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Assessing potential risks of influenza A virus transmission at the pig-human interface in Thai small pig farms using a questionnaire survey

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Running title: Risk of influenza in small pig farms

Summary

Influenza A viruses pose a major public health threat worldwide, especially due to the potential for inter-species transmission. Farmers could be among the first people to be infected with a novel reassortant virus in a pig herd and may serve as a source of the virus for their communities. In this study, the pig production systems of smallholders in rural Thailand were examined in order to qualitatively evaluate the potential risks that may contribute to the spread of influenza A viruses. The investigation was based on questionnaire interviews regarding pig farmers' practices and trading activities. We found that extensive pig-human contacts, commingling of pigs and chickens and suboptimal biosecurity practices adopted by farmers and traders may constitute substantial risks for inter-species influenza virus transmission, thereby posing a threat to pig populations and human public health. The regular practices of using manure as field fertilizer, hiring boars from outside and trading activities could contribute to the potential spread of influenza viruses in the local community. To mitigate the potential risks of influenza A virus transmission and spread in the local community, it is recommended that appropriate public health strategies and disease prevention policies for farmers and traders should be developed including improving biosecurity, encouraging separation of animals raised on farms and minimizing the exposure between pigs and humans. Furthermore, surveillance systems for pig diseases should be targeted around the festival months and on-farm identification of pigs should be promoted.

Keywords: Influenza A viruses, Public health, Pig, Smallholder, Thailand, Risk

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The epidemic of avian influenza A (H5N1) viruses in Asia, Africa and Eastern Europe and the 2009 influenza pandemic have demonstrated that influenza A viruses are a major public health threat worldwide (McCune et al., 2012). The influenza A (H1N1) virus responsible for the 1918 "Spanish flu" pandemic emerged in humans and also established itself among pig populations as the "classical" swine influenza virus (SIV) (Crosby, 1989). Similar to the 1918 pandemic, in 2009, soon after the causative virus (A(H1N1)pdm09) was isolated from humans, it spread to pig populations throughout the world. Furthermore, it has since reassorted with endemic SIVs and some of the resultant viruses have been shown to readily infect humans (Peiris et al., 2012). That has emphasized the complexity of the epidemiology and evolution of influenza viruses in both pig and human populations.

In Thailand, influenza A virus subtypes of H1N1, H3N2, and H1N2 have been found in pig populations for decades. Evidence of virus transmissions between pigs and humans has been reported. The swine-like influenza virus was isolated from a Thai patient (Komadina et al., 2007). The findings of pig-to-human influenza virus transmission were also confirmed among pig farm workers in Thailand (Kitikoon et al., 2011). Conversely, the studies by Takemae et al. (2008) and Lekcharoensuk et al. (2010) suggested that the Thai H3N2 SIVs acquired the hemagglutinin gene by the introduction of human H3N2 virus into the Thai pig populations. Moreover, after the emergence of the A(H1N1)pdm09 virus in humans, the virus and its reassortants have continued to be isolated from Thai pigs (Hiromoto et al., 2012).

In cases of human infections with SIVs documented in the United States of America, Canada, Europe, and Asia, most patients had a history of contact with pigs (Myers et al., 2007). If the first infection with a novel reassortant virus occurred in a pig herd, pig farmers could be among the first people to be infected, and may serve as a source of virus for their communities (Saenz et al., 2006). In particular pig farmers undertaking small scale pig production represent a high-risk population and need to be recognized as key actors in preventing of cross-species pathogen transfer (McCune et al., 2012). In the context of global concern about a public health threat from influenza A viruses, knowledge of inter-species contact on pig farms, biosecurity practices adopted by farmers, and the influence of pig marketing chains is crucial, particularly in rural communities where biosecurity is often suboptimal. Evaluation of the risk of influenza A viruses spread at the pig-human interface would allow development of improved measures to reduce these risks. The aim of this study was therefore to qualitatively evaluate the risks arising from farming practices of pig farmers and from trading activities of traders.

