قسم: طب الحيوان

كلية: الطب البيطري - جامعة القاهرة •

بعض العوامل الموعثرة على الاستجابة المناعية في الدجاج

R MEMOSEPPRESIDMOP NEWCASTE BEISERSESTACTARISM BY TURKEY HERPS VIRUSTARA REK'S THERASE VACCUAS

١- التثبيط المناعي للتحصين ضد مرض النيوكاسل بواسطة لقاح الماريك

أحمد بسيوني ، محمد عوض ، محمد عامر ، صفاء يسن ، منال عفيفي

تحصين الكتاكيت عمر يوم بلقاح الماريك أدى الى تثبيط الاستجابة المناعية عند عمر 1-1 أسابيع مقاسة بانخفاض الاستجابة المناعية للطيور وذلك عند تحصينها ضد مرض

النيوكاسل وذلك في صورة انخفاض الاجسام المناعية المضادة لتلزن الدم والمقاومة لاختبار التحدي كما أدى ذلك الى تغيرات في صورة الدم البيضاء ممثلة بقلة في عدد كرات الدم البيضاء، وهيتروفيل وزيادة اللمفوسيت.

Dept. of Vet. Medicine, Fac. of Vet. Med., Cairo University, Head of Dept. Prof. Dr. A. Bassiouni.

SOME FACTORS INFLUENCES OF IMMUNOSUPPRESION IN CHICKENS I- IMMUNOSUPPRESION OF NEWCASTLE DISEASE VACCINATION BY TURKEY HERPS VIRUS (MAREK'S DISEASE VACCINE) (With Two Tables)

A.A. BASSIOUNI; M.H.H. AWAAD; M.M. AMER; SAFAA YASSIEN and MANAL A.A. AFIFI (Received at 1/6/1987)

SUMMARY

Vaccination of one day old chicks with 0.2 ml S/C with Marek's disease vaccine (HTV), produced a significant suppression of the immune response of chickens at 4 to 6 weeks of age represented by decreased level of HI titre, low protection rate and change in leukogram which characterized by leucopenia, neteropenia and lymphocytosis.

INTRODUCTION

Field as well as vaccinal virus strains of Marek's disease has gross changes in both bursa of Fabricus and thymus glands of chickens with drastic reduction in packed cell volume and hematopoiesis which result in immunosuppressive effects (JAKOWSKI et al., 1969 and SHARMA, 1978).

Reduction in antibody response to Mycoplasma synoviae in chickens inoculated with Marek's disease herps virus has been reported by KLEVEN et al. (1972) and ELLIS (1980).

Due to the pandemic situation of Newcastle disease; vaccination against this infection is undertaken allover the world. However, with vaccination against Marek's disease at the first day of life in baby chicks; an impairment could be expected in vaccination of Newcastle disease. Eventually; this work was planned to study the possible immunosuppression due to Turkey herps virus vaccine (Marek's disease vaccine) in vaccination against Newcastle disease virus.

MATERIAL and METHOD

Strains:

- Newcastle disease virus (NDV) vaccines: Hitchner B_1 (HB $_1$) containing 10 $^{9.4}$ EID 1 ml were giving intraoccularly and in drinking water respectively.
- Newcastle disease challenge strain:
 Velogeinc viscerotropic Newcastle diseas (VVND) virus strain characterized by SHEBLE and REDA (1976) was used.
- Marek's disease (MD) vaccine:
 Turkey herps virus (HTV) vaccine obtained from TAD company was given sub-cutanously (S/C) to vaccinated birds.

A.A. BASSIOUNI et al.

Experimental design:

Two hundered and Twenty five one day old ISL chickens were used in this experiment. At 1st day of life 25 chicks were sacrified for serum collection to determine NDV maternal HI antibodies. The remaining 200 chicks were divided into 10 equal groups (1-10). Birds of only 5 out of the 10 groups (3,5,7,8 and 9) were injected S/C with 0.2 ml of Marek's disease vaccine (HTV).

