

LONDON
SCHOOL of
HYGIENE
& TROPICAL
MEDICINE



LSHTM Research Online

Fournie, G; Guitian, J; Desvaux, S; Cuong, VC; Dung, DH; Pfeiffer, DU; Mangtani, P; Ghani, AC; (2013) Interventions for avian influenza A (H5N1) risk management in live bird market networks. Proceedings of the National Academy of Sciences of the United States of America, 110 (22). pp. 9177-9182. ISSN 0027-8424 DOI: <https://doi.org/10.1073/pnas.1220815110>

Downloaded from: <http://researchonline.lshtm.ac.uk/1229469/>

DOI: <https://doi.org/10.1073/pnas.1220815110>

Usage Guidelines:

Please refer to usage guidelines at <https://researchonline.lshtm.ac.uk/policies.html> or alternatively contact researchonline@lshtm.ac.uk.

Available under license: <http://creativecommons.org/licenses/by/2.5/>

<https://researchonline.lshtm.ac.uk>

Interventions for avian influenza A (H5N1) risk management in live bird market networks

Guillaume Fournie^{a,1}, Javier Guitian^a, Stéphanie Desvieux^b, Vu Chi Cuong^c, Do Huu Dung^d, Dirk Udo Pfeiffer^a, Punam Mangtani^e, and Azra C. Ghani^f

^aVeterinary Epidemiology, Economics and Public Health Group, Department of Production and Population Health, Royal Veterinary College, University of London, Hatfield AL9 7TA, United Kingdom; ^bAnimal and Integrated Risks Management (AGIRs) Research Unit, Cirad, Campus International de Baillarguet, 34398 Montpellier Cedex 5, France; ^cNational Institute of Animal Science, Thuy Phuong, Tu Liem, Hanoi, Vietnam; ^dDepartment of Animal Health, Ministry of Agriculture and Rural Development, Phuong Mai, Dong Da, Hanoi, Vietnam; ^eDepartment of Infectious Disease Epidemiology, London School of Hygiene and Tropical Medicine, London WC1E 7HT, United Kingdom; and ^fMedical Research Council Centre for Outbreak Analysis and Modelling, Department of Infectious Disease Epidemiology, Imperial College, London W2 1PG, United Kingdom

Edited by Robert M. May, University of Oxford, Oxford, United Kingdom, and approved April 3, 2013 (received for review November 29, 2012)

Highly pathogenic avian influenza virus subtype H5N1 is endemic in Asia, with live bird trade as a major disease transmission pathway. A cross-sectional survey was undertaken in northern Vietnam to investigate the structure of the live bird market (LBM) contact network and the implications for virus spread. Based on the movements of traders between LBMs, weighted and directed networks were constructed and used for social network analysis and individual-based modeling. Most LBMs were connected to one another, suggesting that the LBM network may support large-scale disease spread. Because of cross-border trade, it also may promote transboundary virus circulation. However, opportunities for disease control do exist. The implementation of thorough, daily disinfection of the market environment as well as of traders' vehicles and equipment in only a small number of hubs can disconnect the network dramatically, preventing disease spread. These targeted interventions would be an effective alternative to the current policy of a complete ban of LBMs in some areas. Some LBMs that have been banned still are very active, and they likely have a substantial impact on disease dynamics, exhibiting the highest levels of susceptibility and infectiousness. The number of trader visits to markets, information that can be collected quickly and easily, may be used to identify LBMs suitable for implementing interventions. This would not require prior knowledge of the force of infection, for which laboratory-confirmed surveillance would be necessary. These findings are of particular relevance for policy development in resource-scarce settings.

questionnaire survey | transmission model | livestock disease | zoonotic disease

Highly pathogenic avian influenza virus subtype H5N1 (HPAIV H5N1) is endemic in many parts of Asia and in Egypt (1). The wide genetic diversity and the potential for recombination with human influenza strains continue to pose a major public health concern (2, 3). Although combinations of mass vaccination, culling, and movement restrictions have controlled avian influenza epidemics effectively in developed countries, the high financial outlay makes such strategies inappropriate in resource-poor settings where most poultry is raised by small-holder owners. Moreover, if inappropriately implemented, they might have a major, albeit unintended, impact on disease dynamics by creating conditions that favor silent spread of the virus within the poultry sector (4–7). Therefore, there is a real need to design appropriately targeted interventions for the prevention and control of HPAI H5N1, which are both realistic and sustainable in resource-poor settings. To achieve this, a better understanding of the drivers of disease dynamics in these settings is needed.

Live bird trade, common in HPAI H5N1-endemic areas, is known to be a major pathway for disease spread. Along trade routes, live bird markets (LBMs) act as hubs for traders, yet LBMs frequently are found to be contaminated in disease-epidemic and -endemic areas (8–12). Here, poultry traders can

mix and potentially transfer the virus either by trading infected poultry or by sharing contaminated equipment. In the absence of effective disinfection, traders may then act as a major source of exposure to infection for farms (13–18). Once contaminated, some LBMs may even act as viral reservoirs, depending on the poultry management practices of their traders (19, 20). Such markets provide a continuous source of infection for the poultry sector. The network of LBM contacts resulting from trader movements therefore may play a major role in the spread (21, 22) and maintenance of HPAIV H5N1 within poultry production systems similar to the way in which networks of contacts between hosts or host populations have been shown to determine the emergence and endemic levels of other diseases (23, 24).

