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# Antibody response and risk factors for seropositivity in backyard poultry following mass vaccination against highly pathogenic avian influenza and Newcastle disease in Indonesia

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## SUMMARY

A large-scale mass vaccination campaign was carried out in Java, Indonesia in an attempt to control outbreaks of highly pathogenic avian influenza (HPAI) in backyard flocks and commercial smallholder poultry. Sero-monitoring was conducted in mass vaccination and control areas to assess the proportion of the target population with antibodies against HPAI and Newcastle disease (ND). There were four rounds of vaccination, and samples were collected after each round resulting in a total of 27 293 samples. Sampling was performed irrespective of vaccination status. In the mass vaccination areas, 20–45% of poultry sampled had a positive titre to H5 after each round of vaccination, compared to 2–3% in the control group. In the HPAI + ND vaccination group, 12–25% of the population had positive ND titres, compared to 5–13% in the areas without ND vaccination. The level of seropositivity varied by district, age of the bird, and species (ducks *vs.* chickens).

**Key words**: Avian flu, control, Indonesia, serology, vaccination (immunization).

## INTRODUCTION

The first poultry epidemic of highly pathogenic avian influenza A/H5N1 (HPAI H5N1) in Indonesia occurred in late 2003 on the island of Java. Since then, HPAI has become endemic in poultry on Java, and the island is considered the epicentre for expansion of the virus in Indonesia [1].

HPAI has been controlled in some countries through the classical control measures of culling, biosecurity and movement controls. However, this was not successful in countries with extensive backyard poultry systems and high population densities, including Indonesia, Vietnam, Egypt and the People's Republic of China [2]. All of the countries listed have applied mass vaccination to control HPAI H5N1 [3]. However, HPAI became endemic or persisted in all of these countries, despite vaccination and other control measures [4]. Reasons cited for the failure of vaccination to control HPAI include inadequate vaccination coverage, use of low-quality vaccines, inappropriate administration of vaccines, vaccination of immunocompromised birds, high population turnover, cold chain problems [4]. Further, mass vaccination is very costly and resource intensive. Post-vaccination monitoring and surveillance is critical to ensure that vaccination campaigns have been effective at conveying immunity

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and also to detect virus circulation in the face of vaccination [4, 5].

In Indonesia, an extensive Participatory Disease Surveillance and Response (PDSR) programme was developed in response to HPAI in 2006 [6]. Under this programme, government veterinary officers were trained to conduct surveillance on a village-wide basis to diagnose HPAI-compatible events. Outbreaks detected were controlled through measures that included focal culling, carcass disposal, decontamination, and movement control. This was a very large programme with 2100 PDSR officers engaged across much of Indonesia by May 2008. Despite these efforts, large numbers of HPAI cases continued to be reported in poultry.

The Operational Research in Indonesia for More Effective Control of Highly Pathogenic Avian Influenza (ORIHPAI) Project was undertaken between 2007 and 2009 to provide an evidence base to inform decision-making on HPAI control in Indonesia. At the core of this research was a longitudinal study that evaluated two mass vaccination control interventions in smallholder poultry (chickens, ducks, geese, turkeys) within the context of the ongoing PDSR programme [7].

The longitudinal study area comprised 16 districts within three provinces on the island of Java. Within each district, four geographical blocks ('treatment blocks') were randomly selected and randomly assigned to one of four treatment groups. Each treatment block contained 80000–120000 smallholder poultry according to census data.

The treatment groups were selected by stakeholders. They were: (1) a control group, in which only routine PDSR activities were implemented, (2) an HPAI vaccination group, in which there was mass vaccination of smallholder poultry against HPAI with an inactivated A/Ck/Legok/2003 H5N1 vaccine, (3) HPAI and Newcastle disease (ND) vaccination group, in which there was mass vaccination of smallholder poultry using both the A/Ck/Legok/2003 H5N1 vaccine and live eye drop Hitchner B1 (HB1) ND vaccine, and (4) culling with financial compensation provided immediately. The latter group was subsequently dropped because it proved impossible to identify a mechanism to provide the required funds for compensation.

Four vaccination campaigns were conducted; the first campaign was conducted in July 2008, and the subsequent campaigns occurred on a quarterly basis (every 3 months). During each campaign, two sets of vaccination were implemented (i.e. primary and booster), 21 days apart. Vaccination was presented on a voluntary basis, and was completely free of charge. The inactivated A/Ck/Legok/2003 H5N1 vaccine was administered intramuscularly while the HB1 ND vaccine was given as an eye drop. Both vaccines were manufactured by PT Medion (Bandung, Indonesia) and were available commercially.

This paper describes the results of the four serological surveys that were conducted after each mass vaccination campaign to determine the proportion of smallholder poultry with antibodies to H5 and ND virus in control and vaccinated treatment areas.

## METHODS

### Sero-monitoring sampling scheme

The objective of the sero-monitoring study was to estimate the proportion of chickens and ducks with antibodies to HPAI and ND in each treatment group (control, H5N1 vaccinated group, H5N1 and ND vaccinated group). Post-vaccination sero-monitoring was done in three of the 16 districts that participated in the mass vaccination campaign. The three districts were selected randomly on the basis of one per province (Fig. 1).

The expected prevalence of poultry with antibodies in vaccinated areas (design prevalence) was 80%. At a 95% level of confidence with 4% allowable error, the required sample size was 385 samples for a population of 100 000 poultry [calculated with Win Episcope 2·0 software, CLIVE: Computer-aided Learning In Veterinary Education, Edinburgh, UK (http://www.clive.ed.ac.uk/winepiscope)]. This sample size was doubled to account for expected clustering and associated intra-cluster correlation [8]. Thus, the final target was to collect 780 samples per treatment block per district per round of sampling. The same sample size was applied in the control areas where no vaccination was performed, for purposes of consistency and logistics.

