

CHAPTER 4

Results

4.1 Distribution of H5 HI antibody titres

In line with expectations, no signs of adverse effects or illness were detected in any of the vaccinated ducks, indicating that the vaccine was well tolerated. The controls (non-vaccinated ducks) showed no detectable antibody levels throughout the observation period. None of the sampled ducks was positive for H5-specific antibodies by the HI test (HI titre $< 4 \log_2$) prior to vaccination.

Figure 4.1 shows the distribution of antibody levels measured after each of the two AI vaccinations for the vaccinated ducks included in this study. The serologic responses each time could be either negative (HI titre $\leq 3 \log_2$) or positive to the H5-specific antibody and could have a very high HI titre, up to 8 or 9 \log_2 . Twenty-eight out of 166 ducks (nearly 17%) were non-responders to the first single vaccination, with HI titres below 4 \log_2 . In contrast, more than 70% of antibody responses at that time were between 4 and 7 \log_2 . The second vaccination significantly increased antibody titres to where nearly 73% of ducks (119 of 164) produced a high to very high HI titre ranging from 6 to 9 \log_2 . Only 3.67% of vaccinated ducks (6 of 164) showed a negative HI titre after the booster dose.

Over the sample collection period, 18.3% of the ducks (30 of 164) remained at their HI titres induced by the initial vaccination despite the booster. Some 20.7% of the ducks (34 of 164) actually demonstrated a decline in antibody level even after the second dose of vaccine. Of all the vaccinated ducks, 62.2% (102 of 164) showed a higher antibody response after the booster immunization compared to their response after the initial immunization. Some 25.61% (42 of 164) showed a ≥ 4 -fold rise (i.e. a 2 \log_2 difference) in antibody titre after the second vaccination compared to the first vaccination. Thus, increasing HI titre with each of the two vaccine doses was the dominant pattern of the ducks' humoral immune response to the H5N1 vaccination.

