Bread, H.W.(1978): Preparation of inactivated oil emulsion vaccines with avian viral or mycoplasma antigens. Avian Dis., 22 : 666 - 674.

Thornton, D.H. and Maskett (1973): Effect of infectious bronchitis vaccination on the performance of live Newcastle Disease vaccine. Vet. Rec., 86 : 467 - 468.

Winterfield, R.W. and Seadale, E.H. (1957): Newcastile Disease immunization status. 2-The immune response of chickens vaccinated with B1 NDV administrated through the drinking water . Poult. Sci., 36 : 54 - 64.

Table (1) : The parameter that resulted from vaccination of chickens with clone 30 or IB vaccine either alone or combined vaccine (ND + IB) challenged post second dose of vaccination with VVNDV.

Type of the vaccines	Weeks post vaccination						No. of dead birds / Total No.		Protection %	
	2 weeks post 1 st dose of vaccination		2 weeks post 2 nd dose of vaccination							
			HI titer (AM)		NI					
	HI (AMT)	SN (NI)	Sub group (A)	Sub group (B)	Sub group (A)	Sub group (B)	Sub group (A)	Sub group (B)	Sub group (A)	Sub group (B)
ND (Clone 30) group I	2.5	ND	6.5	5.4	6	6	18/20	16/20	89	75
IB (H 120) group II	3.0	ND	5.0	6.9	7.3	7.3	16/20	ND	75	ND
Combined vaccine (IB + ND) group III	2.9	ND	3.9	3.0	5.4	3.5	12/20	14/20	58	34
+ ve Control							0/20		100	100
- ve Control							20/20		0	0

