

قسم: أمراض الدواجن.
كلية: الطب البيطري - جامعة القاهرة.
رئيس القسم: أ.د. / ابراهيم عبدالمعطي.

دراسة مقارنة عن رد الفعل المناعي للدجاج المحصن
بلقاح النيوكاسل المختلفه بطريقة الحقن في العضل

مصطفى بسطامي، محمد عامر، أحمد حموده

تم دراسة رد الفعل المناعي لخمس لقاحات ضد مرض النيوكاسل وهي لقاح
التهشنر ب، واللاسوتا والتهشنر ب، مع هيدروكسيد الألومنيوم والكوماروف ولقاح
النيوكاسل الميت حقنت جميعها في مجاميع من كتاكيت التسمين عمر ٢١ يوم
والتي سبق تحصينها بلقاح التهشنر ب، بالتقطير بالعين عند اليوم السابع من العمر.

وقد استخلص من النتائج ان مقارنة اللقاحات عند حقنها عضليا قياسا
بمستوى الأجسام المناعية المانعة للتلازن في الدم وكذلك نسبة الصد عند اجراء
اختبار التحدى بواسطة عترة ضارية من فيروس النيوكاسل كانت بالترتيب الاتي:

عترة التهشنر ب، يليها لقاح النيوكاسل الميت - ثم عترة التهشنر ب،
مع هيدروكسيد الألومنيوم وعترة اللاسوتا واخيرا عترة الكوماروف.

بسم الله الرحمن الرحيم
الحمد لله رب العالمين
والصلاة والسلام على سيدنا محمد وآله

والصلاة والسلام على سيدنا محمد وآله
والصلاة والسلام على سيدنا محمد وآله

والصلاة والسلام على سيدنا محمد وآله

والصلاة والسلام على سيدنا محمد وآله
والصلاة والسلام على سيدنا محمد وآله
والصلاة والسلام على سيدنا محمد وآله

والصلاة والسلام على سيدنا محمد وآله
والصلاة والسلام على سيدنا محمد وآله
والصلاة والسلام على سيدنا محمد وآله

والصلاة والسلام على سيدنا محمد وآله
والصلاة والسلام على سيدنا محمد وآله
والصلاة والسلام على سيدنا محمد وآله

Dept. of Poultry Diseases,
Fac. of Vet. Med., Cairo University,
Head of Dept. Prof. Dr. I. Abd-Elmoty.

**COMPARATIVE STUDY ON THE IMMUNE RESPONSE
OF CHICKENS VACCINATED INTRAMASCULRLY
WITH DIFFERENT NEWCASTLE DISEASE VACCINES**

(With One Table)

By

M.A. BASTAMI, M.M. AMER and A.S. HAMOUDA

(Received at 25/2/1986)

SUMMARY

In a comparative study on five different types of Newcastle disease (ND) vaccines (Hitchner, B₁, La sota, Aluminium hydroxide (AH) adsorbant Hitchner B₁ strain, Komarov and Inactivated vaccine injected intramuscularly in to 21 day old broiler chicks, which were previously vaccinated with Hitchner B₁ occularly at the age of 7 days. This study revealed that for intramuncular route of vaccination the most suitable vaccine is Hitchner B₁ followed by oil adjuvent inactivated, AH-adsorped Hitchner B₁ then La Sota and finally the local komarov vaccine.

INTRODUCTION

Newcastle disease had been recognised for the first time by DOYLE (1927) in England, while DAUBNEY and MANSI (1947) had reported the enzootic of the disease in Egypt and thus posses a serious threat to the development of the poultry industry.

A variety of vaccines were therefore used in an attempt to control the disease, but it become evident that the most widely used methods of vaccination are drinking water, eye drop and spray with F,B₁ and La Sota types of vaccines, did not aloways give adequete protection against the virulent local disease virus.

The intramuscular (i.m) route of vaccination with lentogenic vaccines had been reported by ASPLIN (1952), MAZZARACCHIO and ORFEI (1956), NILAKANTAN *et al.* (1960) Rizk and CHU (1973) where "F" strain was used. Hitchner B₁ live vaccine was used for i.m vaccination of chickens by NILAKANTAN *et al.* (1960). La Sota vaccinal virus was used as i.m vaccine by BANKOWSKI (1974), VECTEN and LAARHOVEN (1975) as well as SAAD and BASTAMI (1982).

Alluminium hydroxide (AH) adsorped living ND vaccinal strains were intramuscularly used by NAKAMURA *et al.* (1956), SEMERDJIEV (1962) and NEDELCIN *et al.* (1963).

