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Risk factors of Highly Pathogenic Avian Influenza H5N1 occurrence at the village and farm levels in the Red River Delta region in Vietnam

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Worked carried out in Vietnam

Running head

Case control study on HPAI H5N1 in Northern Vietnam

Abstract

A case-control study at both village and farm levels was designed to investigate risk factors for Highly Pathogenic Avian Influenza H5N1 during the 2007 outbreaks in one province of Northern Vietnam. Data related to human and natural environments, and poultry production systems was collected for 19 case and 38 unmatched control villages and 19 pairs of matched farms. Our results confirmed the role of poultry movements and trading activities. In particular, our models found that higher number of broiler flocks in the village increased the risk (OR = 1.49, 95% CI: 1.12-1.96), as well as the village having at least one poultry trader (OR =11.53, 95% CI: 1.34-98.86). To a lesser extent, in one of our 2 models, we also identified that increased density of ponds and streams, commonly used for waterfowl production, and greater number of duck flocks in the village also increased the risk. The higher percentage of households keeping poultry, as an indicator of households keeping backyard poultry in our study population, was a protective factor (OR= 0.95, 95% CI: 0.91-0.98). At the farm level, 3 risk factors at the 5% level of type I error were identified by univariate analysis: a greater total number of birds (P=0.006), and increase in the number of flocks having access to water (p=0.027, and a greater number of broiler flocks in the farm (P=0.049). Effect of vaccination implementation (date and doses) was difficult to investigate due to a poor recording system. Some protective or risk factors with limited effect may not have been identified due to our limited sample size. Nevertheless, our results provide a better understanding of local transmission mechanisms of HPAI H5N1 in one province of the Red River Delta region in Vietnam and highlight the need to reduce at-risk trading and production practices.

Key words: HPAI; H5N1; Vietnam; Risk factors

1. INTRODUCTION

Vietnam, with a poultry population over 200 million (Desvaux and Dinh, 2008), faced its first outbreaks of Highly Pathogenic Avian Influenza (HPAI) H5N1 at the end of 2003 (OIE, 2008). By the end of 2009, 5 epidemic waves had occurred in domestic poultry; with the latest waves being limited to the North or the South regions whereas the first waves had a national distribution (Minh et al, 2009). To limit the number of outbreaks and the risk of transmission to humans, the Government of Vietnam decided to use a mass vaccination strategy at the end of 2005. After a period of about a year without an outbreak, Northern Vietnam faced a significant epidemic in 2007 with 88 communes (administrative level made of several villages) affected in the Red River Delta administrative region (Minh et al, 2009). So far, most of the studies investigating the role of potential risk factors on the occurrence of HPAI outbreaks in Vietnam have been implemented at the commune level using aggregated data from general databases for risk factor quantification (Gilbert et al, 2008; Henning et al, 2009a; Pfeiffer et al, 2007). In Pfeiffer's study of the 3 first waves (Pfeiffer et al, 2007) increased risk was associated with decreased distance from higher density human populated areas, increased land area used for rice, increased density of domestic water birds and increased density of chickens. In the same study, significant interaction terms related to the periods and the regions were also associated with the risk of HPAI emphasizing the importance of spatio-temporal variation in the disease pattern. Gilbert demonstrated that the relative importance of duck and rice crop intensity, compared to human density, on the risk of HPAI was variable according to the waves (Gilbert et al, 2008). Human-related transmission (as illustrated by human density being the predominant risk factor) played an important role in the first wave, whereas rice cropping intensity was the predominant risk factor in the second wave. For the third wave, duck and rice cropping intensity became less strong predictors probably due to control measures targeting duck populations during that period. Those studies provided a general understanding of the main mechanisms involved in the epidemiology of HPAI in this region and their possible evolution over the different waves: in particular the role of human activities in the transmission process and the role of environment (mainly rice-related areas) as an indicator of the presence of duck populations or as a component of the transmission and maintenance processes. Previously, only one published case-control study has been carried out in Vietnam, at the farm level, following outbreaks in the South in 2006 (Henning et al, 2009b). There have been no studies investigating village-level indicators for HPAI infection. In order to define more detailed risk factors at a smaller scale (village and farm), this case-control study was carried out in one province in Northern Vietnam, Bac Giang, located 50 kms northeast of the capital Hanoi (Fig 1). Bac Giang had a poultry population estimated around 10 millions in 2007 (GSO, 2010) of which around 1 million were ducks. The province presents 3 distinct agro-ecological areas with one of them consisting of lowland, typical of the rest of the Red River Delta area in terms of agricultural practices and poultry density (Xiao, 2006; Desvaux and Dinh 2008). We focused our study in this lowland area since it is in this type of agro-ecological area that outbreaks in northern Vietnam were mainly concentrated (Pfeiffer et al, 2007; Minh et al, 2009). The objective of the study was to evaluate the risk factors related to the human and natural environments and the poultry production systems on the introduction; transmission or maintenance of the HPAI virus during the 2007 epidemic wave in Northern Vietnam, at both village and farm levels.