At 7th day of life; birds of groups 2 and 3 were vaccinated with HB₁ vaccine. Birds of groups 4 and 5 were vaccinated with lasota vaccine at 21 days of age while groups 6 and 7 received both HB₁ and Lasota vaccines at 7 and 21 days respectively. Birds of groups 1 and 10 were left as nonvaccinated negative controls. At the 3rd week post-vaccination; birds of all groups were I/m injected with 0.2 ml of VVND. The challenged chickens were kept under daily observation for 21 days for recording symptoms; rate of mortality and post-mortem lesions. Birds with persisted symptoms till the end of the observation period were considered as if dead. Fifteen blood samples were randomly taken from each group at 0, 1, 2 and 3 weeks post-vaccination to determine the HI antibodies. For haematological examination; five blood samples were taken from the wing vein at the 3rd week after vaccination as well as after challenge to determine their total and differential leucocytic count.

Virological examination:

Virus reisolation of challenged dead birds was undertaken by inoculation of five 9-10 day old-embryonating chicken eggs via allantoic sac (CUNINGHAM, 1964). The inoculated eggs were incubated at 37.8°C for six days and specific deaths were identified by the slow HA test as well as HI test with known ND specific immune serum.

Haematological studies:

Blood samples were collected on anticoagulant ethylen-diamintetra acetic acid (EDTA) and subjected to the following:

- Total leucocytic count: was done according to NUTT and HENRICK (1952).
- Differential leucocytic count was done according to the SCHALM et al. (1975).

Data obtained were statistically evaluted according to SNEDECOR and COCHRAN (1967).

RESULTS

Challenge of the non vaccinated control groups as well as birds of groups injected with HTV vaccine developed respiratory clinical signs 2-12 days post challenge while nervous signs appeared at the 10th day post challenge. Mortality started on the 3rd day till the 15th day after challenge. HI titre in negative control birds was significantly higher than in birds received HTV vaccine before and after vaccination. The vaccinated control birds with HB₁ showed higher HI titers before vaccination and at one week post vaccination. Vaccinated groups with lasota and birds vaccinated with both HB₁ and lasota vaccines showed higher HI titre than those received HTV and simillarly vaccinated with HB₁ and lasota at 2 and 3 weeks post-vaccination. The protection rate recorded in control vaccinated birds was significantly higher than birds received HTV vaccine and vaccinated with ND vaccines.

The changes of the white blood cells depended mainly on the kinetic of heterophils and lymphocytes. Statistical analysis of the total and differential leucocytic count in table (2) revealed that no significant difference between control negative (group 1) and (group 8) which reveived HTV (Marek's disease vaccine) as well as control vaccinated with HB (group 2) and birds vaccinated with HB and received HTV at one day old (group 3), at 3rd week post-vaccination and challenge.

NEWCASTILE DISEASE VACCINATION

But control birds vaccinated with lasota (group 4) as well as control birds vaccinated with HB₁ and lasota (group 6) showed significant increase in the total leucocytic count and heterophil values with decreased in the mean values of lymphocytes than birds which vaccinated with lasota and received HTV (group 5) and birds vaccinated with HB₁ and lasota and received HTV (group 7) respectively, at 3rd week post-vaccination and challenge.

DISCUSSION

Taking results of the HI titre as a criterion of immune response determination after NDV vaccination. The obtained results proved that Turkey herps virus (HIV) vaccine (Maek's vaccine) strated to suppress the HI antibodies from the 4th week post-vaccination against NDV and onwards (Table 1). PURCHASE et al. (1968) and EVANS and PETTERSON (1971) concluded that Marek's disease herps (MDH) and HTV inhibit the antibody response to poultry pathogens. KLEVEN et al. (1972) reported tht antibody response to M. synoviae was decreased in chickens vaccinated with HTV and this decrease was most evident during the first 4-6 weeks after inoculation.

Determination of the protection rate after ND challenge of vaccinated birds with either lasota or with both HB₁ and lasota revealed significant high rates than that recorded in groups received HTV vaccine and similarly vaccinated either with lasota or with both HB₁ and lasota respectively (Table 1).

At 3rd week post either lasota vaccination or challenge, groups of birds vaccinated with HTV showed leucocytosis, heterophilia and lymphopenia than non-vaccinated control groups which indicated that HTV vaccination resulted in lymphocytosis, heteropenia and leucopeina after five weeks post inoculation. JAKOWSKI et al. (1969) found that Marek's disease virus (MDV) caused haemopoitic destruction in lymphoid, myloid and erythroid tissues by other viruses falling in the herps virus. CALNEK and HITCHNER (1949) reported that herps like virus of MDV caused increase in the small immature lymphocytes. PAYNE and REMINE (1973), ROSS (1977) and SHARMA (1978) recorded that MDV caused inhibition of peripheral blood leucocytes and lymphoid organs as well as lytic affection on lymphocytes.