In each district, six villages per treatment block were randomly selected from a list of all villages in the treatment and control areas. About 100 chickens and 30 ducks were sampled in each village, which reflects the average ratio of the village chicken:duck population in the 2006 census. Villages can be very large in Indonesia, and so at least three sub-villages were visited in each village to ensure wide geographical representation of samples. Because no sampling


**Fig. 1.** District boundaries and the relative locations of the three districts where sero-monitoring was done on Java Island, Indonesia. ORIHPAI, Operational Research in Indonesia for More Effective Control of Highly Pathogenic Avian Influenza.

frame of poultry-owning households was available, a transect walk was conducted within each sub-village and a maximum of five poultry were sampled from every third household. Sampling continued until 40–50 samples were collected from each sub-village. Poultry were sampled randomly irrespective of vaccination status.

During sampling, the following information was recorded pertaining to each individual sample: species (chicken or duck), sex (male or female) and age [<6 months (young) or adult].

Four rounds of sample collection were completed (one after each vaccination campaign). The timing of sample collection following vaccination was variable due to logistical issues. Serum samples were collected 2 months following the first round of mass vaccination, 3 weeks following the second and third rounds and 1 month after the fourth round. The random selection of villages was repeated each time, employing a sampling with replacement strategy. To ensure a high level of quality and consistency between districts, an initial training course was provided for the persons who performed the sampling and refresher training courses were held to review the sampling protocol before the second and fourth rounds of sample collection. Field-monitoring visits were undertaken in each district during the third round of sample collection.

### Laboratory analysis

Following collection, all samples were processed by the nearest provincial laboratory and then sent to Wates Disease Investigation Centre for analysis. Haemagglutination inhibition (HI) tests were used to measure antibody titres for H5 and ND. Antigen from a virus isolate from Kediri district in East Java was used to perform the H5 HI test; these were produced by Pusvetma, Surabaya. Antigen from the ICHII ND strain was used in the ND test. Both HI tests were performed according to the OIE manual [9, 10]; 4 haemagglutination units (HAU) of antigen was used in the H5 HI test. Results were entered into a database (Microsoft Access, USA).

The cut-off for a 'positive' titre was set at $2^4$ [equivalent to $\log_2(4)$ or inhibition at a serum dilution of 1/16] for both H5 and ND, in accordance with OIE guidelines [9, 10].

### Statistical analysis

All analyses were performed using Stata Release 10·1 (StataCorp LP, USA). Vaccination coverage was calculated by dividing the number of vaccine doses administered (according to financial records) by the number of backyard poultry present in the target population, according to 2006 poultry census data. The proportion of seropositive poultry in different groups was compared using a two-sample proportion comparison with normal approximation for a large sample size. The average titre was calculated based on samples with titre $\geqslant 2^1$ [$\log_2(1)$], and calculated as the geometric mean titre (GMT). The percentage of the population with a titre $\geqslant 2^4$ [$\log_2(4)$] was calculated using binomial estimates and confidence intervals.

Mixed-effects logistic regression models were constructed to study the factors associated with

seropositivity, using random effects to account for the clustering of poultry within villages (Stata command 'xtmelogit'). One model was created to study HPAI seropositivity and a second for ND seropositivity. Data from the first sampling round were not included in the model because there were no data for age of poultry from one district due to a data recording error at the time of sample collection. The models were built with a manual backwards selection procedure, with statistical significance and confounding considered at each step. Interactions of biological importance were tested (treatment*species and treatment*age). The intra-class cluster coefficient (ICC) was calculated according to the latent variable approach in which the error variance is fixed at $\Pi^2/3$ [11]. Model fit was examined using a *Q*-normal plot of village level residuals, and plotting these residuals *vs.* predicted values [11].

## RESULTS

The randomly selected districts were Cirebon in West Java province, Semarang in Central Java province and Kulon Progo in Yogyakarta province (Fig. 1). Using financial records of vaccination and poultry census data, the calculated vaccination coverage ranged from 48% to 100% after each campaign (Fig. 2).

Between 6400 and 7066 serum samples were collected following each vaccination campaign for a total of 27 293 samples over the entire study (Table 1).

### H5 titres

In the mass vaccination areas, 20–45% of poultry sampled had H5 titres $\geqslant 2^4$ after each round of vaccination, compared to only 1–3% in the control group (Table 1). Over the entire study, the proportion of birds with a positive H5 titre was significantly higher in the areas vaccinated against HPAI only compared to areas vaccinated against both HPAI and ND ($z = 8{\cdot}60$, $P < 0{\cdot}001$); however, this was not consistent within the different study areas nor with sampling rounds (Table 1, Fig. 3). There were differences in the proportion that were seropositive after each round; however, there was no consistent pattern and there was no evidence that the proportion of the population with a positive titre increased over time (Fig. 3).

In both vaccination treatment groups, the proportion of chickens with a positive H5 titre was higher than ducks (Table 1). Similarly, a greater proportion of adult birds had positive titres compared to young birds (Table 2).

