Antibody Responses to Avian Influenza Vaccination in Broiler Chickens in Indonesia

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Final Report

SRA	**Antibody Responses to Avian Influenza Vaccination in Broiler Chickens in Indonesia**
prepared by	**Erianto Nugroho**
co-authors/ contributor/ collaborators	**Retno D. Soejoedono** Veterinary Immunologist and Viroligist **Trioso Purnawarman** Poultry Health and Management Specialist **Chaerul Basri** Veterinary Epidemiologist and Biostatistician **Patrick Hermans** Poultry Disease Specialist
approved by	**Arend Jan Nell** **Albertus Teguh Muljono**

FINAL REPORT

CENTER FOR INDONESIAN VETERINARY ANALYTICAL STUDIES (CIVAS)

SUMMARY

Avian Influenza (AI) is a respiratory disease in poultry caused, has great economic significance for the world-wide poultry industry, particularly in countries. 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.

The highest antibody titers for all groups were found on day 1, indicating high levels of maternal immunity. Antibody titers reached their lowest levels (zero) on day 21 for groups 1T to 4T and on day 28 for group 5T. Hereafter, mean titers increased to reach peak levels at day 42 for all groups except for the negative control group after which titers started to decline in all four vaccinated groups. Highest mean antibody titers were found in group 3T but these mean titers were not significantly different from those in group 2T and 4T. Possible reasons for low antibody titers in vaccinated birds are poor vaccine quality, unsuitable vaccination schedules, improper vaccine administration, or impaired immune-competence. 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 and it could well be that it also has had a negative effect on the capacity of the modern broiler to produce antibodies

ACKNOWLEDGMENT

Antibody Responses to Avian Influenza Vaccination in Broiler Chickens in Indonesia

FINAL REPORT

TABLE OF CONTENT

Antibody Responses to Avian Influenza Vaccination in Broiler Chickens in Indonesia

FINAL REPORT

LIST OF TABLE

Antibody Responses to Avian Influenza Vaccination In Broiler Chickens In Indonesia

FINAL REPORT

LIST OF FIGURE

Antibody Responses to Avian Influenza Vaccination in Broiler Chickens in Indonesia

FINAL REPORT

LIST OF ANNEX

Antibody Responses to Avian Influenza Vaccination in Broiler Chickens in Indonesia

FINAL REPORT

INTRODUCTION

1.1 Background

Avian Influenza (AI) is a respiratory disease of poultry caused by type A influenza viruses from the Orthomyxoviridae family. This disease has great economic significance for the world-wide poultry industry, particularly in countries in Asia, Europe, and Africa (Easterday et al., 1997). Highly pathogenic avian influenza (HPAI) viruses cause severe systemic infections in several poultry species and can be present in multiple internal organs, meat, eggs, and blood (Swayne, 2008).

Type A avian influenza viruses were first found to be transmissible from animals to humans (zoonotic disease) in Hongkong in 1997. During this epidemic, 18 people became infected with AI virus of which 6 died (Ligon, 2005). In 2003, another case of AI infection in humans was found in Hongkong and the suspected source of infection was poultry. It was concluded that human AI cases are almost invariably closely associated with AI cases in chickens or other types of poultry (WHO, 2008a).

In Indonesia, avian influenza cases in humans were first detected in 2005 with 20 cases and 13 fatalities (WHO, 2008b). The number of cases and fatalities peaked in 2006 with 55 cases and 45 fatalities. As of 2008, Indonesia has had 137 cases of AI in humans with 112 fatalities resulting in a Case Fatality Rate of 82% (KOMNAS FBPI, 2008). Indonesia is the country with the highest number of human fatalities as a result of AI.

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 after vaccination, possibly related to vaccine quality, vaccine strain or inadequate administration (Swayne, 2008).

Although AI vaccination programs have been implemented on many broiler chicken farms in Indonesia, the optimum vaccination age leading to the maximum

amount of protection of broiler chickens is unknown. Therefore, a study was designed to measure antibody responses of broiler chickens after AI vaccination at different ages.

