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Effect of time vaccination on immunization of broiler chicks against influenza A subtype H_9N_2 virus

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ABSTRACT

The effective time of inactivated influenza A subtype H_9N_2 vaccines, in preventing the disease was evaluated in day-old broiler chicks (150). They were divided randomly into 3 equal groups.

Group A chicks were vaccinated at the age of 2 days. The chicks of group B were vaccinated similar to the group A, but in the age of 8 days. Group C were kept as unvaccinated control group. Blood samples were collected from wing vein of 5 chicks of each groups weekly from day-old up to the end of experiment. Serum antibody titre of influenza A subtype H_9N_2 was determined by haemagglutination inhibition (HI) test. Half of the chicks of group A,B and C were challenged with influenza A subtype H_9N_2 virus at the age of 30 days.

It was concluded that the level of mean HI titre in chicks vaccinated at 2 or 8 days was low. Vaccination on the 8 days of age showed better immune response and protection. The vaccination decreased the severity of clinical signs, morbidity and mortality, but did not protect the birds against infection. Administration of killed avian influenza vaccine has useful effects in broiler chicks against infection in areas where disease is prevalent.

Key words: Broilers, Immunization, Influenza, Vaccine

Avian influenza is an infection caused by any type A virus of the paramyxoviridae family. Type A viruses are responsible for major disease problems in birds as well as in humans and lower mammals. Recently new avian influenza A virus subtype combination H_5N_7 was identified in Danish mallard ducks (Bragstad *et al.* 2005). Severity of signs depends on the species affected, age, sex, concurrent infections, strain of virus, environmental factors, etc. Because of antigenic variation of influenza virus, vaccination with live vaccines is not advised (Esterday *et al.* 2003). Recent epizootics of highly contagious OIE list A diseases, such as foot-and-mouth disease, classical swine fever and avian influenza (AI), have led to the implementation of stamping-out policies resulting in the depopulation of millions of animals. The implementation of a control strategy that is based only on the application of sanitary restrictions and that involves the culling of animals that are infected, suspected of being infected or suspected of being contaminated, may not be sufficient to avoid the spread of infection. This control strategy results inevitably in mass depopulation. There is an increased risk of disease spread, and the financial consequences of the occurrence of an epizotic are severe (Capua and Marangon 2003, Capua and Marangon 2004, Capua and Mutinelli 2001). The slaughter and destruction of large numbers of animals is also questionable from an ethical point of view, particularly when the implication for human health are negligible. Mass depopulation has raised serious ethical concerns among the general public. Inactivated influenza virus vaccines have been used in a variety of avian species, and their effectiveness in preventing clinical signs and mortality is different. However, protection is virus subtype specific. Birds are susceptible to infection with influenza viruses belonging to any of the 15 HA subtypes, and there is no way to predict their exposure to particular one. It is not practical to use preventive vaccination against all possible subtypes. After an outbreak occurs and the subtypes of the virus is identified, vaccination may be useful tool (Esterday *et al.* 2003). Numerous experimental studies have demonstrated that inactivated monovalent and polyvalent viral vaccines, with adjuvants, are capable of inducing antibody and providing protection against mortality, morbidity and egg production declines. No debate has been made that inactivated vaccines have a role in the control of non-H5 and non-H7AI. In Iran recent outbreak of influenza A subtype H_9N_2 in poultry farms caused serious economic loss. The objective of this study was to evaluate effects of vaccination time on the efficiency of inactivated influenza A subtype H_9N_2 vaccine.

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MATERIALS AND METHODS

Vaccination: A monovalent A/chicken/Iran/79 (H_9N_2) oil-emulsion vaccine (prepared by Razi Vaccine and Serum Research Institute) was used.

Virus challenge: A/chicken/Ahvaz/80(H_9N_2), a low-pathogenicity influenza virus isolated from a field case, was used for challenge. Each chicken was challenged orally with 0.1 ml of infected undiluted allantoic fluid containing approximately $10^{7.5}$ EID_{50}/ml.

Chicken: Day-old broiler chicks (150) free from maternal antibody against influenza virus, were used.