In the control group, 6·3% (528/8439) of the poultry sampled had an H5 titre $\geqslant 2^1$ [GMT $2^{3{\cdot}1}$, 95% confidence interval (CI) $2^{2{\cdot}9}$–$2^{3{\cdot}3}$]. In the avian influenza (AI) vaccination group, 64% (5874/9227) of the poultry sampled had an H5 titre $\geqslant 2^1$ (GMT $2^{3{\cdot}8}$, 95% CI $2^{3{\cdot}8}$–$2^{3{\cdot}9}$). In the HPAI and ND vaccination group, 49·5% (4762/9627) of the poultry sampled had an H5 titre $\geqslant 2^1$ (GMT $2^{3{\cdot}9}$, 95% CI $2^{3{\cdot}8}$–$2^{4{\cdot}0}$).

### ND titres

The proportion of the population with a positive ND titre was significantly higher in areas that received mass ND vaccination compared to areas that did not. However, fewer birds had positive titres to ND compared to H5. In the group vaccinated for both HPAI and ND, the proportion of the population with ND titres $\geqslant 2^4$ after each round ranged from 12% to 25% (Table 1).

As with H5 titres, the pattern of the proportion of positive titres over time varied between districts with no consistent pattern (Fig. 4), and the proportion of the population with positive ND titres was greater in adult birds compared to young birds (Table 2).

In the control group, 18·7% (1574/8433) of the poultry sampled had an ND titre $\geqslant 2^1$ (GMT $2^{3{\cdot}6}$, 95% CI $2^{3{\cdot}5}$–$2^{3{\cdot}7}$). In the AI vaccination group, 19·0% (1758/9227) of the poultry sampled had a titre $\geqslant 2^1$ (GMT $2^{3{\cdot}2}$, 95% CI $2^{3{\cdot}1}$–$2^{3{\cdot}3}$). In the group vaccinated against both AI and ND, 52·6% (5060/9626) of the poultry sampled had a titre $\geqslant 2^1$ (GMT $2^{3{\cdot}1}$, 95% CI $2^{3{\cdot}0}$–$2^{3{\cdot}1}$).

### Multivariable analysis

#### *HPAI model*

All predictors presented to the model were significant and so remained in the final model (Table 3). As indicated in the descriptive analysis, poultry located in the treatment areas were much more likely to be HPAI seropositive (titre $\geqslant 2^4$) compared to poultry in the control area. When confounding related to sampling round, species, district and age were controlled for in the model, it became apparent that H5 seropositivity was more likely in the areas receiving only AI vaccination compared to areas that received AI and ND vaccination. There were no significant interactions between treatment and either species or age.

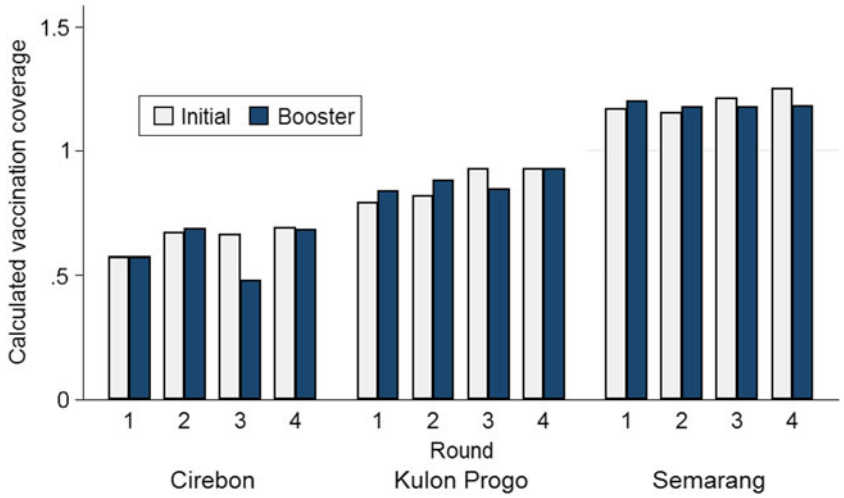


**Fig. 2.** The vaccination coverage calculated for each round of vaccination in the districts where sero-monitoring took place (calculated by dividing the number of vaccine doses provided to the district in each round by the number of poultry in the district, according to 2006 poultry census data).

Chickens were about twice as likely to be seropositive as ducks [odds ratio (OR) 1/0·51 = 1·96] and adults were about twice as likely to be seropositive than young birds. There were also significant differences between the districts, with seropositivity more likely in both Semarang and Kulon Progo compared to Cirebon. The proportion of variance at village level (ICC) was 0·23.

### ND model

There was no significant difference between sampling rounds and so this variable did not remain in the final model. There were significant differences between districts, with birds in Semarang most likely to be ND seropositive (Table 4). In this model, the proportion of variance at village level (ICC) was 0·14.

There were significant interactions between both treatment and species (chicken *vs.* duck) and treatment and age (adult *vs.* young). As expected, birds in areas vaccinated against both HPAI and ND were more likely to be ND seropositive compared to the other two areas. This was true for both chickens and ducks, young and adult birds, but the ORs were higher for ducks and adult birds in the areas vaccinated with ND compared to the other areas (Table 5).

In both the control areas and those areas with ND vaccination, ducks were less likely to be seropositive compared to chickens; however, in areas with AI vaccination ducks were more likely to be ND seropositive. Adults were more likely to be seropositive compared to young birds in all areas (Table 5).

## DISCUSSION

The results of this study revealed moderate levels of seropositivity in smallholder poultry in areas with mass vaccination (25–45% of the sampled population for H5, 12–25% for ND), and very low levels of H5 seropositivity (~2%) in the areas where no vaccination occurred.

