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Risk-based surveillance for avian influenza control along poultry market chains in South China: The value of social network analysis

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ABSTRACT

Over the past two decades, the poultry sector in China went through a phase of tremendous growth as well as rapid intensification and concentration. Highly pathogenic avian influenza virus (HPAIV) subtype H5N1 was first detected in 1996 in Guangdong province, South China and started spreading throughout Asia in early 2004. Since then, control of the disease in China has relied heavily on wide-scale preventive vaccination combined with movement control, quarantine and stamping out. This strategy has been successful in drastically reducing the number of outbreaks during the past 5 years. However, HPAIV H5N1 is still circulating and is regularly isolated in traditional live bird markets (LBMs) where viral infection can persist, which represent a public health hazard for people visiting them. The use of social network analysis in combination with epidemiological surveillance in South China has identified areas where the success of current strategies for HPAI control in the poultry production sector may benefit from better knowledge of poultry trading patterns and the LBM network configuration as well as their capacity for maintaining HPAIV H5N1 infection. We produced a set of LBM network maps and estimated the associated risk of HPAIV H5N1 within LBMs and along poultry market chains, providing new insights into how live poultry trade and infection are intertwined. More specifically, our study provides evidence that several biosecurity factors such as daily cage cleaning, daily cage disinfection or manure processing contribute to a reduction in HPAIV H5N1 presence in LBMs. Of significant importance is that the results of our study also show the association between social network indicators and the presence of HPAIV H5N1 in specific network configurations such as the one represented by the counties of origin of the birds traded in LBMs. This new information could be used to develop more targeted and effective control interventions.
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1. Introduction

China's poultry sector plays an important role in the national economy for commercial and livelihoods reasons. It has experienced vigorous growth in the past two decades. While traditional husbandry systems including backyard operations still represent a significant part of the overall poultry output and still play a key role in people's livelihood, China has experienced a rapid concentration of primary poultry production since the mid-1980s, especially in the eastern provinces where large scale commercial production is becoming increasingly important and the trend for the future is expected to be one of modernisation with increasing emphasis on food safety regulation (Bingsheng and Han, 2008).

Although the epidemiological situation of highly pathogenic avian influenza (HPAI) H5N1 in China has remarkably improved over the past 5 years with no outbreak reported in 2010, the risks for animal and human exposure may still persist in some limited segments of the poultry production and marketing industry. While every link in the poultry market chains interacts with the following link in the same chain, the entire poultry production and distribution system has to be examined to understand the patterns of disease exposure and risk. Along these market chains, live bird markets (LBMs) represent a particularly high-risk element of the industry value chain because poultry and people from different locations have the opportunity to come into contact in one place before dispersing. In South East Asia, these markets are particularly important and are deeply rooted in cultural practices, traditions and consumer preferences for live poultry. In China, HPAIV H5N1 has regularly been detected in LBMs through the national surveillance program for the detection of HPAIV H5N1 circulation (Martin et al., 2011) and sporadic urban human cases have continued to occur in 2009 and 2010. Although the mechanism of human exposure and infection has often not been clearly identified, LBMs have regularly been incriminated as a possible source of human infection. This view is further supported by a case-control study conducted in Hong Kong where exposure to live poultry at LBMs in the week before illness was associated with a 4-fold increased risk in infection (Mounts et al., 1999; Van Kerkhove et al., 2010). A case-control analysis conducted in mainland China also showed that human urban cases were significantly more likely to have visited a LBM compared with rural cases (Zhou et al., 2009).