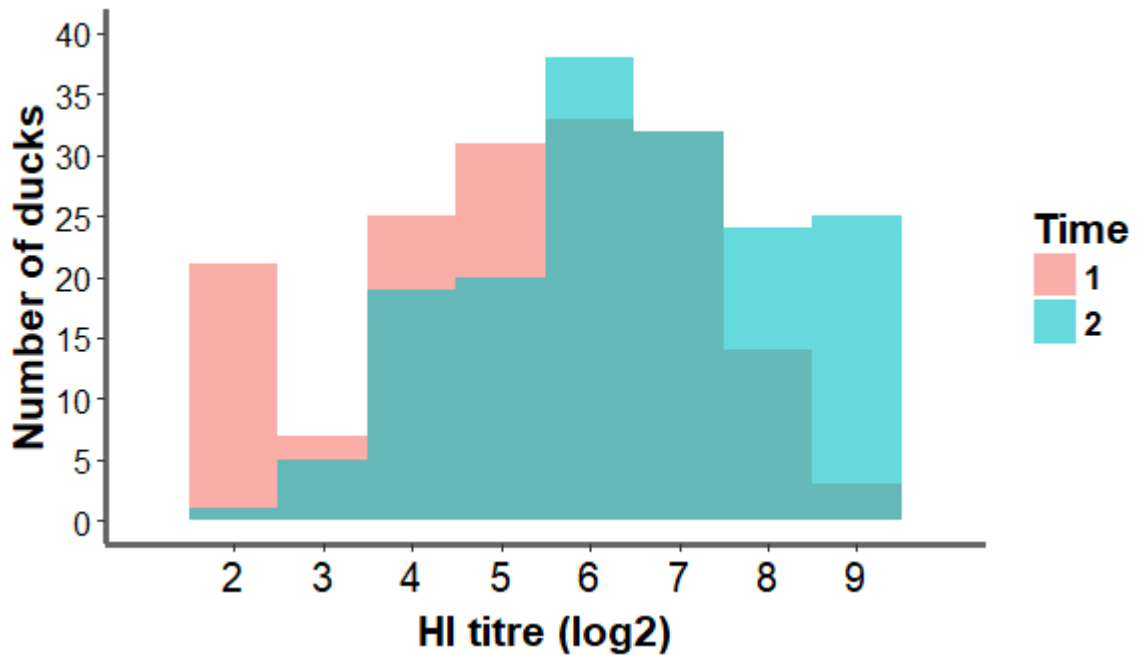


Figure 4.1 Distribution of H5-specific antibody titres in vaccinated ducks. (N = 166 and 164*). Antibody titres were measured by the HI assay at 2 different times: 21 days after the prime vaccination (Time 1), and 21 days after the booster vaccination (Time 2). Distribution of H5-specific antibody titres measured by the HI assay presented as log₂ values ranging from 2 to 9 log₂. Samples without a detectable antibody activity (HI titre < 3 log₂) were assigned at an HI titre of 2 log₂ and HI titre of 4 log₂ is the minimum positive HI antibody titre; *data from 164 ducks at 'Time 2' due to unsuccessful sampling in 2 ducks after the second vaccination.

Among the group of 28 non-responders to the single prime vaccination, 3 ducks remained negative for the H5 antibody even after the second immunization, while HI titres varied considerably (from negative up to 9 log₂) among ducks which responded to the booster dose (Figure 4.2a). Among the 138 ducks demonstrating a seroconversion following the first vaccination, 42 ducks showed higher antibody levels of up to 8 or 9 log₂ after the second vaccination (Figure 4.2b).

There was marked variation in the ducks' antibody response both within each farm and among farms after each vaccine dose (Figure 4.3). Twenty-one days post prime vaccination, several seronegative results (i.e., HI titres < 4 log₂) were observed in 10 out of 11 farms, while the largest HI titre value detected at that time at most of the farms was 8 log₂. There were, however, smaller variations in antibody reaction following the booster

dose: most vaccinated ducks had positive HI titres against the HPAI H5 virus, although a few ducks showed a negative HI titre. Specific values of coefficient of variation (CVs) which indicate the level of variability in HI titres of ducks within single farms can be seen from Table 4.2.

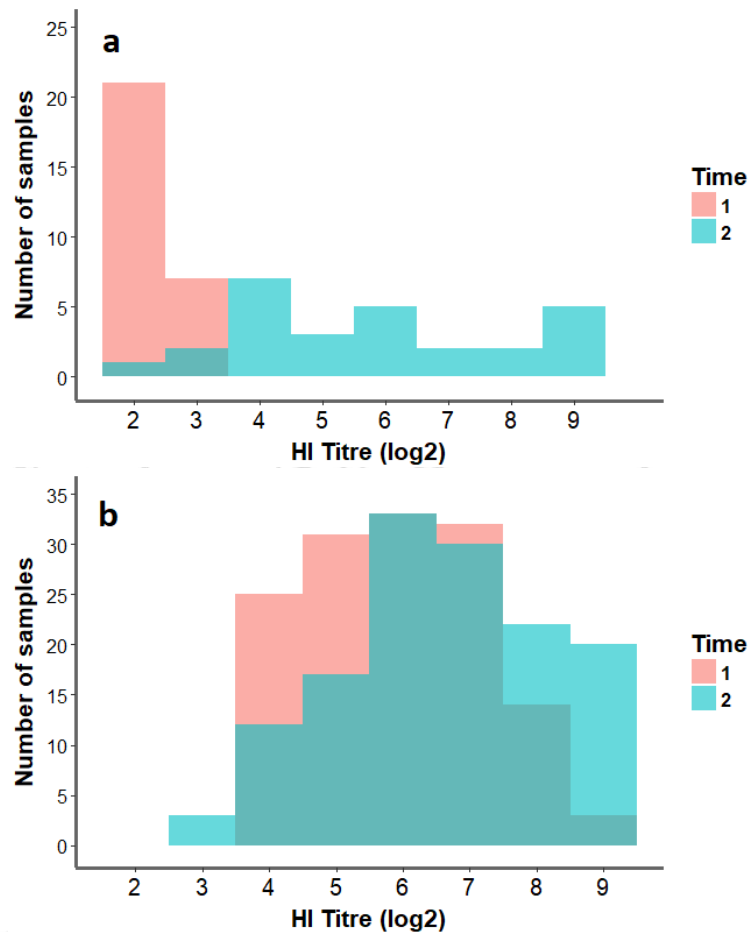


Figure 4.2 Distribution of HI titres against H5 HPAI virus induced by prime and booster vaccination of (a) non-responders and (b) responders to the first vaccination.

