CHAPTER 5

Discussion and Conclusions

5.1 Discussion

In response to the current situation in Vietnam and the desire for increased control and prevention of HPAI, this study was conducted to evaluate the antibody response of naïve domestic ducks to an early course of vaccination against HPAI subtype H5N1. There could be several characteristics of the household farming sector that might influence the effectiveness of vaccination. For that reason, this study was designed not to set out any fixed conditions or to take into account any local factors but rather to reflect the reality of vaccinations applied in the field. Both meat and layer ducks were included in this study as almost all domestic ducks in the region are mixed rather than pure breeds, and many can be raised for either meat or egg production. As mentioned above, the local husbandry practice in smallholder farming system with less than 1500 birds is kindly similar from farm to farm. Furthermore, due to the potential efficacy of the vaccine, the antibody response of birds would be expected to be likely influenced by combination effects rather than single factors. The 'farm' factor in this study was supposed to cover all of the differences among farms.

Although the number of samples per farm included in this study was lower than that prescribed in the national routine serosurveillance post-vaccination, the sample size was calculated to achieve a statistical significance with a consideration of ethical issues. However, ducks were cautiously selected for sampling, individually identified by leg bands and monitored closely that they were believed to be representative of their flock. Therefore, this study would be expected to provide results that reflect the target population.

The antigen strain used in the HI assay of this study is known to be antigenically different from the vaccine strain. Thus, HI titres against the antigen strain would be supposed to be lower than those against vaccine strain (Park et al., 2013), thus this could

be a limitation of this study. However, both strains belong to the H5N1 subtype. A\Ck\Scot\59 has been the only AI antigen commercially available and has been approved to be used for H5-specific antibody detection by HI tests in veterinary diagnostic laboratories throughout the country, which is the purpose of the routine post-vaccination serosurveillance. In practice, protection is provided against the individual HA subtype(s) included within each vaccine (D. E. Swayne & D. R. Kapczynski, 2008). Therefore, the antigen has been considered reasonably used to evaluate protective antibody titres induced by vaccinations.

HI antibody titres and seroprotection rates in response to vaccinations were the main subject of the research. The distribution of antibody HI titres was characterized by GMT and CV, the latter indicating the level of variability in HI titres of ducks within a single farm and among different farms in response to the same vaccination regime. Seroprotection rate determined whether herd immunity had been attained. Five countries, including Vietnam, have conducted post-vaccination surveillance by the HI serological assay to assess the field protection of an AI vaccine using a minimum protective antibody titre of 1:16 (4 $\log_2$) (D. E. Swayne, 2011).

No HPAI outbreaks were reported in the province where this study was conducted during the study period (according to the provincial SDAH). The result that control non-vaccinated ducks remained negative to H5 neutralizing antibodies throughout the observation period and that no ducks were detected as positive prior to vaccination suggests that it is likely that neither maternal immunity, natural infection, nor some other unknown factor influenced the ducks' immune response to vaccinations. Thus vaccination was essential to change the ducks' immune status against the HPAI virus. Furthermore, the finding that there was a significant difference in HI titres of ducks on different farms which had all been vaccinated with the same schedule indicates that the effectiveness of vaccination was highly variable and farm-related, although all vaccine immunizations did induce a certain level of antibody response.

5.1.1 H5-specific antibody HI titres

Antibody response is the principal contribution to protective immunity against AI viruses, and thus the level of neutralizing antibody to the hemagglutinin proteins, which can be measured by the HI assay, can be an indicator of vaccine-induced protection (D.

L. Suarez & Schultz-Cherry, 2000; D. E. Swayne & D. R. Kapczynski, 2008). Results from countries where vaccination programs have been implemented show that if vaccination coverage rates and HI titres of vaccinated poultry are high, vaccination can result in a substantial decrease in AI virus transmissibility and infection. Similarly, groups possessing more low HI titre birds are both more subject to infection and potentially more infectious than groups with many high HI titre birds (Sitaras et al., 2016). For that reason, high antibody titres within the poultry population are expected to be achieved following a vaccination program.