1.2 Objective

This study aims to record the development of AI antibody titers after vaccination in broiler chickens.

1.3 Benefit

This study will provide information on the optimum age for AI vaccination in broiler chickens. Furthermore, this study is expected to support AI control policies in Indonesia.

MATERIALS & METHODS

2.1 Time and Location

The study was conducted for two months, from September to November 2008, in Cilubang Lebak subvillage RT 03 RW 01, Situ Gede village, West Bogor subdistrict.

2.2 Study Design

A total of 1500 Cobb broiler chickens were divided at random into 5 groups of 300 chickens. Group 1 was vaccinated against AI on day 1, group 2 on day 7, group 3 on day 10, and group 4 on day 14. Group 5 was a control group and was not vaccinated against AI. The variable under study was H5 antibody titers on day 1, 7, 14, 21, 28, 35, 42, and 49.

2.3 Vaccination

The AI vaccine used in this study was locally produced H5N1 AI killed oil emulsion vaccine. The vaccine was applied subcutaneously (SC) with a dose of 0.2 ml of vaccine for 1 and 7 days old chicks and 0.25 ml of vaccine for 10 and 14 days old chicks.

Vaccination for Newcastle Disease (ND) was conducted using a live vaccine. The ND vaccine was administered on day 4 through eye-drops and was repeated on day 18 through the drinking water. Vaccination against Infectious Bursal Disease (IBD) was conducted using a live intermediate IBD vaccine. The vaccine was given on day 12 through the drinking water.

2.4 Samples

2.4.1 Sample Type

The types of samples collected in this study were serum samples, tracheal swabs, and cloacal swabs. Serum and swab samples were maintained at 4-8°C during storage and transportation to the laboratory.

2.4.2 Sample Collection

Serum samples were collected from blood taken from the heart of chickens younger than 3 weeks and from wing veins (vena brachialis) of chickens older than 3 weeks. Serum samples were collected from 20 randomly selected chickens within each group. Sampling was conducted on day 1, 7, 14, 21, 28, 35, 42, and 49. Tracheal

and cloacal swab samples were collected from 10 chickens within each group on the last day of the experiment (day 49).

2.4.3   Sample Testing

Serum samples were tested for AI antibodies with the Haemagglutination Inhibition (HI) test according to the procedure described in the OIE Terrestrial Manual (OIE, 2007) using four haemagglutination units (HAU) of H5N1 antigen (A/ch/Legok/03). In addition, the serum samples collected on days 1, 21, and 35 were tested for antibodies against ND using the HI test and those samples collected on days 1, 28, and 42 were tested for antibodies against IBD using an ELISA (LSIVET AVI IBD).

Tracheal and cloacal swab samples were tested using an AI H5 Reverse Transcription Polymerase Chain Reaction (rt-PCR). All AI related tests were conducted at Balai Penyidikan Penyakit Hewan dan Kesmavet, Cikole-Lembang. The serological tests for ND and IBD were carried out at the laboratory of the Veterinary Faculty of the Institut Pertanian Bogor (IPB).

2.5   Case Definition

Vaccination for AI was defined as effective if it produced mean antibody titers considered protective and lasting until the end of the production period (harvest) and negative results for rt-PCR tests. Protective mean titers were taken to be those equal to or greater then 2^5 (1:32). This is based on vaccination – challenge trials of broilers (Kumar et al, 2007) in which it was found that titers of 1:40 or higher gave near complete protection against mortality and virus shedding.

2.6   Data Analysis

Data was analyzed using ANOVA (analysis of variance) tests and Duncan tests (Duncan multiple range test) with a critical probability of 0.05.

2.7   Poultry management

The 5 groups of broiler chickens were raised in open houses on a concrete floor covered with rice hull litter. Chickens were acquired as day old chicks from one breeding farm. The parent stock had been vaccinated against AI. The broiler chickens were managed based on a 7-week broiler management program. Approximately 100 meters from the

poultry house there were domestic houses that had semi-intensive native chicken backyard flocks.