The usage of komarov (K) attenuated i.m vaccine had been reported for immunization of chickens against ND by ZUGDAM (1953), EL-NASSERI (1958), NILAKANTAN *et al.* (1960), EL-SISI (1966), AHMED *et al.* (1967), ACURIA (1975) and RIZK and CHU (1973).

Inactivated oil adjuvent ND vaccine was used for vaccination of chicken's MITCHOLL and WALKEN (1953), HOFSTAD (1953 & 1968), JACOTO and VALLEE (1962), KEEBLE and COID (1962), HOFSTAD and YODER (1966), GOXDON (1971), LEVEY and ZAKAY-RONES (1973),

BOX and FURMING (1975) and EIDSON (1981).

This work was planned to evaluate the protective efficacy of different ND vaccines used intramuscularly against ND by both Hemagglutination inhibition (HI) and challenge tests as comparative criteria.

MATERIAL and METHODS

- **Embryonated chicken eggs:** Commercial fertile chicken eggs were used in this experiment.

- **Experimental chicks:** Two hundreds and ten Cross breed Hubbard chicks as one day old chicks were used.

- **Vaccinal strain viruses:** ND Hitchner B (TAD, Lot. no. 209/1) with $10^{8.92}$ EID₅₀/vial 1000 doses was used for both ocular and i.m injection. La Sota vaccine (TAD, Lot. no. 575) containing $10^{8.51}$ EID₅₀/vial 1000 doses, Aluminium hydroxide adsorped living Hitchner B₁(TAD, Lomavac, Lot. no. 297/ L) Containing $10^{9.23}$ /vial 1000 doses komarov attenuated vaccine (Anim. Res. Inst., Abassia, Cairo, lot No. 8717) with $10^{7.92}$ /vial 1000 doses as well as inactivated oil adjuvent vaccine (Rhone Merreux, Lot No. 40U191) were used for i.m vaccination of experimental chicks. The doses for i.m injection were adjusted to contain 10^6 EID₅₀ for each.

- **Challenge virus:** A velogenic viscerotropic ND virus local strain identified by SHEBLE and REDA (1976) was used.

- **Determination of virus infectivity:** Titration of the used living vaccines and challenge virus before use was carried out according to ANON (1971), while the EID₅₀ was calculated according to REED and MUENCH (1938).

- **Hemagglutination inhibition (HI) test:** The B-procedures of the HI test was employed using the micromethodology according to TAKATSY (1956) and the titres were given titre reference numbers (TRN) according to KALETA and SEIGMANN (1971).

Challenge test: A challenge dose of 10^6 EID₅₀ per bird was intramuscularly injected. The challenged birds were daily observed for symptoms and/or specific mortalities for 3 weeks. Birds with symptoms and survived till the end of the observation period were considered as if dead.

- **Statistical analysis:** Analysis of the obtained data was performed according to SNEDECOR (1956).

NEWCASTLE DISEASE VACCINES

EXPERIMENTAL DESIGN

Two hundred and ten one day Hubbarred chicks were used. At the age of one week, 30 chicks were randomly collected and left as non-vaccinated negative control (Group 1); while the rest of the birds (180) were vaccinated with Hitchner B₁ vaccine via the ocular instillation. At the 3rd. week of life 30 chicks were left as Hitchner vaccinated control (Group 2) and the remaining birds (150) were then divided into 5 equal groups; consists of 30 birds each (Group 3-7); and subjected to i.m vaccination as follows:

- Group 3, injected with Hitchner B₁ vaccine.
- Group 4, injected with La Sota vaccine.
- Group 5, injected with AH-adsorped Hitchner B₁ living vaccine.
- Group 6: injected with Komarov vaccine.
- Group 7, injected with inactivated oil adjuvent vaccine.

Fifteen random blood samples were collected from each group at 1,2,3,4,5,6 and 7 weeks of age for serum collection. These sera were subjected to HI-test for determination of HI-antibody titres. At the 7th. week of age (4 weeks after i.m vaccination) chickens of all groups were challenged and kept under observation for 3 weeks. The obtained results are shown in Table (1).