In the current epidemiological context where the clinical expression of the disease is becoming an exception, the Chinese National Veterinary Authorities face new challenges with the silent circulation and likely persistence of influenza viruses, including HPAIV H5N1, in traditional LBMs or specific ecosystems where free ranging duck rearing systems are dominant (Gilbert et al., 2006, 2008; Pfeiffer et al., 2007; Martin et al., 2011; Shortridge, 1995). To address this challenge and design targeted risk-based surveillance and control interventions, better knowledge of HPAIV H5N1 risk factors is required as well as innovative ways of thinking for better integrating poultry production and marketing systems into risk assessments. Methods initially developed in the social sciences can be applied to epidemiology to assist identifying persistence of infection or points of concentration along poultry market chains. Among these techniques, value chain analysis and social network analysis are playing an increasing role in describing infectious disease transmission patterns and guiding control policies elaborated by health authorities (Kobayashi, 2007; Yi-Da et al., 2007; Zheng et al., 2008; Kitsak et al., 2010). Value chain analysis, originally developed in economics and managerial science, provides an analytical framework that allows characterization of an entire poultry industry as well as inter-linkages among various actors in the industry (Kaplinsky, 2004; Reardon, 2006; Humphrey and Napier, 2005). As an additional tool, social network analysis (SNA) techniques provide a network based-approach and offer new insights on disease transmission dynamics, making it possible to develop more effective strategies for disease control (Klovdahl, 1985). Although the use of SNA for contact tracing dates back to the 1930s, it only received major attention after the emergence of AIDS (Yi-Da et al., 2007) before being further applied successfully to other infectious diseases. More recently, it was used to explain the patterns of the severe acute respiratory syndrome (SARS) transmission taking into account transmission modes in clustered populations (Chen, 2007; Fearnley, 2009) that epidemiological models based on the mean field assumption (equal probability of transmission between all hosts) failed to explain. A public health implication of this finding is that a traditional disease control approach based on random immunization may not be effective (Zheng et al., 2008). In veterinary epidemiology, the application of SNA techniques is much more recent. It was used to examine infection risk for bovine tuberculosis in wild possums in an experimental setting (Corner et al., 2003) and to investigate the spread of foot-and-mouth disease during the 2001 epidemic in the United Kingdom (Ortiz-Pelaez et al., 2006).

In this study, a comprehensive LBM survey was implemented in March and April 2009 in South China in Hunan and Yunnan provinces and Guangxi autonomous region, combining virus surveillance and social network analysis. Placing LBMs at the center of our analysis, our study aimed at investigating associations between poultry trade network characteristics in southern China and HPAI H5N1 infection status represented by the occurrence of poultry outbreaks, human cases or HPAIV H5N1 isolated in LBMs.

2. Materials and methods

2.1. Data

A cross-sectional survey was carried out from March to April 2009 in 30 live bird markets (LBMs) – hereafter referred to as market nodes – in Hunan, Yunnan and Guangxi. Ten LMBs were selected per province. These provinces – which had historical records of disease presence – were selected as representing the existing traditional poultry production and marketing systems, including the traditional LBMs marketing system typical of Southern China. The markets were selected based on characteristics such as size, trade and hygiene practices which best represent the market network characteristics at

the provincial level and the co-existence in each province of wholesale, retail and mixed LBMs. A questionnaire was submitted to market authorities to collect information regarding biosecurity measures applied within the markets. A list of questions including 56 hygiene and 97 trade-related indicators was used to interview the market manager in order to profile each market. This information was then linked to each market node and used in the statistical analysis to identify risk factors related to market hygiene practices contributing to HPAIV H5N1 presence. Within each market, live poultry traders (LPTs) or vendors were interviewed to capture their incoming and outgoing trading activities. An average of 7 LPTs (ranging from 2 in small markets to 12 in wholesale markets) randomly selected within each of the 30 LBM visited were interviewed and questioned about the origin of the birds, their destination, as well as the volume being traded. The poultry network data collection process followed an ego-centric approach whereby we studied the trading activities of each individual LPT. The ego-centric approach studies the networks of relations surrounding individuals rather than focusing on the complete network linking all individuals (Chung et al., 2005). It contrasts with the socio-centric approach where a whole group is studied in order to quantify relationships within subgroups of people. In total, the survey elicited information from 179 LPTs from these 30 markets concerning (1) their poultry trade activities outside and inside the province and (2) their relationship with other intermediaries and poultry flocks. The questionnaire included questions regarding the location of the source flocks, market or trader with whom they had trading relationships and from whom they had collected their poultry. The county of origin of the birds traded in these market nodes is hereafter referred to as source nodes.