2.7.1 Feed and Water

The feed used in this study was always freshly made from the mill with a one month expiry date after it was produced. The composition of the feed was water content (max) 13 %, protein (min) 21.5-23%, fat (min) 5%, crude fiber (max) 4%, ash (max) 6.5%, calcium 0.9-1.2%, phosphorus 0.7-0.9%, and the antibiotic and coccidiostatic agents diclazuril/salinomycin.

Drinking water was provided *ad libitum*. Feeders and watering units were cleaned every day using soap and disinfectants to prevent contamination.

2.7.2 Lighting and Heating

Chickens were given light every night for the first two weeks to help them locate their feed. Also, brooders were operated 24 hours a day during the growth period (2 weeks) to provide warmth.

2.8 Monitoring and Daily Reports

Monitoring was conducted daily. Monitoring activities included checking the health condition of all chickens, ensuring chickens had enough feed, water, and lighting, and checking the brooders for the first 14 days of production. The information obtained through the daily monitoring was recorded in daily reports.

In case chickens were found sick or dead, farm workers would contact monitoring officers. The officers would then conduct clinical examinations and autopsies to diagnose the cause of illness or death. Dead chickens were immediately buried to protect the health of the other chickens within the flock.

Body weight was monitored and recorded on a weekly basis by weighing 20 randomly chosen chickens.

2.9 Biosecurity and Biosafety

Biosecurity and biosafety measures were practiced to prevent the spread of AI virus from poultry houses and the farm environment and also to prevent disease transmission to humans, particularly to sampling officers and farm workers. Standard operating procedures (SOP) were made to standardize monitoring and sampling activities. SOPs created for the study were SOPs for entering a poultry house, collecting

serum and swab samples, labeling of samples, exiting a poultry house, receiving samples, and submitting samples to the laboratory.

Biosafety for monitoring officers, sampling officers, vaccinators, and farm workers was applied through the use of personal protective equipment (PPE). Monitoring officers, sampling officers, equipment and vehicles were also sanitized and disinfected after each visit.

RESULTS

3.1 Body Weight

The weekly development of chicken body weight in the five experimental groups is shown in Figure 1.
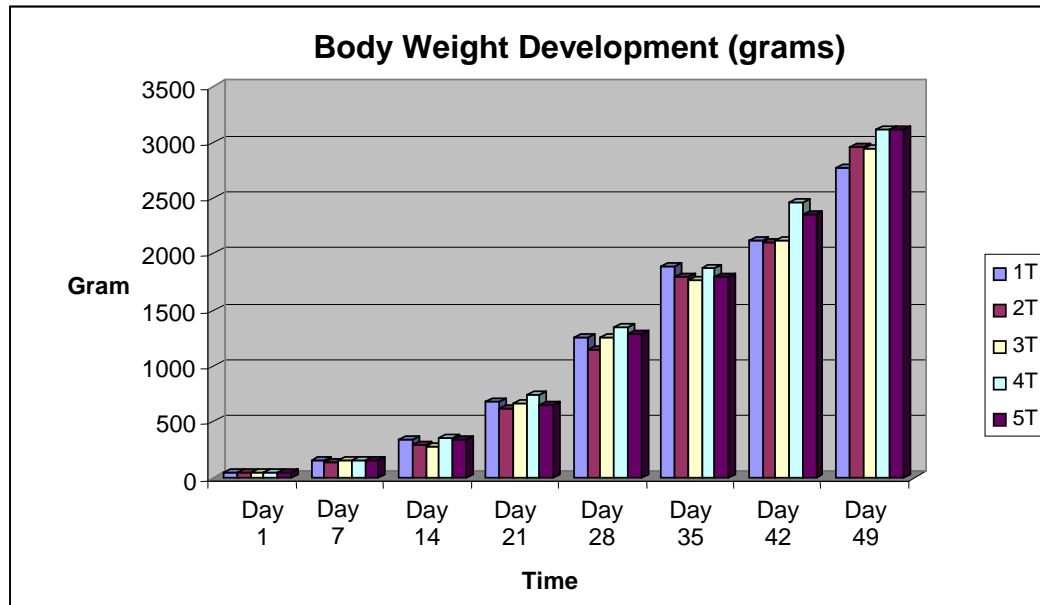
Note : 1T chickens AI vaccinated at day 1, 2T chickens AI vaccinated at day 7, 3T
chickens AI vaccinated at day 10, 4T chickens AI vaccinated at day 14, 5T chickens
not vaccinated against AI (control group)

Figure 1. Weekly Development of Body Weight in 5 Groups of Broiler Chicken

Average body weight of the broiler chickens increased week on end for all groups. At the end of the experiment on day 49, groups 4T and 5T had the highest body weights but these were not significantly different from the other groups.