The impact of the market network topology on the course of livestock disease epidemics was studied previously in production systems in developed countries where detailed data relating to the movements of livestock, farmers, and other stakeholders are readily available (25, 26). Such studies are less common in developing countries, as detailed movement data generally are not available. A deeper understanding of the topology of networks of contacts between livestock populations would allow more appropriate tailoring of surveillance programs and control strategies. This is relevant particularly to the allocation of the limited resources available to control livestock diseases in developing countries. It also is a global public health concern, given that the extended circulation of some pathogen strains through trade networks may promote the emergence of new zoonotic variants (2). The design of strategies for the eradication of livestock diseases, such as foot-and-mouth disease, also would benefit from a network-based approach.

Previous studies in southeast Asia explored the flow of poultry through the Cambodian market chain (27), including the links between some LBMs and the supplying flocks in Vietnam (16) and China (28). However, the topology of the LBM contact network formed by the movements of poultry traders has not been assessed. Here we describe empirically, using social network analysis, the topology of such a network of contacts between LBMs in northern Vietnam based on structured interviews with live poultry traders. A stochastic network transmission model, based on the empirical network, then is used to assess the

Author contributions: G.F., J.G., S.D., V.C.C., D.H.D., D.U.P., P.M., and A.C.G. designed research; G.F., S.D., V.C.C., and D.H.D. performed research; G.F., J.G., D.U.P., P.M., and A.C.G. analyzed data; and G.F., J.G., S.D., V.C.C., D.H.D., D.U.P., P.M., and A.C.G. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Freely available online through the PNAS open access option.

¹To whom correspondence should be addressed. E-mail: gfourmie@rvc.ac.uk.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1220815110/-DCSupplemental.

impact of control measures targeted at central nodes, which were identified using network structural measures.

Results

Characterizing LBM Contact Networks. As most disease events are not detected, two study areas were selected based on demographic features—the province with the highest human population density in northern Vietnam, Hanoi (29), and a rural province with a large poultry population, Bac Giang (30) (Fig. 1A). Live poultry traders were recruited in 30 LBMs ($n = 561$) as well as in a nonmarket site ($n = 6$) (*Materials and Methods*). Of the 567 traders interviewed, 200 reported operating in at least two LBMs (100 of 416 traders in Hanoi and 100 of 151 traders in Bac Giang).

Directed and weighted networks were built with LBMs as the nodes and trader movements as potential pathways for disease transmission among LBMs. Weightings were determined by the number of trader visits connecting the markets. When considering all traders and markets, the network of contacts was composed of 162 LBMs, 18% of which were located outside the study zone, in 10 other Vietnamese provinces (Fig. 1A). A total of 140 LBMs (86%) were encompassed in a giant strong component (GSC). The GSC is the largest subset in which any node can reach any other by following network links, and informs on the maximum epidemic size (31). Additionally, imports of live poultry from China into the Vietnamese LBM network were reported. This suggests that the LBM network can support large-scale, and even transboundary, disease spread, epidemiologically connecting regions that otherwise may have remained isolated.

The two provincial-level networks that incorporated only LBMs and traders interviewed within each province also were characterized by large GSCs. All 49 of the LBMs in the Bac Giang network were included in the GSC. Of the 81 LBMs comprising the Hanoi network, 7 (9%) were isolated and 62 (77%) belonged to the GSC. The Bac Giang network was highly clustered, with a clustering coefficient (0.33) consistently higher than that obtained from simulated random networks with the same number of links and similar link weights (median, 0.08; range, 0.04–0.14). In contrast, the Hanoi network showed a lower level of clustering (0.02) than corresponding random networks (median, 0.03; range, 0.002–0.09) in 84% of simulations.

To identify potential network hubs, principal component analysis and hierarchical cluster analysis were used in combination to partition LBMs based on three centrality measures—degree, betweenness, and closeness—with the resulting clusters used to define LBMs as peripheral nodes, nodes with medium connectivity, and hubs (*Materials and Methods* and *SI Text*). Here “degree” refers to the number of visits to a given LBM by traders operating in several LBMs. Most LBMs in the networks of both Hanoi (61; 82%, excluding isolated LBMs) and Bac Giang (33; 67%) were peripheral (Fig. 1), whereas a few hubs—the largest wholesale LBM in Hanoi and three Bac Giang LBMs—accounted for one-third of the total number of trader journeys within their respective network.

Both networks were resilient to random node removal, but targeted removal of nodes with high centrality measures drastically reduced the GSC. In Hanoi, removing the single hub reduced the GSC by at least 73%, whereas the removal of one to