Our results are in disaggrement with FURIMINGER and WARDEN (1971) who concluded that attenuated NDV vaccine not interfer with the effectiveness of Killed ND vaccine. Contrary to our findings ZANELLA and POLI (1981) also found that the immunosuppressive effect not occur in chickens vaccinated with HTV at one day old and subsequently to MD. FLETCHER et al. (1972) mentioned that HTV did not produce adverse effect on the antibody production.

The alterations in antibody formation, protection to challenge test and changes in the leukogram values are indicating the immunosupprissive effect of HTV vaccine on ND immoune response. A conclusion which is similar to that reported by ELLIS (1980) and KLEVEN et al. (1972). This effect might be attributed to the destructive action of the virus to the bursal lymphocytes (SCHAT and CALNEK, 1978; POWEL and RENINE, 1978 and ELLIS, 1980).

REFERENCES

Anon (1971): Methods for examining poultry biologics and for identifying avian pathogens.

Nat Acad. Science, Washington D.C.

Calnek, B.W. and Hitchner, S.B. (1949): Localization of viral antigen in chickens infected with Marek's disease herps virus. J. Natl. Cancer Inst. 43: 935-949.

Cuningham (1964): Cited by Amer, M.M., Ph.D. (Poultry disease).

A.A. BASSIOUNI et al.

- Ellis, M.M.; Eidson, C.S.; Brown, J.; Fletcher, O.J. and Sitti Klever (1980): Serological responses to mycoplasma synoviae in chickens infected with virulent or A virulant strains of Marek's disease virus poultry Science, 60: 1344-1347.
- Evans, P.L. and Petterson, L.T. (1971): Correlation of immunological responsiveness with lymphocyte changes in chickens infected with marek's disease. Infect. Immun. 4: 564-574.
- Fletcher, O.J.; Eidson, C.S. and Kleven, S.U. (1972): Bursal lesions in chickens inoculated with marek's disease vaccine. Avain disease, 16: 153-162.
- Furminger, I.G. and Warden, D. (1971): Immunization against Newcastle disease in the presence of marek's disease virus. Vet. Rec. 89: 475-479.
- Jakowski, R.M.; Fredrickson, T.N.; Luginbuhl, R.E. and Helmboldt, C.F. (1968): Early changes in Bursa of fabricus from Marek's disease. Avian disease. 215-223.
- Jakowski, R.M.; Fredrickson, T.N.; Chomiak, T.W. and Luginbunl, R.E. (1964): Haematopoiteic destruction in Marek's desease. Avian disease 374-485.
- Kleven, S.H.; Eidson, C.S. and Anderson, P.P. (1972): Immunosupperession effects of infection of chickens with Marek's disease herps virus. American Journal of Vet. Research, 33, No. 10: 2037-2042.
- Nutt, M.P. and Henrick, C.A. (1952): A diluent for counting the leucocytes and erythrocytes of the chickens. Poultry Science, 31: 735-738.
- Payne, L.N. and Rehine, M. (1973): Pathogensis of Marek's disease in chicks with and without maternal antibody. J. Natl. Concer Inst. 1559-1573.
- Powel, P.C. and Renine, M. (1978): Marek's disease tumor specific antigen (MATSA) induced by the herps virus of Turkeys (HTV) in vaccinated chickens. Vet. Rec. 103: 232-233.
- Ross., L.J. (1977): Antiviral T cell-mediated immunity in Marek's disease. Nature (Land.) 268: 644-646.
- Purchase, H.G.; Cubb, R.C. and Biggs, P.M. (1968): Effect of lymphoid leukosis and Marek's disease in the immunological responsiveness of the chicken. J. Vatl. Cancer, Inst. 40: 583-592.
- Sheble and Reda (1976): Personal communication Amer, M.M. (1984).
- Schat, K.A. and Calnek, B.W. (1978): Characterization of apperently oncogenic Marek's disease virus. J. Natl. Concer Inst. 60: 1075-1082.
- Schalm, O.W.; Jain, N.C. and Carroll, E. (1975): Vet. Haematology 3rd Ed., Lea and Febiger, Philadephia.
- Scharma, J.M. (1978): Immunosuppresive effects of lympho proliferative neoplasms of chickens. Avian disease Vol. 23, No. 2: 315-345.
- Snedecor, G.W. and Cochran, W.G. (1967): Statistical methods 6th Ed. Iowa State College Press. Ames, Iowa.
- Takatsy, G.Y. (1956): The use of spiral loops in serological and virological micromethods. Acta Microbiologica Hung, 3: 197.
- Zanella, A. and Poli, G. (1981): Immunosuppressive effect of Marek's disease on immune response to dead and live vaccines in chickens. Clinica Veterinarin J. (10/4) 363-371.