Data on HPAIV H5N1 presence in markets, poultry outbreaks and human cases at county level, hereafter referred to as HPAI H5N1 infection status were collected and used as dependent variables in this study. Poultry HPAI H5N1 outbreak data from 2004 to 2009 were compiled from the Official Veterinary bulletin published on the website of the Chinese Ministry of Agriculture (MoA; http://www.agri.gov.cn/). Data on HPAI H5N1 human cases were obtained from the China Ministry of Health website (http://www.moh.gov.cn/) as well as the WHO Website during the period 2005–2009 (http://www.who.int/csr/don/en/) and geocoded according to the known geographical source of infection. To monitor the presence of HPAIV H5N1 in LBMs, cloacal and tracheal swab samples were collected in March and April 2009 at each market node from domestic poultry by the provincial Center for Disease Control (CDC) Laboratory Staff, the same day LPT were interviewed, and tested by polymerase chain reaction (PCR) for the matrix A influenza gene and H5 subtype in provincial CDCs. All the samples that tested positive to avian influenza were sent to the Harbin National Veterinary Research Institute for further confirmation, subtyping and virus isolation. Samples found positive for HPAIV H5N1 were recorded in a database for each market node and later combined with the network parameters obtained through SNA analysis. In addition, the Ministry of Agriculture in China routinely conducts a monthly, provincial LBM surveillance program consisting of sampling domestic poultry for the detection of HPAIV H5N1. The surveillance protocol for the national surveillance program as well as the laboratory testing method were considered to be sufficiently similar to the methods used in this cross-sectional survey to be able to combine these data with our LBM surveillance virological results without introducing an unacceptable bias.

The administrative level "county" was the epidemiological unit for determining infection disease status for source nodes. Therefore, for each source node and study market nodes (90 counties; median size 1921 km^2, interquartile range 1585 km^2), the county centroid and its infection status was extracted using a geographical information system software (GIS; ArcView 9.2© ESRI).

2.2. Analysis

The association between LBM biosecurity attributes and each source node infection status outcome variable (i.e. HPAI H5N1 poultry outbreaks or human case in the same county and HPAIV H5N1 presence in the LBM) was examined. The risk factors included "market type" ("wholesale" vs. "retail" markets), "segregation", "disposal of dead poultry in trash", "disposal of poultry blood in trash", "disposal of feathers in trash", "disposal of feed in trash", "manure processing", "market disinfection" and "market cleaning". The effect of clustering due to the presence of several LBMs in the same province was assessed by including 'province' as a random effect in the models.

The statistical analysis was carried out in two phases using the market nodes as the unit of analysis. First, all LBM biosecurity attributes were screened for association with market node infection status using univariable logistic regression based on $P < 0.20$ when applying the likelihood-ratio test (Stata 9.2. Stata® Corporation 2005). All continuous-scale variables were transformed to their natural logarithm when the variables were not normally distributed. Second, all factors significant in the screening phase were considered for inclusion through a manual backward stepwise variable selection process using multivariable logistic regression. The criterion for removal of risk factors was based on the likelihood ratio statistic $P > 0.05$. The presence of confounding variables in the final model was assessed by stepwise removal of variables which at some stage were not significant ($P > 0.05$), and noting the impact on the coefficients of the remaining variables in the model. If one of the coefficients of the other variables changed by more than 25% the eliminated variable was assumed to be a confounder and forced into the model (Dohoo et al., 2003). Model assumptions and goodness of fit were assessed following the methods described in Hosmer and Lemeshow (2000).