2. MATERIALS AND METHODS

2.1. Study design overview

Two epidemiological units of interest were considered in this study: the village and the farm. Risk factors were investigated using a non matched case-control study for the villages and a matched case-control study, based on farm production type and location, for farms. Questionnaires were designed and administered between April and May 2008 and were related to outbreaks occurring in 2007. The epidemic wave period was defined as a window between February 2007 and August 2007 (DAH, 2008).

2.2. Data source and case and control selection

The initial data source used was provided by the Sub Department of Animal Health of Bac Giang province where the study was based. The data included information on 2005 and 2007 H5N1 outbreaks aggregated at the village level and included both villages with disease outbreaks and villages where only preventive culling had been performed. There was no precise indication of the number of farms infected or culled in the villages. In addition, some outbreaks were based on reported mortalities only whereas others also had laboratory confirmation of H5N1 infection. Laboratory confirmation was performed either by the Veterinary Regional Laboratory or the National Centre for Veterinary Diagnosis. Given these parameters, a village case was therefore initially defined as a village having reported H5N1 mortality and/or a village with laboratory confirmation reported.

2.2.1. Case and control selection at village level

In order to further refine the list of village cases, the list of infected village obtained was checked by field visits and discussion with local veterinary authorities (district and commune veterinarians) before the study commenced. When local veterinary authorities agreed on the HPAI status of a particular village, it was confirmed as a case. Where a discrepancy was found between our list and their reports, details were requested on the mortality event in the village farms involved. A case-definition was then applied on the description of symptoms provided by the local veterinarians and the village was defined as a case if the following criteria were met in at least one farm in the village:

- per acute or acute disease (time from observed symptoms to mortality less than 2 days)
- mortality over 10 % within 1 day

  - neurological signs in ducks if ducks were involved in the outbreak (head tilt, uncoordinated movements)
  - a positive result for a rapid diagnostic H5N1 test on sick birds if such a test had been applied (usually not reported on our initial list).

At the end of the field interviews and before analysis, a final check of the case villages included was carried out based on the answers to the village questionnaires. This enabled case villages where mortalities had occurred outside the epidemic wave period to be removed from the study.

The villages from communes with outbreaks in 2005 or 2007 were also excluded to take into account pre-emptive culling sometimes organized at a large scale. Control villages were randomly selected from the remaining villages in the study area. Two controls were selected for each case. The selection of control was stratified at the district level for administrative reason and to balance the number of case and control per district. A last check on the selection of controls was performed based on the answers to the questionnaire. Control villages reporting unusual poultry mortality in 2007 (anytime in 2007) were excluded from the analysis.

2.2.2. Case and control selection at farm level

The case farms were the first farms that had an outbreak in each of the case village. This was designed to investigate risk factors of introduction. If this farm was not available, the nearest farm (geographically) to be infected in 2007 was selected.

The matched control farms were selected among farms that never experienced an HPAI outbreak in the same village as the case farm (matched by location) and were also matched by species and by production type (broiler, layer or breeder).

2.3. Data collection

2.3.1. Questionnaires

Two questionnaires were developed, for the village and the farm levels. The village questionnaire, targeted at the head of the village, included general information about the village (number of households, presence of a live bird market within or near the village, presence of wild birds), the list of poultry farms in the village in 2007, the origin of day-old-chicks (DOC) in 2007, the vaccination practices, the description of mortality events that had occurred in previous years and a description of the HPAI outbreak for the village case (timeline, reporting, control measures). Where mortality events had occurred in previous years, we asked for estimates of the percentage of households involved and the date of this mortality event. The latter information was used to confirm the case or control status of the villages by eliminating cases with mortalities outside the defined epidemic period and controls with reported poultry mortality in 2007 (any report of poultry mortality by the head of the village was considered as an unusual event since only significant mortality event are generally noticed by local authority).

At the farm level, the questionnaire was targeted at the farmer or his/her family. The questions included information on the composition of the farm poultry population in 2007, trading practices (to whom they were selling and buying their birds), vaccination practices, and housing systems and for the cases, a description of the HPAI outbreak event. General opinions of the farmers were also collected regarding thoughts on why the farm had or did not have an HPAI outbreak.