RESULTS

Results in table (1) are showing that:

a) HI-antibody titres

- Nonvaccinated control group (1) showed undetected titres at the 3rd. week of life and still negative up to the age of challenge.
- Hitchner B₁ vaccinated control group (2) showed the highest titre at the 4th. week of age (3.00) followed by deresing titres to be 1.07 at the 7th. week.
- Birds injected with Hitchner B₁ (Gr.3) and inactivated vaccine (Gr. 7) showed increasing HI-titres to reach the level of 3.46 and 5.20 at the 7th week; respectively.
- La Sota (Gr. 4) and AH-adsorped Hitchner B₁ (Gr. 5) injected birds showed the highest titre 3.73 and 3.86 at the 6th week of life (3rd post-injection) and decreased to 3.47 and 3.46; respectively at the 7th week.
- Birds of group 6, those received komarov vaccine showed the highest titre 3.53 at the 5th week then decreased to 2.00 at the 7th week of age.
- Comparing the HI-results obtained from the injected groups (3-7) birds vaccinated with inactivated oil vaccine showed the highest titres followed by those vaccinated with AH-adsorped Hitchner B₁, La Sota, Komarov and finally Hitchner B₁ vaccine.

b) Protection rates

The protection rate against the challenge virus was the highest (86.67) in group 3 (vaccinated i.m with Hichner B₁) followed by 83.33 in group 7 (vaccinated with oil adjuvent), 73.33 in group 5 (vaccinated i.m with AH-adsorped), 63.33 in group 4 (that vaccinated with La Sota) and 60 in group 6 (vaccinated with the i.m komarov). moreover, the Hitchner B₁ eye drop vaccinated control (Gr. 2) showed 46.67% protection and the nonvaccinated control

(Gr. 1) showed Zero per cent protection.

Statistical analysis of the challenge test results proved that group 2 is significantly decrease than those of groups 3, 5 and 7 ($P/ \underline{\quad} 0.05$), and groups 4 and 6 are significantly decrease than those of group 3 and 7 at $P/ \underline{\quad} 0.05$.

DISCUSSION

In Egypt, it is well known that ND is highly epidemic and causes a serious losses among poultry. Also the individual methods of vaccination (eye drop and injection) are the most suitable methods to face this epidemics ALLAN *et al.* (1973) and RIZK and CHU (1973). So our study was carried out to detect the most effective ND vaccine it is i.m administered.

Results of HI-test showed the HI-titres in sera of birds i.m vaccinated with lentogenic strains (Hitchner B₁, La Sota and AH-adsorped B₁ vaccine) are higher than those in vaccinated with i.m komarov, these results disagree with those reported by MAZZARACJOP and ORFEI (1956), NILAKANTAN *et al.* (1960) and ALLAN *et al.* (1973). Oil adjuvent vaccinated birds showed highest and perisistant HL-level this finding is simillar to those reported by ALLAN *et al.* (1973), CERSI and NARDELLI (1974) and BOX and FUNMINGER (1975). Birds vaccinated with AH-adsorped vaccine (Gr. 5) showed slightly higher HI-antibodies than those injected with Hitchner B₁ vaccine only, this does not completely agree with results of SOMERDJIEV (1962) who found that birds vaccinated with AH-adsorped komarov resulted in HI-titres 4-14 times higher; 14 days after injection, than those injected with komarov vaccine alone.

The challenge test showed that the injection of lentogenic vaccines resulted in higher protection rates, 86.67, 73.33 and 63.33 in group 3,5 and 4; respectively; than the komarov vaccinated (60%) these results agree with those reported by SAAD and BASTAMI (1982) who reported that La Sota i.m vaccine showed higher protective rate than the komarov, and disagree with those reported by NILAKANTAN *et al.* (1960) and ALLAN *et al.* (1973). The 83.33% protection obtained in group 7 vaccinated with inactivated oil vaccine is so far simillar to those reported by HOFESTAD and YODER (1966) who repourted 86% protection rate, while our result is higher than those reported by LOMBARDI and COAVO (1976) who reported 60% protection rate in birds with HI-titres 5.12. Protection rate of birds vaccinated with AH-adsrped vaccine (Gr. 5) is lower than those vaccinated with vaccine without aluminium hydroxide (Gr. 3), this can be supported with those reported by PALATKA and TOTH (1966) who mentioned that immunogenicity of B₁ strain with AH was lower than that of B₁ strain in saline.

From the present investigation it can be conculuded that for intramuscular route of vaccination the most suitable vaccine is Hitchner B₁ followed by oil adjuvent, Aluminium hydroxide adsorped live vaccine, La Sota and finally the fosal komarov.