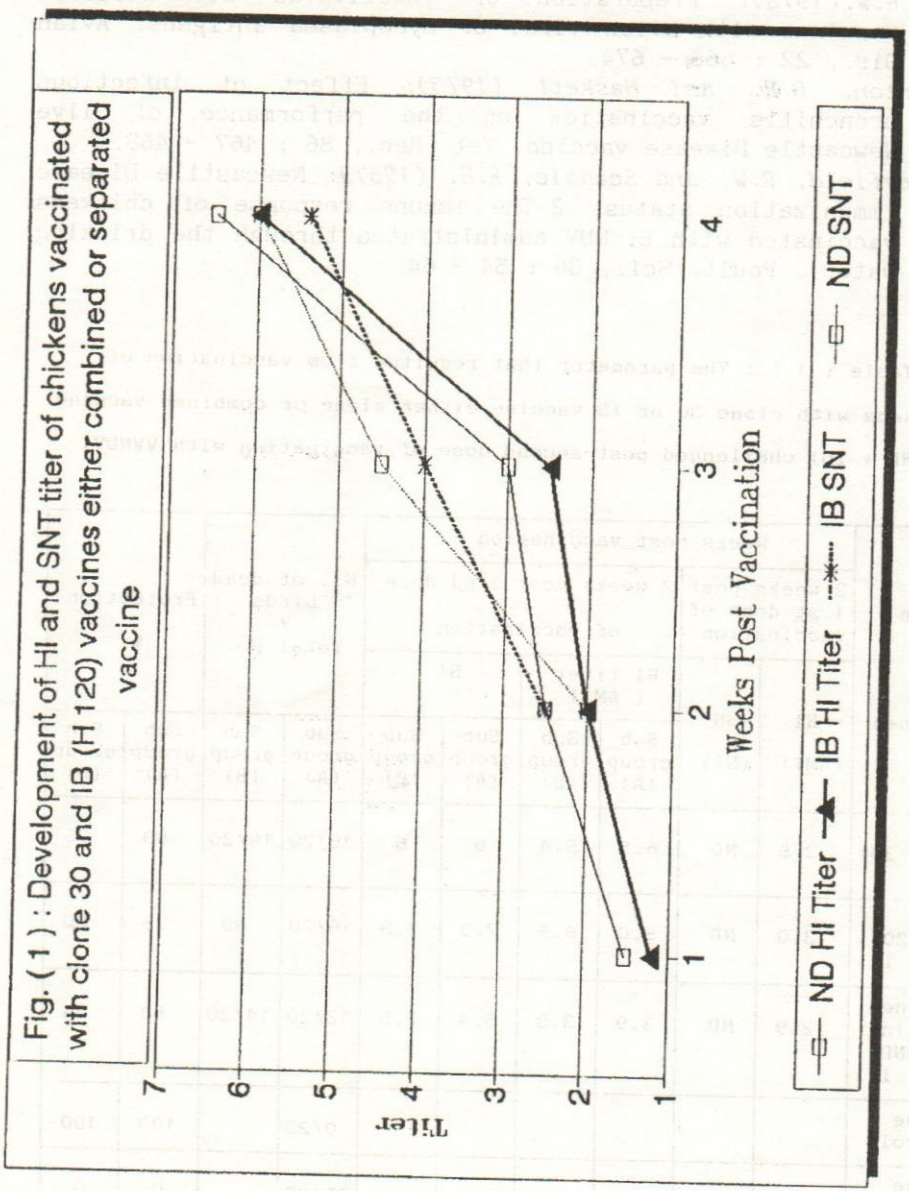

Fig. (1) : Development of HI and SNT titer of chickens vaccinated with clone 30 and IB (H 120) vaccines either combined or separated vaccine

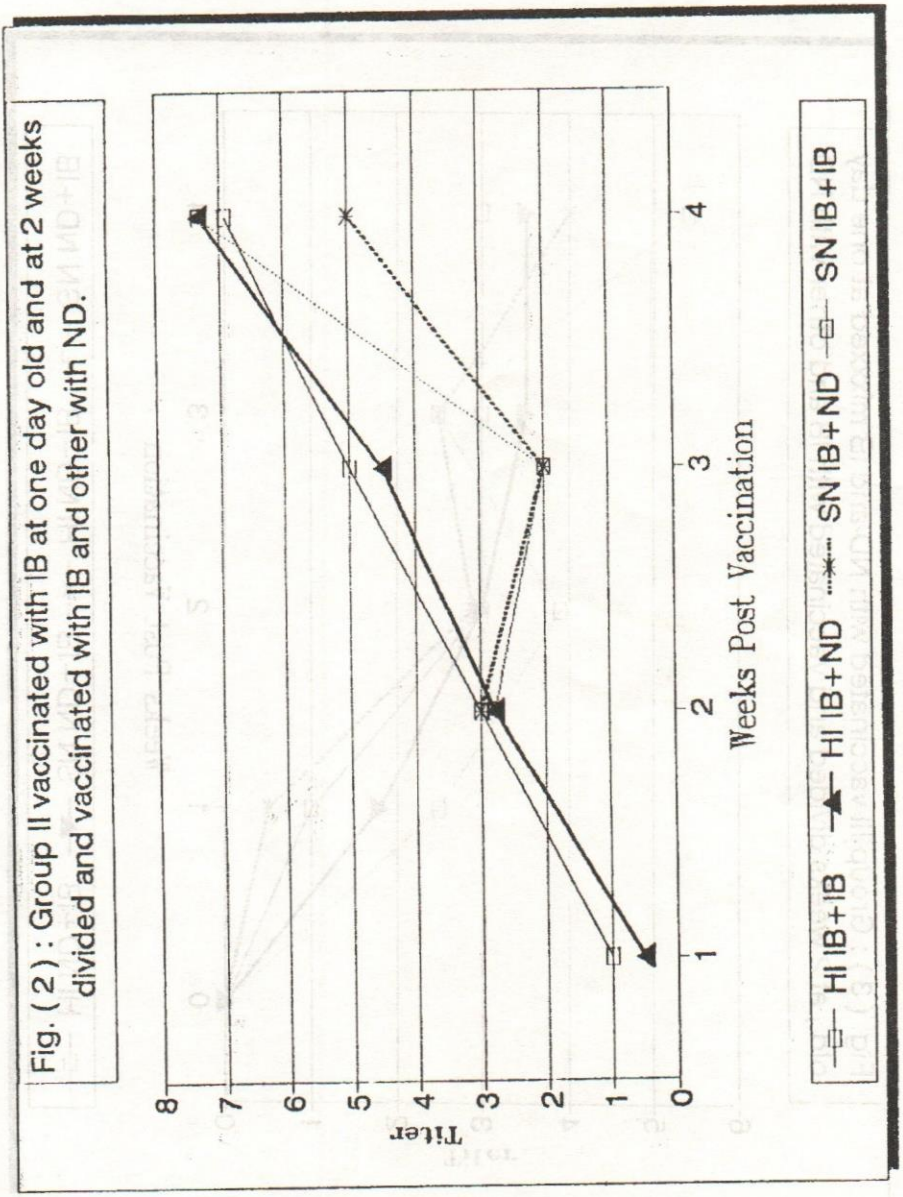

Fig. (2) : Group II vaccinated with IB at one day old and at 2 weeks divided and vaccinated with IB and other with ND.