Time 1 and 2 represent HI results at 21 days after the prime vaccination (21 dpv) and 21 days after the booster vaccination (21 dpbv), respectively.

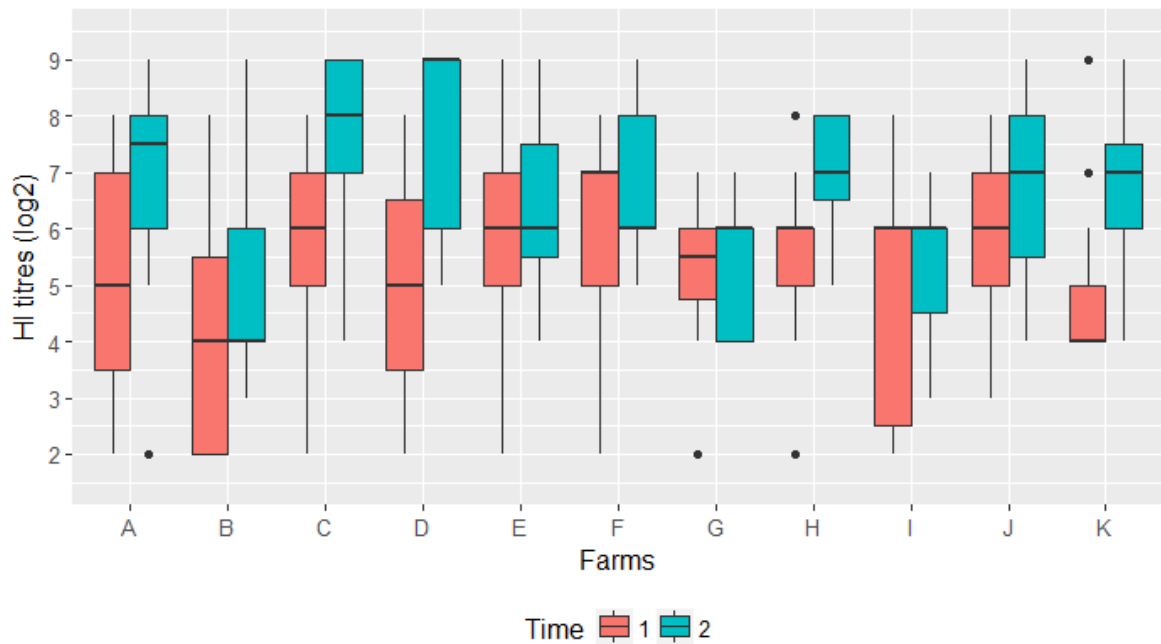


Figure 4.3 Distribution of HI titres against H5 HPAI virus by individual farm after each of the two vaccinations. Farms are coded A to K. Times of sample collection is referred to as “Time”. Time 1 and 2 represent HI results at 21 days after the prime vaccination (21 dpv) and 21 days after the booster vaccination (21 dpbv), respectively.

4.2 Geometric mean titre (GMT)

Test using the GLS method with the *ar1* covariance structure indicated statistically significant differences in antibody titres of ducks from different farms and between times of vaccination ($p < 0.0001$). The considered potential confounding factors regarding of farms’ categories such as location, the purpose of duck production or farm scale did not show statistically significant differences. Geometric mean HI titres of combination data are shown in Table 4.1. Prior to vaccination, none of the ducks had a detectable level of antibodies against H5 AI virus (thus, no GMT was calculated for pre-vaccination). A significant increase in antibody level over the two vaccinations was observed in our study when the first vaccination made a big change in ducks’ immune status against the virus while the second vaccination showed a greater than two-fold rise (i.e. 1 log₂) in GMT of H5-specific antibodies compared to the first one. This increasing antibody reaction to the H5N1 vaccine was observed consistently in all sampled farms (Figure 4.4). At some individual farms, GMTs measured at 21 days after the first vaccination remained relatively low, some being just slightly above the cut-off level, ranging from 4.13 to 6.07

\log_2 . After the second vaccination, GMTs increased on all farms, ranging between 4.87 and 7.87 \log_2 . Although the GMT of farm B increased somewhat during the observation period, it was always lower than those of other farms. On farm G, there was almost no difference in GMTs in spite of the booster (GMT difference = 0.14). In contrast, several farms exhibited a considerable increase in their GMTs of H5-specific antibodies during the observation period. The greatest difference in GMTs recorded was 2.8 \log_2 at farm D.