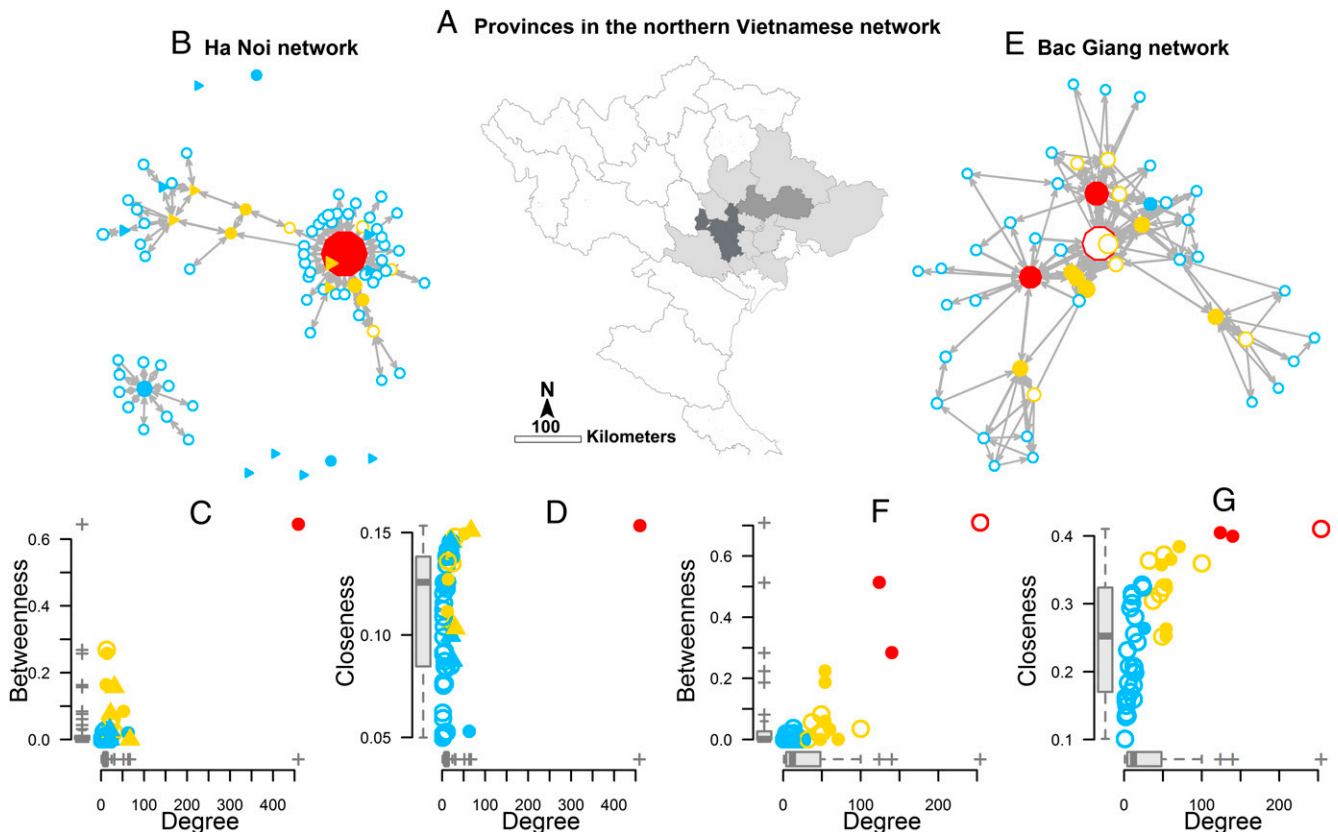


Fig. 1. Northern Vietnamese network, province-level networks, and centrality measures. (A) Location of provinces included in the northern Vietnamese network: Hanoi (dark gray), Bac Giang (medium gray), and other provinces included in the network but not studied in the survey (light gray). Networks and distributions of centrality measures are shown for Hanoi ($n = 81$) (B–D) and Bac Giang ($n = 49$) (E–G). Peripheral nodes are colored in blue, nodes with medium connectivity in yellow, and hubs in red. ○, nonsurveyed markets. Depending on their seller composition (20), markets included in the survey had the potential to sustain the virus circulation (▲) or not (●).

three other nodes had only a limited added impact. To reduce GSC in Bac Giang by at least 50%, three to four nodes would need to be removed.

Traders who might promote conditions favorable for sustaining HPAIV H5N1 in LBMs (20) were predominant in 13 of the Hanoi LBMs included in the survey (solid triangle in Fig. 1). In contrast, traders operating in the Hanoi hub, and all traders operating in Bac Giang markets, kept their poultry in LBMs for only a short period, so virus maintenance was unlikely (20). Most of these 13 Hanoi markets with the potential to act as viral reservoirs were either isolated from or connected only weakly to the Hanoi network (five isolates, four peripheral nodes, and four nodes with medium connectivity). Some of these isolates were linked only to Bac Giang LBMs. Therefore, disconnecting LBM networks would result in the epidemiological isolation of these potential viral reservoirs, reducing their contribution to virus perpetuation in the poultry sector.

Modeling HPAIV H5N1 Spread Within the LBM Network. In Bac Giang, several markets were open periodically and clustering of the network was high. Temporal changes in contact patterns and the trajectory of each trader within the network therefore would need to be captured to (i) assess whether centrality measures were good predictors of the importance of LBMs in disease transmission (32, 33) and (ii) explore ways in which the network could be fragmented. An individual-based model, in which each trader and each market was explicitly modeled, was developed to simulate the spread of HPAIV H5N1 through the Bac Giang LBM network. In contrast to Bac Giang, the Hanoi network was structured around a single hub. As a result of the low level of clustering, 45% of nodes encompassed in the GSC were linked solely to this hub. Analysis of the Hanoi network clearly highlighted the central role of this hub in virus spread.

Bac Giang traders kept poultry for only a short period in LBMs and thus were unlikely to permit virus perpetuation in these LBMs (20). However, a trader whose poultry was infectious or whose equipment was contaminated might potentially transfer viruses to the market environment. Other traders then might become contaminated through contact with the contaminated environment (with a probability of P_M) or through contacts with contaminated traders visiting the same market (with a probability of P_T), including the handling and purchase of infectious poultry and the sharing of contaminated equipment, such as cages, weighing scales, and force-feeding tools. Contaminated traders would act as fomites, spreading virus through the market network for a period, T_V , depending on the survival of the virus in the environment and the frequency and effectiveness of hygiene measures. Based on these parameters, simulations of an individual-based model were run, starting at the seeding of infection into an LBM in the Bac Giang network. Market susceptibility was defined as the proportion of simulations for which a given market was contaminated. Market infectiousness was the proportion of other markets in the

network that were contaminated if the infection was seeded in a given market.

