Immunological studies on turkeys vaccinated with both turk rhinotracheitis (TRT) and Newcastle disease (ND) vaccine using differe vaccination programs

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Abstract
Spotting the light on the immune response of turkeys against two of the m important respiratory diseases vaccines was ND and TRT administrat simultaneously and separately. The cell mediated immune response vaccinated turkeys evaluated, beside, the humoral immune response by ELIS SNT, HI and challenge tests. Obtained results revealed that inoculation of vaccine at the same time with TRT vaccine was the program of choice for turk poults.
These vaccines were administrated simultaneously, separately and in the sa time.

Introduction
The diseases that may result from avian pneumovirus infection of turkeys h been termed turkey rhinotracheitis (TRT). It is now universally accepted that conditions referred to as TRT can occur as a result of infection with av pneumovirus. Anon (1996)
Pneumovirus infections are associated with serious economic and ani welfare problems particularly in commercial turkey flocks. Avian pneumovi are members of subfamily movirinae, belonging to the family Paramyxoviri (Richard E. Gough).
On the other hand, turkeys are susceptible to NDV infection by direct con between birds through the air borne route via aerosols and dust particles and contaminated feed and water.
The role of dual infection of NDV and TRT resulted in increased morbidity mortality of turkey poults Turpin et al (2002)
In Egypt, the initial observation suggested the existence of infection pneumovirus was reported by Ahmed (1991). S. Abd El Rahman (2 prepared an inactivated oil adjuvant vaccine against TRT. So, preparation local vaccine against two of the most serious diseases of turkeys (TRT and as a live vaccine was important.
After many records about wide spread of respiratory diseases of turkey different locations of Egyptian governorates, a trial for preparation evaluation of egg adapted live vaccine against two of the most impor respiratory diseases of turkeys (TRT) and (ND) was necessary.

Material and Methods

1. Viruses:

1.1. TRT virus (Local strain):

A strain of avian pneumovirus isolated from turkey by S. Abd El Rahman (2
It was used for vaccine preparation after egg adaptation and tissue cu
adaptation.

1.2. Virulent TRTV strain:

Virulent strain of avian pneumovirus isolated from turkey by. Hafez (1992)
Universitate, Berlin, Germany) designated as VCO3 was used for challen
vaccinated birds via nasal route used (105 TCID50/ml) 0.2 ml /bird.

1.3. NDV (LaSota strain):

A freeze dried live virus vaccine produced in NDV Department, Veter
Serum and Vaccine Research Institute, Abbasia, Cairo. was used
preparation of ND vaccine.

2. Antiserum:

TRT reference hyperimmune serum lot No. Pro-1.97-OC F
(Weybridge, UK) was kindly supplied by Prof. Dr. Fekria El-Bordiny, Veteri
Serum and Vaccine Research Institute, Abbasia, Cairo. Was used for
identification

3. Laboratory host system:

3.1. SPF embryonated chicken eggs:

One hundred specific pathogen free embryonated chicken eggs were obta
from Koum Osheim SPF Farm, Fayoum. The eggs were used for ND and
virus propagation and titration.

3.2. Cell cultures:

A reference green monkey kidney cell line (Vero):

The cells were established by Yasamora and Kawatika (1963) obta
from Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo and u
for TRT virus propagation, titration and SNT.

3.3. Turkeys:

75 one day old, bronze turkeys were obtained from El-Wafaa F
Giza, Egypt. The birds were raised under hygienic measures up to 21 day
and then transferred to special isolates.

4. Reagents used for enzyme linked immunosorbent assay (ELISA):

Avian rhinotracheitis antibody test kit (Bio-Chek) Code, (K120, lot
FS3705, Holland). The kit was supplied with the required buffers and reagent

ELISA was carried out according to Grant et al. (1987) for se
samples.

ELISA positive titre > 1656

1. Samples positive ratio (S/P) =

$$\frac{\text{Mean test sample} - \text{mean of negative control}}{\text{Mean of positive control} - \text{mean of negative control}}$$

2. Calculation of antibody titre:

$$\text{Log10 titre} = 1.0 (\text{log10 S/P}) + 3.52$$

3. Titer = anti-log

5. Vaccines:

5.1. TRT egg adapted living vaccine:

Was prepared during ph. D study by S. Abd El Rahman (2003) f
egg adapted local strain of TRT (106.83 EID50/ml was testing the prepa

vaccine proved to be sterile without any bacterial or fungal contaminants wh tested on specific bacterial and fungal media and free from extraneous viruses

5.2. NDV vaccine:

Egg adapted LaSota strain of Newcastle disease virus with a titre EID_{50}/ml was used for vaccine preparation.