NEWCASTLE DISEASE VACCINES

REFERENCES

- Acuria, H., McGergor, C. and Lora, C. (1973): Immunogenicity and safety testing of komarov strain Newcastle disease vaccine in broiler and breeding flocks. *Revista del Instituto de Zoonosis e Investigacion Pecuaria*, 2, 86.
- Ahmed, A.A.S., Reda, I.M., Abdasi, K.H. and El-Sisi, M.A. (1974): Studies on the immune response to vaccination against Helcafille disease in U.A.R. *J. Vet. Sci. U.A.R.* 4, 115.
- Allan, W.H., Lancaster, J.E. and Tjoth, B. (1973): The production and use of Newcastle disease vaccines, FAO, United Nations, Rome.
- Anon (1971): Methods for examining poultry biologics and for identifying and quantifying avian pathogens. National Academy of Science, Washington, D.C.
- Asplin, F.D. (1952): Immunization against Newcastle disease a virus of low virulence (Strain F) and observation on subclinical infection in poultry resistant fowls. *Vet. Rec.* 64, 245.
- Bankowski (1974): Anamnestic response to revaccination as measured by hemagglutination inhibition antibodies to Newcastle. *Develop. Biol. Standard.* 25, 357.
- Box, P.G. and Furminger, I.G.S. (1975): Newcastle disease antibody levels in chickens after vaccination with oil emulsion adjuvant killed vaccine. *Vet. Rec.* 96, 108.
- Daubnuy, R. and Mansi, W. (1947): The occurrence of Newcastle disease in Egypt. *J. Comp. Path.* 58, 189.
- Doyle, T.M. (1927): A hithero unrecorded disease of fowls due to filterpassing virus. *J. Comp. Path. Therap.* 40, 144.
- Eidson, S.C. (1981): Vaccination of breeder chickens and their progeny with alive or with an inactivated oil emulsion Newcastle disease vaccine. *Develop. Biolog. Standard.* 51, 191.
- El-Nasseri, B.B. (1958): Control of Newcastle disease in Egypt. Immunity status. *Agr. Res. Rev. Egypt, Ministry of Agr.* 36, 426.
- El-Sisi, M.A. (1966): The role of different existing vaccines in the control and prevention of Newcastle disease. MD. Vet., Thesis, Facult. Vet. Med., Cairo UNI.
- Gordon, R.F. (1971): Newcastle disease. *Vet. Rec.* 88, 261.
- Hofstad, M.S. (1954): The secondary immune response in chickens revaccinated with Newcastle disease virus vaccines. *Am. J. Vet. Res.*, 15, 604.
- Hofstad, M.S. (1968): Comparative immunogenicity of three strains of Newcastle disease virus used in inactivated vaccines. *Av. Dis.* 12, 665.
- Hofstad, M.S. and Yoder, H.W. (1966): Immunogenicity of Newcastle disease virus preparations inactivated with beta-propiolacton, nitrosoguanidine, Ethylsulphate or urothane. *Av. Dis.* 5, 83.
- Jacotot, I. and Vallee, A. (1963): Immunization against Newcastle disease with formalized virus in oil. *Bull. Acad. Vet. Fr.* 32, 373.
- Kaletka, E.F. and Siegmann, O. (1971): Comparative studies on the demonstration of hemagglutination inhibiting and virus neutralizing antibodies after vaccination against Newcastle disease. *Archi. Geflugelk.* 35, 79.
- Keeble, S.A. and Coid, C.R. (1962): Duration of immunity Newcastle disease in chicks vaccinated with beta-propiolactone-inactivated vaccine. *Vet. Rec.* 74, 1112.
- Levy, R. and Zakay-Rones, Z. (1973): Immunization of chickens with an inactivated oil adjuvant Newcastle disease vaccine. *AV. Dis.* 17, 598.
- Lombardi, D. and Coava, R. (1976): Simultaneous immunization of day old chicks against Newcastle disease with hyperconcentrated inactivated oil emulsion and live B₁ vaccines. *Clinica Veterinaria*, 99, (8) 380.

M.A. BASTAMI, et al.

