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IMMUNOLOGICAL STUDIES ON A BIVALENT INACTIVATED OIL ADJUVANTED VACCINE AGAINST TURKEY RHINOTRACHITIS VIRUS AND PASTEURELLA MULTOCIDA

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SUMMARY

The immunogenecity of combined Turkey RhinoTrachitis Virus (TRTV) and *Pasterella multocida* inactivated oil emulsion vaccine were evaluated in comparison to both the monovalent (TRT) and (P. Multoocida) vaccines.

Sixty turkeys poults were divided into four groups, group 1, 2, and 3 were vaccinated with (TRTV), Pasterella multocida and combined (TRTV + Pasterella multocida) vaccines respectively while group 4 was kept as non vaccinated control, obtained results of cellular (Detected by lypmphocyte blastogenesis assay) and Humoral (Detected by indirect haemagglutination IHA, Enzyme linked Immuno Sorbant assay ELISA, and Serum neutralization test S.N.T) immune responses of experimentally vaccinated poults revealed that 2 successive doses of the prepared vaccines with 21 days apart produce protective immune response started at 3 weeks post vaccination and lasted for 12 weeks covering the whole fattening period.

INTRODUCTION

Respiratory diseases have always been a feature of intensive poultry production and awide variety of syndromes of known etiology has been associated with such disease in turkey. A distinct novel upper respiratory disease of turkey termed turkey rhinotrachities (TRT) has been reported for the first time in late 1970s in South Africa (Buys and Dupreeze 1980).

TRT is caused by a pneumo virus a member of the sub family Pneumoviridae, belonging to the family Paramyxoviridae (Alexander, 1993), characterized clinically by nasal, ocular

discharge, swollen infra orbital sinuses, sneezing and head shaking (Lister and Alexander 1986), the economic impact of the disease comes from its wide spread in most countries where turkeys are commercially kept, with high morbidity rate reached 100%, mortality rate ranged from 0-40% and drop in egg production up to 70% (Hafez, 1993).

In Egypt, the initial observation suggested the existence of infection with pneumo virus was reported by (Ahmed, 1991), later on many serological studies allover the Egyptian Governorates revealved the prevalence of TRT in both turkeys and chickens as reported by (Dessokey 1997) who detected TRTV antibodies in 19 out of 20 tested broilers and broiler breeders flocks. Also, the antibodies were reported in sera of turkey meat type and turkey breeders by (Hanaa, 2000). P.multocida was reported by (Gergis 1978 and Zienab 1999) where the disease appeared in an epidimec form among turkeys poults on a farm belonging to the general poultry organization.

The hazard of TRTV infection depends greatly on the extent of infection with other pathogen specially pasteurella species (Stuart, 1989) where co-infection with TRT virus and P.multocida occurs. Fowl cholera, a contagious disease affecting domesticated and wild birds is usually appear as a-peracute or subacute septicemic disease associated with high morbidity and high mortalities (Glisson, 2003) with petcheail hemorrhage on coronary and abdominal fats and heamoroghic enteritis as pathognomonic lesion (Fram et al., 1994).

Turkeys are much more susceptible than chickens to infection with P.multocida. Acute or chronic pneumonia is an especially common lesion in turkeys, infection of conjunctiva and adjacent facial tissues occur. Chronic localized infection can involve the middle ear with yellowish caseous exudate in air spaces of cranial bones and menengities leading to death (Olson et al., 1966).

Since TRT infection can not be controlled by medication, the main approach to control through the use of attenuated vaccines in young turkey and inactivated vaccines in layer, however, since TRTV is RNA virus and genetically unstable, live vaccines are liable to show some reservation to virulence as well as they only protect against infection and not against drop in egg productic (Alexander, 1993), accordingly this study w planed to prepare a bivalent inactivated oil emulsion vaccine against both TRTV and P.multoci to evaluate their suitability in order to save gua turkeys from such dangerous infectious disea in one shot vaccine.