6. Vaccination and challenge:

Susceptible turkey poults of 3 weeks old where their sera were tes by HI test and ELISA for NDV and TRTV antibodies respectively and proved be free from maternally derived antibodies; were divided into five grou vaccinated with 0.25 ml for each bird I/N with either LaSota vaccine or T vaccine as follows:

Group (1): Vaccinated with the locally prepared living attenuated LaS vaccine.

Group (2): Vaccinated with TRT egg adapted living vaccine.

Group (3): Vaccinated with living attenuated simultaneously vaccine of (LaSota) and TRT in the same time.

Group (4): Vaccinated by TRT vaccine at first then followed by ND vaccine days later.

Group (5): It was kept as non-vaccinated negative control group.**15** bird /grou

Blood samples were obtained from turkey poults through the jugular v two weeks intervals and tested by the following parameters:

- Serum neutralization test (SNT) :for TRT virus antibodies, as described Hichner et al (1985)
- Haemagglutination inhibition test (HI) : for ND virus antibodies,as describec Anon (1971)
- Enzyme linked immunosorbent assay (ELISA) :for TRT virus antibod according to wyeth et al (1987).
- Cell mediated immunity by lymphocyte proliferation transformation test. (A 11, 17 and 21 days post vaccination) according to Cook et al (1989b)

7. Challenge test:

Four weeks post vaccination five poults of groups (1, 3, 4 and received 0.5 ml I/M of VVNDV for NDV challenge, according to Anon (1971)

Another five poults of groups (2, 3, 4 and 5) received 0.2 ml of EID50/ml TRTV 6 weeks post vaccination intraocular (I/O) and kept for sco clinical signs ten days post challenge as recorded by Cook et al. (1989).

Results

Table (1): Cell mediated immune response of vaccinated turkeys lymphocyte transformation expressed by optical density

Turkey groups	Optical density			
	4th day	11th day	17th day	21th day
1	0.128	0.219	0.270	0.172
2	0.269	0.585	0.248	0.244
3	0.239	0.366	0.233	0.227
4	0.016	0.020	0.006	0.003

Δ Optical density value:
(OD of PHA – OD of media) – (OD of cells – OD of media)

Table (2): Geometric mean of serum neutralizing antibody (SNT) in TR vaccinated turkeys

Turkey Groups	*ND -antibodies					
	2wpv**	4wpv	6wpv	8wpv	10w pv	12wpv
(2) vaccinated with (Single TRT)	5	6	7.5	7.4	6	5
(3) vaccinated with (ND+TRT)	-	8	9	7.5	6	5
(4) vaccinated with (TRT then ND)	4	5	6.6	8	7	-
(5) Control	2	-	-	-	-	-

*Serum neutralizing antibody titres = the reciprocal of final serum dilution whic neutralize and inhibit the CPE of 100-200 $TCID_{50}$ of TRT virus.
**wpv = weeks post vaccination

Table (3): ELISA antibodies in sera of vaccinated turkeys

Turkey groups	ELISA titer				
	4 wpv*	6 wpv	8 wpv	10 wpv	12 wpv
(2) Vaccinated with(Single TRT)	3122	3361	4227	1209	0.305
(3) Vaccinated with (ND+TRT)	407	943	4071	1206	241
(4) Vaccinated with (TRT then ND)	1650	1846	2816	1158	-
(5) Control	302	161	219	629	508

*wpv =weeks postvaccination
Antibody status:
Negative: 0.349
Suspected: 0.350 – 0.499
Positive: 0.500 or greater

Table (4): Humoral immune response to NDV of turkey as measured by test

Turkey Groups	Mean log2 HI titre					
	2 wpv*	4 wpv	6 wpv	8 wpv	10 wpv	12 wp
(1) vaccinated with (ND)	4.2	7.1	5.3	4.1	4.0	-
(3) vaccinated with (ND+TRT)	5.0	6.6	7.2	5.0	3.3	-
(4) vaccinated with (TRT then ND)	-	7.0	6.8	6.1	5.2	-
(5) Control	-	-	-	-	-	-