2.3.2. Environmental and infrastructure data

As no Geographic Information System (GIS) map layers were available for the village administrative level, the density of variables possibly related to the transmission of virus (transport network, running water) or the persistence of virus (presence of rice fields and non running water) was calculated for a 500 m radius buffer zone from each village center using GIS software (ESRI ArcGIS^{TM}, Spatial Analyst, Zonal statistics as table function). GIS layers including transport networks, hydrographic networks, lakes and ponds were bought from the National Cartography House in Hanoi. The density of transport feature (national roads and all roads) and animal production-related water features (canals, ponds and streams) were calculated within each buffer zone by dividing the number of pixels occupied by a specific feature by the total number of pixels in the buffer. The size of a pixel was defined as 20 x 20 meters. A land cover map derived from a composite SPOT (Satellite Pour l'Observation de la Terre) image supervised classification (Fig 1) was produced, validated by field visits and used to characterize the landscape of our study area (Tollis, 2009). The density of 5 different land cover types (water, rice, forest and fruit-tree, upland culture and residential areas) was calculated within each buffer.

2.4 Data analysis

2.4.1. Univariate analyses

Statistical analyses were conducted using Stata 10 (StataCorp. 2007. *Stata Statistical Software: Release 10*. College Station, TX: StataCorp LP) and R 2.11.1 softwares. The association between the outcomes (being a case or a control) and each explanatory variable was assessed using exact logistic regression (Hosmer and Lemeshow, 2000) (with the exlogistic command in Stata). A matched procedure was undertaken for the matched case-control study at the farm level. P-values for each variable were estimated using the Wald test (Hosmer and Lemeshow, 2000). Variables having a p-value ≤ 0.1 were candidates for inclusion in the multivariable model. All continuous variables were tested for linearity assumption by comparing two models with the Likelihood Ratio test: a model using a categorical transformation and a model with the same transformation but the variable treated as an ordinal variable. Different categories were tested: either a transformation based on quintile (or quartile depending on the distribution) or using equal range of values of the variable.

2.4.2. Multivariate analyses

For the unmatched case-control study at the village level only, an investigation of multivariate models was undertaken. The first step was to build a model including all the explanatory variables selected during the univariate step. We also included into this model one environmental variable with a p-value of less than 0.2. We then checked for collinearity among the variables in this model using -collin command in Stata, checking that tolerance was of more than 0.1 (Chen et al, 2010). In order to take into account our small sample size we used a backward stepwise selection method based on the second-order bias correction Akaike Information Criteria comparison (AICc) (Burnham, 2004). Variables were removed sequentially. At each step, the variable which removal resulted in the largest AICc decrease was excluded. Goodness-of-fit of the final multivariate models was assessed using Pearson’s chi square test.

3. RESULTS

3.1. Study population

After initial field visits for infected village selection and confirmation, we ended up with a total number of 22 villages which had experienced an HPAI outbreak in Bac Giang in 2007. Among those 22 villages, 20 were targeted for interview (the 2 remaining ones belonged to 2 districts from more remote areas not targeted in our study as not representative of the Red River Delta region) and 40 control villages were selected. One village could not be interviewed and after reviewing the mortality criteria, a final total of 18 villages were included in our analysis as cases. The same procedure was followed to check control villages and 6 were omitted because they did not meet the definition for a control (unusual poultry mortalities was reported in 2007). In total, 18 case villages and 32 control villages were included in the final analysis.

Using the established criteria, a total of 18 pairs of matched farms remained for the analysis.

3.2. Characteristics of the study population

The village study population (18 cases and 32 controls) were located within 6 districts and 32 different communes. On average, the number of households per village was 218 (range 21-600).

The farm study population consisted of 18 pairs of case and control farms totaling 74 flocks, with farms having on average 2.1 flocks (range 1-4, median2) of mixed poultry types. Duck flocks (N=34) had numbers of birds ranging from 10 to 1050 (mean 351; median 200) with the main breeds being Tau Khoang (N=11) and Super Egg (N=9). Chicken flocks (N=28) ranged from 10 to 2500 birds (mean 363; median 230) with the main breeds being local (N=26). Muscovy duck flocks (N=12) ranged from 20 to 400 birds (mean 160; median 200) with all flocks derived from the French breed.

3.2.1. Description of the case farms

Outbreaks had occurred in the farms between 7th April 2007 and 23rd June 2007. Among the 18 case farms, clinical signs and mortality were reported from 63 % of the flocks (24/38). At the farm level between 25 and 100% of the flocks were showing clinical signs and mortality. On average, 45% of the birds in the infected flocks died before the remaining ones were culled (n=24, range 5-100). The description of infected flocks by species, production type and age is given in Table I. The average age of infected birds was 66 days (range 20-120 days, median 60). Fourteen case farms out of 18 were reported to have been vaccinated against HPAI. The disease occurred on average 48 days after vaccination (range 7-92, n=7).

3.2.2. Description of the report and culling delay

On average the farmers declared the disease to official veterinarians 2.8 days (range 1-8, n=18) after the onset of the disease. There were on average 8.9 days between the onset of the disease at the farm and the culling of the flock (range 1-31, n=16).