The data generated by LPTs' interviews were analysed using SNA techniques. The aim was to understand the direct and indirect connections between markets in the region as well as to quantify the network density, connectivity and geographical catchment area represented by this network. This information was also analysed taking into account the HPAI H5N1 infection status of the market nodes or their source nodes. SNA was used to describe the connectivity

pattern within the dataset consisting of records of paired trading events. Each pair represented the link between a particular LBM (or market nodes) and the source county (or source nodes) in which the flock of the purchased poultry was located. Two symmetric 2-mode networks – valued and binary (Network 1) – were built linking market nodes and source nodes so that two source nodes are linked via a LBM if the LPT (in that LBM) reported buying poultry from flocks in both counties during the study period. In the case of the valued network, it also included the number of links a source node establishes with a given market node. The 2-mode market–source network was converted into two separate 1-mode binary symmetric networks: one 1-mode binary symmetric network of source nodes linked via a common LBM (Network 2; source–source network) and a 1-mode binary symmetric network of market nodes linked via a common county (Network 3; market–market network).

To test the association between network parameters and the HPAI H5N1 infection status of source nodes, several statistical network metrics were estimated and compared. The degree represents the absolute number of unique links of a given node to another one. The *k*-core represents the maximal group of nodes, all of whom are connected to a number (*k*) of other nodes. These two network indicators are important for describing the levels of connectivity and centrality that exist between the different elements of a network and play a key role while identifying the most influential spreaders within a network (Kitsak et al., 2010). The means of the degree distribution as well as the *k*-core values between infected and non-infected counties were compared using the *t*-test with a permutation-based significance test involving 10,000 random permutations. In addition, the association between the density of links in Networks 2 and 3 and infection status of source nodes was tested by applying a randomization test of autocorrelation for a symmetric adjacency matrix using two classes and 10,000 random permutations. The test of autocorrelation for Networks 2 and 3 compared the observed number of links between two groups of nodes and the expected number obtained through random permutations of the network. The use of randomization tests of autocorrelation within symmetric adjacency matrices allows statistical significance testing of associations between dyadic binary variables such as represented by the links within the network and the disease status attributes of the source nodes. The three types of links based on the different variables of HPAI H5N1 infection status are: Type 1 – between infected source nodes, Type 2 – between infected and non-infected source nodes, and Type 3 – between non-infected source nodes. All social network analyses were performed using UCINET 6.135 (©Analytic Technologies, Inc., 1999).

3. Results

3.1. LBM biosecurity and HPAI H5N1 infection status

A total of 7050 cloacal and tracheal swabs, 2415 environmental and 610 water samples were collected from 30 market nodes for the detection of HPAIV H5N1.

The results – presented as odds ratios – of the uni- and multivariable analyses of LBM attributes associated with HPAI H5N1 poultry outbreaks during 2004–2009 and with HPAIV H5N1 isolated in LBMs are presented in Tables 1 and 2, respectively. None of the tested biologically plausible first-order interactions resulted in improvement of model fit. A total of 3 market biosecurity factors were considered ($P < 0.20$) for multivariable analysis for association with HPAIV H5N1 market infection and with poultry outbreaks during the period 2004–2009. None of the network parameters was significantly associated with the HPAI H5N1 infection status ($P > 0.20$). The only exception was for the number of links whereby our results suggest that markets located in counties with a previous history of H5N1 poultry outbreaks have significantly fewer links compared to markets located in counties with no history of H5N1 poultry outbreaks.

Only two factors (manure disposal and cage disinfection) were retained in a multivariable model and tested for association with HPAIV H5N1 in markets and none for the presence of poultry outbreaks in the county where these markets were located (Tables 1 and 2, respectively). For HPAIV H5N1 in markets – as measured by the isolation of HPAIV H5N1, and controlling for the confounding effect of disposal of dead birds in the trash – our results suggested that markets which utilize manure processing services are at reduced risk of being found positive for HPAIV H5N1 (Table 1). Although marginally significant, markets which implemented daily cage disinfection were also at lower risk of detecting HPAIV H5N1.