MATERIAL AND METHODS

1- Laboratory animals :

a) **Turkey:-** A total of 60 one day old tur poults obtained from EL-Wafaa Farm, C Egypt were assigned into 4 groups, re

under hygenic measures till reach 6 weeks, old. They were screened for anti- *P.multocida* serotypes A and D antibodies and anti TRTV antibodies. All proven to be free from the screened antibodies.

b) **Mice:** A total of 33 Swiss albino mice weighing about 18-20g, were used for vaccine safety

2- Bacterial strains:

Four vaccinal bacterial strains of P. multocida sero types (A : 5,8,9 and D:2) from veterinary serum and vaccine research institute, Abbassia, Cairo, Egypt were used for monvalent and bivalent vaccine preparation. Each serotype was propagated separately on casien hydrolysate medium (Oxoid). Bacteria were adjusted to a density of approximately 2 x 10^9 colony forming unit (c.f.u)/1ml (Kucera et al., 1981) and inactivated with 0.3% formalin for 18-24 hours.

3- Viral strains:

TRT virus vaccinal strain (Vco3), kindly supplied by central laboratory for evaluation of veterinary biologics was used for preparation of both monovalent and bivalent vaccines, the virus was propagated in specified pathogen free embryonated chicken eggs via yolk sac according to (Allan et al. 1973), allantoic fluids and embryos were collected, The virus titer was (10^6 EID_{50} / 0.1ml) and inactivated at a final concentration of 0.1% formalin for 6 hrs.

4- Prepration of monovalent & bivalent oil adjuvant TRTV & *P.multocida* vaccines:

These were prepared according to (Stone et al., 1978), by mixing equal volumes of (TRTV and *P.multocida*) inactivated fluids, then emulsified in oil with 1:2 aquas to oil ratio, Monovalent vaccine were adjusted to contain the same concentration of antigen as used in bivalent vaccine.

5- Quality control of the prepared vaccines:

a- **Sterility, safety and potency:** The prepared vaccines were tested for sterility for any contaminants, safety in lab. mice and potency (sero conversion, challenge exposure to virulent *P.multocida*) following the standard international protocols as described by (Code of American Federal Regulation, 1985) .

b- **Physical characterization**: The prepared vaccines were subjected to drop test (Roshdy, 1996), emulsion viscosity (Becher, 1965) and emulsion stability (Cessi and Nardelli, 1973).

c- **Evaluation of immuno response:**

1- **Cellular immune response:** Assay of Lymphocyte blastogenesis was applied (Lucy, F.L.,1974). Evaluation of the test using MTT according to (Mosmann, 1983). Results of the test were expressed as delta optical density (ΔOD).

2- Humoral immune response:

- Serum neutralization test (SNT) was done on serum samples of turkeys for estimation of antibodies against TRT virus according to (Cunningham, 1973).
- Indirect haemagglutination (IHA) was done on serum samples for estimating antibodies against *P.multocida* (Solano et al 1983).
- Enzyme linked immunosorbant assay (ELISA) was done for estimating antibodies against TRT virus using commercial kits (Biocheck -Holand) according to (Grant et al 1987) while ELISA was carried out to evaluate antibody response against *P.multocida* according to (Marshall et al 1981).

d) Challenge exposure: The protective efficiency of *P.multocida* vaccines were evaluated by virulent challenge exposure. Vaccinated and non vaccinenated control turkeyes were inoculated I/M with 0.1 ml / bird containing 10 lethal dose fifty (LD_{50}) for both types of *P.multocida* A and D as was suggested by (Heddleston and Rebers 1968).

Experiment design:

Sixty Turkey poults, six week old were divided into 4 groups as follows:

Group No.	Type of vaccine	No. of poults	Dose	route	Age of vaccination		Challenge
					1st dose	Booster dose	
1	Inactivated oil emulsion TRTV	15	1/2ml	I/M	6 weeks	9 weeks	------
2	Inactivated oil emulsion *P.multocida*	15	1/2ml	I/M	6 weeks	9 weeks	12 weeks
3	Combined oil emulsion TRTV + *P.multocida.*	15	1/2ml	I/M	6 weeks	9 weeks	12 weeks
4	Control	15	------	------	------	------	12 weeks

* Blood samples were collected at 4, 11, 17 and 21 days for cellular immune response and at 3,4, 5, 6, 8, 10 and 12 weeks post vaccination for humoral immune response.

RESULT AND DISCUSSION

Table (1): Results of cell mediated immune response of turkeys vaccinated with monoval and combined oil adjuvanted inactivated TRTV and *P.multocida* vaccines.