The strength of the positive linear correlation between susceptibility and infectiousness increased with longer virus survival periods, T_V (Fig. 2). Although the ranking of most LBMs according to their susceptibility or infectiousness varied with parameter values, LBMs with the highest susceptibility or infectiousness remained unchanged. For each simulation set, the four markets with the highest susceptibility always belonged to a group of five markets located in the provincial capital city, including one hub and four nodes with medium connectivity. Likewise, the three hubs always combined high susceptibility and infectiousness. Therefore, the LBMs in which to implement surveillance, namely those with high susceptibility, could be chosen even without prior knowledge of the level of transmission. The same is true for LBMs considered suitable targets for disease control interventions, namely those with both high susceptibility and infectiousness.

These LBMs could be identified based on the number of visits by traders also operating in other LBMs. Indeed, a generalized additive model (GAM) (34, 35) with degree as predictor explained a high proportion of the null deviance for both susceptibility (0.53–0.69, depending on parameter values) and infectiousness (0.46–0.76). Similar results were obtained with closeness as a predictor (susceptibility, 0.70–0.73; infectiousness, 0.50–0.74), whereas GAMs with betweenness as a predictor explained less than 0.20 and 0.32 of the null deviance for susceptibility and infectiousness, respectively.

To reduce disease spread through the Bac Giang network, daily disinfection could be applied simultaneously to the LBM environment and traders' vehicles and equipment in the three hubs. This intervention reduced the median epidemic size, defined as the fraction of contaminated markets, by 0.80–0.89 (depending on input parameters, and for parameter sets in which the fraction of contaminated markets reached 0.10 without disinfection). However, as the impact on the upper bound of the epidemic size was limited, substantial epidemics still might occur. In an extreme case scenario where $P_T = P_M = 1$, daily disinfection of the three hubs still reduced the median epidemic size by 0.68–0.72.

However, as disinfection was sequentially applied less frequently and less thoroughly, the benefit of this intervention was lost (Fig. 3 for $P_M = 0.1$ and $P_T = 0.1$). This loss occurred more rapidly as P_M and P_T increased. When disinfection was applied every 2 d, the median epidemic size was reduced only by 0.30 for high values of P_M and P_T , and by 0.79 for low values of P_M and P_T . Weekly disinfection reduced the median epidemic size by 0.04–0.25. In addition to its frequency, the impact of disinfection on epidemic size also depended on the ability to disinfect traders leaving the markets. Daily disinfection of 80% of traders leaving the three hubs reduced the median epidemic size by 0.50–0.77, whereas disinfection of 50% of these traders resulted in a reduction by 0.23–0.68. When only the traders leaving these hubs without birds could be disinfected daily

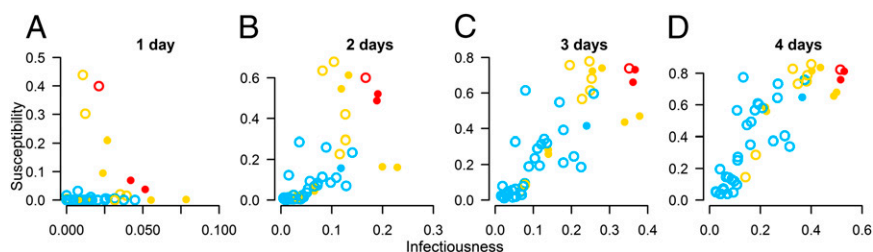


Fig. 2. Association between susceptibility and infectiousness, shown for $P_T = 0.1$, $P_M = 0.1$, and $T_V = 1$ d (A), 2 d (B), 3 d (C), and 4 d (D). LBMs are partitioned into peripheral nodes (blue), nodes with medium connectivity (yellow), and hubs (red). ○, nonsurveyed LBMs; ●, surveyed LBMs.

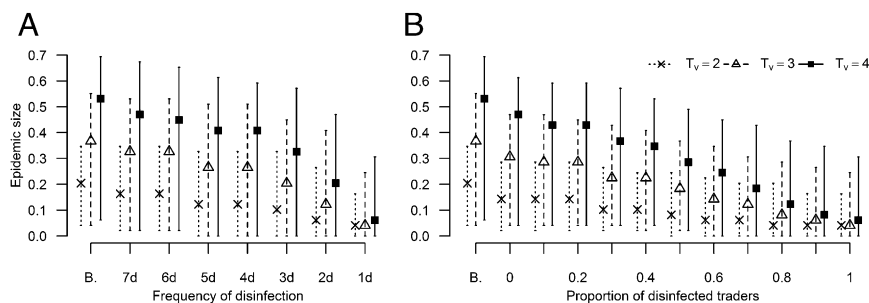


Fig. 3. Impact of disinfection on the final epidemic size. The relation between the epidemic size (fraction of contaminated markets) and (A) the disinfection frequency, and (B) the proportion of disinfected traders, is shown for $P_T = 0.1$, $P_M = 0.1$, and $T_V = 2$ d (dotted line), 3 d (dashed line), and 4 d (solid line). Median and 95% range are presented. B, baseline, no disinfection; 2d, disinfection every 2 d.

(37% of traders leaving the three hubs), the median epidemic size was reduced by 0.23–0.55.

Although the model suggests that disinfection should be applied frequently and thoroughly to have a substantial impact on disease spread, the actual frequency of application of hygiene measures in LBMs included in the survey was very low. Although cleaning of LBMs was reported to be undertaken daily by all interviewed market managers ($n = 20$), disinfectants actually were applied daily in only two markets, and only to the market environment. In 12 other markets, application frequency ranged from once per week to once every 2 mo. Additionally, the sale of live poultry was supposed to be banned in three markets located in the Bac Giang provincial capital city, namely the hub with the highest centrality measures and two other markets among those with the highest susceptibility. Live poultry trade also was supposed to be banned in Hanoi inner districts, where 22 markets that shared traders with the largest Hanoi wholesale LBM were active.

