ABSTRACT

Doses of 500, 125 or 62.5 µl of two inactivated influenza vaccines prepared using the virulent A/tern/S. Africa/61 (H5N2) strain were injected subcutaneously into five week old SPF chicks.

HI antibody appeared faster but also waned faster after vaccination with vaccine A, in aqueous solution, than with the oil emulsion based vaccine B. This was associated with a slightly better protection against challenge with the virulent live virus by vaccine A 3 weeks after vaccination. Challenge was not followed by a significant increase of HI titres or appearance of precipitating antibody. Virus titres were approximately the same in faecal samples from all vaccinated groups and from a non-vaccinated control group at the third day after challenge.

Birds had higher mean HI titres 11 weeks after vaccination with vaccine B than with vaccine A, and were better protected against challenge. An increase in HI titres and the appearance of precipitating antibody was observed after challenge of the less well protected groups vaccinated with 500 or 125 µl of vaccine A.

While most birds that died or became sick had no circulating antibody at the time of challenge, some had titres of 3, 4 or 5. However, many vaccinated birds without circulating antibody overcame challenge without developing symptoms.

INTRODUCTION

Although in case of an influenza outbreak an eradication policy would be followed to control the disease in The Netherlands, it was necessary to know to what extent vaccination with an inactivated vaccine against influenza serotype H5 would result in protection.

We were interested in the potency of such a vaccine with respect to production of circulating antibody, protection against mortality and morbidity and to virus recovery after challenge with a virulent virus. The strain A/tern/S. Africa/61 (H5N2) was chosen as a challenge virus since it causes a high morbidity and mortality. The disadvantage of this strain is that while it grows to high titres, it produces only low titres of haemagglutinin (HA). Two vaccine formulations (A and B) were tested. B was known to stimulate high haemagglutination inhibition (HI) titres in
turkeys with another subtype of influenza virus. Vaccine A, an aqueous solution, was expected to stimulate an earlier but not a higher antibody response than vaccine B.

MATERIALS AND METHODS

**Vaccine preparation**

The A/tern/S. Africa/61 influenza virus was propagated in SPF chicken eggs. Eggs were inoculated in the allantoic cavity at day 9 or 10 of incubation; all embryos had died within 24 h. The allantoic fluids of the individual eggs were pooled, clarified by centrifugation at 3000 g for 10 minutes and tested for bacteriological sterility.

The HA titre of the pool was 1:64 and the virus titre measured by titration in hatching eggs was $10^{8.5}$ ELD$_{50}$ per 0.2 ml.

The virus in one litre of this suspension was inactivated at room temperature by adding (dropwise during 15 minutes) diluted reagent grade formalin to 0.445% final concentration. The suspension was stirred in a sterile Erlenmeyer flask during the addition of formalin. Stirring was continued for a further 5 minutes after the last formalin had been added. Thereafter the suspension was transferred to another sterile Erlenmeyer flask and left for 24 h at room temperature whereupon it was kept at +4°C.

Inactivation was controlled by inoculation of 0.2 ml of the suspension into the allantoic cavity of each of 30 ten days incubated SPF eggs. Although embryos dying within 24 h were regarded as non-specific, their allantoic fluids were tested for absence of HA activity. The allantoic fluids from embryos that had eventually died up to 96 h p.i. and those from the surviving embryos were harvested separately and inoculated into another series of 10 days incubated eggs. The allantoic fluids from the latter were tested for absence of HA activity 96 h later. The influenza virus appeared to be inactivated.

Vaccines A and B were made from the inactivated suspension by incorporation into the appropriate adjuvants and emulsion. The antigen concentration in the finished products was 10 per cent by volume.

**Experimental design**

Three groups of 30 five week old SPF chicks were injected individually in the neck with 500, 125, 62.5 µl of vaccine A. Three other groups of 30 birds were injected similarly with vaccine B. Birds were housed in an isolated pen together with 30 non-vaccinated birds.