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RESEARCH

Evaluation of Turkey humoral immune response to Avian Influenza and Turkey Rhinotracheitis viruses' vaccines

Rana A. Rabiea[1], Mohamed Fawzy[2], Mohamed H. Khodeir[3] and MokhtarM. EL-Tarabili[2]

ABSTRACT

Background: Turkeys are susceptible to many virus diseases such as turkey rhinotracheitis (TRT) and avian influenza (AI) that affect their populations in a dramatic form. Successful control of these viral diseases depends on the implementation of well-designed vaccination programs using highly potent and safe vaccines.
Aim: The present study investigated the effect of simultaneous administration of inactivated AI vaccine and live attenuated TRT vaccine on the immune response of turkey chickens.
Methods: Four groups of bronzy turkey chickens were used in this study; group-1 received live attenuated TRT vaccine, group-2 received inactivated AI vaccine, group-3 received TRT and AI vaccines and group-4 served as non-vaccinated control. The humoral immune response evaluated serologically by using hemagglutination inhibition test (HI), serum neutralization test (SNT), and indirect ELISA.
Results: There was detectable TRT antibody titers by SNT in the first week post vaccination as 4 $\log_2$ in group-1 and 2 $\log_2$ in group-3. Both groups showed peak SNT titer (256 $\log_2$) of TRT virus by the third-week post administration of the second dose and remain stable up to 24 weeks post vaccination. Follow up avian influenza antibody titers using HI test, peak titer (64 $\log_2$) was detected in group-2 and group-3 by the second week after receiving the second dose then began to decrease in both groups by third-week post the second dose to reach the zero level at week twenty-four. ELISA test confirm the results of SNT and HI.
Conclusion: There is no antagonizing effect between the two vaccines on the immune response of turkey chickens against each other as all vaccinated birds exhibited good levels of specific TRT and AI antibodies. Therefore, it is possible to vaccinate turkeys simultaneously against TRT and AI safely and potently.

Key Words:
Turkey, Avian Influenza, turkey rhinotracheitis, turkey chicks.

BACKGROUND

Turkey called *Meleagris gallopavo* belongs to the family Meleagrididae raised mainly for meat production or for hatching eggs production. Turkey Rhinotracheitis (TRT), caused by avian metapneumovirus (aMPV), is an acute highly infectious upper respiratory tract disease mainly in turkeys and chickens. It called turkey rhinotracheitis in turkeys and swollen head syndrome (SHS) in chickens. The avian metapneumovirus is a single-stranded negative-sense non-segmented RNA virus belonging to genus *Metapneumovirus*, family *Paramyxoviridae.* It causes high economic losses in turkey and chicken flocks specially when accompanied by secondary pathogens. aMPV is grouped into four antigenically different subtypes A, B, C, and D (Ferreira, 2015). Turkey rhinotracheitis infect turkeys of all ages showing sneezing, tracheal rales, frothy ocular or nasal discharge, foaming conjunctivitis, submandibular edema, swelling in the infraorbital sinuses (Pringle, 1998), and loss in egg production (Seifi and Boroomand, 2015).

Correspondence: mohamed_mohamed4@vet.suez.edu.eg
Full list of author information is available at the end of the article

Inactivated oil adjuvant vaccines, and live attenuated vaccines used in the control of turkey rhinotracheitis infection in turkeys (Cook, 2000).

All types of birds are susceptible to avian influenza disease with species differences in susceptibility. AI signs in birds varying from mild sickness to a highly infectious and fatal disease resulting in severe epidemics called "highly pathogenic avian influenza" which characterized by sudden onset, severe illness, rapid death, and mortality rate can reach 100 % (FAO, 2005). Wild aquatic birds are considered the natural reservoir of influenza type A viruses and play a major role in the spread of AI infection among the mammalian and avian species causing severe outbreaks (Webster *et al.*, 1992).

Avian influenza caused by single-stranded, negative-sense RNA virus belong to genus *influenzavirus A*, family *orthomyxoviridae* with a segmented genome (eight segments) (Swayne *et al.*, 2003). Influenza A viruses are divided into subtypes according to the two surface proteins of the virus: hemagglutinin (HA) of known 18 subtypes and neuraminidase (NA) of known 11 subtypes (CDC 2017). Low pathogenic avian influenza (LPAI) in turkeys characterized by sudden onset of dullness, coughing, and anorexia; male turkey shows milder clinical signs (Reid *et al.*, 2016). Inactivated vaccines used in the control of avian influenza (AI) in order to manage, prevent or eradicate AI from poultry and other birds. AI vaccines usually produced from low pathogenic AI virus strains and rarely produced from high pathogenic AI virus strains (Swayne, 2009).