Discussion

Northern Vietnamese LBMs appeared to be well connected via the movements of their traders, with most LBMs grouped in a single GSC. The LBM network therefore might support large-scale, and even transboundary, disease spread, epidemiologically connecting geographically distant areas.

Similar to other anthropogenic systems (36), each provincial-level network was characterized by the heterogeneity of contact patterns among LBMs. Most LBMs had a small number of neighbors, whereas there were few highly connected hubs. This topology may render these networks more vulnerable than random networks to disease invasion, even if the linkage density and the transmission rates are low (37–39). In previous studies, clustering was observed to increase the likelihood of disease extinction by reducing the local number of susceptible nodes (35). Such a scenario is unlikely to apply to the spread of HPAIV H5N1 in these LBM networks, however, because a contaminated LBM either remains contaminated or returns to a susceptible state.

Although the LBMs that were more likely to become viral reservoirs were small markets that were connected only weakly to the network, some hubs were shown to be potential interfaces between these LBMs and the poultry sector. These hubs increased the probability of LBM contamination, resulting in their becoming viral reservoirs. Measures aiming to fragment the networks could epidemiologically isolate these potential viral reservoirs and, consequently, limit their impact on disease maintenance within the poultry sector. Implementing hygiene measures, such as market rest days (40), in all potential viral reservoirs no longer would be necessary.

In an effort to control the spread of HPAI H5N1, official banning of LBMs has been attempted in Egypt (41) and some Vietnamese urban areas (42). Although such measures may have reduced live bird trade somewhat, the activity has not ceased

completely. Official closure has not resulted in the termination of live bird trade in some markets in northern Vietnam. Despite the ban, these markets were still very active and likely to have a substantial impact on disease dynamics. These included the most influential hub of the Bac Giang network, two other Bac Giang markets identified by the model to be suitable for targeted surveillance programs, and 22 markets located in Hanoi inner districts. Although the traders in these unauthorized markets were not interviewed, some unofficial LBMs in Hanoi inner districts were visited. They presented demographic features similar to those of the Hanoi markets identified as potential viral reservoirs (20), and also could act as potential viral reservoirs themselves. Such prescriptive policies actually might promote the proliferation of informal gathering points for traders outside the LBM system. Although official markets may allow rapid disease dissemination, they also are focal points where disease spread can be monitored and controlled, in contrast to unauthorized and informal markets.

Instead, disconnecting the market network should be achieved through the daily disinfection of LBMs and of the vehicles leaving them. Implementing this intervention in only a few hubs would be effective in fragmenting the entire network. As in previous studies of the spread of pathogens in human populations (35, 43), nodes that should be targeted could be identified easily based on their degree (i.e., the number of journeys made by traders to other markets). Degree is an egocentric measure that does not require the overall network to be captured. Variations in the probabilities of disease transmission had only a limited impact on the strength of the association among susceptibility, infectiousness, and degree, and on the identification of highly susceptible and infectious markets. Therefore, a prior knowledge of the level of transmission, which would require laboratory-based surveillance, would not be necessary to identify markets that should be targeted by hygiene measures and surveillance programs.

In the case of network hubs also acting as potential viral reservoirs, market disinfection programs should be complemented by measures aiming to break the virus amplification cycle (19). In our simulations, market disinfection had only a limited impact on the maximum epidemic size because of the high level of clustering in the province of Bac Giang. Although the three hubs mediate most of the traders' movements, substantial epidemics involving traders who do not visit these hubs still may occur.

The practical applications of mitigation strategies based on empirical networks need further investigation. To increase the uptake of such studies by policy makers, field trials might be conducted to demonstrate the efficacy and assess the feasibility of selected strategies (*SI Text*). Indeed, the behavioral changes required may make such interventions unfeasible. Disinfection of traders' vehicles and equipment may be particularly challenging. Additionally, some markets have particular physical characteristics that make environmental elimination difficult,

such as nonsealed, earthen floors that would first require reinforcement. To ensure a high level of compliance and to minimize the negative impact on trading activities, the design of such interventions must involve all stakeholders.

Both the Hanoi and Bac Giang networks were only samples of wider networks, as only a fraction of the nodes and links were captured through the survey. Moreover, the markets included in the survey were not selected randomly. The results of the network analysis should be interpreted somewhat cautiously. Indeed, the sampling design may affect the structure of the observed networks and thus influence network parameter distributions (44). Such bias may have been introduced into the Hanoi network, in which the hub was the mediator in most contacts among other markets. The high impact of its removal on the network connectivity resulted from the low clustering: most of its neighbors were connected only to this hub and not to one another. In most markets of this network, traders were not interviewed. Although it is possible that additional market contacts might have been identified through further interviews, 22 of the network markets were visited and were observed to be small, with only one to six traders. Therefore, it is a realistic assumption that these markets were supplied by only one market. The centrality of the hub also is consistent with its role as a poultry supplier, being a wholesale market. Without doubt, it is the biggest market in northern Vietnam in terms of the number of traders and volume of sales. Contrary to all other investigated markets, in which all or almost all traders operating in them were included in the survey, only a fraction of traders in this hub were interviewed. Therefore, it is possible that only a fraction of its contact markets were identified.

In contrast, half the markets classified as nodes with medium connectivity in the Bac Giang network were not included in the survey, and the most “important” hub was not surveyed. This suggests that the observed Bac Giang network indeed reflects some characteristics of the true network. A higher proportion of traders were thought to have been interviewed in the Bac Giang network than in the Hanoi network, and most markets in which poultry was sold regularly likely were included in the network. Markets from the provincial capital city and from all surrounding districts were visited, and because of the much lower human population density, the total number of markets and traders in Bac Giang likely is less than in Hanoi. Trader movements were driven by the opening schedules of periodic markets, so most traders were highly mobile. Although not all periodic markets were visited, traders likely were interviewed in other markets with alternative opening days.

