Short Paper

The efficacy of inactivated oil-emulsion H9N2 avian influenza vaccine

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Summary

An experimental inactivated oil-emulsion H9N2 avian influenza vaccine was formulated with 3 parts of inactivated avian influenza antigen A/Chicken/Iran/101/1998(H9N2) emulsified in 7 parts of oil adjuvant. Twelve week-old specific pathogen-free (SPF) chickens were divided into seven groups of 10 birds. Six groups were vaccinated with 1, 1/10th, 1/50th, 1/100th, 1/200th and 1/400th field dose of the experimental avian influenza vaccine (EAIV). The last group, was injected with saline and served as the control group. The mean titer in haemagglutination inhibition (HI) test (log 2) on the vaccinated groups, 21 days post-vaccination were 6.0, 4.4, 3.83, 3.3, 3.0 and 2, respectively. Prevention of virus shedding through cloaca was used as the potency test which revealed that the protective doses 50% (PD50) of full, 1/10th and 1/50th of the field dose of the experimental vaccine were 100, 100 and 96.25%, respectively. Those groups that received <1/50th dose could not prevent virus shedding. So it can be concluded that EAI vaccine could even be entirely protective and efficient in 1/10th dose and got a desirable immunity in experimental SPF chickens.

Key word: Avian influenza H9N2, Inactivated oil-emulsion vaccine, Efficacy

Introduction

Influenza viruses belonge to the family Orthomyxoviridae and are comprised of three immunological distinct types, A, B and C. Type A viruses are regarded as the most significant pathogens in terms of morbidity and mortality in poultry (Slemons *et al*., 1974; Graves, 1992). Control of avian influenza by vaccination is complicated by the antigenic diversity of the viruses (Bean *et al*., 1985; Alexander, 1987; Brugh and Purdue, 1991). Many studies have shown that the extent of protection induced by avian influenza vaccines primarily depends on the type of viral haemagglutinin antigen (Brugh *et al*., 1979). However, some farmers think usage of influenza inactivated vaccine is helpful to combat the disease; on the other hand, eradication of influenza disease could be potentially too costly or even not successful. Therefore, vaccination as a desired alternative or at least a supplemental control procedure may need to be considered (Stone, 1987). In this paper we intended to demonstrate the results of a research that may lead to production of an acceptable experimental inactivated water-in-oil emulsion avian influenza vaccine which was contained local avian influenza antigen and its immune response in specific pathogen-free (SPF) chickens.

Materials and Methods

Eggs

SPF eggs were purchased from Lohmann Co. (Valo, Lohmann, Cuxhaven, Germany).

Chickens

Seventy 12-week-old chickens were hatched from the above-mentioned SPF eggs.

Antigen

Influenza virus A/Chicken/Iran/101/1998(H9N2) was used as the antigen. It was isolated from infected chickens in Karadj area and identified by Weybridge Center Veterinary Laboratory. The virus was propagated in the allantois of 11-day-old embryonating chicken eggs. The amnio-allantoic fluids (AAF) were harvested after 72 hrs. The harvested material was clarified and inactivated by treatment with 0.1% formalin for 16 hrs at 37°C while the fluid being continuously shaken. The absence of inactivated viruses were confirmed by inactivating test by inoculation in susceptible embryonated egg (Slemons *et al*., 1974; Stone, 1987). Antigen was stored at -70°C before homogenizing with oil adjuvant (Brugh *et al*., 1979).

Adjuvant

Water-in-oil adjuvant Montanide ISA-70 (SEPPIC, Commits/Pharmacy Division, Paris, France) was used for production of this experimental vaccine.

Preparation of EAIV

Inactivated oil-emulsion vaccine was prepared by homogenizing 3 parts (v/v) of antigen with 7 parts (v/v) of Montanide ISA-70. Concentration of antigen in the aqueous phase was retained at least to the equivalent of $10^{8.5}$ EID50/dose (embryo infective dose 50) (Brugh and Siegel, 1978). Details of preparation and methods used for assessment of emulsion viscosity and stability have been described earlier by Stone *et al*., (1978). Controlling tests of the vaccine were carried out according to FAO Series No. 10 and 89.