Groups	Days post vaccination			
	4	11	17	21
1	0.116	0.132	0.152	0.018
2	0.105	0.123	0.133	0.108
3	0.085	0.124	0.177	0.102
4	0.016	0.095	0.006	0.003

• **ΔOD:** delta optical density value =

[(OD of pHA-OD of media) - (OD of cells - OD of media)]

* **PHA** = Phyto hemagglutinin

Group (1): Turkeys vaccinated with monovalent inactivated TRTV vaccine.

Group (2): Turkeys vaccinated with monovalent inactivated *P. multocida* vaccine.

Group (3): Turkeys vaccinated with combined inactivated TRTV+ *P.multocida* vaccine.

Group (4): Non vaccinated control turkeys.

Table (2) : Mean serum neutralizing antibody titers in serum of turkeys vaccinated with oil adjuvanted inactivated TRTV vaccines.

Weeks post vaccination	Groups		
	1	3	4
3	4.66	24	2
4	12	72	2
5	64	100	2.82
6	32	128	0
8	36	72	2
10	21.33	16	0
12	10.66	16	0

Mean value neutralizing antibody titres = reciprocal of final serum dilution which neutralize and inhibit the CPE of 100-200 $TCID_{50}$ of the TRTV virus.

Group (1) : Turkeys vaccinated with single inactivated TRTV vaccine

Group (3) : Turkeys vaccinated with combined inactivated TRTV+ *P.multocida* Vaccine.

Group (4) : Non vaccinated control turkeys.

Table (3): Indirect haemagglutination (IHA) geometric mean antibody titers (GMT) in sera of turkeys vaccinated with inactivated oil adjuvanted monovalent and bivalent *P. multocida* vaccines.

Groups of turkey	Antigen	Weeks post vaccination					
		3w	5w	6w	8w	10w	12w
Group (2) Monovalent vaccine *P.multocida.*	A	610	755	844	903	1280	2560
	D	485	610	686	844	970	181
Group (3) Bivalent vaccine	A	903	970	1040	1280	1372	1470
	D	788	844	905	970	1040	1194
Group (4) Control	A	21	28	25	26	30	26
	D		26	23	21	28	

Table (4): Anti TRTV antibodies in sera of turkeys vaccinated with both monovalent and bivalent vaccines using ELISA.

Weeks post -vaccination	No. of Groups		
	1 TRTV vaccine	3 TRTV+ *P.multocida* vaccine	4 Non vaccin
3	10541	14884	1800
5	13670	11215	1290
8	17929	12538	1066
10	14834	11281	838
12	18170	17571	1390

* ELISA positive titer ≥ 1656.

1- Sample positive Ratio (S/P))= $\frac{\text{mean of test sample-mean of negative control}}{\text{mean of positive control - mean of negative control}}$

2- Calculation of antibody titer

Log10 titer = 1.0 (Log10 S/P) + 3.52.

3- Titer = antilog

Table (5) : Anti Pasteurella multocida antibodies in sera of turkeys vaccinated with both monovalent and bivalent inactivated oil adjuvant vaccines using ELISA.

Groups of turkey	Antigen type	Weeks post vaccination					
		3W	5W	6W	8W	10W	12W
Group (2) *P.multocida* vaccine	A	945	1521	2122	3599	3645	5473
	D	846	1273	1521	2530	3599	3921
Group (3) combined(*P.multocida*.+TRTV) vaccine	A	1121	1490	1859	2145	2432	4599
	D	996	1122	1248	1553	1859	2576
Group (4) Control	A	136	165	143	122	155	164
	D	123	129	118	132	140	124

1- sample positive Ratio (S/P) = $\frac{\text{mean of test sample - mean of negative control}}{\text{mean of positive control - mean of negative control}}$

2- Calculation of antibody titer

Log10 titer = 1.08 (Log10 S/P) + 3.82

3- Titer = antilog

Table (6): Challenge exposure test results of turkeys vaccinated with monovalent and bivalent *P. multocida* inactivated oil adjuvanted vaccine using virulent *P. multocida* serotypes A and D.

Groups of Turkey	No. of birds / group	No. of dead / survived birds after challenge exposure to virulen serotypes of *P. multocida*		Protection %	
		Type A	Type D	Type A	Type D
Group (2) Monovalent vaccine	10	0/5	0/5	100%	100%
Group (3) Bivalent vaccine	10	0/5	0/5	100%	100%
Group (4) Control	10	5/5	5/5	0%	0%

Turkey Rhinotrachitis (TRT) and Pasteurella multocida (*P. multocida*) are the most important epizotic diseases that threatens turkey industry in Egypt, epidemics of P.multocida causing great economic losses for large projects in the field of chicken, ducks and Turkey breeding, this due to rapidity of spread and the extra ordinary virulence of P.multocida serotyps (Zeinab, 1999) since TRT virus infection can not be controlled by medication, the main approach to control was through the use of live vaccine in young Turkey poults and inactivated vaccine for fattening or breeder Turkey (Jone, 1996).