Serological procedure: Hemagglutination inhibition (HI) test was done as per OIE (2000) and Swayne *et al.* (1998). The HI test was carried out in U-shaped microtitre plates using 4 HA units of antigen per well. The HI results were averaged into a geometric mean titre (GMT) for each treatment (Swayne *et al.* 1998).

Experimental design: Day-old broiler chicks (150) were divided into 3 equal groups of 50 each. Group A and B chicks were vaccinated at 2-and 8-day-old subsequently. Each chick received 0.5 ml of the vaccine subcutaneously in the anterior dorsal cervical region. Group C were kept as unvaccinated control chicks. At 30 days of age half of the each vaccinated group and unvaccinated control group were separated randomly and kept in 3 isolation rooms (A', B' and C') and challenged orally with 0.1 ml of influenza virus. All the birds were kept under high hygienic condition on the floor and had access to complete sterile feed.

Following oral live virus challenge, the chicks were closely observed daily for the clinical signs.Blood samples were collected from wing vein of 5 chicks of groups A, B, C at weekly interval and from chicks of groups A, B and C at 0, 7, 14 and 21 days after challenge. Sera were separated immediately and kept at –20°C until test was done.

RESULTS AND DISCUSSION

Serology: The influenza vaccine administered at 2- and 8-day-old induced measurable antibody against hemagglutinin antigens of the virus as detected by HI test (Table 1).

Clinical signs: Chicks of group A, B and C 5 days after challenge with influenza virus showed signs of depression, decreased feed consumption, ruffled feather and sneezing,

Table1. Geometric mean titre of chicks vaccinated for influenza

	Days post vaccination					
	7	14	21	28	35	42
A*	0	1.15	1.7	2.72	2.6	2.42
B**	1.8	2.15	2.75	3.11	3.3	3.95
C	0	0	0	0	0	0

*Vaccinated at 2 days of age, **vaccinated at 8 days of age.

Table 2. Geometric mean titer of vaccinated and unvaccinated chicks challenged at 30 days of age with influenza virus

	Days post infection			
	0	7	14	21
A'	2.5	4.37	5.75	5.25
B'	2.75	4.75	5.7	6
C'	0	3.3	4.3	5.75
C	0	0	0	0

but clinical signs in group C was more severe than chicks of groups A'and B'. In each group A' and B' mortality of one chick and in group C' mortality of 4 chicks were observed in first week after challenge.

Cross protection between different haemagglutinin subtype appears to be minimal (Karunkaran *et al.* 1987, Newman *et al.* 1981). The role of immune status of the chicken in regulating susceptibility to influenza infection and response to vaccination has not been clearly understood, usually, influenza subtype H9N2 in Iran is seen mostly concurrently with other viral and/or bacterial agent specially *E.coli* and *Mycoplasma* infections or concurrently with a prolonged environment stress at the time of viral exposure (Karunkaran *et al.* 1987). For these reasons, there has been no good challenge method available to determine the efficacy of a vaccine against infection and/or disease with the mild strains. The degree of protection offered against infection and/or clinical disease depends on the degree to which the immune system has been stimulated by vaccine. In present study low serological response of the chickens to the influenza vaccines were seen in both groups, which was correlated with reports of Brugh *et al.* (1979) and Karunakaran *et al.* (1987). They found that the HI response was influenced by the viral strain, antigen concentration, and species vaccinated. Immune response in chicks vaccinated at 8 days of age was more than chicks vaccinated at 2 days of age. This may be related to immune system that will be developed with increasing the age of birds.

After challenging with influenza virus, the birds of 3 groups were infected and clinical signs were seen after 5 days but clinical signs and mortality were more severe in chicks of unvaccinated control group. These results showed that vaccination with influenza virus could not result in absolute protection against infection but vaccine protected chickens against severity of morbidity, mortality and clinical signs and in addition to humoral response, probably secretory antibody in the mucosal immune response plays an important role in the recovery of infected birds and providing protection from further infections (Suarez and Schultz 2000). It was concluded that the level of mean HI titre in chicks vaccinated at 2 or 8 days was low. Vaccination on the 8 days of age has better immune response and protection. The vaccination decreased severity of clinical signs, morbidity and mortality,

but did not protect the birds against infection. Administration of killed avian influenza vaccine has useful effects in broiler chicks against infection in areas where disease is prevalent.

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