Experimental design

Seventy 12-week-old chickens were divided randomly into seven 10-bird groups (A, B, C, D, E, F and G). The first group received one dose of experimental avian influenza vaccine, while the rest of the groups were injected with different fractions of a single dose; 1/10th, 1/50th, 1/100th, 1/200th and 1/400th, respectively (FAO Animal Production and Health Series No. 10). The control group was injected with 0.5 ml of saline.

Vaccination route

The SPF chickens were injected subcutaneously in the dorsal anterior of the neck. The chicken groups were housed in the same area and were fed nutritionally with complete diets. The control group was kept in the same area.

Haemagglutination inhibition (HI) test

Individual serum samples were collected 21 days after vaccination. The HI titers were determined using standard method. The HI responses were measured using 4 units of homologous (H9N2) avian influenza virus antigen (FAO Animal Production and Health Series No. 89).

Protective dose 50%(PD50) assay

Challenge-exposure could not be carried out in its normal way because the H9N2 is non-highly pathogenic avian influenza virus. Therefore, protection of virus shedding through cloaca was used as a potency test (Vasfi Marandi *et al*., 2002). After the second serum sampling, 0.1 ml ($10^{7.0}$ EID50) of the field avian influenza virus A/Chicken/Iran/102/1999(H9N2) was injected directly into the right lung in all chicken groups (Moghaddam Pour *et al*., 2000). The birds were observed for 10 days, then swab samples were collected from cloaca. The viral recoveries were carried out by Swayne *et al*., (1998) procedure; three passages were conducted in a same way by inoculation in 10-day-old SPF embryonated eggs. The PD50 was calculated through probit analysis (FAO Animal Production and Health Series No. 89) on the number of chickens that were not shedding avian influenza virus after ten days.

Results

The mean HI antibody titers in SPF chickens are shown in Table 1. All the chickens showed no antibody titer against

Fig. 1: Comparison of the mean HI titer after administration of full and fractions of one field dose of the vaccine

avian influenza before vaccination. The mean HI antibody titers of the groups A, B, C, D, E and F which were received full and different fractions of a single dose of the vaccine 21 days after vaccination, varied as follows: 6.0, 4.4, 3.83, 3.3, 3.0 and 2.0 (Table 1, Fig. 1). Groups A and B could show 100% PD50 while group C had 96.25% PD50 value. The immunity against H9N2 induced by vaccination of groups D, E and F of chickens could not prevent virus shedding. Nonetheless, no symptoms of influenza were observed in the vaccinated groups but all chickens of the control group and only one in the group C, showed depression over 10 days post-challenge.

Table 1: The maen HI titer (log 2) in SPF chickens

Chicken group	Injection material	Dose	Days after inoculation	
			0	21
A	EAIV	1	0	6.0
B	EAIV	1/10th	0	4.4
C	EAIV	1/50th	0	3.83
D	EAIV	1/100th	0	3.3
E	EAIV	1/200th	0	3.0
F	EAIV	1/400th	0	2.0
G (Control)	Saline	0.5 ml	0	0.0

Discussion

Little is known about the importance of antigenic drift in field condition to escope the immunity provided by inactivated vaccines against low pathogenic avian influenza. Successful control by vaccination will be contingent upon periodic surveillance of poultry to determine the antigenic characteristics of avian influenza virus involved in the disease (Brugh *et al*., 1979). Since such vaccines are used in some areas on a routine basis (Le Gros, 1999) and in addition, eradication may be too costly, so Razi Vaccine and Serum Research Institute with responsibility of vaccinology made a plan to produce a commercial type of vaccine to decrease economic loss in poultry farms. It was obvious, that the type and concentration of antigen in oil-emulsion avian influenza vaccine are major determinants of immunogenecity of the vaccine (Brugh *et al*., 1979). Therefore, a comparative study among different groups was carried out. It showed that chickens which were vaccinated with one field dose of the experimental vaccine possessed the highest antibody titer (group A); even those in group B which were inocculated with 1/10th of a single dose of the vaccine developed good immunity. Moreover, after challenge-exposure test, it was found that vaccination with 1/10th of a single dose of the vaccine, like its full dose, can provide full protection (100%) of virus shedding. It can be accepted that experimental vaccine would be succeeded and efficient to reduce symptoms of the disease and economic loss in chickens. Also it induced protection of virus shedding.

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