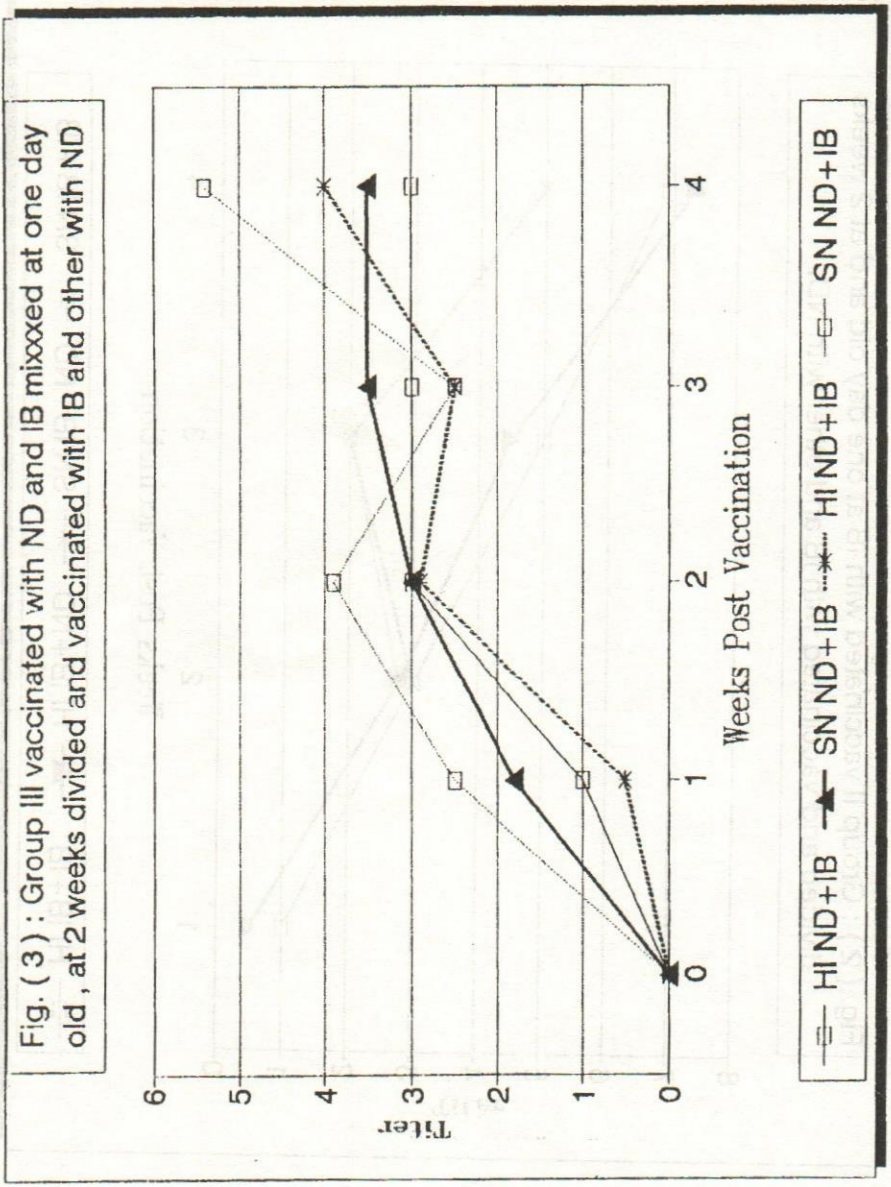
Fig. (3) : Group III vaccinated with ND and IB mixxxed at one day old , at 2 weeks divided and vaccinated with IB and other with ND
Titer
0
1
2
3
4
5
6
Weeks Post Vaccination
0
1
2
3
4
HI ND+IB
SN ND+IB
HI ND+IB
SN ND+IB

SUMMARIES OF THESIS

Assiut Vet. Med. J. Vol. 32 No. 64, January 1995.