Protection of quail against avian influenza.

Abd Elaziem, M.A.[1]; El-Bagoury, G.F.[2]; Sharawy, S. [2], Amani, A. Saleh[3] and Khodeir, M.H.[3]

[1]General Organization for Veterinary Services, [2]Department of Virology, Faculty of Veterinary Medicine, Benha University, [3]Veterinary Serum and Vaccine Research Institute, Abassia, Cairo.

A B S T R A C T

An attempt has been under taken to evaluate and investigate the humeral immune response of quail to the inactivated avian influenza (AI) vaccine. Fifty quail were divided into 2 groups where group-1 of 40 birds was vaccinated with inactivated avian influenza vaccine (H5N2) while group-2 of 10 birds was kept without vaccination as control. The induced AI antibodies in vaccinated quail were followed up using HI and ELISA up to 28 weeks post vaccination. All quail remained healthy all over the experimental period showing no abnormal clinical signs. The exhibited AI antibodies reached their peak (64 by HI and 145 by ELISA) by the 4th week post vaccination then begin to decrease by the 8th week (16 by HI and 122 by ELISA). This finding suggest the successful of quail vaccination against AI but more booster doses may be required and further studies are in need to investigate the role of quail in AI epidemiology and immunization.

Keywords: avian influenza, Quail, HI, ELISA.

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1. INTRODUCTION

Quail are members of the kingdom Animalia; phylum Chordata; Class Aves; order Galliformes; family Phasianidae; genus Coturnix having the scientific name Coturnix Coturnix. The term "influenza" originally referred to epidemics of acute, rapidly spreading catarrhal fevers of humans caused by viruses in the family Orthomyxoviridae (Kilbourne, 1987). The viral genome is composed of eight segments of single- stranded, negative-sense RNA that code for 10 proteins. Eight proteins are constituents of the virus (HA, NA, NP, M1, M2, PB1, PB2, and PA), and the two nonstructural proteins (NS1 and NS2) are located in the host cell cytoplasm. Recently, NS2 has been shown to also be a minor constituent of virions (Lamb and Krug, 1996). It was suggested that quail may share as an intermediate host and reservoir of avian influenza viruses (AIV). To elucidate this question, European quail were experimentally challenged with two highly pathogenic AIV (HPAIV: H7N1/HP and H5N1/HP) and one low pathogenic AIV (LPAIV) (H7N2/LP). Contact animals were also used to assess the viral transmission among birds. Severe neurological signs and mortality rates of 67% (H7N1/HP) and 92% (H5N1/HP) were observed. Although histopathological findings were present in both HPAIV-infected groups, H5N1/HP-quail displayed a broader viral antigen distribution and extent of microscopic lesions. Neither clinical nor pathological involvement was observed in LPAIV-infected quail. Consistent long-term viral shedding and effective transmission to naive quail was demonstrated for the three studied AIV. Drinking water arose as a possible transmission route and feathers as a potential origin of HPAIV dissemination. The study demonstrated that European quail may play a major role in AI epidemiology, highlighting the need to further understand its putative role as an intermediate host for avian/mammalian reassortant viruses (Kateri, 2013). Transmission of virus from intranasally infected birds to birds placed in contact varied considerably with both host and infecting virus and the various combinations of these (Alexander et al., 1986). In 1972, wild waterfowl of the order Anseriformes (ducks and geese) were demonstrated to be a principal reservoir and the natural host for mild pathogenic avian influenza (MPAI) viruses (Slemons et al., 1974). In 1979, the cleavability of the haemagglutinin (HA) protein was identified as the major determinant of virulence in HPAI viruses (Bosch et al., 1979). In 1981, the first International Symposium on avian influenza was convened in Beltsville, Maryland, United States of America (USA), and the term 'fowl plague' was abandoned for the more, accurate term 'highly pathogenic avian influenza' (Bankowski, 1981)

The present work was designed to investigate the possibility of quail protection against AI infection through evaluation of their immune response to AI vaccine.

2. MATERIAL AND METHODS

2.1. Quail :

Fifty quail were obtained from a commercial market. These quail were found to be healthy and free from external and internal parasites. Also, all quail were screened serologically and found to be free from AI and Newcastle antibodies through application of haemagglutination inhibition test (HI). They were housed under hygienic measures receiving balanced ration and adequate water. These birds were divided into 2 groups housed separately where group (2) of 40 birds were vaccinated with AI vaccine receiving 2 doses (0.5ml/bird) inoculated intramuscular with one-month interval. The second group of 10 quail was kept without vaccination as control. Serum samples were obtained from all birds on week intervals up to 28 weeks post vaccination and subjected for HIT to follow up the levels of induced AI antibodies.

2.2. Avian influenza vaccine:

Inactivated oil adjuvant avian influenza vaccine type-A, subtype H5N2 A/chicken/Mexico/232/94/CPA under the trade name Volvac AIKV of a titer $10^{7.6}EID_{50}$/dose and 32HAU/dose. It was supplied by Boehringer Igelheim Vetmedica, GmbH, Germany. The recommended dose is 0.5ml for each bird inoculated intramuscularly.

2.3. Virus Antigen:

H5N2 antigen of avian influenza virus was supplied by ID.VET Company for innovative diagnostics and used in HIT and ELISA. ND antigen was kindly supplied by Veterinary Serum and Vaccine Research Institute (VSVRI) Abassia, Cairo and used in HI tests to screen the experimental quail for ND antibodies.