3.2 Mortality

The weekly mortality rates of the broiler chickens in the five experimental study groups are shown in Table 1.

Table 1 Weekly Mortality rates (%) in five groups of Broiler Chickens

Vaccinated	1T (Day 1)	2T (Day 7)	3T (Day 10)	4T (Day 14)	5T (Control)
Week I	0 (0.0)	0 (0.0)	1 (0.3)	3 (1.0)	3 (1.0)
Week II	1 (0.3)	1 (0.3)	1 (0.3)	1 (0.3)	1 (0.3)
Week III	1 (0.3)	1 (0.3)	2 (0.6)	2 (0.6)	1 (0.3)
Week IV	0 (0.0)	2 (0.6)	4 (1.3)	0 (0.0)	2 (0.6)
Week V	12 (4.0)	2 (0.6)	1 (0.3)	2 (0.6)	2 (0.6)
Week VI	0 (0.0)	2 (0.6)	8 (2.6)	1 (0.3)	1 (0.3)
Week VII	4 (1.3)	5 (1.6)	0 (0.0)	3 (1.0)	1 (0.3)
Total	18 (6.0)	13 (4.3)	17 (5.6)	12 (4.0)	11 (3.6)

The highest mortality rate was found in the group vaccinated on day 1 (1T) with 6 % followed by the group vaccinated on day 10 (3T) at 5.6 %. Specifically, high mortality occurred during week 5 in group 1T and during week 6 in group 3T

3.3 Antibody Response

Mean antibody titers before and after vaccination are shown graphically in Figure 2. Mean antibody titers with standard errors, percentage of birds with zero titers and percentage of birds with protective titers ($\geq 2^5$) are shown in Table 2.
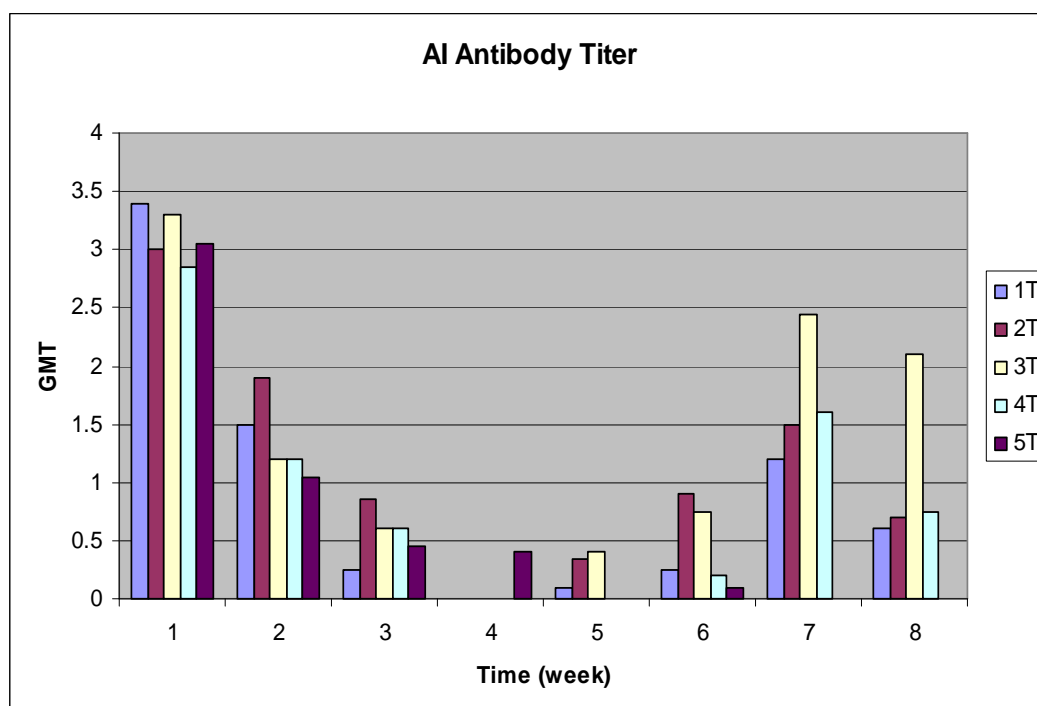