Network analysis carried out in other livestock production systems has confirmed livestock markets as the main hubs for livestock movements (25, 26) and their contamination as a prerequisite for large epidemics (45). However, some farms also might act as bridges connecting markets. In Vietnam, a nonquantified proportion of live poultry transactions are mediated outside markets at informal locations. These informal markets will modify the structure of the trader movement network.

In conclusion, although the northern Vietnamese LBM network may create conditions for maintaining HPAIV H5N1 and its spread across large areas, opportunities for targeted surveillance and control do exist. These strategies might be implemented effectively in a small number of hubs. Their identification might be based on egocentric measures without prior knowledge of the force of infection. These findings are particularly relevant for resource-poor settings where LBM systems are well developed.

Materials and Methods

Data Collection. Markets where live birds are sold are ubiquitous in Vietnam and heterogeneous in terms of the volume of poultry sold. Live bird trade is an irregular and minor activity in most markets. Therefore, sampling was conducted in a purposive manner, targeting the largest LBMs in the selected

areas in terms of the amount of poultry sold. These LBMs were identified through interviews with traders. In addition, six traders were interviewed in Bac Giang province in a location outside the market system where poultry was traded. This site was identified by traders interviewed within an LBM located in its vicinity. Details on market and trader selection are provided in Fournié et al. (20). The sampling methodology may be described as a labeled star sampling approach (44): a set of markets, where traders were interviewed, allowed the identification of connections with other markets that did or did not belong to this set. The refusal rate was 8%, the principal reason being that some traders were too busy to participate. Informed oral consent was sought before interviewing. Ethical approval was granted by the Royal Veterinary College Ethics and Welfare Committee.

Social Network Analysis. A timescale of 10 d was chosen for constructing networks because of the periodicity of market opening days: several markets were periodic and their sequence of opening days was fixed, repeating every 10 d. Most traders reported visiting LBMs every opening day; however, 46 traders (23%) visited markets less regularly. The number of days these traders operated in each market during a 10-d period and the specific days these markets were visited were unknown; therefore, they were defined stochastically from the number of days these traders visited markets in the week preceding the interview, and during a usual month. For each set of traders and markets, 1,000 stochastic networks were generated. Further details of the network construction and an assessment of its influence on network structure are provided in *SI Text*.

For each network, the GSC was assessed. For the Bac Giang and Hanoi networks, the “weighted” clustering coefficient was calculated (46) and compared with the clustering coefficient of 1,000 random networks generated with the same number of links and similar weight links. The LBM’s “importance” in the network was assessed by centrality measures: degree, betweenness, and closeness. “Unweighted” in- and out-degrees, defined as the number of markets sending or receiving traders from a given market, were highly correlated to the weighted degree, i.e., the number of visits to a given LBM by traders operating in several LBMs (Pearson’s correlation coefficient $\rho > 0.85$). Therefore, only weighted degrees were considered. Betweenness characterizes the extent to which a node is located between other pairs of nodes, and closeness measures how close one node is from others. Similar to degree, betweenness and closeness accounted for link weights and directions, as detailed in *SI Text*. The median estimate of each network parameter is presented. The 95% bounds of estimates from stochastic realizations closely follow the median.

Based on their centrality measures, LBMs were classified using principal component analysis (PCA) and hierarchical cluster analysis (HCA) (47). PCA may be used to reduce the dimensions of multivariate data and create a smaller number of uncorrelated synthetic factors (components) accounting for most data variability. HCA allows the grouping of LBMs into clusters according to their level of similarity in the created components. Similarity between two markets was assessed by the calculation of the Manhattan distance. The algorithm was agglomerative, and Ward’s criterion for linkage was adopted.

To assess the impact of node removal on the size of the GSC while accounting for the weights of the links, “epidemiological networks” were simulated (31). The probability Γ_i of a link i with a weight W_i transmitting the virus was given by $\Gamma_i = 1 - (1 - \gamma)^{W_i}$, with γ the probability of a trader transmitting virus from one market to another. At each simulation, a Bernoulli process was applied to each link i with probability Γ_i , so the resulting simulated network was composed of “truly infectious links” if a given node was infected. A thousand epidemiological networks were constructed for each investigated values of the probability γ , $\gamma \in (0.1, 0.3, 0.6, 1)$.

Individual-Based Model. Poultry trade activities took place in most Bac Giang markets during a period of only a few hours per day, so it was assumed that traders visiting the same market on the same day were in contact with one another. In general, traders operating in the same markets visited each market in the same order, although there were a few exceptions. For instance, a trader might visit market A and then market B, whereas another would visit B and then A. These traders could have been in contact only in A or B, not both. The market in which they met was defined stochastically such that the number of contacts between traders was maximized (*SI Text*).

At a given time t , a market j was characterized by its contamination status $M_{j,t}$ (equal to 1 if the market environment was contaminated, 0 if not) and the number $N_{j,t}$ of contaminated traders operating there. The market environment became contaminated once this market was visited by at least one contaminated trader. Markets and traders remained contaminated for the length of time before virus inactivation, T_v , unless

disinfection occurred. The probability $P_{i,t}$ that a trader i visiting a market j at time t will become contaminated as the result of contact with the contaminated market environment or with contaminated traders was defined as $P_{i,t} = 1 - (1 - P_T)^{M_{j,t}}(1 - P_M)^{M_{j,t}}$, where P_T is the probability of a contaminated trader transferring virus to a trader visiting the same market at the same time, and P_M is the probability of a trader being contaminated via the market environment.