3.2.3. Farmers' behavior and thoughts regarding HPAI source

Of 14 farmers who answered the question, 12 tried to cure their birds, 6 buried the dead birds, 4 threw the dead birds into a river, channel or fish pond, 1 ate the dead birds and 1 tried to sell the sick birds. The following possible causes of HPAI in the farm were quoted by the farmers:

- introduction from neighboring infected farms (3 answers)

- contact with wild birds (2 answers)
- scavenging in rice fields (2 answers)
- contamination of the channel water due to animal burying nearby (1 answer)
- poisonous feed in rice field (1 answer)

Five farmers out of 18 did not believe their farm had HPAI even following veterinary authorities' confirmation of the diagnosis.

3.3. Vaccination practices in the village study population

Twelve percent (6/50) of the heads of village declared that vaccination was not compulsory, whereas it is; but only one head of village declared that no AI vaccination had been used in the village. In the majority of the villages (94% = 45/48), the small size farms had to take their birds to a vaccination center. Those farms usually had less than 50 birds (56%=27/48 of the villages) or between 50-100 birds (35%=17/48). One village declared that farms up to 200 birds had to bring birds to the vaccination center. The vaccination center was located within each village. In most of the villages (90%) the head of the village declared that there was only one injection of HPAI vaccine per bird per campaign. Heads of villages also reported that the vaccination coverage was not 100% due to difficulty in catching some birds in the farms and also because certain farmers with small number of birds did not want to vaccinate them.

3.3. Analyses at the village-level

Twenty eight potential risk factors were individually tested using simple exact logistic regression method. Table II presents odds ratio (OR) estimation and their confidence intervals (CI). Then, eight variables with $p \leq 0.1$ and the only environmental variable with a p-value less than 0.2 were included in the initial multiple logistic regression model. Hatchery in the village (p-value of less than 0.1) was not included in the model because of the limited number of units in one category, which caused a problem with parameter estimation (Table II). The variable related to the number of flocks of more than 100 birds was of concern regarding collinearity (Tolerance=0.12). We tested the selection without this variable in the full model and came to the same result. . Table III provides a summary of the 2 models obtained from the backyards selection based on the AICc. Those 2 models have an AICc that did not differ by more than 2 points and can thus be considered as describing the data with equivalent quality (Burnham, 2004). The lowest AICc model included three main predictors: percentage of households keeping poultry, presence of at least one poultry trader in the village and number of broiler flocks. The second lowest AICc model allowed the identification of risk factors of moderate effect. Indeed, model 2 identified two additional risk factors at the limit of significance: number of duck flocks and the percentage of village area occupied by ponds and small streams. These two final models fitted the data adequately (model 1: Pearson's chi square = 37.33, df= 34, p value=0.3185; model 2: Pearson's chi square = 25.66, df=37, p value=0.9198)

3.4 Analysis at the farm-level

Three factors were significantly influential at the 5% level: the total number of birds in 2007 (p=0.005), number of flocks having access to water (p=0.027), and the number of broiler flocks in the farm in 2007 (p=0.049). Two factors could be considered as significantly influential at the 10% level: the presence of more than one species in the farm (p=0.065) and the total number of flocks in 2007 (p=0.089) (Table IV). No multivariate model was built due to limited sample size.

4. DISCUSSION

Our results confirm the role played by poultry movements and trading activities, detailed by different indicators both at village and farm levels. Our results also suggest the role played by certain water bodies in virus transmission or as a temporary reservoir. The precise influence of vaccination was difficult to investigate due to limited data available.

4.1. Methodology

Both studies suffered from low statistical power that probably led to conclude that some potential risk factors did not have effect whereas they had one (type II error). We especially faced some limitations in the analysis of the matched case-control study at farm level. Indeed, the effective sample size is reduced by the matching procedure with only discordant pairs included into the analysis (Dohoo et al, 2003). The number of farm cases could not be increased since we had initially targeted all cases in our study area, but we should have tried to increase the number of matched controls per case in order to increase the effective sample size. We also recognize that for some questions recall bias may have occurred. This is particularly obvious for the questions related to the detailed implementation of the vaccination (date and number of injections). However, for most of the questions related to the structure of the village or the farm, no bias was suspected in the answers. The selection biases were limited by our checking of the status at different steps of the study: field verification after initial selection and elimination criteria based on mortality events after interviews and before inclusion into the analysis.