A.A. BASSIOUNI et al.

Table (1): The effect of Marek's disease vaccine (HVT) on the immune response of chickens vaccinated with Newcastle disease vaccines.

No.	birds	Vaccination of of ND	Vaccination of MDV				На	ema	ggl	uti	nat	tia	In:	hibiti	on t	est										12.31	c	hall		
Group No.	1 500	ina io	cina of M		1	Pos	t v	acc	ina	tio	n		CT (I				Poi	st	chs	11	eng	8					f	- 1	No.of '	Proctect.
Gro	of .	Vacc	Vaco	VOW	Jo c	D	ist	rib	. 0	f H	- I	ti	reg	- =	WPC	No. of	1	-	tri	b.	of	H-I	-	-	e	N/N	No.of	No. of	rv1	100 t
	No.			-	No. of	0	2	3	14	5	6	17	8 8	AB	*	N. S	C	2	3	4	5	6	7	В	9	4		ž	N a	a.
1	80 80 20	-4.	Θ	0 1 2 3	25 20 20 15	1 2 4 6	1 8 9 5	8 5 5 3	11 3 2 1	4 2		-		3.60 2.65 2.05 1.53	0 1 2 3	15	6	4	3	2			-			1.66	15	15		0
2	120 80 80 20		Θ	0 1 2 3	25 20 20 15	1	1 3 2	3 4 2	11 5 7 3	4 6 5 3	3 2	2 4		3.60 4.15 4.55 4.33	0 1 2 3	15 10 10 10	1	1	2 2 2	1	3	3	1	3	2	4.33 6.30 7.20 6.30	15		15	100
3	120 80 80 20	•	0	0 1 2 3	25 20 20 15	2	3 3	8 6 5 2	8 10 4 6	2 4 3	2	1		3.04 4.00 4.10 3.40	0 1 2 3	15 10 10 10	1 1 1	3	2	3	1:	1	1		2	3.80 5.80 6.60 6.40	15	2	13	86
4	80 20 20 20	**	Θ	0 1 2 3	20 15 15 15	2	9 4 4 2	5 5 3 3	2 3 2 2	1 2 2	2 3 3	1		2.05 3.40 4.00 3.70	0 1 2 3	15 10 10 10	2 4		3 7 2	1		2 2	-	A Comment works of the	6	3.70 5.10 6.20 5.90	15	2	13	86
5	80 20 20 20 20	**	•	0 1 2 3	20 15 15 15	6 2 4 3	7 2 1	4 6 1 7	3 1 3 3	2	2 3 1	3		1.90 3.20 3.70 3.40	0 1 2 3	15 9 9	3		7 3 3 1 3 2 1	1	12	1 1		THE REAL PROPERTY.		3.40 4.80 5.20 5.00	15	6	:9	60
6	80 20 20 20	++1	٥.	0 1 2 3	20 15 15 15	0	3	3 2	7 5 2	6	2 2 2	6 3		4.55 4.60 5.20 4.90	0 1 2 3	15 10 10 10			2 2	1	-	2 2 2 2 2 2	2	1	1 8	4.90 3.20 7.90 7.10	15		15	100
7	80 20 20 20	***		0 1 2 3	20 15 15 15	2	3 1 1	5 3 3 2	4 5 2 2	3	3 2 2 3	1 3 3 3	1	4.10 4.30 4.80 4.70	0 1 2 3	15 10 10 10	2		2 2 2 2 3	1	1	3 3	1 2	1	1 1	4.70 5.40 5.80 5.20	15	4	11	73
8	120 80 80 20	215		0 1 2 3	25 20 20 15	2 4 6 6	5 5 7 5	8 7 4 3	8 4 3 1	2				3.40 2.35 1.90 1.50	0 1 2 3	15 d d	6	5	3 1	and the property of the last	Property of the Print of the Pr	The same of the same of the same of		OCCUPANT OF PERSONS	1	1.50	15	15		0
9	80 20 20 20 20	- 101 9-101	•	0 1 2 3	20 15 15	6 6 12 15	7 5 3	4 3	3	-	The same	W. China, Wynam o		1.90 1.50 0.40 0.00	0 1 2 3	15 d d	15		per une de la consequence que man	A THE PARTY AND PARTY AND PARTY AND PERTY AND	Complete was published in the	Control of the Contro	Single of the last	AND AND SECTION		0	15	15	0	0
10	80 20 20 20 20	. s. s.	Θ	0 1 2 3	20 15 15 15	4 6 11 15	9 5 3 0		2					2.05 1.53 0.60 0.00	0 1 2 3	15 d d d	14	ı		Mercel and and and and		-			C	0.06	15	15	0	0