Coefficients of variation (CV) of the ducks' overall antibody titres induced by the two vaccinations are presented in Table 4.1 In detail, the majority of farms had a CV of between 26.77% and 40% after the first vaccination and from 19.5% to 26.41% following the booster dose. All individual farms exhibited a decline in the variation of the ducks' antibody response after the second vaccination (see in Table 4.2).



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Table 4.1 Humoral immunity of ducks at 2 different times: 21 days after the prime vaccination and 21 days after the booster vaccination.

Time	N	GMT (log₂) ± SE	CV (%)	Seroprotection rate (%)
21 dpv	166	5.30 ± 0.14 ^a	34.87	83.00
21 dpbv	164*	6.48 ± 0.13 ^b	26.30	96.30

The average mean and variability of HI titres and the proportion of vaccinated ducks showing seropositivity are presented as values of GMT, CV and seroprotection rate, respectively. Samples without a detectable antibody activity (HI titre < 3 log₂) were assigned at an HI titre of 2 log₂ in the calculation of GMTs.

21 dpv = 21 days after the prime vaccination

21 dpbv = 21 days after the booster vaccination

N = total number of ducks in each observation

**data from 164 ducks due to unsuccessful sampling in 2 ducks after the second vaccination*

GMT = geometric mean titre (log₂) of total number of vaccinated ducks

SE = standard error

CV = coefficient of variation, indicating the level of variability of HI titres within each flock. As individual bird HI titres ranged from 2 to 9, CV (%) was obtained using the formula CV (%) = 100 x Standard Deviation/GMT.

Values between rows with differing superscripts denote differences in GMT (GLS, $p < 0.0001$)