4.2. Intensity of poultry movements and trading activity at the village and farm level

A higher number of broiler flocks was found to be a significant risk factor for HPAI outbreaks at both the village and farm levels. Broiler production is characterized by a high turnover of birds because of the short production cycle and by a high number of trading connections and poultry movements, with several DOC supplies per year and visits by multiple traders when a flock is being sold. Furthermore, H5N1 vaccination in Vietnam is normally carried out during 2 main campaigns per year, in March-April and October-November (FAO, 2010). In some areas vaccination is also organized between those campaigns to better suit the production cycles but Bac Giang province was following the bi-annual vaccination strategy in 2007. Thus, some broiler flocks could have been produced between the main vaccination campaigns and thus not protected against the infection as demonstrated by serological study of the vaccination coverage (Desvaux et al, 2010). Therefore, we can hypothesize that in

Vietnam the number of broiler flocks is a risk factor of H5N1 introduction because of the high poultry trading movements related to this production type and because of the low vaccination coverage. Broiler flocks may also better reveal virus circulation than layer flocks that are better vaccinated as illustrated by the distribution of flocks affected in the case farms (Table I). Indeed, infected not vaccinated flocks show a more typical HPAI clinical picture. Paul et al (2010) found that density of broiler and layer ducks and, to a lesser extent, density of boiler and layer chickens was associated with the risk of HPAI in Thailand where vaccination against HPAI is not applied. In our study we found that only the number of broiler flocks is associated with this risk.

The presence of at least one poultry trader in the village was found to be significantly associated with the risk of HPAI at the village level. This variable is an indicator of the poultry movements within the village that may contribute to disease introduction and transmission. Traders are usually carrying poultry on their motorbikes or on small trucks without significant biosecurity measures (Agrifood Consulting International, 2007). They also often bring birds at home for few days in order to gather enough animals for selling. Those practices probably contribute to the introduction of virus within the village which can then be easily transmitted to village farms by animal and human movements. The presence of a trader was not tested as a potential risk factor in previous studies.

We also found that a higher percentage of households keeping poultry was a protective factor at the village level. In our sample of villages there was no correlation between the number of poultry farms and this percentage meaning that it is more an indicator of the percentage of backyard poultry in the village. Backyard production is defined as a poultry production of small size with low level of investment and technical performance (Desvaux and Dinh, 2008). Thus, villages with high percentage of households keeping backyard poultry are probably more rural and with a smaller human density than others (human density figures were not available for our villages but we found a tendency for negative correlation between household density and this percentage in our sample). The protective effect of low human density on the risk of HPAI has been reported in previous studies (Minh et al, 2009; Paul et al, 2010; Pfeiffer et al, 2007). Another observation that can be made from this result is that even if the percentage of households keeping backyard poultry increases in a village, the risk of HPAI does not increase. This could be explained by the backyard production system having less trading activities and connections than semi-commercial farms. This result is also in accordance with Paul et al's (2010) results. It is also possible that people keeping backyard poultry pay less attention to their birds than larger farmers. Thus, we cannot exclude the possibility that detection of HPAI suspect cases is less efficient in this sector.

Finally, all the variables found positively associated with the risk of HPAI outbreaks in our study explain how the disease can be spread form one village or farm to another, thus they are indicators of the distribution mechanism.

4.3. Farm-level factors

Apart from a higher number of broiler flocks, , an increased number of birds and a greater number of all poultry flocks were both also identified as potential risk factors by the univariate analysis at the farm level. Size of the farm has already been described as a risk factor for HPAI infection (Thompson et al, 2008). This may be explained by an increased frequency of potentially infectious contacts (e.g. by traders, feed or DOC suppliers). Furthermore, viral transmission was also found to be dependent on an increased number of birds (Tsukamoto et al, 2007). Thus a big farm may have more chance to develop a typical H5N1 case with most of the birds being infected and showing symptoms and subsequently being detected as a HPAI case.

The presence of more than one species in the farm was also positively associated with the risk of HPAI. This variable may simply be an indicator of a farm having several flocks or an indicator of the role of waterfowl in the increased risk of HPAI as discussed later.

Most of the farmers declared that their flocks were vaccinated against H5N1, but we can suspect a bias in this answer since, as the vaccination was compulsory, the tendency might be to declare that the flocks were vaccinated. Furthermore, there were too many missing data related to the date of vaccination or the number of injections received to categorize the farms according to those criteria or to observe this having an influence on the protection of the birds. The poor recording system, both at farm or veterinary services levels, did not allow us to fully investigate the influence of vaccination except indirectly by showing that broiler flocks, known to be less vaccinated, are also related to an increased risk of infection.