3.2. Poultry trade networks and HPAI H5N1 infection status

3.2.1. 2 mode market–source network (Network 1)

The 2-mode network represents the links between market nodes i.e. surveyed LBMs ($n = 30$) and source nodes ($n = 222$) (Fig. 1).

Among these 222 source nodes, only 60% of the source locations reported in the questionnaires by the LPTs could be linked to Chinese county names. The remaining 40% could not be included in the analysis since they only referred to the provincial or prefecture capital city as the origin of birds, preventing the identification of the exact location of origin within the county.

There were three types of source nodes: poultry farms ($n = 82$), LBMs ($n = 163$) and poultry traders ($n = 7$).

This first network was composed of 4 components: a giant weak component containing 160 nodes, a second component containing 82 nodes and two small components with five nodes (Fig. 2). A giant weak component is defined as a component containing the majority of the nodes of the network while the links established between nodes have an equal or "weak" strength due to the absence of direction between them.

The average degree for the studied LBM nodes was 10 (SD 8.7, range 1–37) and the average degree of the source nodes 1.4 (SD 1, range 1–9). A total of 17 market nodes had a *k*-core of 2; 5 market nodes had a *k*-core of 1, 4 had a *k*-core of 3 and 4 a *k*-core of 4 (Fig. 3). A total of 14 (47%) and 5 (17%) market nodes were located in counties with a

Table 1
Live Bird Market biosecurity attributes in southern China in 2009 tested for association with market node infection (defined as Highly Pathogenic Avian Infection H5N1 viruses isolated in LBMs) using univariable and multivariable analysis.

	OR	SE	z	$P>\|z\|$	95% CI	
A. Univariable analysis						
Network parameters						
Length of catchment area (Log)	1.15	0.22	0.73	0.464	0.79	1.68
Number of links	1.02	0.02	1.09	0.275	0.98	1.07
Biosecurity indicators						
Dead in trash	2.92	2.42	1.29	0.197*	0.57	14.82
Blood in trash	2.17	1.74	0.96	0.335	0.45	10.44
Feather in trash	3.12	2.50	1.42	0.156*	0.65	15.03
Feed in trash	0.88	0.70	−0.17	0.868	0.18	4.21
Manure disposal	0.07	0.07	−2.7	0.007*	0.01	0.48
Cage disinfection	0.25	0.24	−1.46	0.144*	0.04	1.59
Cage cleaning	0.31	0.25	−1.45	0.148*	0.06	1.51
B. Multivariable analysis						
Manure disposal	0.05	0.05	−3.14	0.002*	0.007	0.32
Cage disinfection	0.11	0.13	−1.94	0.053*	0.013	1.02

* Statistically significant.

previous history of HPAI H5N1 poultry outbreaks and human cases, respectively. In addition, 11 (36.7%) of the market nodes had positive market surveillance results for HPAIV H5N1. A total of 33 (15%), 13 (6%) and 79 (37%) source nodes were located in counties with a previous history of poultry outbreaks, human cases or HPAIV H5N1 positive in LBMs, respectively.

3.2.2. 1 mode source–source network (Network 2)

This network is represented by source nodes connected via a LPT. It was found to be as equally fragmented as Network 1, consisting of 4 components (Fig. 4).

The symmetric binary 1-mode source–source network included 222 nodes. The average degree was 21.2 (SD 12.9, range 2–75) so that a source node was connected to more than 21 others via common LPTs. The giant weak component included 140 nodes, a second large component included 74 nodes while two small size components contained 4 nodes each (Fig. 4).

Applying significance tests to the county attribute data, we found a significant difference in the mean degree and k-core of the nodes of Network 2 between the ones located in counties which had HPAIV H5N1 positive in LBMs (Table 3), with the mean degree and k-core of nodes located in infected counties being higher than that of nodes located in non-infected counties. The mean degree and k-core in counties which had experienced human cases were also higher, but this difference was not found to be statistically significant.