In the present study, although several vaccinated ducks had no seroconversion, most vaccinated ducks possessed a titre from 4 to 7 $\log_2$ induced by a prime vaccination, higher than those found in two previous studies of ducks and chickens with the same vaccine conducted in other provinces of the Mekong Delta (Phan & Tran, 2016; V. T. Tran, 2016). As a consequence, the overall GMT achieved after the first vaccination was above 5 $\log_2$, far higher than that found in the two mentioned studies. The difference in antibody mean titres in the different studies can be linked to different times of vaccination and to timing of post-vaccination sample collection. In this study, ducks were vaccinated and blood sampled at an older age, thus their immune system was able to make a stronger response to the prime vaccination compared to that in studies of younger birds. It has been suggested that pre-existing maternal immunity may interfere with the effect of vaccination on young poultry (Maas et al., 2011; E. Spackman & Swayne, 2013), but that was not detected in this study. Antibody titres measured at 21 days after the prime immunization in the present study were still higher than those found in another study of ducks and Muscovy ducks. In that research, birds were vaccinated by subcutaneous injection in the neck at 14 days (Pham, 2015). It has been proved that vaccination route will affect presentation and processing of antigen by host immune cells when specific antibody response provoked (D. E. Swayne & D. R. Kapczynski, 2008). In addition, the study of Pham was conducted in only one farm thus it could hardly reflect the general scenario. On the other hand, H5-specific antibodies of Muscovy ducks in that study were concentrated at 4$\log_2$ and did not distribute to higher levels, thus this poultry species was found to have a slower immune response compared to ducks. Differences in responses to vaccination has been observed in different species of domestic ducks (Cagle et al., 2011).

On almost every farm there were a number of ducks that did not show a seroconversion after the first vaccine immunization while rest of the flock did. Various endogenous factors such as differences in specific immune reaction, health status, or the prevailing disease situation could be the reason for that (Marangon & Busani, 2007; McLaws et al., 2015). That could also explain the wide distributions of the ducks' vaccine-induced HI titres found in this study, findings which correspond with those of other studies mentioned above. However, in a species of bird such as ducks, a minimum of 2 vaccinations may be necessary to produce protective HI titres (D. E. Swayne & D. R. Kapczynski, 2008). Indeed, in most ducks which were non-responsive to the prime vaccination, a booster dose provoked a change in their immunity status and most become seropositive to H5-specific antibodies. Moreover, the second vaccine immunization resulted in higher HI titres in the majority of vaccinated ducks overall, the most common pattern of increasing antibody response found in this study: a higher GMT (above 6 $\log_2$) following the booster vaccine dose. This is consistent with the findings of Tung and his co-workers which suggest that two administrations of an H5N1 vaccine can elicit a significantly higher GMT value of H5-specific antibodies in domestic poultry as ducks (Tung et al., 2013). However, after 2 doses, the vaccine Re-6 strain used in the present study provided a far higher GMT in ducks compared to the vaccine strain NIBRG-14 used in the study of Tung. This big difference may possibly be due to the different type of vaccines used. Bertelsen and Lecu and their co-workers also reported that two administrations of an H5N1 vaccine can elicit marked HI antibody titres in birds. (Bertelsen et al., 2007; Lecu et al., 2009). In addition, Sitaras reported that the immune response of vaccinated birds (measured in HI titres) is a direct consequence of the quantity of the vaccine administered (Sitaras et al., 2016). Therefore, two vaccinations would be expected to be more likely to raise a higher immune response than a single one. In contrast to the vaccination responders, some vaccinated ducks in this study were seronegative or demonstrated a decline in their HI titres after two vaccine administrations. A possible explanation for that negative serologic response is that antibody levels can wane in the interval between vaccination and sample collection for serology due to endogenous factors (McLaws et al., 2015).