TRT and *P. multocida* live vaccines have been reported to be unstable and their efficiency is not fully proven and those are suspection that they have contributed in spread of the disease (Parntner et al., 1990 and Alexander, 1993).

Studies on the use of oil adjuvant in the preparation of inactivated vaccines show that parentally inoculated antigens adjuvated with oil emulsion generally stimulate a higher and more persistent antibodies titer (Zanella, 1969), Moreover combined vaccines given in one shot have the advantage of protecting against more than one pathogen and can save effort, time and cost.

Based on the previously reported studies the present work was conducted for the preparation of combined inactivated oil adjuvant vaccine against both TRTV and P.multocida.

Results of cellular immune response by lymphocytes blastgenesis assay as represented in table (1) revealed that a maximum response of T. cells expressed as ΔOD were 00.152, 0.133 and 0.177 at 17 days post vacciantion then decline to 00.0018, 0.108 and 0.102 at 21 days post vaccination for groups 1,2 and 3 respeectively compared with very low ΔOD of control gorup (0.006 and 0.003. The results of cellular immune responses agreed with (Timmes and Bracemell, 1983) as they stated that once the humoral immune response became established, there was acorresponding decrease in the cellular immune response.

Both utilized vaccines single and combined proven to be effective in inducing serum neutralizing antibodies against TRTV antigen as shown in table (2), it was noticed that group (3) which received combined vaccine gave a higher SNT titer (128) than group (1) SNT titer (32), which received single inactivated TRT vaccine at six week post vaccination. This protective titer suggested to be important for protection of breeding and laying turkeys against viremic virus which might effect in egg production (Jone, 1996).

(Solano et al., 1983) developed indirect haemagglutination and indirect ELISA to measure humoral antibody response against P.multocida, There was a gradual increase in GMT antibody titers (table 3) in groups 2 & 3 which were vaccinated with monovalent and bivalent vaccines from 3rd

12th week post vaccination for both Sero types of P.multocida (A and D). Same results obtained by (Rahman et al., 2004) as they observed increased in Haemagglutination antibody titers following vaccination with formaline inactivated fowl cholera vaccine.

Using of serological assays, especially ELISA has become a practical method for evaluating the immunological response of poultry to various vaccination programmes (Briggs and Skeels, 1984) and (Eteradossi et al., 1995), it was clear that the ELISA titer of groups 1 and 3 begin with protective ELISA level 10541 and 14884 respectively at 3 weeks post vaccination and still protective with the titer of 18170 and 17571 at the 3rd month post vaccination.

Table (5) revealed that there was an increase in anti P multocida antibody titers in both monovalent and bivalent vaccines for both types of P.multocida A and D. ELISA titers increased gradually from 3rd week post vaccination (945, 846 and 1121, 996) and reached maximum titer at 12 week post vaccination (5473, 3921 and 4599, 2576) compared with control group with law titers ranged from 123 - 165. These results are in agreement with (Sharaf et al., 1988) and (Kedrark et al., 2000) as they concluded that there was an increase in anti-P.multocida IgG levels following vaccination with P.multocida vaccine in both turkeys and geese respectively.

Detection of degree of protection of birds vaccinated by P.multocida against virulent challenge using virulent strain considered most valuable method for evaluation of immune response of the tested vaccine (Zeinab, 1999), from the presented data in tables (3,5) it was clearly that there was a correlation between antibody titers and protection as both vaccines gave good protection against virulent challenge as reported by (Abd El Hady, 1992). Also fowl cholera oil adjuvant vaccine contained serotypes 5, 8, 9 A and D : 2 gave higher protection rate as reported by (Farid et al., 1987).

The present study represent preparation of inactivated monovalent and combined bivalent vaccines for TRT and P.multocida which revealed neither competition nor interference between the two antigens, Moreover the vaccine offered a good protective immunity against the used antigens.

In conclusion, application of such vaccine (combined TRTV + P,multocida) is greatly useful, economic and potent for protection of fattening and breeding Turkeys against such disease.