The present study was planned to investigate the effect of inactivated AI vaccine and live attenuated TRT vaccine on the immune response of turkey chickens against each of them when administrated in a mutual manner.

MATERIALS AND METHODS

Avian influenza and TRT vaccines

Inactivated oil adjuvant avian influenza type A subtype H_5N_2 vaccine (A/chicken/Mexico/232/94/CPA) under the trade name Volvac AIKV of a titer $10^{7.6}$ egg infective $dose_{50}$ (EID_{50})/dose and 32 HAU/dose. It was supplied by Boehringer Ingelheim Vetmedica, GmbH, Germany.

Live attenuated cell culture TRT vaccine was supplied under the trade name Aviffa RTI by MERIAL, Lyon, France in the form of freeze-dried pellet for nasal, ocular and oral suspension. Each dose contains $10^{2.3}$ tissue culture infective $dose_{50}$ ($TCID_{50}$).

Avian influenza and TRT antigens

The H_5N_2 antigen of the avian influenza virus was supplied by ID.VET Company for innovative diagnostics and used in HI.

TRT antigen was prepared by infection of Vero cells with the virus followed by two cycles of freezing and thawing when complete CPE was obtained, centrifugation at 3000 rpm for 5 minutes then at 100,000 rpm for 1 hour at 4^oC. The virus pellet was suspended in PBS with pH 7.2.

African Green Monkey Kidney (VERO) cell culture

Vero cells were established by Yasummara and Kawatika (1963), and obtained from the Department of Pet Animals Vaccine Research, Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo, Egypt. These cells were used in SNT to follow up TRT antibodies in vaccinated turkeys.

Birds and Vaccination schedule

One hundred one-day bronzy turkey chickens were obtained from Animal Production Research Institute, Mahalet Mousa Station, and divided into four groups, (25 each) as follows:

* Group-1: vaccinated at 7-day old with TRT vaccine through the drinking water, receiving a booster dose on 21st day of age.
* Group-2: vaccinated at 7-days old with 0.5 ml S/C injection of AI vaccine, then received a booster dose on the 35th day according to the producer instructions.
* Group-3: vaccinated at 7-days old with TRT vaccine in drinking water and AI vaccine 0.5ml S/C injection then received a booster dose of TRT vaccine on the 21st day of age then a booster dose of AI vaccine on the age of 35 days.
* Group-4: served as non-vaccinated control group.

All birds were housed under hygienic measures receiving balanced ration and adequate water.

Sampling

Blood samples were obtained from the experimental birds through the jugular vein puncture under complete aseptic conditions according to Lennete (1964) and allowed to form clots at 4°C overnight. The serum was separated and centrifuged at 2000 rpm for 15 minutes then kept in sterile screw-capped vials at -20°C till subjected for serological examination. Serum samples were obtained on week intervals for 4 weeks then month intervals up to 6 months post-vaccination.

Hemagglutination (HA) and Hemagglutination inhibition (HI) test

The HA test was carried out according to Allan *et al.,* (1978) to determine the HA units of AI antigen required to apply HI test. HI test was done using the Beta procedure according to the standard method of examining poultry biologics (Anon 1971).

Serum Neutralization test (SNT)

SNT was carried out using the microtiter technique according to Bass *et al.,* (1982). The antibody titer was expressed as the reciprocal of the final serum dilution that neutralized and inhibited completely the CPE of 100 $TCID_{50}$ of the used virus according to Singh *et al.,* (1967).

Indirect Enzyme-Linked Immuno-Sorbent Assay (ELISA):

ELISA were done according to Voller *et al.,* (1976).

RESULTS

Monitoring of TRT serum neutralizing antibody titers in vaccinated turkey chickens

After administration of the first dose of live attenuated TRT vaccine, there are detectable serum neutralizing antibody titers (as demonstrated by SNT) by the first week post vaccination as 4 log_2/ml in group-1 (receiving TRT vaccine alone) and 2 log_2/ml in group-3 (receiving TRT and AI vaccines). Both vaccinated turkey's groups showed peak titer of TRT serum neutralizing antibody titer (256 log_2/ml) by the third-week post administration of the second dose that remain stable up to 24 weeks post vaccination as in table (1). Indirect ELISA showed that the first dose of TRT vaccine resulted in detectable antibodies in vaccinated turkey chickens by the first-week post vaccination as 20 log10/ml in group-1 and 30 log10/ml in group-3. Both groups reached the Peak TRT-ELISA titer (155 log10/ml) by the third week after the second dose up to 24 weeks post-vaccination. No antagonizing effect of AI vaccine on the turkey's immune response to TRT vaccine as in table (1). All non-vaccinated birds remain seronegative all over the experimental period.

Table 1: Mean TRT-serum neutralizing antibody titers in vaccinated turkey chickens.