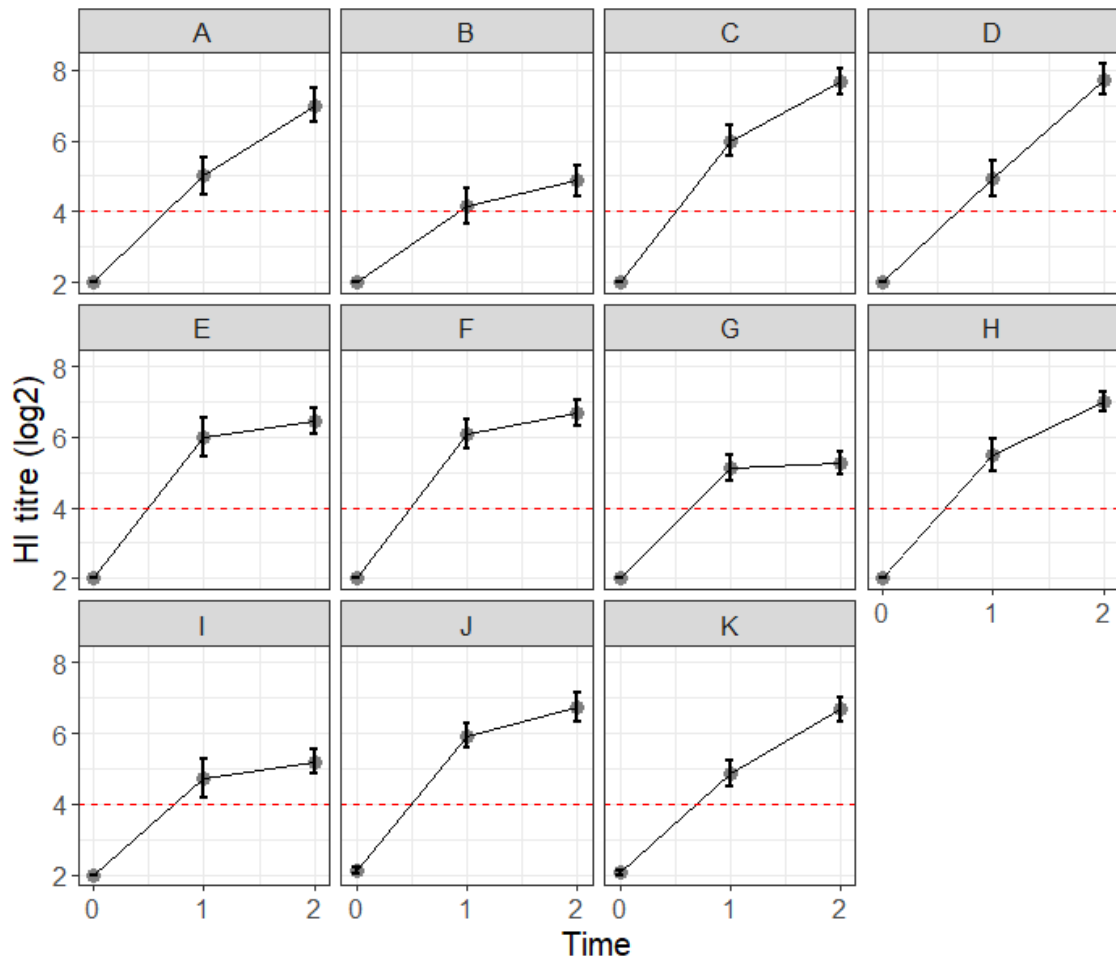


Figure 4.4 Immunogenicity profiles of the 11 sampled farms. GMTs of H5-specific antibodies are plotted for individual farms as a function of time of sample collection. Time 0, 1 and 2 represent HI titres pre-vaccination, 21 days after the prime vaccination (21 dpv) and 21 days after the booster vaccination (21 dpbv), respectively. Antibody titres are expressed as GMT with error bars which represent the standard error of measurements for 15 vaccinated ducks in each of 11 farms ($n = 15$). Dashed line indicates the cut-off level of the HI assay ($4 \log_2$).

Table 4.2 Coefficients of variation (CV) of anti-H5 HI titres in individual farms.

Farm	CV (%)	CV (%)
	Time 1 (21 dpv)	Time 2 (21 dpbv)
A	40.00	27.45
B	47.40	33.73
C	26.73	18.88
D	40.15	21.56
E	35.63	21.76
F	26.77	20.96
G	29.27	24.30
H	32.33	15.27
I	45.50	26.41
J	23.38	19.50
K	29.95	20.18

The CV was expressed as percentages (%) illustrate the variability of HI antibody titres of ducks in 11 sampled farms responding to the 2 vaccinations: 'Time 1' at 21 days after the prime vaccination (21 dpv) and 'Time 2' at 21 days after the booster vaccination (21 dpbv).

4.3 Seroprotection rate

The proportion of ducks demonstrating seropositivity (in other words, showing a protective antibody titre), was calculated based on the cut-off level HI titre at 4 log₂ as mentioned above. Prior to vaccination, 0% of the sampled duck population showed a positive level of antibodies against the H5 AI virus. Over all, the first vaccination induced a positive antibody response in 83.13% of the vaccinated ducks. This proportion increased to 96.34% with the second vaccination (see in Table 4.1). Considering the farms individually after the prime immunization, seroprotection rates ranged between 60% and 100%, while 2 out of 11 farms had a seroprotection rate lower than 70%. None of the farms' seroprotection rates were lower than 80% following the second vaccination (see in Table 4.3).

Table 4.3 Seroprotection rates of the 11 farms following the 2 vaccinations.

Farm	Seroprotection rate (%)	Seroprotection rate (%)
	Time 1 (21 dpv)	Time 2 (21 dpbv)
A	73.33	92.86
B	66.67	86.67
C	93.33	100.00
D	73.33	100.00
E	86.67	100.00
F	93.33	100.00
G	87.50	100.00
H	86.67	100.00
I	60.00	80.00
J	93.33	100.00
K	100.00	100.00

'Time' 1 at 21 days after the prime vaccination (21 dpv) and 'Time 2' at 21 days after the booster vaccination (21 dpbv). The proportion (%) of ducks demonstrating seropositivity was calculated with the cut-off level HI titre at 4 log₂.

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