REFERENCES

Abdel Hady, N.F. (1992): Studies on pasteurella infection in turkeys. M.V.Sc. Thesis. Cairo Univ.

Ahmed, A.A.S. (1991): Newcastle disease and other avian paramyxoviridae infection in: Disease of poultry. 10th ed. Calnek, B.W.; Barnes, H.J; Beard, C.W.;

McDougald, L.R. and Saif, Y.M., State University Press, Ames Lowa. 541-569.

Alexander, D.J. (1993): Pneumoviruses (Turkey rhinotracheitis and Swollen head syndrome of chickens). In J.B. McFerran and M.S. MCNATTY (eds) virus infections of birds elsvier science publishers: B.V. 375-382.

Allan, W.H.; Lancaster, J.E and Toth, B. (1973): The production and use of ND vaccines. P.35 Food and Agriculture Organization, Rome, Italy.

Becher, P. (1965): Theory of emulsion stability in: Becher, P. (Ed.) emulsion: Theory and practice. 2nd ed Rheinold publishing corporation, New York. PP. 95-149.

Briggs, D.J. and Skeels, J.K. (1984): An enzyme linked immunosorbent assay for detecting antibodies to *P.multocida* in chicken. Avian Dis, 28 (1): 208-215.

Buys and Dupreeze (1980): A preliminary report on the isolation of virus causing sinusitis in turkeys in South Africa and attempts to attenuate the virus. Turkeys, 28: 36-46.

Cessi , D. and Nardelli, L. (1973): Requirement for testing oil emulsion inactivated Newcastle disease vaccine". Proc. 42nd Symp. On Requirement, for poultry virus vaccines lyon, PP. 325-328.

Code of American Federal Regulation (1985): Published by the office of the federal register National Archives Records Service. General Services Administration.

Cunningham C.H., (1973): Immunologic methods in avian research: Neutralization test. Avian Dis. (62) 227-235.

Dessokey, M.K.M. (1997): Studies on swollen head syndrome in some chickens. Ph.D. Vet. Thesis, Suez Canal, Univ.

Eterradossi, N.; Toquin, D.; Guittat, M. and Bennejean, G. (1995): Evaluation of different turkey rhino tracheitis viruses used as antigens for serological testing following line vaccination and challenge. J.Vet. Med. B, 42: 175-186.

Farid, A. H.; Torky, H. A; EL-Nimr, M. M., Abeila, M. and Essat, M. (1987): Studies on P.multocida of birds 4.New poly valent oil adjuvanted vaccine. Archiv for Experimentalle Vet., 41(2), 208-211.

Frame, D. D.; Clark., F. D. and Smart, R. A. (1994): Recurrent outbreak of a cuetaneous form of P.multocida infection in turkeys. Avian Dis., 83 (2), 390-392.

Gergis, S. M. (1978): Some immunizing properties concerning P.multocida in poultry MVSc Thesis. Cairo Univ.

Glisson. R. John (2003): In diseases of poultry . 11th Edition Saif Y.M, Barnes, H.J, Glission J.R, Fadly A.m, Macdougald. L.R and Swayne D.E. Iowa State Press. 627-674.

Grant, M.; Baxter-Jones, C. and Wilding G.P. (1987):

An enzyme linked immunosorbent assay for the serodiagnosis of turkey thintracheitis infection. Vet. Rec., 120: 279-280.

Hanaa. A. Mohamed (2000): Prevalence of pneumovirus infection in some turkey flocks in Egypt. Ph.D. Vet. Thesis, Cairo, Univ.

Hafez, H. M. (1993): Pneumovirus infection in Turkeys and chickens. Present status. Proc. 3rd Sci. Conf.. Egypt Vet. Poult. Assoc. : 3-13.

Heddleston, K. L. and Rebers, P. A. (1968): Fowl cholera active immunity induced in chickens and turkeys by oral administration of killed P.multocida. Avian. Dis., (12) 129-134.

Lester, S. A. and Alexander, D. J. (1986): Turkey Rhino Tracheitis. A review Vet. Bull. 56: 637-663.

Lucy, F.L., (1974): Invetro assay of mitogen stimulation of peripheral lymphocyte. Avian Dis, 18: 602-608

Jones, R.C. (1996): Avian pneumovirus infection. Question still unanswered.Avian pathol, 25: 639-648.