This study was conducted in Mukdahan, a rural province in the north-eastern part of Thailand (see Supporting Information). It is considered a priority area to study as local smallholders usually raise domestic pigs which cohabit with free-range chickens. Structured questionnaires for face-to-face interviews were developed. These were designed to identify and describe the practices among farmers and traders and the interactions between humans and pigs that could be involved in the transmission of influenza A viruses (see the supporting information for details). A two-stage, household-based cluster sampling technique was employed. The first stage was selecting Wanyai district in Mukdahan based on its high density of small pig farms. The second stage consisted of selecting four of the 53 subdistricts in Wanyai district because these were located close to Kaeng-Kabao where a high number of pig smallholders are located to provide pig products for tourists.

Participating pig farmers and traders were recruited through Wanyai livestock officers. Before conducting interviews, 20 livestock service providers (paravets) from the Wanyai livestock office were trained and questionnaires were assessed to ensure their comprehensibility, practicability and validity. Ninety-eight farmers and five traders were visited at home by the trained paravets. Verbal consent was obtained from all participants. Descriptive analyses were carried out with SPSS version 17 (SPSS Inc., Chicago, IL, USA), including percentages for categorical data and means, SE, and ranges for continuous data. The 95% confidence intervals for the percentage were calculated based on the exact binomial method (Ross, 2003).

Interviewed pig farmers in the study were predominantly male (67.7%, 95%CI: 57.4%-76.9%). Sixtynine per cent of farmers had graduated from primary school (95%CI: 59.3-78.3%). More than half of them had less than 3 years pig farming experience (58.2%, 95%CI: 47.8-68.1%). Generally, participants practiced mixed farming, with rice-growing as a principal component (77.6%, 95%CI: 68.0-85.4%) and pigs providing additional cash income (97.9%, 95%CI: 92.7-99.7%). In this area, the type of pig production was predominantly farrowing-to-weaning and most pigs were a Thai indigenous breed. The average number of pigs per farm was seven. Raising pigs together with chickens was commonly seen on the interviewed farms (93.6%, 95%CI: 85.7-97.9%). Other animals present on the farms included cattle, dogs, and cats (see Supporting Information for details).

A number of husbandry characteristics that may increase the potential for virus spread on farms were observed. Firstly, pig-raising utilized household waste e.g. swill (59.4%, 95%CI: 48.9-69.3%), and contributed to crop production through manure (78.7%, 95%CI: 69.1-86.5%). Secondly, most pig production was performed in conventional sties with wooden walls (93.5%, 95%CI: 86.5-97.6%) and roofs made of leaves or metal sheets (87.6%, 95%CI: 79.4-93.4%). Twenty-four per cent of farms

were constructed to allow piglets to escape and roam freely (95%CI: 15.4-34.1%). Thirdly, pigs were mostly raised on dirt (54.8%, 95%CI: 44.2-65.2%) and excessive water from rain could enter most pig pens (85.5%, 95%CI: 75.0-92.8%). Fourthly, sweeping was the most common method of cleaning (84.9%, 95%CI: 76.0-91.5%). Disinfectants were rarely used (8.6%, 95%CI: 3.8-16.2%). Lastly, farmers rarely identified individual pigs by tattoo, ear notching or ear tags (see Supporting Information for details). Farmers usually recognized their pigs by their physical attributes (92.3%, 95%CI: 84.8-96.9%).

Biosecurity risks that could facilitate spread of viruses were identified. Quarantine procedures were not applied in any of the studied farms. Hiring boars from other farmers for breeding was commonly adopted (66.7%, 95%CI: 55.5-76.6%). Almost half of the farms allowed vehicles to drive close to the pig pens (49.5%, 95%CI: 38.9-60.0%) and most farms had no on-farm policies for visitors, such as regulations on cleaning hands or shoes (91.6%, 95%CI: 84.1-96.3%). Although most farmers wore protective boots (77.9%, 95%CI: 68.2-85.8%), less than 10% of farmers wore gloves and masks (9.5%, 95%CI: 4.4-17.2% and 6.3%, 95%CI: 2.4-13.2%, respectively) and 22% did not use any protective clothing on farm (95%CI: 14.2-31.8%). Generally, they fed pigs twice a day and spent approximately 30 minutes each time with their pigs. Eighty-two per cent of farmers frequently cleaned their hands after touching their pigs (95%CI: 68.6-91.4%). Washing hands with soap was common among farmers (94.0%, 95%CI: 83.5-98.7%). Most farmers had never been vaccinated against the A(H1N1)pdm09 virus (93.2%, 95%CI: 85.7-97.5%). When they were sick, twenty-six percent of them said they would continue to work on their farms (95%CI: 11.9-44.6%).