*wpv=weeks post vaccination

Table (5): Potency of TRT vaccine in turkeys 6 weeks post vaccination

Turkey Groups	No. of diseased / challenged birds	Protection %
(2) vaccinated with (Single TRT)	0/5	100 %
(3) vaccinated with (ND+TRT)	1/5	90 %
(4) vaccinated with (TRT then ND)	0/5	100 %
(5) Control	5/5	0 %

Table (6): Potency of NDV vaccine in vaccinated turkeys four weeks vaccination

Turkey groups	No. of diseased / challenged birds	Protection %
(1) vaccinated with (Single ND)	0/5	100 %
(3) vaccinated with (ND+TRT)	1/5	90 %
(4) vaccinated with (TRT then ND)	0/5	100 %
(5) Control	5/5	0 %

Discussion

Turkey rhinotracheitis (TRT) is an acute contagious disease of the upper respiratory tract of turkeys, and highly spread to all ages through flocks causing mortality ranged from 2-50% and usually greater in younger turkeys (McDougall and Cook, 1986).

The hazard of the disease depends greatly on the extend infection with other respiratory pathogens such as Newcastle disease virus (NDV) and Pasteurella species (Stuart, 1989).

Since avian pneumovirus infection can not be controlled by medication, the main approach to control was through the use of attenuated vaccine in young stocks of turkeys and inactivated vaccines in layers (Jone, 1996).

Records of wide spread of the disease in different localities in Egypt beside several serological investigations through Egyptian governorates have been reported using commercial ELISA kits confirming the existence of respiratory virus infection of TRT in Egyptian farms (S. Abd El Rahman, 2003). Because of existing of natural exposure by the virus in most kinds of poultry farms, so there is a need to establish a control strategy based on vaccination programs.

Evaluation of the vaccine by cell mediated immune response of poults demonstrated that it reached its maximum value 11 days post vaccination and it was superior in simultaneously vaccinated group (0.585), while in group 4 (which vaccinated with TRT vaccine first then ND vaccine later) reached (0.366) and in single vaccinated group record the lowest value (0.219), laboratory studies suggested that cell mediated immune response provides the main resistance to infection of respiratory tract infection in turkeys (Jones et al., 1992).

Obtained results showed that : results of Haemagglutination inhibition test (HI) demonstrated that group (1) record (27) four weeks post vaccination and gradually decreased till reached (24) ten weeks post vaccination while group (3) of simultaneously vaccinated was (26.6) four weeks post vaccination and (23) ten weeks post vaccination on the other hand group vaccinated firstly with TRT vaccine then ND vaccine ten days later (group 4) record (26) and (26.6) at four and eight weeks post vaccination and reached (25) ten weeks post vaccination.

To confirm successful application of the vaccine and an adequate immune response by birds serum neutralizing antibodies were measured by SNT against TRTV, results in table (2) showed that serum neutralizing antibodies were detected at 4, 8 and 10 weeks post vaccination with mean titre of 7.5, 7.4 and 5.0 for group (2), 9, 7.5, 6 for group (3) and 6.6, 8, 5 for group (4) respectively. These results agree with Cook et al (1996) who mentioned that neutralizing antibodies titer necessary for complete protection of birds. The results are in harmony with results of HI titre.

ELISA used for measuring TRTV antibodies is considered the most common reliable and practical assay (Grant et al., 1987 and Eteradossi et al., 1995), ELISA against TRTV proven the effectiveness of both single TRT vaccine group (2) and simultaneously TRT and ND vaccine group (3) eight weeks post vaccination with a value of 4227 and 4071 while in groups received TRT vaccine then ND vaccine ten days later record a value of 2816 eight weeks post vaccination , results proved that the employed ELISA didn't demonstrate false negative and confirm results, previously observed by Boxter-Jones et al (1989)

In the present study, one can notice SNT to TRTV was detected ea than ELISA titre and this agree with Baxter-Jones et al. (1989).

Concerning the protection of vaccinated birds post challenge VVNDV results as presented in table (6) it reached to 100% by the 15 days challenge, in group (1 and 4) followed by group (3) (90%) while death challenged vaccinated and non-vaccinated poults with virulent TRT revealed that group (2) and group (4) is equal in efficacy with 100% protec and better than group (3).

In conclusion, one can notice that inoculation of attenuated TRT vac at the same time with live attenuated ND vaccine was the program that sa effort, money besides decreasing stresses conducted on birds du vaccination.