Weeks post vaccination	Mean TRT serum neutralizing antibody titers* (log_2/ml) in vaccinated Turkey chickens groups			Mean TRT-ELISA antibody titers (log_{10}/ml) in vaccinated Turkey chickens groups		
	Group-1	Group-3	Group-4	Group-1	Group-3	Group-4
0 (at 7 days old)	0	0	0	0	0	0
1WP1st V**	4	2	0	20	30	0
2 WP1st V	8	8	0	45	45	0
3 WP1st V	32	32	0	102	106	0
1 WP2nd V***	64	64	0	144	110	0
2 WP2nd V	128	128	0	148	122	0
3 WP2nd V			0			0
4 WP2nd V			0			0
8 WP2nd V		↑	0		↑	0
12 WP2nd V	←	256 →	0	←	155 →	0
16 WP2nd V		↓	0		↓	0
20 WP2nd V			0			0
24 WP2nd V			0			0

Group-1= turkey chickens vaccinated with TRT vaccine at 7 days old and receiving the second dose at 21 days old.
Group-3= turkey chickens received TRT and AI vaccines on the 7th day of age and the second dose of TRT vaccine on the 21st day of age then AI vaccine at the age of 35 days.
Group-4= non-vaccinated control group.
* TRT serum neutralizing antibody titers = the reciprocal of the final serum dilution which neutralized and inhibited the CPE of 100 $TCID_{50}$ of TRT virus
**WP1stV= week post first vaccination. ***WP2nd V= week post second vaccination.

Following up avian influenza antibody titers in vaccinated turkey chickens:

Vaccinated turkey chickens exhibited detectable antibodies against AI (using HI test) by the first week post vaccination as 4 log_2/ml in group-2 (vaccinated with AI vaccine) and 2 log_2/ml in group-3 (vaccinated with TRT and AI vaccines). Both vaccinated turkey's groups showed peak AI-HI titer (64 log_2/ml) by the second week after receiving the second dose then began to decrease by third-week to reach the zero level by the 24th week as shown in the table (2). The first dose of AI vaccine-induced detectable ELISA titers in vaccinated turkey chickens by the first-week post vaccination as 20 log_{10}/ml in group-2 and 30 log_{10}/ml in group-3. These levels of AI-ELISA titer recorded peaks of 148 and 150 log_{10}/ml in group-2 and group-3 respectively by the second week after administration of the second dose of AI vaccine then began to decrease by third-week post second dose vaccination to reach the zero level by the 24th week later as shown in the table (2). All unvaccinated birds remain seronegative all over the experimental period.

Table 2: Mean AI-HI antibody titers in vaccinated Turkey chickens.

Weeks post vaccination	Mean AI-HI antibody titers* (log_2/ml) in vaccinated Turkey chickens groups			Mean AI-ELISA antibody titers (log_{10}/ml) in vaccinated Turkey chickens groups		
	Group-2	Group-3	Group-4	Group-2	Group-3	Group-4
0 (at 7 days old)	0	0	0	0	0	0
1WP1st V**	4	2	0	20	30	0
2 WP1st V	8	4	0	45	45	0
3 WP1st V	16	16	0	102	106	0
1WP2nd V***	32	32	0	144	145	0
2 WP2nd V	64	64	0	148	150	0
3 WP2nd V	48	48	0	110	122	0
4 WP2nd V	32	40	0	98	112	0
8 WP2nd V	16	20	0	100	102	0
12 WP2nd V	8	8	0	60	48	0
16 WP2nd V	2	4	0	45	30	0
20 WP2nd V	2<	2<	0	30	20	0
24 WP2nd V	0	0	0	0	0	0

Group-2= turkey chickens vaccinated with AI vaccine at 7 days old, receiving the second dose on the day 35 of age.
Group-3= turkey chickens received TRT and AI vaccines on the 7th day of age and the second dose of TRT vaccine on the 21st day of age then AI vaccine at the age of 35 days.
Group-4= non-vaccinated control group.
* HI titers of AI antibodies= the reciprocal of the final serum dilution which inhibited 4 HA units of AI antigen.
**WP1stV= week post first vaccination. ***WP2nd V= week post second vaccination.