Due to their potential for disease dissemination and threats to public health, biosecurity risks posed by the market chain were studied. Traders bought pigs at farms within their subdistrict more

commonly than from different provinces (65%, 95%CI: 40.8-84.6% and 5%, 95%CI: 0.1-24.9%, respectively). After traders purchased pigs from farms, they would commonly transport the live pigs by motorcycles (31.0%, 95%CI: 17.6-47.1%) or pick-up trucks (66.7%, 95%CI: 50.5-80.4%) to restaurants at Kaeng-Kabao. The volume traded per time varied from 20 to 100 head. As the price increased during the New Year and Thai traditional New Year festivals, a high number of pigs were sold at that time. Regarding biosecurity practices, the risk was heightened by the fact that vehicles were rarely cleaned and disinfected. More importantly, if pigs were not delivered to the customers on the same day they were purchased, they were usually stored by traders on their own farms or at collection yards. An illustration of possible pathways for the interspecies transmission of influenza A viruses is provided in Figure 1. Potential modes of influenza transmission vary depending on the farmers' practices on-farm and the traders' behaviors during their trading activities.

The questionnaire surveys revealed that most pigs had not been vaccinated (85.2%, 95%CI: 76.1-91.9%). The most common clinical sign observed in pigs was diarrhea (32.6%, 95%CI: 23.2-43.2%). Influenza-like signs were also reported, including fever (8.7%, 95%CI: 3.8-16.4%), cough or sneezing (7.6%, 95%CI: 3.1-15.1%), and rhinorrhea (4.3%, 95%CI: 1.2-10.8%). Farmers believed that pigs were more likely to be sick during the hot (April and June) and rainy seasons (August). When interviewed farmers found sick pigs, most would contact someone to get help and in most cases they contacted a paravet (see Supporting Information for details).

Our study provides new information on potential risks of influenza A virus transmission on farms in rural Thailand based on a questionnaire survey regarding farm management and pig trading activities (See Supporting Information for complete results). As the study was conducted in one community, the possible lack of representativeness of the general small pig farm population is a major limitation of this study. However it does represent pig farms in rural communities where farmers raise pigs with few resources for a secondary income and their practices have generally been influenced by socio-economic considerations. The findings of this study are consistent with the direct observations on inter-species interactions in small swine farms in Peru, including mixed farming of swine with chickens, common human-pig contacts and suboptimal biosecurity and hygiene practices adopted by farmers (McCune et al., 2012).

On most studied farms, the intermingling of species was commonly found and free-ranging chickens were observed. Although avian influenza viruses seems to be poorly transmissible to swine, a study by Suriya et al. (2008) found that mammalian pets on pig farms may serve as potential carriers to spread the viruses to pigs. Keeping pigs close to other species should therefore be discouraged to diminish the risk of cross-species transmission and the possibility of reassortment of influenza A viruses. Generally, pigs were raised in the traditional pig pens that could facilitate the dissemination of air-borne pathogens through close contact between animals from different pens. In addition, the high humidity and poor hygiene of pig pens may favor the survival and transmission of influenza viruses in the environment (Kyriakis et al., 2011). The use of readily available products in households that are able to kill the A(H1N1)pdm09 virus, such as 1% bleach, 10% vinegar and 0.01% washing up liquid, should be encouraged for cleaning pens in this low resource setting (Greatorex et al., 2010). Encouragingly, hand-washing with soap was performed among most farmers. If carried out properly, such a practice is effective in removing the A(H1N1)pdm09 virus from the hands (Grayson et al., 2009).