A simulation was started at a randomly chosen day, k , with $1 \leq k \leq 10$ and was run for T_V . Infection was seeded in market j , such that $M_{j,t} = 1$, and for the first visit $P_{i,t} = 1 - (1 - P_T)(1 - P_M)$. A thousand simulations were run for each combination of values of T_V , with $T_V \in \{1d, 2d, 3d, 4d\}$, of P_M with $P_M \in \{0, 0.1, 0.2, 0.3\}$, and of P_T with $P_T \in \{0, 0.1, 0.2, 0.3\}$ (SI Text). Univariable GAMs were fitted for each simulation set, with the response variable being either the susceptibility or the infectiousness and the predictor variable being the degrees, the betweenness, or the closeness. The proportion of the

null deviance explained by the GAMs was used as a measure of the strength of the association between variables (35). All analyses were run using R 2.12.0 (48) and the package “sna” (49). The package “tnet” (50) was used to calculate the clustering coefficient.

ACKNOWLEDGMENTS. The authors are grateful to the study participants and the interviewers. They also express their thanks to Rowland Kao, James Wood, Richard Kock, Angel Ortiz-Pelaez, and Thibaud Porphyre for their suggestions, which helped improve the analysis; Anna Dean for her constructive comments on the manuscript; Raphaëlle Métras and Kim Stevens for providing maps; Jeff Gilbert and Andrew Bisson for their support in the implementation of the field survey in Vietnam; and two anonymous reviewers for their constructive comments. G.F. thanks the Bloomsbury Consortium and the University of London Central Research Fund for their support. A.C.G. acknowledges support from the UK Medical Research Council.