DISCUSSION

Regarding vaccination of turkey chickens with live attenuated TRT vaccine, the antibody titers provide evidence for the effectiveness of the used vaccine that is necessary for the complete protection of adult birds (Cook *et al.,* 1996). SNT was used by Cook *et al.,* (1988) for detection of TRT antibodies in commercial flocks of chickens in Britain after initial appearance of TRT virus from day 31 to week 56. Williams *et al.,* (1991) showed that Vero cell preparation of TRT vaccine was satisfactory able to protect turkeys against clinical disease signs. Equal protection was recorded against virulent challenge when used in the presence or absence of maternal antibodies using Vero attenuated TRT virus (Naylor and Jones, 1993). In disagreement with Sowa *et al.,* (2000) who showed that no distinct increase in TRT antibody titers after vaccination twice orally with Aviffa-TRT lives vaccine. However, Gulati *et al.*, (2001) prepared a live attenuated TRT vaccine by 7 passages in CEF and 34 passages in Vero cells and when administrated by nasal, ocular and drinking water routes induced protection against virulent virus challenge for at least 10 weeks. Jirjis *et al.,* (2001) found that vaccination of turkeys with a dose of $4x10^3$ $TCID_{50}$/ml protect them against virus infection.

Monitoring TRT antibodies by ELISA proven the effectiveness of the used TRT vaccine, concerning ELISA as the most common reliable and practical assay as concluded by Grant *et al.*, (1987); Chettle and Wyeth (1988); Mandour *et al.,* (2014), O'Loan *et al.*, (1990), Elfeil *et al.,* (2012), and Eteradossi *et al.*, (1995). Detected TRT antibodies in this study are specific and due to vaccination where unvaccinated control birds showed negative results, confirming the usefulness of ELISA in measuring TRT immune response as cited by Baxter-Jones *et al.,* (1987). It was noticed that both results of SNT and ELISA came in a parallel manner in contrast to those of Baxter-Jones *et al.,* (1987) who suggested that SNT detected TRT antibodies earlier than ELISA. Ahmed (2003) showed that such vaccine is safe inducing no illness signs on vaccinated birds and potent as measured its ability to induce specific anti-TRT antibodies by serum neutralization test and indirect ELISA. He recorded TRT serum neutralizing antibodies similar to our present obtained results till 18 weeks post vaccination. In addition, he added that there was no antagonizing effect between live TRT vaccine and Newcastle disease vaccine on the immune response of vaccinated turkeys vaccinated simultaneously with the two vaccines.

Regarding vaccination of turkey chickens with AI vaccine, Narayan *et al.,* (1970) stated that the immune response of turkeys to inactivated AI virus at any level of detectable antibodies by HI or SNT induce protection for at least 42 days. It was clear that the booster dose increased the level of induced AI antibodies in agreement with Shieh *et al.,* (1993) who reported that AI vaccine was administrated to chickens at 3 or 5 weeks old then at 8 weeks producing high titers of HI antibodies remain to a long time. In the same way Bertelsen *et al.,* (2007) found that inactivated AI-H5N9 developed 76% protection in vaccinated birds with HI titer ≥32 with geometric mean titer 137. Tian *et al.,* (2005) and Kumar *et al.* (2007) supposed that HI antibody titers of $4log_2$ or higher of vaccinated chickens were completely protective from virus challenge. Adair *et al.,* (1989) and Kodihalli *et al.,* (1993) concluded that ELISA is a rapid, economical, sensitive and specific method for screening of avian sera for AI antibodies. Similarly, Shafer *et al.,* (1998) and Jin et al. (2004) used indirect ELISA for detecting AI antibodies in chicken sera

and noticed that it was more sensitive than HI test with agreement 82%. On the other side it was concluded that typical assay to measure vaccine responses against AI include HI test and SNT as standardized tests are easy to perform and provide a quantitative measure of antibodies based on their ability to neutralize virus particles as stated by Rowe *et al.,* (1999). The use of inactivated AI H5N2 vaccine, Ellis *et al.,* (2004) concluded that vaccination of chickens with such vaccine could be a tool to enhance bio-security measures and intensive surveillance for control of high pathogenic AI virus in Hong Kong in 2002. Similar results confirming the present results in vaccinated turkeys with inactivated AI-H5N2 were obtained by Samah *et al.,* (2009) using HI and ELISA.

Spotting the light on simultaneous vaccination of turkey chickens with TRT and AI vaccines there are no available data discuss this point but other studies carried out on simultaneous vaccination of turkeys with ND and AI-H5N2 vaccines revealed no antagonizing effect between the two vaccines on the turkey immune response to any of them as demonstrated by Samah *et al.,* (2009).

AUTHOR DETAILS

[1]Veterinary Directorate, Port Said, Egypt.
[2]Department of Virology, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt.
[3]Veterinary Serum and Vaccine Research Institute, Abbassia, Cairo, Egypt.

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Authors' contributions:

Fawzy M., Rabiea R.A., organized the whole process and drafted the manuscript. Fawzy M., Khodeir M. H., El-tarabilli M. M. A., designed the work. Rabiea R. A., Fawzy M., and Khodeir M. H., performed the work. Fawzy M., Rabiea R. A., performed the data analysis. Fawzy M., and Rabiea R. A., written the work. All authors read and approved the final manuscript.

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