In order to assess the feasibility and impact of mass vaccination of poultry in the Indonesian context, the campaign was co-designed and implemented by the Indonesian veterinary services, with support from FAO, using protocols similar to those that would be used if mass vaccination for HPAI were to be conducted by the Indonesian veterinary services as an ongoing programme.

Because of the mass vaccination campaign design, the vaccination status of individual birds in the study area was unknown. Thus, birds were sampled irrespective of their vaccination status, and in fact the vaccination status of individual birds was unknown. This sampling strategy allowed for an estimate of seropositivity in the population, but not a direct assessment of the seroconversion rate due to vaccination. However, birds were also sampled in control areas, where there was no mass vaccination campaign. Assuming that the birds in these areas were similar to birds in the mass vaccination areas, a reasonable assumption because areas were randomly chosen and assigned to treatment and control groups, it may be concluded that the difference in seropositivity levels in the vaccinated areas compared to the control areas was due to vaccination.

Table 1. *The percentages of chickens and ducks with titres $\geqslant 2^4$ against H5 and Newcastle disease (ND) following each round of mass vaccination*

| | | No. sampled | | Percent with HPAI titre $\geqslant 2^4$ (95% CI) | | | Percent with ND titre $\geqslant 2^4$ (95% CI) | | |
|---|---|---|---|---|---|---|---|---|---|
| Treatment | Round | Chicken | Duck | Chicken | Duck | Overall | Chicken | Duck | Overall |
| Control | 1 | 1 753* | 161 | 2·9 (2·2–3·8) | 4·3 (1·7–8·6) | 3 (2·3–3·9) | 10·5 (9·1–12) | 11 (6·6–16·8) | 10·5 (9·2–12) |
| | 2 | 1 903 | 69 | 2 (1·5–2·8) | 0 (0–5·2) | 2 (1·4–2·7) | 13·4 (11·9–15) | 7·2 (2·4–16·1) | 13·2 (11·7–14·7) |
| | 3 | 2 096 | 110 | 1·3 (0·9–1·9) | 1·8 (0·2–6·4) | 1·4 (0·9–1·9) | 5·3 (4·4–6·3) | 0·1 (0·0–5·0) | 5·1 (4·2–6·1) |
| | 4 | 2 043 | 304 | 3·0 (2·3–3·9) | 0·3 (0·01–1·8) | 2·7 (2·1–3·4) | 9·9 (8·6–11·2) | 1·3 (0·3–3·3) | 8·7 (7·7–10·0) |
| | Overall | 7 795† | 644 | 2·2 (1·9–2·6) | 1·2 (0·5–2·4) | 2·1 (1·8–2·5) | 9·5 (8·9–10·2) | 4·0 (2·7–5·9) | 9·1 (8·5–9·7) |
| AI vaccination | 1 | 2 026 | 164 | 28·1 (26·1–30·1) | 13·3 (8·5–19·5) | 27 (25·1–28·9) | 8·5 (7·3–9·8) | 7·3 (3·8–12·4) | 8·4 (7·3–9·6) |
| | 2 | 2 169 | 139 | 31·4 (29·5–33·4) | 14·4 (9–21·3) | 30·4 (28·5–32·3) | 7·2 (6·2–8·4) | 7·9 (4–13·7) | 7·3 (6·2–8·4) |
| | 3 | 2 323 | 35 | 44·9 (42·8–46·9) | 22·9 (10·4–40·1) | 44·5 (42·5–46·6) | 6·5 (5·5–7·6) | 11·4 (3·2–26·7) | 6·6 (5·6–7·6) |
| | 4 | 2 364 | 7 | 30·2 (28·4–32·1) | 28·6 (3·7–71·0) | 30·2 (28·4–32·1) | 7·7 (6·7–8·9) | 0·0 (0·0–41·0) | 7·7 (6·7–8·8) |
| | Overall | 8 882 | 345 | 33·8 (32·8–34·8) | 15·1 (11·5–19·3) | 33·1 (32·2–34·1) | 7·4 (6·9–8·0) | 7·8 (5·2–11·2) | 7·4 (6·9–8·0) |
| AI and ND vaccination | 1 | 1 998‡ | 301 | 26·2 (24·3–28·2) | 22·9 (18·3–28·1) | 25·8 (24–27·6) | 12·7 (11·2–14·2) | 8·6 (5·7–12·4) | 12·1 (10·8–13·5) |
| | 2 | 2 011 | 221 | 25·6 (23·7–27·6) | 36·9 (30·6–43·7) | 26·7 (24·9–28·6) | 18·3 (16·6–20) | 12·6 (8·5–17·7) | 17·7 (16·1–19·3) |
| | 3 | 2 204 | 150 | 36·9 (34·9–39·0) | 32·0 (24·6–40·1) | 36·6 (34·7–38·6) | 26·0 (24·1–27·8) | 12·0 (7·3–18·3) | 25·1 (23·3–26·9) |
| | 4 | 2 233 | 109 | 21·0 (19·3–22·8) | 4·6 (1·5–10·4) | 20·2 (18·6–21·9) | 19·9 (18·2–21·6) | 2·7 (0·6–7·8) | 19·1 (17·5–20·7) |
| | Overall | 8 846§ | 781 | 27·4 (26·5–28·4) | 26 (22·9–29·2) | 27·3 (26·4–28·2) | 19·3 (18·5–20·2) | 9·5 (7·5–11·7) | 18·5 (17·7–19·3) |

AI, Avian influenza; CI, Confidence interval.
* Samples for ND = 1747.
† Samples for ND = 7789.
‡ Samples for ND = 1997.
§ Samples for ND = 8845.