4.4. Environmental and infrastructure variables at village and farm level

At the village level, a higher percentage of the village surface occupied by ponds and small streams (defined as a 500 meters radius buffer zone around the village centroids) was found to increase the risk of H5N1 outbreak in one of our models. At the farm level, a higher number of flocks having a housing system with access to outdoor water was found to be a risk factor by the univariate analysis. The farm level result corroborates the result at the village level since the water bodies involved in the poultry farming of ducks and Muscovy ducks in Vietnam are usually ponds, canals or small streams, with the birds being kept in a restricted area (around a pond or within part of a canal or small river) or with the ducks ranging in the rice fields, canals and rivers during the day (Desvaux and Dinh, 2008). It was also known, and reported by one of our interviewed farmers, that dead birds may be thrown into canals or rivers by farmers, contributing to contamination of this possible reservoir of virus. In our study, the density of canals within the 500 m buffer zone was not identified as a significant risk factor probably because canals are more frequent outside the village than inside contrary to the ponds. Direct and indirect contact with wild birds through the aquatic environment can also be hypothesized even if in Vietnam infection from wild birds to domestic poultry has not been proven. Our results support the previous work that faecal-oral transmission by contaminated water is a mechanism of avian influenza transmission (Brown et al, 2007), and our results suggest that contaminated water can play a part in the transmission of the virus within a flock and also between flocks sharing the same environment at the same time or at different periods (Brown et al, 2007; Brown et al, 2009; Tran et al, 2010).

Our study area was limited to few districts in one province and thus the heterogeneity of spatial variables was limited. This may explain why we did not find any significant relationship between our outcome and variables related to transport networks as shown in previous studies (Fang et al, 2008) (Paul et al, 2010).

Density of waterfowl was recognized previously as a risk factor for disease occurrence, possibly due to their potential role as a reservoir of infection (Biswas et al, 2009; Fang et al, 2008; Gilbert et al, 2006; Paul et al, 2010; Pfeiffer et al, 2007). Nevertheless, in our study, the number of duck flocks was at the limit of significance at the village and farm levels, indicating that this species was not a predominant risk factor for disease occurrence in 2007 in our study area. This might be explained in the Vietnamese context by the prevention measures applied to that species (vaccination) and also to the H5N1 strains circulating in North Vietnam. Indeed, as ducks were recognized as a silent carrier in a study conducted in 2005 (National Center for Veterinary Diagnosis, 2005) the veterinary services took the decision to vaccinate this species. Thus, in 2007 ducks in Vietnam were better protected against infection than in the earlier waves of infection. Another significant change relates to the predominant strains circulating in North Vietnam in 2007 (clade 2.3.4) (Nguyen et al, 2008) which are more pathogenic for ducks than the original clade 1 strain (Swane and Pantin-Jackwood., 2008) and may limit the role of silent carrier played by non-vaccinated ducks.

5. CONCLUSIONS.

Our results provide a better understanding of the local transmission mechanisms of the HPAI H5N1 virus in one province of the Red River Delta region by confirming and detailing the role played by poultry movements and trading activities as well as water bodies in the introduction and transmission of the H5N1 virus at the village and farm levels. Despite limited statistical power and possible unrecognized risk factors of more limited effect, we were able to characterize the villages that may be more at risk of H5N1 outbreaks based on the structure of their poultry production (a higher number of broiler flocks), the presence of a poultry trader and a higher surface area of ponds or small streams. It was interesting to note that broiler flocks are also those known to be less well vaccinated against H5N1 due to their short production cycle. Thus, despite intensive mass communication and awareness campaigns organized in Vietnam by different programs since HPAI first occurred, there are still considerable at-risk behaviors and local disease transmission is still difficult to avoid. Nevertheless, it should also be noted that detection of an H5N1 case may also be more challenging for farmers and local veterinarians since clinical expression is probably altered in partially immunized populations. We also recognize the limitation of classical epidemiological studies for investigating the effect of vaccination in the absence of good recording systems. Use of modeling approaches to test effect of different vaccination strategies on populations or capture-recapture methods using different information sources may be more suitable techniques in that context. Finally, it is vital that the scientific knowledge acquired is transformed into appropriate actions in terms of prevention and surveillance. In this respect, better use of sociological approaches could also help to change high risk practices.

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Figure Legend

Figure 1. Bac Giang province land cover map derived from composite SPOT image supervised classification

