Swine influenza in Vietnam: preliminary results of epidemiological studies

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Swine influenza in Vietnam: preliminary results of epidemiological studies

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Introduction

In Vietnam, the modelling of the pandemic H1N1 progression estimates that 460 000 (260 000–740 000) pigs might be exposed to the virus on the basis of 410 000 cases among swine owners (220 000–670 000).[1] A poor level of biosecurity, high animal densities, and a mix of species could increase the risk of influenza virus flow, persistence, and emergence on swine and poultry farms. This study was set up in the Red River Delta, where a third of the national pig husbandry is produced.[2] The aims are to give preliminary information of the epidemiological state of swine influenza and in order to further assess the risk of infection of SwIV, through cross-species transmissions from poultry to pigs. This paper will present the preliminary results on SwIV and the risk factors of pig seropositivity in Vietnam.

Materials and methods

A cross-sectional study was conducted in two provinces of the Red River Delta in April 2009. Pig farms were randomly selected from nine communes representative of at risk area of avian H5N1. In each farm, pig and poultry were sampled and collected to virological and serological analyses. Interviews were conducted in all farms by trained interviewees. Questionnaires included closed and open questions on

livestock husbandry/management and household characteristics, such as herd size and structure, health history and vaccination, pig housing, watering and feeding system, reproduction, purchasing of animals, biosecurity measures, pig contact with poultry, and environmental factors.

The virological detection assay was performed on pools of nasal swab specimens from pigs. We investigated whether real-time RT-PCR assay could detect gene M on pools of nasal swab specimens before attempting virus isolation from individual nasal swab specimens. The poultry and pig sera were tested against influenza type A with an Enzyme-like immunosorbant assay (ELISA) competition test IDVET©. This commercial kit is designed to specifically detect antibodies directed against the NP protein antigen of influenza type A viruses. The positive serum samples were examined in hemagglutination inhibition (HI) to determine antibody titers and subtypes. The HI test was tailored for H1, H3, and H9 subtypes in pigs and H6 and H9 subtypes in poultry. Seroneutralization tests by pseudo particles were used to test the presence of antibodies directed against H5 subtype.

We analysed the data for relationships between Influenza A serological status (the outcome variable) and possible risk factors using R version 2·11·1 (R Development Core team). The statistical unit was the individual. Initially, the quantitative variables were encoded into categorical variables according to the quartiles or median. Descriptive statistics (e.g., means or medians, proportions, standard deviations) were calculated for all herd-level and commune level predictors to assist in the subsequent modeling process. We also performed the independence test among all variables to determine if variables were dependant. Then, univariate analysis of potential risk factors for the pigs being positive for SwIV and estimation of odds ratios were performed using generalised linear mixed models with binary outcome and logit link function for each herd-level and commune-level variable to determine which variables were individually associated with influenza A seropositivity at a significance level of $P < 0{\cdot}30$. Herd and commune of residence were included as a random effect to account for the correlation of observations at the herd level.

The third stage of the analyses included the four herd-level variables found to be significantly ($P < 0{\cdot}30$) associated with Influenza A seropositivity. An automatic process using all possible associations between the selected variables was computed into a mixed logistic regression models, with random effects. When two variables were collinear, as determined before, only one variable was likely to enter the multivariable model, and therefore, the selection of which collinear variable to enter the model was guided by biological plausibility and statistical significance.

Results

All of the 146 pools of nasal swabs were RT-PCR negative. The maximal possible prevalence considering perfect diagnostic tests would be of 2·03% at a confidence level of 95%, in an infinite population within these regions (WinEpiscope 2·0).

Six hundred-and-nine pig sera were tested in 76 non-vaccinating farms. The herd seroprevalence of swine influenza in the commune previously infected by the avian H5N1 in the Red River Delta raised by 17·1% [8·7; 25·6] in April 2009. But among 13 seropositive farms, only four had at least two seropositive pigs. The within-herd seroprevalence is very low, and no seropositivity was detected in the majority of farms. Estimates had large confidence intervals due to small sample sizes. The individual seroprevalence raised 3·62% [1·98; 5·27]. The subtyping of seropositive sera is still in process.