The OIE Manual suggested that the minimum HI serological titre in field birds should be 1:32 (5 $\log_2$) for a good probability of protection against mortality from HPAI

infection or greater than 1:128 (7 $\log_2$) for a reduction in virus shedding in infected birds (OIE, 2017), while there have been some studies suggesting that protection can be afforded by lower titres than those specified by the OIE (Hill et al., 2016). Nearly 85% of the ducks in this study achieved an antibody level equal to or higher than 5 $\log_2$ after receiving the booster vaccination, while that proportion was only around 68% following the prime vaccination. According to the OIE, ducks receiving the booster vaccination have a higher probability of being protected from mortality if an outbreak occurs. In addition, there have been studies suggesting that to produce protective immunity and prevent the transmission of AI viruses in ducks and geese, more than one vaccination is needed (David E Swayne, 2009; van der Goot, van Boven, de Jong, & Koch, 2007).

Although high GMTs are always expected after vaccination, the level of variability of the immune response has usually been brought to the attention of veterinary authorities when vaccinations were implemented. The presence of some low HI titre poultry represents a risk to the rest of the flock because the lower HI titre birds are more likely to be infected with the disease than those with higher HI titres. The CV is commonly used to evaluate poultry humoral immune response and vaccination programs (Opengart, 2003). High CV indicates lack of uniformity in antibody titre in the flock. The lower the CV, the more uniform the distribution of titres and the better the vaccination. For most poultry diseases, the CV after a correctly applied vaccination should be less than 40% (Crespo & Shivaprasad, 2014; Greenacre & Morishita, 2014). A course of vaccination would not be considered to be effective if there were a high variability in the poultry serological response. Indeed, post-vaccination surveillance programs have shown that HPAI outbreaks are more likely to occur in poultry flocks with a CV of 40% or higher after vaccination (personal communication with veterinary authorities of the SDAH of Ben Tre province). Thus, the goal is to have a CV value as low as possible. In the present study, although the mean titres were not low, there was a variability in antibody responses amongst ducks both within farms and between farms following the primary vaccination as evidenced by the relatively large CVs. Some ducks were able to develop high HI titres against the vaccine virus; however, a number of ducks possessed low HI titres or were even seronegative to the antibody. This result concurs with the finding of Tarigan et al. that the outcome of a field H5N1 vaccination is highly variable and farm-related when the HI titres of individual birds in each flock differ significantly from birds in other flocks

(Tarigan et al., 2018). In fact, field conditions are often dissimilar. Such inter-farm outcome variations can be linked to different environmental factors and rearing practices, immunization techniques, vaccine storage, vaccinator's skill and incentive, etc., which vary with individual farms (Mc et al., 2015). However, the second vaccine administration was observed to provide better results with a smaller variation of antibody response amongst ducks both within farms and between farms, even though that can be partly explained by the contribution of the primer dose to the outcome of the booster dose. The initial dose could induce certain initial humoral immune responses, then the later dose could boost antibody reaction to higher levels even though the maximum response in individuals may differ. If some individuals respond very strongly to the first vaccination, the second dose might not make a big change in their HI titres. Previously, Swayne et al. reported that the use of a single vaccination for short-lived broilers and meat ducks did not provide consistent immunity and protection (D. E. Swayne, 2011). Therefore, achieving lower variability in antibody response following a booster immunization has important implications for the effectiveness of a vaccination program.

5.1.2 Seroprotection rate – Flock immunity assessment

The most important goal of an AI vaccination program is flock immunity which is proportional to the level of protection achieved by all birds in a vaccinated flock. In Vietnam, national regulations stipulate that to achieve herd level immunity after vaccination, 70% of the poultry in each flock must demonstrate a seroprotection (HI titres of ≥ 4 log2) to be considered protected, and 80% of the poultry flocks in each province or region must show flock level immunity because in theory there are not enough susceptible individuals to propagate an epidemic (MARD – DAH, Circular No. 07/2016/TT-BNNPTNT).