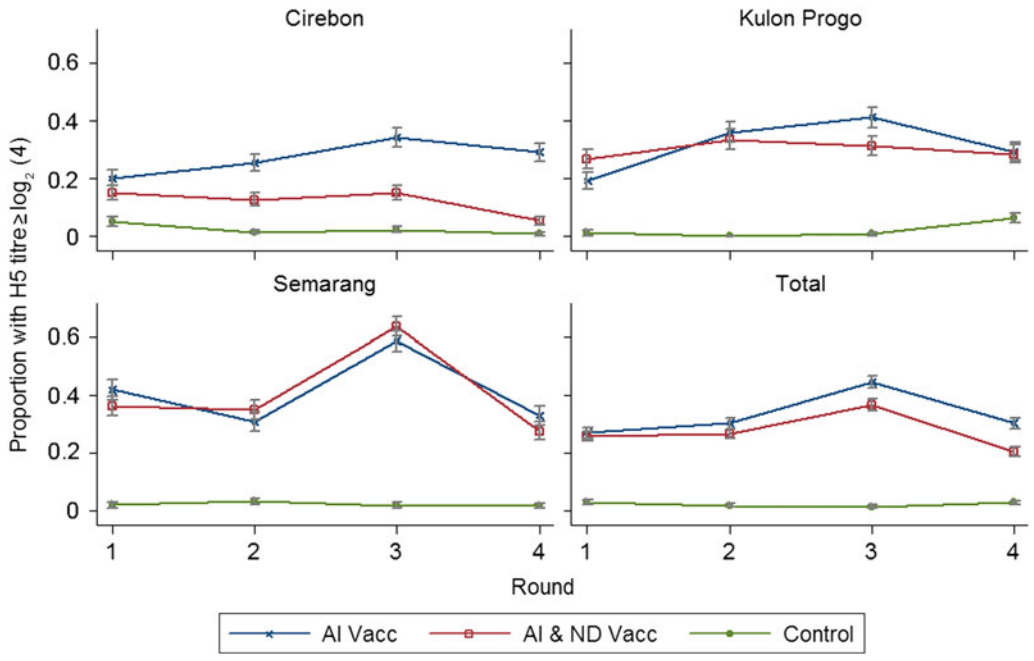


**Fig. 3.** Proportion of poultry with positive H5 titres ($\geqslant 2^4$) by district and overall, over the four rounds of sero-monitoring.

Table 2. *Percentage of poultry in each treatment group with positive H5 and Newcastle disease (ND) titres ($\geqslant 2^4$), stratified by age (adult vs. young birds) over the entire study period*

| | Percent of population with positive titre (95% CI) | | | |
|---|---|---|---|---|
| | H5 | | ND | |
| Treatment block | Young | Adult | Young | Adult |
| Control | 1·0 (0·6–1·4) | 2·4 (2·0–2·9) | 5·5 (4·69–6·4) | 10·2 (9·3–11·1) |
| AI vaccination | 23·0 (21·4–25·0) | 39·2 (37·8–40·5) | 3·0 (2·3–3·6) | 9·4 (8·6–10·2) |
| AI and ND vaccination | 19·5 (17·9–21·1) | 31·6 (30·3–32·9) | 12·5 (11·2–13·8) | 22·7 (21·6–23·9) |

AI, Avian influenza; CI, Confidence interval.

There are several possible reasons for the low level of H5 seropositivity observed in the control areas. Explanations include that some birds may have been vaccinated outside of this study, there may have been some circulation of low pathogenic avian influenza strains resulting in antibodies that cross reacted in the HI test, false-positive laboratory results and/or rare cases of chickens that survived natural HPAI infection. By contrast, substantial numbers of birds in the areas not receiving mass vaccination against ND (control and HPAI vaccination groups) had ND titres. This reflects the fact that infection with lentogenic and mesogenic ND strains will induce antibodies but is often not fatal, and so birds will recover from natural infection. As with H5, some of these titres might also be due to vaccination outside of the study, movement of birds from areas with vaccination, or be caused by false-positive results.

There are four general factors that could limit the level of seropositivity achieved in vaccinated areas. First, not all birds eligible for vaccination will have been vaccinated. This almost certainly played an important role in this study, because free-ranging backyard poultry are difficult to catch to administer the vaccine. Moreover, reports from the field indicate that insufficient vaccine was supplied to some areas, which was attributed to an underestimation of the population size in treatment blocks due to inaccurate