Descriptive statistical analyses on five major risk factors of SwIV: farm size, breeding vs. fattening, purchasing, percentage of family income, and poultry production, were conducted. Based on this analysis, three types of farming systems were identified and included in mixed models (Table 1). Percentage of family income by pig production and poultry production were not differentiating factors for this typology. Whereas types 1 and 2 seem to be specialized in fattening, the type 3 produces and might sell piglets on the farm site.

The exploration of the different variance components indicated that the random effect variances were mainly associated with the herd, while the commune did not seem to have any effect. Therefore we included in all models only the herd as a random effect. The random effect term for herd was modelled, assuming a normal distribution with a

Table 1. Typology of farming system

Type 1: Large fattening farms	Largest scale production, with more than 40 pigs per year Specialized in fattening, and purchase more than 20 pigs per year
Type 2: Small fattening farms	Small scale of production, with less than 20 pigs per year Specialized in fattening, and purchase less than 20 pigs per year
Type 3: Medium breeding-fattening farms	Medium scale of production, with less than 40 pigs per year Breeding and fattening piglets, with rare purchase

Table 2. Seroprevalence of SwIV and univariate analysis with typology as fixed effect and herd as random effect

	Seroprevalence (%)	IC95%	OR	*P*-value
Type 1	1·93	0·53–4·87	1	–
Type 2	4·76	1·77–10·08	3·11	0·39
Type 3	6·47	3·00–11·94	5·26	0·18

common variance [$\sim N(0,\sigma 2herd)$].[3] The univariate analyses were conducted on 22 variables and typology variables, with herd as random effect. Some coefficient or confidence intervals were inconsistent because of small effectives, especially for the percentage of self-product culture or the pig free-grazing because of the lack of positive results in the dataset. The only one significant (P value < 0·1) parameter was the percentage of pig sales in the familial annual income. Surprisingly, common risk factors of swine influenza infection, such as farm size, animal movements, and sanitary parameters got low odds ratio individually (without being significant); the typology provides the hypothesis of complex interactions effects that increase the risk of infection. As shown in Table 2, the farming system type 3 got a higher seroprevalence of 6·47% [3·00–11·94] and a higher risk indicator, with OR = 5·26 (P-value = 0·18) in comparison with type 1. This finding was not significant. In the multivariate mixed model, the percentage of familial income provided by pig production was the only one significant variable, with OR = 0·22 [0·04–1·25].

Discussion

The focus on diseased animals in the winter-time is usually required in order to increase the likelihood to isolate the virus, although the isolation rate on healthy or clinical samples never exceed 6%.[4] The season and the lack of disease reports might explain the difficulties to detect influenza viruses. Additionally, the pooling method tends to decrease the isolation rate because of a dilution effect, potential presence of PCR assay inhibitors, or uneven distribution of virus in the sample.[5]

Our seroprevalence results must be confirmed and the subtypes identified, especially because we found only one positive animal in a few farms that could be attributed to false positive results of the ELISA test (performances are not known). These preliminary results are in favor of a virus circulation at low level in the spring, but must be completed by further surveys in the winter and before the New Year (Têt celebration) when pig production, trade, and movement increase at their maximum.

No clear prior information on the expected prevalence of swine influenza in Vietnam, tests sensitivity, and specificity could be obtained from literature or reliable sources. Bayesian methods will be carried out in the future in order to compute prevalence and/or to estimate the probabilities of freedom.

The risk factors analysis was limited by the lack of positive results. Further studies are necessary to identify the at-risk season and type of farming systems at risk of swine influenza infection. However, this investigation of risk factors leads to the hypothesis that medium size breeding-fattening farms had a higher risk than large or small size fattening farms. Further investigation are needed to precise this typology. The risk of SwIV infection increases with a combination of three major factors. Poultry production does not seem to play any role on swine infection. The generalized linear mixed model afforded to take into account all the non investigated parameters at the herd level. Although we investigated the most common risk factors of swine influenza infection covering different kind of fields, the herd random effect might explain risk variations. Mixed models have become a frequently used tool in epidemiology. Due to software limitations, random effects are often assumed to be normally distributed. Since random effects are not observed, the accuracy of this assumption is difficult to check.[6]

Further studies, such as case-control or cohort studies could help to identify more precisely risk factors of swine influenza seropositivity, as these study designs are more adapted than cross-sectional studies.

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