Kedrak, A.; Borkowska - Opacka, B.; Samorek- Salamonowi Q., E.; (2000) : Use of the ELISA for determination of IgG antipasteurella multocida serum level in gees vaccinated against Pasteurellosis. Bull. Vet . Inst. Putway 44 (2) 133-160.

Kucera, C. J. Won, J. C. S. and Eis, R.L. (1981): Development of a chemically altered P.multocida vaccinal starin. Am. J. Vet. Res., 42(8): 1389-1394.

Marshall, M.S., Robisom R.A and M.M Jensen (1981):Use of an enzyme - linked immuno sorbant assay to measure antibody responses in turkeys against *P. multocida* Avian Dis. 25 (4) 964-971.

Mosmann J. (1983): Rapid colormetric assay for cellular growth and cytotoxicity assays. J. Immunol. Methods, 65 : 55.

Olson, L. D.; McCume, M.L. and Moseley B. L. (1966): Gross and histopathological description of cranial form of chronic fowl cholera in turkeys. Avian Dis., (10) 518-529.

Parntner, M.M.; Harman, B. G.; Glisson, J.R. and Mahaffey, E.A. (1990): The pathogenesis of P.multocida sero type A: 3,4 infection in turkeys. A comparison of two vaccines strain and field isolates. Avian Dis 34 : 260-266.

Rahman M. K.; Rahaman, M. B., Siddiky M. N.A; Kafi, M.A. Islam, M.A. and Choudhury, K. A. (2004): Efficacy of formalin killed fowl cholera vaccine in experimentally immunized Fayoumi chicken. Bangladish J. Vet. Med. 2(1) 23-25.

Roshdy, O. H. (1996): Studies on inactivated oil FMD vaccine. Ph.D., Vet. Cairo Univ.

Sharaf M. M., Nestok K. F., Saif Y. M., Sacco R. E. and Havenstein G. B. (1988): Antibody response to Newcastle disease virus and *Pasteurella multocida* of two strains of turkeys. Avian Dis. (32) 1372 - 1377.

Solano W.; Giambrome J. J. and Panangala V. S. (1983): Comparison of enzyme- linked immunosorbant assay and indirect haemagglutination test for quantitating antibody responses in chicken against Pasteurella multocida. Avian Dis. 27. (4): 1034-1042.

Stone, D.; Brugh, M.; Hopkins, S.R.; Yoder, H.W and Bread C.W. (1978): Preparation of inactivated oil emulsion vaccines with avian or viral or mycoplasmal antigen. Avian Dis., 22 (4): 666- 674.

Stuart, T.C. (1989): Turkey rhinotracheitis (TRT) in Great Britain. Recent advances in turkey. Poult. sci. symp. series No.21, London, UK, Butterworth.

Timmes L.M. and C.D. Bracemell. (1983): Cell mediated and humeral immune response of chickens to inactivated oil emulsion infectious bronchitis vaccine. Res. Vet. Sci., 34: 224-230.

Zanella (1969): Research on use of inactivated oil-emulsified in the control of the most important avian diseases. Proc. Fourth congr. World Vet. Poult. Assoc., Belgrade, PP.69-78.

تحضير ودراسات مناعية للقاح ثنائى مثبط ضد إلتهاب القصبة الهوائية الرغامى والباستيريلا ملتوسيدا فى الرومى

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تم تحضير ثلاث أنواع من اللقاحات الزيتية الميتة وهى لقاحين فرديين والثالى مزدوج ضد مرض إلتهاب القصبة الهوائية رغامى والباستيريلا ملتوسيدا ، وتم تقييم هذه اللقاحات الثلاثة بحقنهم فى طيور الرومى بحقنتين متتاليين بينهما ٢١ يوم ياس المناعة الخلوية بواسطة إختبار تحور الخلايا الليمفاوية والمناعة الخلطية بواسطة إختبار المصل المتعادل والإليزا ضد ض إلتهاب القصبة الهوائية الرغامى وإختبارات التلزن الدموى الغير مباشر والإليزا وإختبار التحدى ضد مرض الباستيريلا وسيدا وأضهرت النتائج أعلى مستويات للمناعة بعد ٢١ يوم من حقن الجرعة الثانية ، وإستمرت فى الوقاية ضد المرضين ى الإسبوع الثانى عشر بعد التحصين (على مدار فترة التسمين كاملة)