No limiting of visits and biosecurity protocols were applied in the studied farms. Uncontrolled access could be associated with a high seroprevalence to influenza viruses (Simon-Grife et al., 2011). Using manure as field fertilizer may play a role in transmission of influenza viruses in the region (Desrosiers

et al., 2004). The regular practice of hiring boars from outside could also be a significant contribution to disease transmission in the local community. To illustrate, the hired boars could carry viruses and boar owners may transmit the viruses back to their premises through their boots, clothing or vehicles. Limiting visits and improving biosecurity practices are key in preventing virus introduction and lower the chance of virus spread between farms.

Most farmers generally spent one hour feeding pigs per day. Since the A(H1N1)pdm09 virus could become established in pig populations following primary contact with infected humans (Brookes and Brown, 2011), this pig-to-human interaction should be reduced. Whilst working with pigs, most farmers only used rubber boots for protection and some wore no protective footwear. Without proper protective clothing, there is a high likelihood of exposure to influenza viruses from pathogenic animal secretions (Kumarsean et al., 2009). Thus, personal protective measures should be adopted to reduce virus transmission between pigs and humans on farms. Furthermore, a policy of annual influenza vaccination of swine workers may lower the possibility of reassortment events occurring (Myers et al., 2007). Unfortunately, pandemic vaccine recommendations in Thailand do not include pig farmers. During pandemics, agricultural workers should be included in influenza surveillance efforts and be considered as a priority group for annual influenza vaccination, and influenza vaccines and antivirals (Gray et al., 2007).

The study found that the potential spread of the virus through the movements of pig traders was mainly local. Transmission of influenza A viruses in the community could easily occur from one farm to another by indirect contact through vehicles, humans and fomites. Noteworthy, local pigs were slaughtered at an early age which may facilitate disease spread in the community through frequent trading; however, the risk of virus circulation in the pig population may conversely be reduced due

to rapid replacement. In a surveillance context, trading seasonality should be taken into account. It was suggested that surveillance systems for pig diseases should be targeted around the festival months. In most farms, pig identification was lacking and this could compromise disease control and surveillance efforts. With animal identification, if an outbreak occurred, control and eradication processes could be timely implemented. On-farm identification should therefore be promoted in order to ensure traceability of animals.

Overall, risk behaviors for influenza virus transmission from pigs to humans and vice versa that could arise from pig farming practices and trading activities have been demonstrated in this study. The results have emphasized the need to develop public health policies to prevent the spread of influenza A viruses on pig farms. Strengthening collaboration between the relevant authorities responsible for the human health, animal health, and food safety sectors, i.e. following the One Health approach, will improve surveillance activities and control of inter-species transmission of influenza A viruses. An interdisciplinary approach involving epidemiologists, economists and sociologists should be carried out to refine the strategies to the actual context. To account for complete mechanisms of human infection with SIVs, more investigations of the key parameters, including pig-human contact rate, the disease prevalence in pigs and the probability of human infection is needed in the future.

Supporting Information

Additional supporting information may be found in the online version of this article:

Fig. S1. Map of Thailand showing Mukdahan province

Questionnaire S1. Pig Health and Husbandry in Mukdaharn Province, Thailand

Questionnaire S2. Pig Marketing among Traders in Mukdaharn Province, Thailand

Table S1. Type of animals raised in the surveyed households, rural Thailand

Fig. S2. Bar chart presenting the per cent of different identification methods adopted for sows

Table S2. Actions taken by farmers when they found sick pigs

Summary S1. Summary of results of Questionaire S1 and S2

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Legend:

Fig. 1. Potential pathways of spread of influenza A viruses onto pig farms



Fig. 1. Potential risk pathways of influenza A virus transmission on pig farms. The risk pathways were developed based on a questionnaire survey to determine ways that influenza A viruses can spread on pig farms through husbandry practices and trading activities. The number in brackets () means the percentage of the surveyed farms performed for each pathway. Potential sources for pig infection that were identified included infected humans, infected pigs, infected chickens and contaminated environment, facilitated by suboptimal biosecurity and poor hygiene practices adopted by farmers and traders.