Figure 2. Weekly AI Antibody Titers in five groups of broiler chickens

Table 2 Mean log$_2$ AI Antibody titers with standard errors, Percentage of zero titers and Percentage of protective antibody titers ($\geq 2^5$) in five groups of broiler chicken

	Vaccinated	1T (day 1)	2T (day 7)	3T (day 10)	4T (day 14)	5T (control)
Day 1	Mean titer ± SE	3.4 ± 0.27^a	3.0 ± 0.30^a	3.3 ± 0.28^a	2.8 ± 0.38^a	3.0 ± 0.37^a
	% zero titers	5	5	0	5	15
	% titers $\geq 2^5$	55	40	45	25	40
Day 7	Mean titer ± SE	1.5 ± 0.25^{ab}	1.9 ± 0.34^b	1.2 ± 0.27^{ab}	1.2 ± 0.17^{ab}	1.0 ± 0.26^a
	% zero titers	20	30	40	20	35
	% titers $\geq 2^5$	0	15	0	0	5
Day 14	Mean titer ± SE	0.2 ± 0.20^{ab}	0.8 ± 0.23^c	0.6 ± 0.24^{bc}	0.6 ± 0.23^b	0.4 ± 0.13^{abc}
	% zero titers	90	55	70	70	60
	% titers $\geq 2^5$	5	0	5	0	0
Day 21	Mean titer ± SE	0.0 ± 0.0^a	0.0 ± 0.0^a	0.0 ± 0.0^a	0.0 ± 0.0^a	0.4 ± 0.10^b
	% zero titers	100	100	100	100	75
	% titers $\geq 2^5$	0	0	0	0	0
Day 28	Mean titer ± SE	0.1 ± 0.10^{ab}	0.3 ± 0.21^{ab}	0.4 ± 0.13^b	0.0 ± 0.0^a	0.0 ± 0.0^a
	% zero titers	95	80	65	100	100
	% titers $\geq 2^5$	0	5	0	0	0
Day 35	Mean titer ± SE	0.2 ± 0.2^{ab}	0.9 ± 0.3^b	0.7 ± 0.36^{ab}	0.2 ± 0.11^{ab}	0.1 ± 0.70^a
	% zero titers	90	65	80	85	90
	% titers $\geq 2^5$	5	5	15	0	0
Day 42	Mean titer ± SE	1.2 ± 0.41^b	1.5 ± 0.41^{bc}	2.4 ± 0.47^c	1.6 ± 0.40^{bc}	0.0 ± 0.0^a
	% zero titers	65	55	30	45	100
	% titers $\geq 2^5$	30	20	30	20	0
Day 49	Mean titer ± SE	0.6 ± 0.28^a	0.7 ± 0.28^a	2.1 ± 0.41^b	0.7 ± 0.48^b	0.0 ± 0.0^a
	% zero titers	80	70	30	65	100
	% titers $\geq 2^5$	5	5	10	5	0

Note: different superscripts in the same row indicate significant statistical differences

The highest antibody titers for all groups were found on day 1, indicating high levels of maternal immunity. Antibody titers reached their lowest levels (zero) on day 21 for groups 1T to 4T and on day 28 for group 5T. Hereafter, mean titers increased to reach peak levels at day 42 for all groups except for the negative control group after which titers started to decline in all four vaccinated groups. Highest mean antibody titers were found in group 3T but these mean titers were not significantly different from those in group 2T and 4T.

