

ANTIBODY RESPONSES TO AVIAN INFLUENZA VACCINATION IN BROILER CHICKENS IN INDONESIA

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Introduction

Avian Influenza (AI) is a respiratory disease of poultry caused by type A influenza viruses from the Orthomyxoviridae family. In Indonesia, avian influenza cases in humans were first detected in 2005 with 20 cases and 13 fatalities. The number of cases and fatalities peaked in 2006 with 55 cases and 45 fatalities. To control this disease, one of the policies implemented by the Indonesian government is vaccination of poultry in high risk areas (targeted vaccination). The World Animal Health Organization (OIE) has recommended vaccination as a way to control AI, although acknowledges that this program alone will not succeed without the support of other control measures, such as biosecurity implementation, surveillance, and management of poultry trade. There is evidence that AI vaccination reduces virus shedding (van der Goot et al, 2005, Poetri et al, 2009) which in turn would reduce virus spread and the risk of human exposure. However, there has been some concern regarding the inconsistency of field protection of poultry after vaccination, possibly related to vaccine quality, vaccine strain or inadequate administration (Swayne, 2008). This study aims to measure the development of AI antibody titers after vaccination of broiler chickens at different ages during the production cycle.

Material and Methods

The study was conducted for two months, from September to November 2008, in West Bogor subdistrict. A total of 1500 Cobb broiler day-old chicks were divided at random into 5 groups of 300 chicks. Group 1 was vaccinated against AI on day 1 (1T), group 2 on day 7 (2T), group 3 on day 10 (3T), and group 4 on day 14 (4T). Group 5 (5T) was a control group and was not vaccinated against AI. The AI vaccine used in this study was locally produced H5N1 killed oil emulsion vaccine. Serum samples were collected from 20 randomly selected chickens on days 1, 7, 14, 21, 28, 35, 42 and 49 and were tested for AI H5 antibodies with the Haemagglutination Inhibition (HI) test. Tracheal and cloacal swabs were collected from 10 chickens of each group on the last day of the experiment (day 49) and were tested using an AI H5 Reverse Transcription Polymerase Chain Reaction (rt-PCR) test. Data was analyzed using ANOVA (analysis of variance) tests and Duncan tests (Duncan multiple range test) with a critical probability of 0.05.

Result and Discussion

The highest antibody titers for all groups were found on day 1 (range $2^{2.8} - 2^{3.4}$), indicating relatively high levels of maternal immunity. Antibody titers reached their lowest levels (zero) on day 21 for the vaccinated groups and on day 28 for the control group. Mean titers increased to reach peak levels at day 42 for all groups except the control group after which titers started to decline in all four vaccinated groups. Highest mean antibody titers were found in the group vaccinated on day 10 ($2^{2.4}$) but these mean titers were not significantly different from those in the group vaccinated on day 7 ($2^{1.5}$) or those in the group vaccinated on day 14 ($2^{1.6}$). From the 100 swab samples that were tested with the rt-PCR on day 49, none were positive for H5N1. In this

study we assumed a titer of 2^5 or higher to be required to protect an individual bird against a challenge of H5N1 virus (Kumar *et al* 2007) with at least 80% of birds needing to acquire this titer to obtain herd immunity (Tiensin *et al* 2007). Even at peak antibody levels on day 42, only a maximum of 30% of serum samples had titers of 2^5 or higher, which was far below levels required for herd immunity. Possible reasons for low antibody titers in vaccinated birds are poor vaccine quality, unsuitable vaccination schedules, improper vaccine administration, or impaired immune-competence. According to Vui *et al.*, (2002) poor vaccine quality is a common problem in developing countries and could be the result of poor manufacturing standards, lack of storage facilities (cold chain), and use of expired batches. Because the manufacturing standards of the vaccine and the quality of the storage facilities at the manufacturer and distributor were outside of our control, it cannot be excluded that this had an effect on the results of this experiment. Impaired immune-competence can be a result of immunosuppressive diseases, immunosuppressive substances in the feed such as mycotoxins or a poor innate immune response of the host. Serological results demonstrated antibody titers for Infectious Bursal Disease (IBD) which were higher than would be expected from vaccination alone in all experimental groups. No clinical signs of IBD were observed during the course of this experiment but to what extent a concurrent sub-clinical IBD infection had impaired AI antibody production is unknown. Feed was not tested for mycotoxins but the overall performance of the birds (i.e growth, morbidity, mortality), did not suggest that these were present at significant levels. An alternative explanation of the poor titer development in this experiment could be the innate immune system of the host. Broiler chickens have been genetically programmed towards high performance (fast growth, high feed efficiency). There is some evidence that this genetic selection has adversely affected some of the innate immune responses of broilers (Kirschermann *et al*, 2006) and it could well be that it also has had a negative effect on the capacity of the modern broiler to produce antibodies. However, Ka Oud *et al.*, (2008) reported titers of $2^{5.2}$ and $2^{6.2}$ after vaccination of broiler chickens with an inactivated H5N1 vaccine given on day 7 and day 10 respectively, indicating that at least some broiler chicken strains are able to mount sufficient immune responses after vaccination. Differences in experimental set-up, vaccine manufacturer, vaccine dose or broiler strain might well be possible explanations for these differences in study results and it highlights the need for further studies.

Conclusion

One-time vaccination of broiler chickens on day 1, 7, 10 or 14 with an inactivated H5N1 vaccine did not result in mean levels of antibody titers which are considered to be protective. Negative results from the PCR tests and absence of clinical signs in the unvaccinated control group indicate there was no AI virus circulation at the study site which could have possibly affected the results of the experiment. More studies are needed to determine the optimum AI vaccination protocol for broilers in Indonesia. Ideally, these should be combined with challenge tests in order to obtain a more accurate assessment of the afforded protection against AI.

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