The test of autocorrelation for Network 2 also showed that the observed number of links between infected counties was significantly higher than expected for all infection status variables (Table 4). Conversely, the proportion of links between infected and non-infected counties (Type 2) was significantly lower than expected for all infection status variables ($P<0.002$) while the number of Type 3 links (between non-infected counties) was significantly different and higher for the same variables.

3.2.3. 1 mode market–market network (Network 3)

The symmetric binary 1-mode LBM-LBM network included 30 nodes. The average degree was 5.9 (SD 3.3, range 0–13) so that a market node was connected to more than five others via common LPTs. The giant weak component contained 20 nodes, a second component contained 8 nodes while there were two other components of one node each (Fig. 5).

Applying significance tests to the county attribute data, there was no significant difference in the mean degree or k-core of the nodes of Network 3 between the ones located in counties which had HPAI H5N1 poultry outbreaks

Table 2
Live Bird Market biosecurity attributes in southern China in 2009 tested for association with the presence of Highly Pathogenic Avian Infection H5N1 outbreaks in the same location (county) as the LBM using univariable analysis.

	OR	SE	z	$P>\|z\|$	95% CI	
A. Univariable analysis						
Network parameters						
Length of catchment area (Log)	0.83	0.131	−1.17	0.244	0.609	1.134
Number of links	0.99	0.005	−1.35	0.176*	0.981	1.003
Biosecurity indicators						
Dead in trash	3	3.40	0.97	0.333	0.325	27.703
Blood in trash	4	2.32	2.39	0.017*	1.281	12.482
Feather in trash	1.5	1.84	0.33	0.742	0.134	16.727
Feed in trash	1.66	0.37	2.3	0.021*	1.079	2.575
Manure disposal	0.33	0.17	−2.17	0.03*	0.123	0.897
Cage disinfection	0.15	0.22	−1.32	0.187*	0.009	2.460
Cage cleaning	0.31	0.49	−0.74	0.46	0.014	6.846

* Statistically significant.