- Food and Agriculture Organization of the United Nations (2011) *Approaches to Controlling, Preventing and Eliminating H5N1 Highly Pathogenic Avian Influenza in Endemic Countries*. (Food and Agriculture Organization of the United Nations, Rome) Animal Production and Health Paper No 171.
- Fournié G, de Glanville WA, Pfeiffer DU (2011) Epidemiology of highly pathogenic avian influenza virus strain type H5N1. *Health and Animal Agriculture in Developing Countries*, eds Zilberman D, Otte J, Roland-Holst D, Pfeiffer D (Springer, New York).
- Li C, et al. (2010) Reassortment between avian H5N1 and human H3N2 influenza viruses creates hybrid viruses with substantial virulence. *Proc Natl Acad Sci USA* 107(10): 4687–4692.
- Meleigy M (2007) Egypt battles with avian influenza. *Lancet* 370(9587):553–554.
- Peyre M, et al. (2009) Avian influenza vaccination in Egypt: Limitations of the current strategy. *J Mol Genet Med* 3(2):198–204.
- Yupiana Y, de Vlas SJ, Adnan NM, Richardus JH (2010) Risk factors of poultry outbreaks and human cases of H5N1 avian influenza virus infection in West Java Province, Indonesia. *Int J Infect Dis* 14(9):e800–e805.
- Walker PG, et al. (2010) A Bayesian approach to quantifying the effects of mass poultry vaccination upon the spatial and temporal dynamics of H5N1 in Northern Vietnam. *PLoS Comput Biol* 6(2):e1000683.
- Shorridge KF (1999) Poultry and the influenza H5N1 outbreak in Hong Kong, 1997: Abridged chronology and virus isolation. *Vaccine* 17(Suppl 1):S26–S29.
- Chen J, et al. (2009) Characterization of highly pathogenic H5N1 avian influenza viruses isolated from poultry markets in central China. *Virus Res* 146(1-2):19–28.
- Abdelwhab EM, et al. (2010) Circulation of avian influenza H5N1 in live bird markets in Egypt. *Avian Dis* 54(2):911–914.
- Indriani R, et al. (2010) Environmental sampling for avian influenza virus A (H5N1) in live-bird markets, Indonesia. *Emerg Infect Dis* 16(12):1889–1895.
- Wan XF, et al. (2011) Indications that live poultry markets are a major source of human H5N1 influenza virus infection in China. *J Virol* 85(24):13432–13438.
- Paul M, et al. (2010) Farming practices and the risk of highly pathogenic avian influenza H5N1 in backyard poultry: A case-control study in Thailand. *Proceedings of the Society for Veterinary Epidemiology and Preventive Medicine*, eds Alban L, Kelly LA (Society for Veterinary Epidemiology and Preventive Medicine, Roslin, UK) pp 249–257.
- Biswas PK, et al. (2009) Risk factors for infection with highly pathogenic influenza A virus (H5N1) in commercial chickens in Bangladesh. *Vet Rec* 164(24):743–746.
- Biswas PK, et al. (2008) Avian influenza outbreaks in chickens, Bangladesh. *Emerg Infect Dis* 14(12):1909–1912.
- Soares Magalhães RJ, et al. (2010) Associations between attributes of live poultry trade and HPAI H5N1 outbreaks: A descriptive and network analysis study in northern Vietnam. *BMC Vet Res* 6:10.
- Desvaux S, et al. (2011) Risk factors of highly pathogenic avian influenza H5N1 occurrence at the village and farm levels in the Red River Delta Region in Vietnam. *Transbound Emerg Dis* 58(6):492–502.
- Kung NY, et al. (2007) Risk for infection with highly pathogenic influenza A virus (H5N1) in chickens, Hong Kong, 2002. *Emerg Infect Dis* 13(3):412–418.
- Fournié G, Guitian FJ, Mangtani P, Ghani AC (2011) Impact of the implementation of rest days in live bird markets on the dynamics of H5N1 highly pathogenic avian influenza. *J R Soc Interface* 8(61):1079–1089.
- Fournié G, et al. (2012) Identifying live bird markets with the potential to act as reservoirs of avian influenza A (H5N1) virus: A survey in northern Viet Nam and Cambodia. *PLoS One* 7(6):e37986.
- Davis CT, et al. (2010) Detection and characterization of clade 7 high pathogenicity avian influenza H5N1 viruses in chickens seized at ports of entry and live poultry markets in Vietnam. *Avian Dis* 54(1, Suppl):307–312.
- Kim HR, et al. (2010) An outbreak of highly pathogenic H5N1 avian influenza in Korea, 2008. *Vet Microbiol* 141(3-4):362–366.
- Salathé M, Jones JH (2010) Dynamics and control of diseases in networks with community structure. *PLoS Comput Biol* 6(4):e1000736.
- Newman ME (2002) Spread of epidemic disease on networks. *Phys Rev E Stat Nonlin Soft Matter Phys* 66(1 Pt 2):016128.
- Ortiz-Pelaez A, Pfeiffer DU, Soares-Magalhães RJ, Guitian FJ (2006) Use of social network analysis to characterize the pattern of animal movements in the initial phases of the 2001 foot and mouth disease (FMD) epidemic in the UK. *Prev Vet Med* 76(1-2):40–55.
- Robinson SE, Christley RM (2007) Exploring the role of auction markets in cattle movements within Great Britain. *Prev Vet Med* 81(1-3):21–37.
- Van Kerkhove MD, et al. (2009) Poultry movement networks in Cambodia: Implications for surveillance and control of highly pathogenic avian influenza (HPAI/H5N1). *Vaccine* 27(45):6345–6352.
- Martin V, et al. (2011) Risk-based surveillance for avian influenza control along poultry market chains in South China: The value of social network analysis. *Prev Vet Med* 102(3):196–205.
- General Statistics Office of Vietnam (2010) Population and population density in 2009 by province. Available at http://www.gso.gov.vn/default_en.aspx?tabid=467&idmid=3&itemID=9882. Accessed March 10, 2011.
- General Statistics Office of Vietnam (2010) Number of poultry by province 2009. Available at <http://www.gso.gov.vn/default.aspx?tabid=503&itemID=13032>. Accessed March 10, 2011.
- Kao RR, Danon L, Green DM, Kiss IZ (2006) Demographic structure and pathogen dynamics on the network of livestock movements in Great Britain. *Proc Biol Sci* 273(1597):1999–2007.
- Vernon MC, Keeling MJ (2009) Representing the UK's cattle herd as static and dynamic networks. *Proc Biol Sci* 276(1656):469–476.
- Keeling MJ, Danon L, Vernon MC, House TA (2010) Individual identity and movement networks for disease metapopulations. *Proc Natl Acad Sci USA* 107(19):8866–8870.
- Wood SN (2006) *Generalized Additive Models: An Introduction with R* (Chapman & Hall/CRC, London).
- Christley RM, et al. (2005) Infection in social networks: Using network analysis to identify high-risk individuals. *Am J Epidemiol* 162(10):1024–1031.
- May RM, Lloyd AL (2001) Infection dynamics on scale-free networks. *Phys Rev E Stat Nonlin Soft Matter Phys* 64(6 Pt 2):066112.
- Pastor-Satorras R, Vespignani A (2001) Epidemic dynamics and endemic states in complex networks. *Phys Rev E Stat Nonlin Soft Matter Phys* 63(6 Pt 2):066117.
- Keeling MJ (1999) The effects of local spatial structure on epidemiological invasions. *Proc Biol Sci* 266(1421):859–867.
- Woolhouse ME, et al. (2005) Epidemiological implications of the contact network structure for cattle farms and the 20-80 rule. *Biol Lett* 1(3):350–352.
- Lau EHY, et al. (2007) Effect of interventions on influenza A (H9N2) isolation in Hong Kong's live poultry markets, 1999–2005. *Emerg Infect Dis* 13(9):1340–1347.
- International Society for Infectious Diseases (2008) Avian Influenza, Human (07): Egypt. WHO, ProMED-mail post. Available at <http://www.promedmail.org>, archive number: 20080113.0171. Accessed December 8, 2009.
- Burgos S, Hinrichs J, Otte J, Pfeiffer D, Roland-Holst D (2008) *Poultry, HPAI and Livelihoods in Viet Nam—A Review*. (Food and Agriculture Organization of the United Nations, Rome) Mekong Team Working Paper No. 2.
- Ghani AC, Garnett GP (2000) Risks of acquiring and transmitting sexually transmitted diseases in sexual partner networks. *Sex Transm Dis* 27(10):579–587.
- Kolaczyk ED (2009) *Statistical Analysis of Network Data Methods and Models* (Springer, New York).
- Green DM, Kiss IZ, Kao RR (2006) Modelling the initial spread of foot-and-mouth disease through animal movements. *Proc Biol Sci* 273(1602):2729–2735.
- Opsahl T, Panzarasa P (2009) Clustering in weighted networks. *Soc Networks* 31: 155–163.
- Manly BFJ (2005) *Multivariate Statistical Methods: A Primer* (Chapman & Hall/CRC, London), 3rd Ed.
- R Development Core Team (2010) *R: A Language and Environment for Statistical Computing* (R Foundation for Statistical Computing, Vienna).
- Butts CT (2010) sna: Tools for Social Network Analysis. R package version 2.2-0. Available at <http://CRAN.R-project.org/package=sna>. Accessed February 1, 2011.
- Opsahl T (2009) Structure and evolution of weighted networks. PhD thesis (Univ of London, Queen Mary College, London).