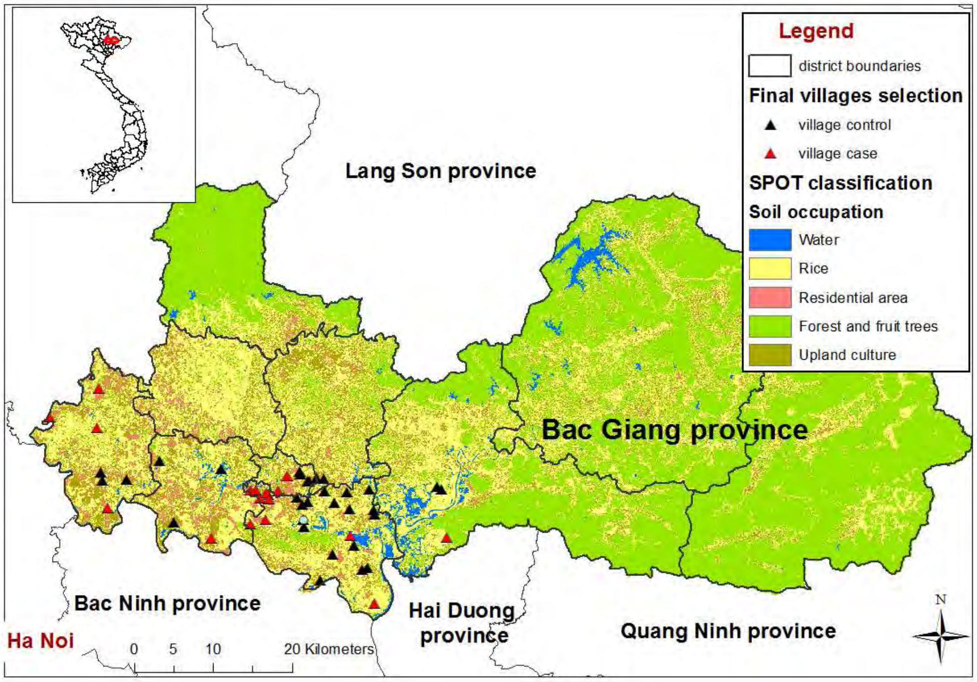

Figure 1. Bac Giang province land cover map derived from composite SPOT image supervised classification
296x210mm (96 x 96 DPI)

Tables

Table I Description of the infected flocks in the case farms

Species	No. flocks	No. flocks with clinical signs or mortality	No. broiler flocks with clinical signs or mortality	No. breeder or layer flocks with clinical signs or mortality	Mean age of the affected flock in days (min-max)
Chicken	15	10	10/13	0/2	78 (30-120)
Duck[1]	16	10	7/9	1/5	53 (20-90)
Muscovy Duck	7	4	4/7	0/0	71 (45-90)
	38	24	21/29	1/7	

[1] The production type of 2 duck flocks with clinical signs was not recorded because the farmer answered globally for all his duck flocks

Table II Results of univariate analysis using exact logistic regression for variables potentially associated with HPAI outbreaks at the village level.

Variable	**Category**	**Case (mean)**	**Control (mean)**	**OR**	**95% CI**	**p value**
General information on the village						
No. households in the village in 2007 (N=49)		18 (260)	31 (195)	1	1-1.01	0.094
Percentage household keeping poultry (N=44)		16 (65%)	28 (83%)	0.98	0.96-1.00	0.053
Wild birds present in rice fields around the village (N=50)	A few	9	23	1		
	A lot	9	9	2.51	0.65-10.03	0.216
Wild birds present in the village (N=50)	A few	13	23	1		
	A lot	5	9	0.98	0.21-4.16	1
Live bird market present in the village in 2007 (N=50)	Yes	5/18	3/32	33.6	0.60-26.84	0.197
Presence of at least one poultry trader in the village in 2007(N=50)	Yes	10/18	5/32	6.45	1.40-32.08	0.009
Presence of at least one bird hunter in the village in 2007 (N=49)	Yes	8/17	8/32	2.61	0.64-11.00	0.214
Presence of at least one hatchery (N=50)	Yes	3/18	0/32	7.55	0.77-inf	0.083
Poultry production in the village in 2007						
No. flock (from farms) of more than 100		18	32	1.31	1.11-1.58	0.001

birds (N=50)		(6.6)	(4.4)			
Percentage of farms vaccinated against HPAI (N=43)		14 (74%)	29 (79%)	0.98	0.95-1.02	0.341
Species						
No chicken flocks (from the farms) (N=50)		18 (4)	32 (2.7)	1.18	0.95-1.48	0.141
No. duck flocks (from the farms) (N=50)		18 (4.3)	32 (2.3)	1.25	1.02-1.58	0.029
Presence of Muscovy duck flock(s) in the village (N=50)		13/18	8/32	7.43	1.81-35.98	0.003
Production type						
No. broiler flocks (N=50)		18 (7.1)	32 (3.2)	1.38	1.14-1.71	<0.001
No. breeder flocks (N=50)		18 (0.5)	32 (0.3)	1.30	0.56-3.00	0.606
No. layer flocks (N=50)		18 (2.2)	32 (1.8)	1.06	0.83-1.35	0.662
Housing system						
No enclosed flocks (N=50)		18 (2.2)	32 (3.3)	0.85	0.65-1.07	0.207
No. fenced flocks (outdoor access) (N=50)		18 (5.8)	32 (1.8)	1.49	1.18-1.98	<0.001
Presence of scavenging flock(s) (N=50)		6/18	4/32	3.4	0.67-19.64	0.165
Spatial [a]						
Percentage of pixels with canals (N=50)		18 (0.8%)	32 (0.6%)	1.16	0.72-1.80	0.559
Percentage of pixels with ponds and streams (N=50)		18 (1.8%)	32 (1.1%)	1.25	0.91-1.75	0.170
Percentage of pixels with national roads (N=50)		18 (1.2%)	32 (1.1%)	1.04	0.77-1.38	0.773
Percentage of pixels with all kind of roads (N=50)		18 (2.4%)	32 (1.9%)	1.07	0.85-1.33	0.571
Percentage of pixels with water using SPOT (N=50)		18 (6.2%)	32 (5.5%)	1.01	0.95-1.06	0.790
Percentage of pixels with rice using SPOT (N=50)		18 (54.6%)	32 (59.1%)	0.99	0.96-1.02	0.452
Percentage of pixels with residential area using SPOT (N=50)		18 (23.6%)	32 (25.5%)	0.99	0.95-1.03	0.671
Percentage of pixels with forest and		18	32	1.02	0.99-1.06	0.228