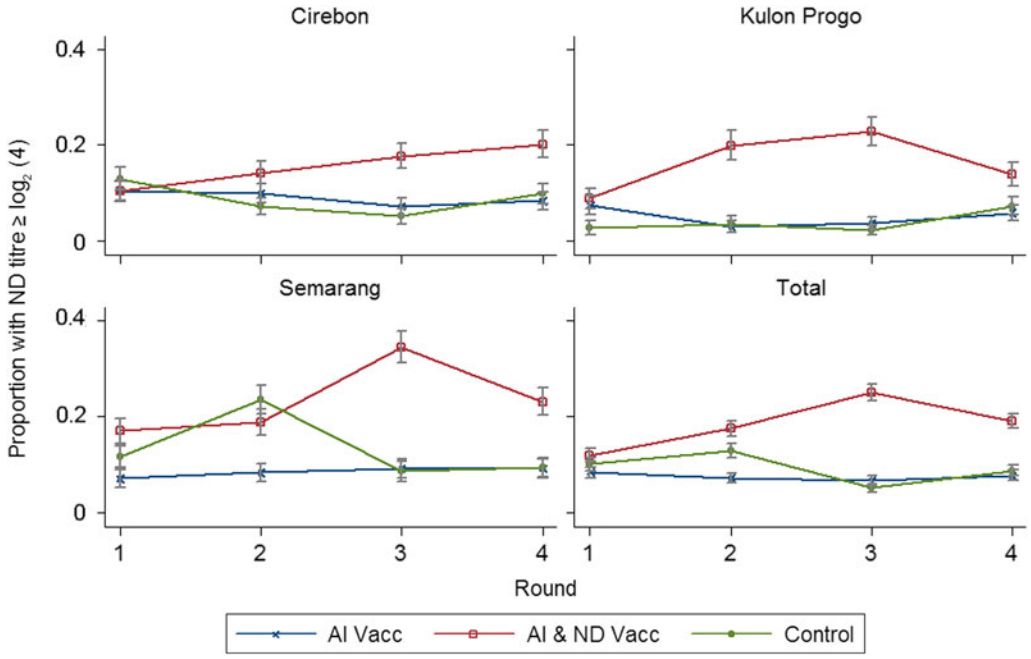


**Fig. 4.** Proportion of poultry with positive ND titres ($\geqslant 2^4$) by district and overall, over the four rounds of sero-monitoring.

Table 3. *Results of random effects logistic regression model comparing poultry that sampled positive for H5 (titre* $\geqslant 2^4$*) to those that sampled negative for H5 (titre* $< 2^4$*) (n = 20 490 poultry sampled)*

| Variable | | OR (95% CI) | *P* |
|---|---|---|---|
| Sampling round | Round 2 | Reference | |
| | Round 3 | 1·57* (1·4–1·76) | <0·01 |
| | Round 4 | 0·66 (0·59–0·75) | <0·01 |
| Species | Chickens | Reference | |
| | Ducks | 0·51 (0·42–0·62) | <0·01 |
| Age | Young | Reference | |
| | Adult | 2·16 (1·97–2·36) | <0·01 |
| District | Cirebon | Reference | |
| | Kulon Progo | 2·21 (1·32–3·72) | <0·01 |
| | Semarang | 3·47 (2·05–5·88) | <0·01 |
| Treatment block | Control | Reference | |
| | AI vaccination | 64·92 (36·32–116·03) | <0·01 |
| | AI and ND vaccination | 30·12 (16·99–53·39) | <0·01 |

OR, Odds ratio; CI, confidence interval; AI, avian influenza; ND, Newcastle disease.
Model log likelihood = −8150; variance of village random effect = 0·97, S.E. = 0·18.
* OR indicates that the odds of a sample being H5 seropositive (titre $\geqslant 2^4$) were 1·57 times greater in round 3 compared to reference round 2.

census data. Further, because vaccinators were paid to achieve a target number of birds vaccinated per day, they may have lacked incentive to continue working once that target was met.

A second cause limiting seropositivity post-vaccination is that backyard poultry have a high population turnover rate, which would result in a high proportion of the population being seronegative when sampled because they were too young to be vaccinated at the time of vaccination [12].

The third cause is that not all vaccinated birds will become seropositive. Backyard poultry might be expected to have a particularly poor response to vaccination due to reduced immunocompetence caused by concurrent disease (e.g. infectious bursal disease, mycoplasma infections). Some will seroconvert to a low titre, and others will not mount a measurable immune response. The extent of seroconversion to a low titre is illustrated in this study by the finding that more than 50% of the poultry in the treatment areas had an H5 titre $\geqslant 2^1$, compared to only 6% in the control group. Vaccinated birds might also fail to seroconvert due to poor immunization technique, or if the vaccine injected was not sufficiently potent or of low quality. This could occur due to issues in vaccine production, storage and/or delivery. The ND vaccine used was a modified live vaccine, which is more sensitive to breaks in the cold chain than the inactivated vaccine used for HPAI vaccination. The HB1 vaccine strain was selected because it is safe to

Table 4. *Results of random effects logistic regression model comparing poultry that sampled positive for Newcastle disease (ND) (titre $\geqslant 2^4$) to those that sampled negative for ND (titre $<2^4$) (n = 20 490 poultry sampled)*

| Variable | | OR (95% CI) | *P* |
|---|---|---|---|
| Species | Chickens | Reference | |
| | Ducks | 0·15 (0·08–0·28) | <0·01 |
| Age | Young | Reference | |
| | Adult | 1·5† (1·22–1·86) | <0·01 |
| District | Cirebon | Reference | |
| | Kulon Progo | 0·66 (0·45–0·98) | 0·04 |
| | Semarang | 1·78 (1·22–2·62) | <0·01 |
| Treatment block | Control | Reference | |
| | AI vaccination | 0·57 (0·35–0·93) | 0·02 |
| | AI and ND vaccination | 2·43 (1·58–3·75) | <0·01 |
| Interactions | Adult*AI vaccination | 1·74 (1·25–2·44) | <0·01 |
| | Adult*AI and ND vaccination | 1·46 (1·12–1·89) | 0·01 |
| | Ducks*AI vaccination | 7·92 (3·34–18·75) | <0·01 |
| | Ducks*AI and ND vaccination | 2·38 (1·16–4·91) | 0·02 |

OR, Odds ratio; CI, confidence interval; AI, avian influenza. Model log likelihood = −6791; variance of village random effect = 0·54, S.E. = 0·10.
† OR indicates that the odds of a sample being ND seropositive (titre $\geqslant 2^4$) were 1·5 times greater in adult compared to young birds. Because of interactions, this value depends on the treatment group (see Table 5).