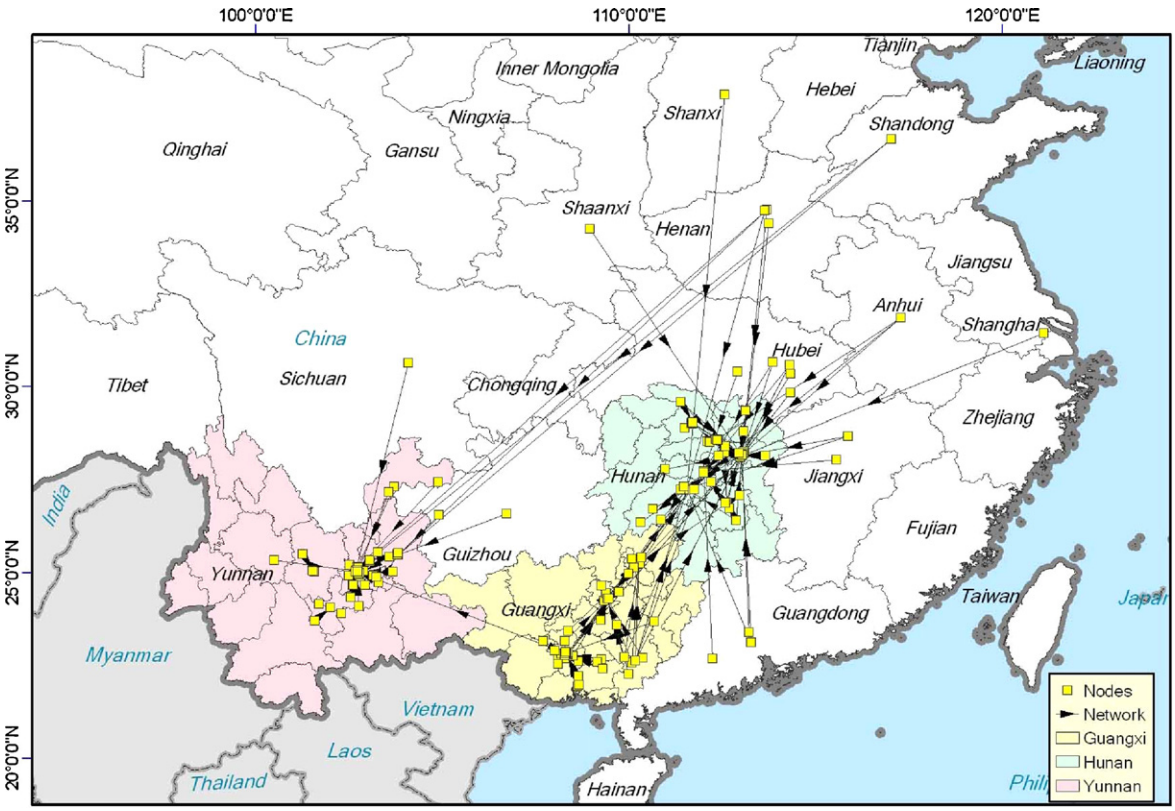

Fig. 1. Representation of the 2 mode "market–source node" network within the three provinces of southern China. The 2 mode network illustrates the links between market nodes represented by surveyed Live Bird Markets ($n = 30$) and source nodes ($n = 222$) from which poultry originate.

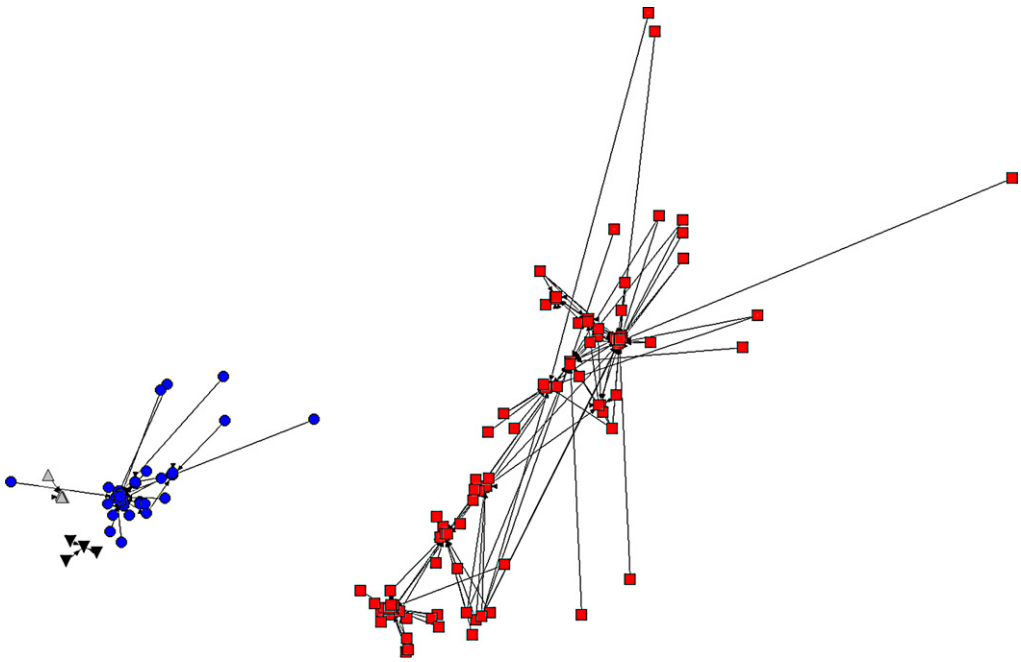

Fig. 2. Representation of Network 1 (2 mode market–source) network and the relative geographical position of its 4 components in southern China, composed of a giant weak component (red squares) and secondary components (blue circles, black down-triangles and grey up-triangles). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