3.4 AI Virus Detection with PCR

From the 100 swab samples that were tested with the rt-PCR on day 49, none were positive for H5N1.

DISCUSSION

The modern-day broiler should easily be able to achieve body weights above 2 kg at 42 days of age (Butcher and Nilipour, 2008) which was the case in the present study. This, combined with mortality rates which were not excessive, indicates a normal broiler grow-out period during the course of this experiment. Although the mortality in groups 1T and 3T was elevated during the fifth and sixth week of the experiment respectively, this was most likely caused by humid weather and heavy rains during this period, resulting in leakage and wet litter in the poultry house. The treatment groups located near the entrance (1T) and in the middle of the house (3T) were most affected by these adverse environmental conditions. However, daily monitoring of culled and dead birds, negative rt-PCR results at the end of the experiment, and normal mortality in the unvaccinated control group, ruled out AI as a possible factor which could have affected the results of this study.

To control AI, one of the policies implemented by the Indonesian government is targeted vaccination in high risk areas. Whereas AI vaccination regimes for laying birds are relatively well established, little is known about optimum AI vaccination strategies for broiler birds. This study was designed to determine a vaccination protocol for broilers which would deliver protective antibody titers for the entire grow-out period. Although the level of antibodies required to confer protection is under debate, challenge experiments in broilers seem to suggest that titers need to be higher than 1:40 to prevent mortality and reduce viral shedding (Kumar et al, 2007). In this study we assumed a titer of 1:32 (2^5) or higher to be sufficient to protect against a challenge. In addition, the spread of an infectious disease within a flock can be quantified using the basic reproduction number (R_0). R_0 is the expected number of secondary cases that arise form a primary case in an entirely susceptible population. If $R_0 > 1$, the infection will spread whereas if $R_0 < 1$ the infection will die out. Vaccination helps to reduce R_0 to a value below 1 and the critical proportion of the susceptible flock that needs to be immunized in order to do this is determined by $1-1/R_0$. Based on data from the AI epidemic in Thailand during 2004, the calculated R_0 in layer and broiler chickens ranges from 2.30 to 3.17 with corresponding lower and upper 95% confidence limits of 1.92 and 5.00 respectively (Tiensin et al, 2007). Assuming a R_0 of 5, the critical proportion of broilers that needs to acquire titers of at least 2^5, provided this titer level confers full

immunity, is 80%. The results of this study show that none of the applied vaccination strategies were successful in achieving this target.

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 indicated high antibody titers for Infectious Bursal Disease (IBD) in all experimental groups (data not shown), a poultry disease well-known for its immuno-suppressive effects. To what extent a concurrent sub-clinical IBD infection has impaired AI antibody production in this experiment is not clear. 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 mean log2 titers of 5.2 and 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 but it also highlights the need for further studies.

Vaccination can be an effective tool in controlling AI. However, the results of this study demonstrate that broiler flocks remain potential risk factors for the spread of the disease due to the difficulties encountered in achieving sufficient protective

coverage after vaccination. Therefore, any vaccination control program needs to be integrated with other control measures such as strict biosecurity, proper disinfection, quarantine, controlled depopulation, education of people in direct contact with poultry, elimination of infected birds, and adequate surveillance.

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

1. 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

2. The highest mean antibody titer was achieved after vaccination on day 10

3. 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

Recommendation

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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FINAL REPORT

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FINAL REPORT

ANNEX

TEAM PROJECT

Expert Panel : Prof. Dr. Drh. Retno D. Soejoedono (Veterinary Immunologist & Virologist)

: Drh. Trioso Purnawarman, MSi (Poultry Health & Management Specialist)

: Drh. Chaerul Basri, M. Epid. (Veterinary Epidemiologist & Biostatistician)

: Patrick Hermans, PhD (Poultry Disease Specialist)

Principal Investigators : Drh. Albertus Teguh Muljono (CIVAS)

Arend Jan Nell (Wageningen International)

Project Coordinator : Drh. Erianto Nugroho

Field Staff : Roby

: Ega

: Ivone

Annex 2 Documentation

FINAL REPORT

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