Table 5. *Interpretation of interaction terms in the logistic regression model comparing poultry that sampled positive for Newcastle disease (ND) (titre $\geqslant 2^4$) to those that sampled negative for ND (titre $<2^4$) (n = 20 490 poultry sampled)*

| Variable | Comparison | Interaction group | Odds ratio |
|---|---|---|---|
| Species | Ducks *vs.* chickens (reference: chickens) | Control | 0·15* |
| | | AI vaccination | 1·16 |
| | | AI and ND vaccination | 0·35 |
| Age | Adult *vs.* young (reference: young) | Control | 1·50 |
| | | AI vaccination | 2·62 |
| | | AI and ND vaccination | 2·19 |
| Treatment | AI vaccination *vs.* control (reference: control) | Chickens | 0·57 |
| | | Ducks | 4·54 |
| | | Young | 0·57 |
| | | Adult | 1·00 |
| | AI and ND vaccination *vs.* control (reference: control) | Chickens | 2·43 |
| | | Ducks | 5·80 |
| | | Young | 2·43 |
| | | Adult | 3·55 |

AI, Avian influenza, ND, Newcastle disease.
* Odds ratio indicates that in the control group, the odds of ducks being seropositive for ND were 0·15 times the odds of chickens being seropositive.

employ in all ages of poultry; however, it is not as immunogenic as other ND strains [13].

The fourth general cause of limited seropositivity is that antibody levels could wane in the interval between vaccination and sample collection for serology. This would be particularly important if the birds did not receive a booster vaccine.

Vaccination coverage estimates calculated using vaccinator records indicated that between 48% and 100% of the target population was vaccinated in each campaign, which is higher than the level of seropositivity in the vaccinated areas. In addition to the fact that not all vaccinated birds will seroconvert, it is also likely that the national census data used to calculate the vaccination coverage were inaccurate. In fact, in one district the coverage calculated using national census data and vaccinator records was greater than 100%. This illustrates the need for accurate population records for post-vaccination monitoring purposes.

The level of post-vaccination seropositivity is somewhat higher than that reported from backyard poultry vaccinated in other countries. Less than 20% of backyard poultry surveyed had H5 antibodies in Egypt [14] and 20% H5 seropositivity was reported in Vietnam [15]. The higher levels of seropositivity in this study may be because the vaccination campaigns were carried out with additional, external resources dedicated to the implementation, including the recruitment of over 1000 community vaccinators and investment to upgrade the cold chain [7].

Although the level of seropositivity post-vaccination was higher than in other countries, it is still much lower than level of immunity generally believed to be required to stop the transmission of HPAI within and between poultry flocks, which has been estimated to be at least 50% [3, 16]. These findings support the principle that HPAI cannot be controlled by vaccination alone, but rather it must be part of an broader programme that includes movement control and enhanced biosecurity [4].

We examined factors associated with seropositivity through multivariable mixed-effects logistic regression models. As expected, birds located in areas where mass vaccination was offered were much more likely to be seropositive. Levels of seropositivity were higher in adults than young birds for both HPAI and ND. This is probably because many of the young birds sampled were not vaccinated. Because the population turnover rate is very high, the young bird population represents about 50% of village chickens at any one time [17]. It is also possible that adult birds had a better response to vaccination compared to younger birds.

The proportion of the population with antibody levels $\geqslant 2^4$ varied significantly between the sampled districts for both H5 and ND. This suggests varying levels of implementation quality were achieved in the vaccination campaign in different districts.

The treatment group offering both HPAI and ND vaccination was included on the basis of the assumption that provision of ND vaccine would increase the poultry owners' participation in the mass vaccination campaign [7]. However, in this study, poultry were more likely to be seropositive for H5 in areas receiving only AI vaccination compared to areas that received both AI and ND vaccination and so this hypothesis was not substantiated by our results. This was an unexpected finding, and the reason for the increased seroconversion in areas with HPAI vaccination only is unknown. It may be related to social factors such confusion about the provision of two different vaccines among poultry owners in the AI and ND treatment areas, and/or the payment scheme of the vaccinators. Vaccinators were paid to vaccinate birds until a fixed quota was reached, at which point there was no incentive for them to continue even if there was still demand from poultry owners.

There is no evidence that immunity to HPAI or ND increased over successive rounds of vaccination. This was probably primarily because of the high population turnover rates, but could also reflect the fact that immunity in individual birds is expected to wane over time, particularly if no booster vaccination is given. A modelling study demonstrated that vaccination of 100% of the population every 4 months was insufficient to achieve immunity levels greater than 30% due to natural flock population turnover rates [12].

For both H5 and ND, the proportion of the population with positive titres was higher in the chicken population compared to the duck population. This may be explained by biological differences, factors related to the laboratory test and/or social issues. Biologically, ducks have been reported to lack a detectable H5 antibody response following vaccination, despite resultant protection against the development of clinical disease [18, 19]. With respect to the H5 HI test, another study found that the test was more sensitive in chickens compared to ducks, based on comparison with the reference tests [20]. However, this result cannot be directly extrapolated to the HI test used in this study because a different antigen type was used; direct comparison between the HI tests used would be required to draw conclusions. It is also possible that poultry owners were more likely to present chickens for vaccination because they develop clinical disease more often than ducks when exposed to many strains of both HPAI and ND [21, 22].

Clustering at the village level was controlled for in the models with the inclusion of a random effect. There might have also been clustering at the household level (a maximum of five birds per household were sampled); however, this was not accounted for in the model because data were not available at the household level.