2.4. Erythrocytes :

Chicken RBCs were obtained from healthy unvaccinated chickens; prepared and diluted to be 1% to be used in HA and HIT (Allan et al., 1978).

2.5. Sampling:

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

2.6. Hemagglutination test (HA):

HA test was carried out to determine the HA titer of AI antigen used in HI test. The test was carried out using the micro-titer technique according to Capucci et al. (1996).

2.7. Hemagglutination Inhibition (HI):

HI test was carried out for titration of AI antibodies in vaccinated quails according to Vander (1980). The HI antibody titer was calculated as $\log_2$/ml.

2.8. Indirect ELISA:

The indirect method of ELISA was carried out to estimate AI antibodies in vaccinated quail according to the comminuted methods of Hubschle et al. (1981) and Voller et al. (1976) using anti=chicken conjugate with horse radish peroxidase. The antibody titer was calculated using the following equation:

$$\frac{\text{OD Serum sample–OD (NCX)}}{\text{OD (RCX) - OD (NCX)}} X \quad \text{End dilution of reference serum}$$

* NCX = Negative control mean
* RCX = Reference control mean

3. RESULTS

The present work revealed that the used inactivated AI (H5N2) vaccine is safe for quail showing no local or systemic post vaccination reaction. Monitoring the level of induced AI antibodies in vaccinated quail using HI test and ELISA (table-1 and 2) demonstrated that AI-HI antibody titer reached 64 at the 4th weeks post vaccination and decreased to 2 at the 20th week then declined to 0 at the 24th week and the mean AI-ELISA antibody titers increased to 145 at the 4th weeks post vaccination and decreased to 30 at 24th weeks.

All vaccinated quail showed negative HI and ELISA results by the 28th week post vaccination. Also, all control quail remain sero-negative all over the experimental period.

Table (1): Mean AI-HI antibody titers in vaccinated quail

Quail groups	Mean AI-HI antibody titers*/WPV**											
	0	1W PV	2W PV	2nd Dose	3W PV	4W PV	8WP V	12W PV	16W PV	20W PV	24WP V	28W PV
1	0	8	16		32	64	16	8	4	2	0	0
2	0	0	0		0	0	0	0	0	0	0	0

* HI titers of AI antibodies= the reciprocal of the final serum dilution which inhibited 4 HA unites of AI antigen.
**WPV= week post vaccination

Table (2): Mean AI-ELISA antibody titers in vaccinated quail

Quail groups	Mean AI-ELISA antibody titers*/WPV**											
	0	1W PV	2W PV	2nd Dose	3W PV	4W PV	8WP V	12W PV	16W PV	20W PV	24WP V	28W PV
1	0	60	98		112	145	122	112	102	48	30	0
2	0	0	0		0	0	0	0	0	0	0	0

* ELISA titers of AI antibodies= calculated according to Hubschle et al., 1981 and Voller et al., 1976. **WPV= week post vaccination

4. DISCUSSION

The humeral immune response to the inactivated AI vaccine (H5N2) was evaluated in quail by HI test showing results demonstrated in table (1). This test revealed that AI-HI antibody titer reached 64 at the 4th weeks post vaccination and decreased to 2 at the 20th week then declined to 0 at the 24th week. The data presented in table (2) shows the results of AI antibody titer among sera of quail vaccinated with inactivated AI vaccine using ELISA. Mean AI-ELISA antibody titers increased to 150 at the 8th weeks post vaccination and decreased to 30 at 28th weeks. Bertelsen et al. (2007) reported that 540 birds in 3 zoos were vaccinated twice against avian influenza with at 6 week intervals using an inactivated H5N9 vaccine. Serological response was evaluated by hemagglutination inhibition test 4-6 weeks following the second vaccine administration, 84% of the birds sero-converted, and 76% developed a titre ≥32. It was reported that in four major Swiss zoos carried out the vaccination of selected zoo birds with the adjuvant inactivated vaccine H5N2 Nobils influenza. Pre- and post- vaccination antibody titers were determined either by HI test or by ELISA at week 0,5,10 and 26 (day 0-1, 35-36, 70-71, and 18 respectively) to determine the humoral immune response to H5 antigen. Based on the antibody titer profiles of all investigated species, they recommend at least annual revaccination for the species that investigated (Maria et al., 2008). Regarding application of ELISA using anti-chicken conjugate; Meulemans et al. (1987) and Adair et al. (1989) reported that ELISAs detect antibody only to the nucleoprotein. There were no available anti-quail conjugated with horse radish peroxidase, so ELISA using an anti-chicken conjugate may make the test species specific. It was suggested that AC-ELISA is a rapid, economical, sensitive and specific sero-diagnostic method for screening large number of avian sera for antibodies of AI (Kodihalli et al., 1993). Shafer et al. (1998) and Jin et al. (2004) used NP-based type specific indirect ELISA for detecting antibodies of AI from chickens and said that it was more sensitive than HI with agreement 82%.

Depending on the obtained results it could be concluded that the inactivated AI (H5N2) vaccine is safe and immunogenic for quails but further studies are in need to investigate other points related to the quail role in the epidemiology of AI especially they are reared now as source of animal protein of low price instead of beef or chicken meat.

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