WPV: Week post vaccination. AM: Arithmatic mean. WPC: Week post challenge.
 -: Non-vaccinated. () Non-vaccinated with Marek's disease. *: Marek's disease vaccination at one day old (0.2 ml S/C).

^{+:} Vaccinated with Hitchner B₁ at 7th day. ++: vaccinated with La Sota at 21st day.

^{+++ :} Vaccinated with Hitcher B, at 7th day and La Sota at 21st day.

	No.	Group	ч	N	ω	4	U	6	7	8	9	10
	birds	20 No. of bird ↑ ↑ ↑ ↑ ↑ Vaccination ① ① ① ① ① ① ① Vaccination										
ND	ion of	Vaccinat	1	+	+	:	:	‡	‡	1	1	_
MDV 3rd	ion of	Vaccinat	0	0	0	0	0	0	•	0	0	0
	of ple	No. C	U	U.	U.	v	٥.	U.	O.	U	U	U)
	Total	count/	28.6±4.7	40.0±1.1	32.0±2.5	33.6±2.8	22.6±5.3	47.6±1.7	29.0±4.9	25.0±2.3	11.8±5.6	13.1±5.1
		Eosino- philes	7.7±1.34	1.1±0.15	n.3±0.07	0.9±0.14	0.7±0.93	0.6±0.21	0.0±0.00	2.3±1.42	0.0±0.00	0.0±0.00
week post vaccination	Absolute value	Hetro- philes	8.7±1.95	27.6±2.90	24.3±3.10	0.9±0.14 25.9±1.80	6.5±1.90	0.6±0.21 31.3±2.43	13.211.78	6.8±0.93	4.1±0.39	5.9±±.26
	alue X 10 ³	Baso- philes	0.1±0.19	0.9±0.28	1.2±0.73	1.1±0.39	1.0±0.33	1.6±0.37	0.6±1.20	0.0±0.0	0.0±0.00	0.0±0.00
	3	Lympho- cytes	81.6±2.45	68.3±2.85	71.4±3.60	69.2±2.10	89.9±1.27	64.6±2.42	83.7±3.20	88.912.90	93.2±0.60	89.9±1.20
		Mono- cytes	1.9±0.39	2.1±0.41	3.8±0.28	2.9±0.41	1.9±0.39	1.9±0.32	2.5±0.92	2.0±0.60	2.7±1.90	3.9±0.27
1	of les	No. o	۵	u	C ⁵	5	O.	U	C)	a	Δ	d
1	Total leucocytic	count/	1	36.0±2.1	27.4±3.5	30.1±1.9	19.2±2.5	43.4±2.8	24.9±2.6	1	1	1
		Eosino- philes	1	0.3±2.8	0.0±0.0	0.2±2.6	0.3±1.6	0.9±0.32	0.0±0.00	1	!	1
000000000000000000000000000000000000000	Absolute value	Hetro- philes	1	33.0±5.71	27.3±2.80	31.2±3.90	17.2±2.90	34.6±1.90	21.8±2.77	;	:	1
	×	Baso- philes	1	1.4±0.33	2.0±0.81	0.8±0.82	0.1±0.19	1.9±1.42	0.9±1.20	1	1	1
	103	Lypho- cytes	!	62.5±5.76	68.6±3.60	64.2±1.90	79.5±2.30	61.4±1.83	75.5±5.60	1	;	1
		Mono-	1	2.8±0.06	2.1±0.41	3.6±0.±0	2.9±0.10	1.2±0.92	1.8±0.28	1	1	1.

Table (2): The effect of Marek's disease vaccine on the total and differential leucocytic count on the chickens vaccinated with Newcastle disease vaccines (mean + S.D).

Assiut Vet.Med.J.Vol. 19, No. 37, 1987.