References

Ahmed, A.A.S. (1991): Newly emerged diseases of chickens in Egypt. Aerosols, No. 4, Sept. 1

Alexander, D.J. (1997): Newcastle disease and other avian paramyxoviridae infection. Disea Poultry. 10th Ed. Calnek, B.W.; Barnes, H.J.; L.R. and Saif. Iowa State University Ames, Iowa. 541-569.

Anon (1971): Methods of examining poultry biologics for identifying and quantifying pathogens. Nat.Acad. sci. Washington, D.C.

Anon (1996): Avian pneumovirus infection; questions still unanswered Avian pathology. 25:639

Baxter-Jones et al. (1989): A comparison of three methods for detecting antibodies to rhinotracheitis virus. Avian Pathology, 18: 91-98.

Brant, M.; Boxter-Jones, C. And Wilding, G.P. (1987): An enzyme linked immunosorbent for the serodiagnosis of turkey rhinotracheitis infection. Vet. Rec., 120: 279-280.

Eteradossi, N.; Toquin, D.; Guittat, M. And Benneyean, G. (1995): Evaluation of different rhinotracheitis virus used as antigens for serological testing following live vaccination.

Gough, R.E.; Ox, W.J. and Alexander, D.J. (1990): Examination of sera from game bir antibodies against avian viruses. Vet. Rec., 127: 110-111.

Hafez, H.M. (1992): Comparative investigation on different turkey rhinotracheitis (TRT) virus is from different countries. Deutsch Tieraztliche Wochenschrif, 99: 486-488.

Hank's and Wallace (1949): Relation of oxygen and temperature in the preservation of tiss refrigeration. Proc. Soc. Exp. Boil. Med., 71: 196-200.

Jone, R.C. (1996): Avian pneumovirus infection. Question still unanswered. Avian Pathol., 25 648.

Jones, R.C.; Naylor, C.J.; Al-Afaleg and Jones, R. (1992): Effect of cyclophosph immunosuppression on the immunity of turkeys to virus rhinotracheitis. Res. Vet. Sc 38-41.

Lennette, E.H. (1964): Diagnostic procedures for viral and rickettsial diseases. 3rd Ed., Ann., Health Assoc. Inc., Broadway.

McDoughall, J.S. and Cook, J.K.A. (1986): Turkey rhinotracheitis, preliminary investigation Rec., 118: 206-207.

S. Abd El Rahman (2003): Preparation and evaluation of inactivated vaccine against rhinotracheitis virus. Ph.D. Thesis, Fac. Vet. Med., Cairo Univ.

Stuart, T.C. (1989): Rhinotracheitis in Great Britain. Recent Advanced in turkey science, Science Symposium series No. 21, London, UK, Butter Worths.

Yasamora, and Kawatika (1963): Studies on SV40 virus in tissue cultures. Ivihon Rinsho, 21, 1215.

Turpin,E.A.;Perkins,L.E. and Swayne,D. (2002): Experimental infection of turkeys with pneumovirus and New Castle disease virus or E .Coli . Avian Dis., 36 (2):412-422

الملخص العربى

دراسات مناعية لأستخدام لقاحى التهاب القصبة الهوائية الرغامى والنيوكاسل فى الرومى باستخدام برامج تحصين مختلفة

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** المعمل المركزى للرقابة على المستحضرات الحيوية البيطرية - العباسية – القاهرة

أظهرت عدة تقارير من محافظات مختلفة فى جمهورية مصر العربية انتشار اعراض تنفسية فيروسية فى عديد من مزارع طيور الرومى . وقد اجريت هذه الدراسة كمحاولة لتحصين طيور الرومى ضد فيروس التهاب القصبة الهوائية الرغامى من عترة معزولة محليا وايضا ضد فيروس مرض النيوكاسل كلا على حدة فى مجموعتين مختلفتين وحقن احداهما قبل الأخر فى مجموعة ثالثة والتحصين المتزامن فى مجموعة رابعة بالاضافة الى المجموعة الضابطة .

عند تقييم اللقاحات فى المجموعات المختلفة من طيور الرومى بقياس المناعة الخلوية بواسطة اختبار تحور الخلايا الليمفاوية وقياس المناعة الخلطية بواسطة اختبار المصل المتعادل (SNT) والاليزا (ELISA) ضد مرض التهاب القصبة الهوائية الرغامى واختبار قوة منع التلازن الدموى (HI) واختبار التحدى أظهرت النتائج أعلى مستويات للمناعة بعد اربعة اسابيع من الحقن واستمرت الوقاية ضد المرضين حتى الاسبوع الثامن.