In this study, both single and booster vaccinations provided some protection to most of the vaccinated ducks when the majority were serologically protected (83.13% and 96.34% seropositivity after the prime and the booster vaccination, respectively). Although the achieved seroprotection rate varied considerably between sampled farms, the first vaccination was observed to elicit an acceptable antibody response in ducks in terms of seroprotection rate according to the targets of the national vaccination strategy for prevention and control of HPAI. This finding may partly explain the fact that although

local farmers often give a single vaccination to their livestock, HPAI outbreaks have not occurred in the province since 2014 when the Re-6 vaccine was introduced. The seroprotection rate achieved by ducks at that time were higher than those reported by all above-mentioned studies with a same vaccine in Vietnam (Pham, 2015; Phan & Tran, 2016). Some similar results were reported in the country which indicated that a lower percentage of vaccinated poultry had H5-specific antibodies at more than 3 weeks post-vaccination with different vaccine strains (Henning et al., 2011; Tung et al., 2013). However, a booster vaccination similarly provided a significantly high achievement in the seroprotection rate in most of the studies on ducks in line with expectations. The seroprotection rate of higher than 90% detected following the second immunization in the current study correspond with the observation of the national veterinary authorities in 2017 (MARD-DAH, Dispatch No.2904/TY-DT, 2017) and in the studies of Pham in 2015 and Phan & Tran in 2016, with the same vaccine Re-6 strain. On the other hand, Zeng and his co-workers reported comparable seropositivity achieved in chickens after a single immunization with the same Re-6 vaccine, as that protocol was prescribed for that species (Zeng et al., 2016). Both studies used a heterologous antigen for the HI assay. Thus, the Re-6 vaccine appears to be highly immunogenic in domestic poultry species. All 2-dose vaccinated flocks showed seroconversion rates of ≥ 80% in the present study. Therefore, based on the current national targets, the second vaccination appears to offer a better level of immunity. In terms of practical significance, results from the last sampling done 21 days after the booster vaccination could reflect the vaccine-induced serological immunity of ducks immediately before the common finishing time of the meat duck production cycle in the field, i.e., 63 day old birds.

It was interesting that one farm had a distinctively lower GMT and seroprotection rate but a higher variation in antibody response of vaccinated ducks compared to the other farms. This farm was the only one which moved their ducks for grazing onto fields of 3 other households in the same district. Additionally, the owner of that farm and his workers vaccinated the ducks themselves rather than asking help from a local veterinarian. Difficulties in duck grazing management and vaccination practices in this case might be a partial explanation for the lower immunity outcome from vaccination on this farm compared with the others.

In general, the relatively high serologic immune response to the H5N1 vaccination found in this study may partly be due to the high level of effort and attention given to the implementation of vaccination at the farms involved by both farmers and veterinarians. Duck production is highly concentrated in the area where this study was conducted, so farm owners probably have more understanding of the disease as well as the importance of H5N1 vaccination; they also showed a high level of cooperation and preparedness for vaccination. Many farm workers can vaccinate properly and can assist in sample collection. On the other hand, this study utilized materials, procedures of serological assay, and methods of expressing and interpreting HI results as well as evaluating criteria similar to those used in the national routine post-vaccination surveillance program. Findings of this study are expected to reflect the current situation in the local area as nowadays, the veterinary service at commune level is very positive, people's awareness of poultry disease prevention and control has been improved and the fact that many farmers actively purchase influenza vaccines instead of waiting for free vaccine distribution from the veterinary authorities. For that reason, these results could potentially provide ideas for vet authorities in the country regarding possible improvement of the field effectiveness of the H5N1 vaccination program.

5.2 Conclusions

Both the prime and booster vaccinations are immunogenic. Two vaccinations are needed to more fully provoke protective antibody levels in ducks. Variations in antibody response of vaccinated ducks suggests that the effectiveness of vaccination in the field is variable, so proper attention should be given to the vaccination process. A single prime vaccination with the Re-6 vaccine strain elicits an acceptable antibody response in domestic ducks in terms of protective HI titres and seroconversion rate according to the targets of the national vaccination strategy for prevention and control of HPAI. There is, however, considerable variability in response between farms. The results support the tenet that a second immunization significantly improves ducks' serological immunity against HPAI virus compared to a single immunization. Booster vaccination results in increased HI-antibody titres in most ducks and increases the proportion of ducks showing serological evidence of protection, although there is a small variation in the individual immunological response.