- Mazzaracchio, V. and Orfei, Z. (1956): The attenuated F strain of New castle disease. R.C: 1st. Sup. Sanit. 19, 807.
- Mitchell, C.A. and Walker, R.V.L. (1953): Newcastle disease vaccines employing an adjuvant. proc. 15th. Int. Vet. Congr., Stockholm, 1, 256.
- Nakamura, J., Mayamoto, T. and Nagashimakh. (1956): Studies on Newcastle disease vaccine added with aluminium hydroxide gel. N.I.B.S. Bull. Biol. Res. Toxyo. 1, 69.
- Nilakantan, P.R., Sakkubani, P.R. and Dhanda, M.R. (1960): Observations on the effect of immunization of fowls with different vaccine strains of Rainkhet (Newcastle) disease. IND. Vet. J. 37, 503.
- Palatka and Toth (1966): Sited by Allan, W.H., Lancaster, J.E. and Toth, B. (1973): The production and use of Newcastle disease vaccines, FAO, United Nations, Rome.
- Reed, L.J. and Muench, H. (1938): A simple method of estimating 50 per cened points. Amer. J. Hyg. 27, 493.
- Rizk, J. and Chu, H.P. (1973): Control of Newcastle disease in Lebanon with lenthogenic and mesogenic vaccines. 4th, Europ. Cong., London, 58.
- Saad, F.E. and Bastami, M.A. (1982): Evaluation of vaccine type, route of vacoination and thime of interforence in the emergency control of experimental Newcastle disease in chickens. Vet. Med. J. Facult. Vet. Med., Cairo. uni., 30, 167.
- Semerdjiev, B. (1962): Immunization against Newcastle disease with a live attenuated strain of virus adsorped on aluminum hydroxide. Zbl. Bakt. I, 185, 160.
- Sheble, A. and Reda, I.M. (1977): Cited by Ghafagy, A.K. (1977): M.V.Sc., Thesis, Facult. Vet. Med., Cairo Univ.
- Snedecor, G.M. (1956): Statistical methods. 4th. Ed. the Iowa State College Press., Am. Iowa.
- Takatsy, G.Y. (1956): The use of spiral loops in serological and virological micro-methods. Acta Microbiol. Hung. 3, 191.
- Vasilu, G., Suhaci, Petrovsky, M. (1963): Adsorption of Newcastle disease virus upon different sorts of aluminium hydroxide II. Bid. 343.
- Voeten, A.C., Laorhoven, P.Y.M., Van (1975): Immunization against Newcastle disease by intramuscular injection of La Sota strain vaccine, a field trial, tijdschrift Voor Bierge-neeskunde 100 (6) 88.
- Zigdam, D.M. (1953): Vaccination against Newcastle disease. pro. 15th. World Vet. Cong., Stockholm, 252.

Table (1)

Results of hemagglutination inhibition and challenge test of birds intramuscularly vaccinated with different Newcastle disease vaccinas

Gr. No.	Vaccine	Age / week	Menagglutination Inhibition (HI) Test								Arth. Mean	Challenge Test			
			No. of Samples	TRN - Distribution								Total No.	No. of + ve	No. of - ve	Protect - ion %
0	2	3		4	5	6	7	8							
1	Non vaccinated	1	15	5	6	4					1.60	30	30	0	0.00
		2	15	8						1.00					
		3	15	15						0.00					
		4	15	15						0.00					
2	Mitchnar B ₁ occular	1	15	5	6	4					1.60	30	16	14	46.67*
		2	15	4	10	1				1.73					
		3	15	2	4	7	2			2.47					
		4	15	-	5	6	3	1		3.00					
		5	15	1	3	9	1	1		2.80					
		6	15	5	4	3	2	1		2.00					
		7	15	9	3	2	1			1.07					
3	B ₁ i.m	4	15	1	2	6	5	1			3.13	30	4	26	86.67*°
		5	15	1	4	3	4	2	1	3.27					
		6	15	2	1	10	1	1		2.73					
		7	15	-	4	6	3	2		3.46					
4	La Sota im	4	15	2	6	4	3				2.20	30	11	19	63.33°
		5	15	-	4	5	3	3		3.33					
		6	15	-	1	6	5	2	1	3.73					
		7	15	1	2	4	5	2	1	3.47					
5	AH-B ₁ im	4	15	-	4	6	3	2			3.20	30	8	22	73.33*
		5	15	-	2	5	4	3	1	3.73					
		6	15	-	1	4	7	2	1	3.86					
		7	15	-	3	3	8	1		3.46					
6	Komarov im	4	15	3	2	5	3	2			2.60	30	12	18	60.00°
		5	15	2	2	1	5	4	1	3.53					
		6	15	2	1	6	4	2		3.07					
		7	15	5	4	3	2	1		2.00					
7	Inactivated i.m	4	15	3	5	5	2				2.20	30	5	25	83.33*°
		5	15	-	1	1	2	3	1	4.93					
		6	15	-	1	1	2	3	6	2	5.20				
		7	15	-	-	1	3	6	3	1	1				

TRN = Titre reference number.

* , ° = Significant P / <u> 0.05.

Table 11

Results of investigation... (mirrored text)

No.	Name	Age	Sex	Height (cm)	Measurements (mm)										Remarks		
					1	2	3	4	5	6	7	8	9	10			
1
2
3
4
5
6
7
8
9
10

Table 11 - This table number

Table 11 - This table number