Any mass vaccination campaign should be carefully monitored in order to assess its efficacy and guide future disease control policy [23]. Despite the fact that there are many mass vaccination programmes implemented against different diseases of poultry and livestock in different countries around the world, there are relatively few reports in the literature describing the results of these campaigns in terms of serological titres. To improve the success of vaccination in the field to control and/or eradicate infectious diseases, more work needs to be done to understand the resultant pattern of immunity in the population and explore approaches to making vaccination programmes more successful.

In conclusion, this study demonstrates an approach to post-vaccination monitoring for a real-world campaign. The results indicate that, given population and disease dynamics, vaccination alone is unlikely to be sufficient to halt the transmission of HPAI. An explicit understanding of national disease control objectives, recognition of the required human, technical and financial resources, and understanding of the socio-economic dynamics of the target disease(s) is essential to design disease control programmes appropriate to the context and the disease control objective.

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## DECLARATION OF INTEREST

None.

## REFERENCES

1. **Takano R, *et al.*** Phylogenetic characterization of H5N1 avian influenza viruses isolated in Indonesia from 2003–2007. *Virology* 2009; **390**: 13–21.
2. **Domenech J, *et al.*** Experiences with vaccination in countries endemically infected with highly pathogenic avian influenza: the Food and Agriculture Organization perspective. *Scientific and Technical Review* 2009; **28**: 293–305.
3. **Hinrichs J, Otte J, Rushton J.** Technical, epidemiological and financial implications of large-scale national vaccination campaigns to control HPAI H5N1. *Animal Science Reviews 2010* 2011; **73**.
4. **Capua I, Cattoli G.** Prevention and control of highly pathogenic avian influenza with particular reference to H5N1. *Virus Research* 2013; **178**: 114–120.
5. **Peyre M, *et al.*** Avian influenza vaccines: a practical review in relation to their application in the field with a focus on the Asian experience. *Epidemiology and Infection* 2009; **137**: 1–21.
6. **Azhar M, *et al.*** Participatory disease surveillance and response in Indonesia: strengthening veterinary services and empowering communities to prevent and control highly pathogenic avian influenza. *Avian Diseases* 2010; **54**: 749–53.
7. **Bett B, *et al.*** The effectiveness of preventative mass vaccination regimes against the incidence of highly pathogenic avian influenza on Java Island, Indonesia. *Transboundary and Emerging Diseases*. Published online: 23 May 2013. doi:10.1111/tbed.12101.
8. **Martin SW, Meek AH, Willeberg P,** *Veterinary Epidemiology. Principles and Methods*. Ames: Iowa State University Press, 1987.
9. **OIE.** Newcastle disease. In: *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2009*. Paris: OIE, 2009, pp. 576–89.
10. **OIE.** Avian Influenza. In: *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2009*. Paris: OIE; 2009, pp. 1–20.
11. **Dohoo IR, Martin SW, Stryhn H,** *Veterinary Epidemiologic Research*, 2nd edn. Charlottetown: VER Inc., 2009.
12. **Lesnoff M, *et al.*** A simple model for simulating immunity rate dynamics in a tropical free-range poultry population after avian influenza vaccination. *Epidemiology and infection* 2009; **137**: 1405–1413.
13. **Bell JG.** A comparison of the different vaccine available for the control of Newcastle disease in village chickens. In: Alders RG, Spradbrow PB, eds. *SADC Planning Workshop on Newcastle Disease Control in Village Chickens. Proceedings of an International Workshop. ACIAR Proceedings No. 103*. Maputo, Mozambique, 2001, pp. 56–60.
14. **Swayne DE, *et al.*** Assessment of national strategies for control of high-pathogenicity avian influenza and low-pathogenicity notifiable avian influenza in poultry, with emphasis on vaccines and vaccination. *Revue Scientifique et Technique de l'Office international des Épizooties*. 2011; **30**: 839.
15. **Desvaux S, *et al.*** Evaluation of the vaccination efficacy against H5N1 in domestic poultry in the Red River Delta in Vietnam. *Epidemiology and Infection* 2013; **141**: 776–788.
16. **Walker PGT, *et al.*** Estimating the transmissibility of H5N1 and the effect of vaccination in Indonesia. *Transboundary and Emerging Diseases*. Published online: 4 June 2012. doi:10.1111/tbed.12108.
17. **ILRI.** Final report: operational research in Indonesia for more effective control of highly pathogenic avian influenza. International Livestock Research Institute, 2011.
18. **Kim JK, *et al.*** Pathogenicity and vaccine efficacy of different clades of Asian H5N1 avian influenza A viruses in domestic ducks. *Journal of Virology* 2008; **82**: 11374–82.
19. **van der Goot JA, *et al.*** Transmission of highly pathogenic avian influenza H5N1 virus in Pekin ducks is significantly reduced by a genetically distant H5N2 vaccine. *Virology* 2008; **382**: 91–97.
20. **Desvaux S, *et al.*** Evaluation of serological tests for H5N1 avian influenza on field samples from domestic poultry populations in Vietnam: consequences for surveillance. *Veterinary Microbiology* 2012; **156**: 277–284.
21. **Onapa MO, *et al.*** A preliminary study of the role of ducks in the transmission of Newcastle disease virus to in-contact rural free-range chickens. *Tropical Animal Health and Production* 2006; **38**: 285–289.
22. **Gilbert M, *et al.*** Free-grazing ducks and highly pathogenic avian influenza, Thailand. *Emerging Infectious Diseases* 2006; **12**: 227–234.
23. **Alders RG, *et al.*** Challenges and constraints to vaccination in developing countries. *Developments in Biologicals* 2007; **130**: 73–82.