fruit trees using SPOT (N=50)		(11.5%)	(5.7%)			
Percentage of pixels with upland culture production using SPOT (standardized) (N=50)		18 (4%)	32 (4.2%)	1	0.92-1.07	0.982

^{a} variables are expressed for a 500m radius buffer around village centroids

Table III Result of the final logistic regression models at village level using two selection methods

		Model 1 (AICc =40.14)		**Model 2 (AICc =40.61)**	
Variable	**Category**	**OR (95% CI)**	**p value**	**OR (95% CI)**	**p value**
Percentage household keeping poultry		0.95 (0.91-0.98)	0.006	0.94 (0.09-0.98)	0.006
Presence of at least one poultry trader in the village	yes	11.53 (1.34-98.86)	0.026	9.69 (0.93-100.89)	0.057
No. duck flocks (from the farms)				1.39 (0.96-2.01)	0.079
No. broiler flocks		1.49 (1.12-1.96)	0.006	1.60 (1.14-2.24)	0.007
Percentage of pixels with ponds and streams				2.35 (0.79-6.98)	0.125

Table IV. Results of univariate analysis using exact logistic regression for variables potentially associated with HPAI outbreaks at the farm level.

Variable	**Category**	**Case (mean)**	**Control (mean)**	**OR**	**95% CI**	**p value**
General information on the farm						
Presence of more than one species in the farm	yes	14/18	7/18	4.5	0.93-42.80	0.065
The different species are separated	yes	2/14	0/8	1	0.03-inf	1
The farmer vaccinates against Newcastle disease	yes	9/17	9/18	1.33	0.22-9.10	1
The farmer vaccinates against the main poultry diseases	yes	16/18	16/17	2	0.10-117.99	1
The farm used H5N1 vaccination	yes	14/18	17/18	0.26*	0-0.41	0.25

Person in charge of the H5N1 vaccination	farmer	2	2	1		
	veterinarian or paravet.	12	15	0.5	0.01 -9.61	1
Trading activity of the farm						
The farm is trading with a trader	yes	10/14	17/18	0.25	0.01-2.53	0.375
The farm is trading with a market	yes	2/16	2/18	1	0.07-13.80	1
Percentage of poultry product sold to a collector		14 (59%)	18 (76%)	0.99	0.96-1.01	0.313
Percentage of poultry product sold to another farmer		14 (29%)	18 (17%)	1.01	0.99-1.05	0.311
Percentage of poultry product sold to a market		14 (4%)	18 (7%)	0.99	0.93-1.03	0.625
The farmer has a trading activity	yes	0/18	1/18	1*	0-39	1
No. of laying and breeding flocks in the farm in 2007		18 (0.5)	18 (0.5)	1	0.29-3.38	1
No.of broiler flocks in the farm in 2007		18 (1.9)	17 (1.7)	3.27	1-24.87	0.049
Total no. of flocks in the farm in 2007		18 (2.4)	18 (1.7)	1.98	0.92-5.51	0.089
No. of chicken flocks in the farm in 2007		18 (0.9)	18 (0.7)	2.49	0.52-23.06	0.359
No. of duck flocks in the farm in 2007		18 (1.1)	18 (0.8)	3.36	0.74-31.09	0.148
No. of Muscovy duck flocks in the farm in 2007		18 (0.4)	18 (0.3)	2	0.29-22.11	0.688
Total no. of birds in 2007		18 (954)	18 (406)	1	1-1.01	0.006
Total no. of production cycles in 2007		18 (2.8)	18 (2.2)	1.32	0.80-2.43	0.324
Housing and feeding system and water source						
No. of flocks having housing without access to water		18 (0.6)	18 (0.7)	0.86	0.22-3.07	1
No. of flocks having housing with access to water		18 (1.7)	18 (1.1)	5.81	1.11-236.82	0.027
Source of drinking water	well	11	15	1		
	pond or river	7	3	5.28*	0.66-inf	0.125

* Median unbiased estimates (MUE) reported instead of the conditional maximum likelihood estimates (CMLEs)