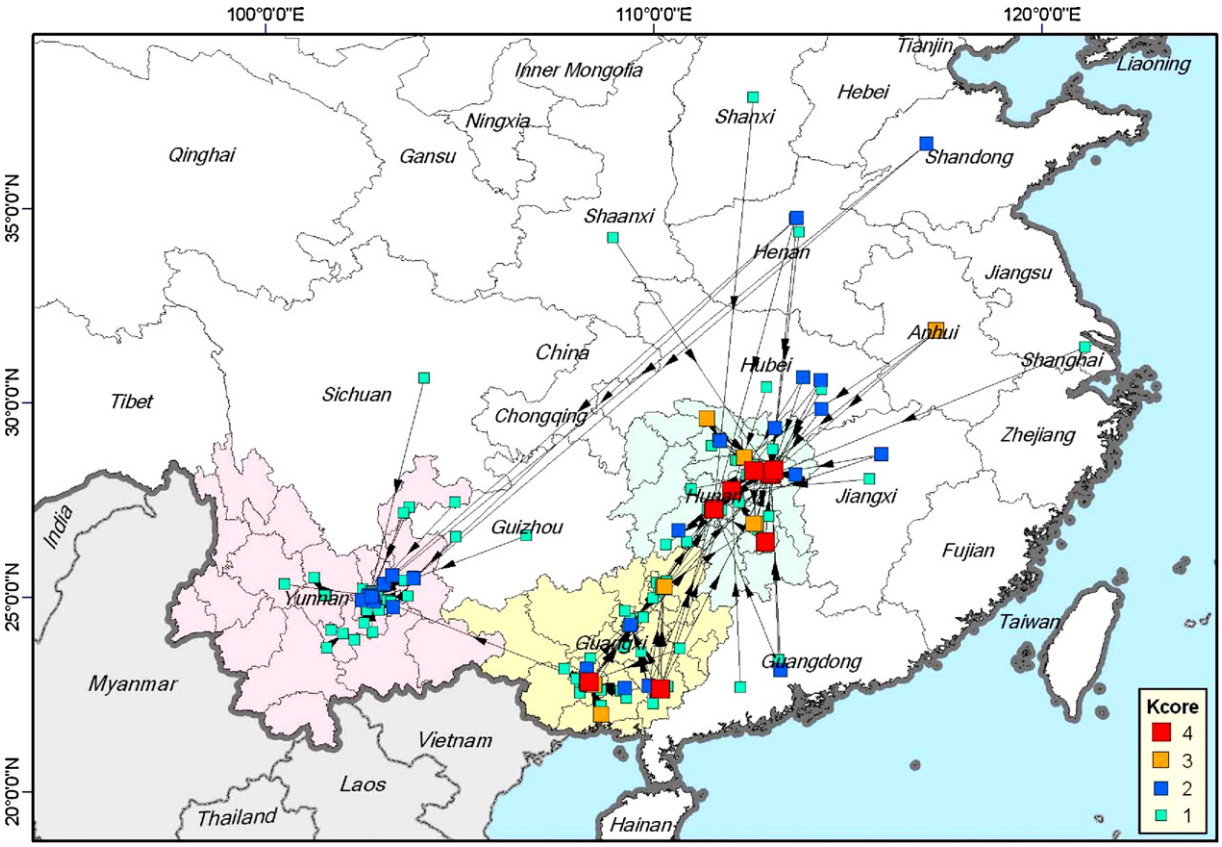

Fig. 3. Representation of the 2 mode "market–source node" network of poultry movement in southern China according to the *k*-core value. The *k*-core is a network parameter that measures the centrality of a node within a network. Some Live Bird Markets have a higher *k*-core than others, especially in Hunan and Guangxi provinces, where some LBMs displayed a maximum *k*-core value of 4 and could play a greater role in HPAIV maintenance.

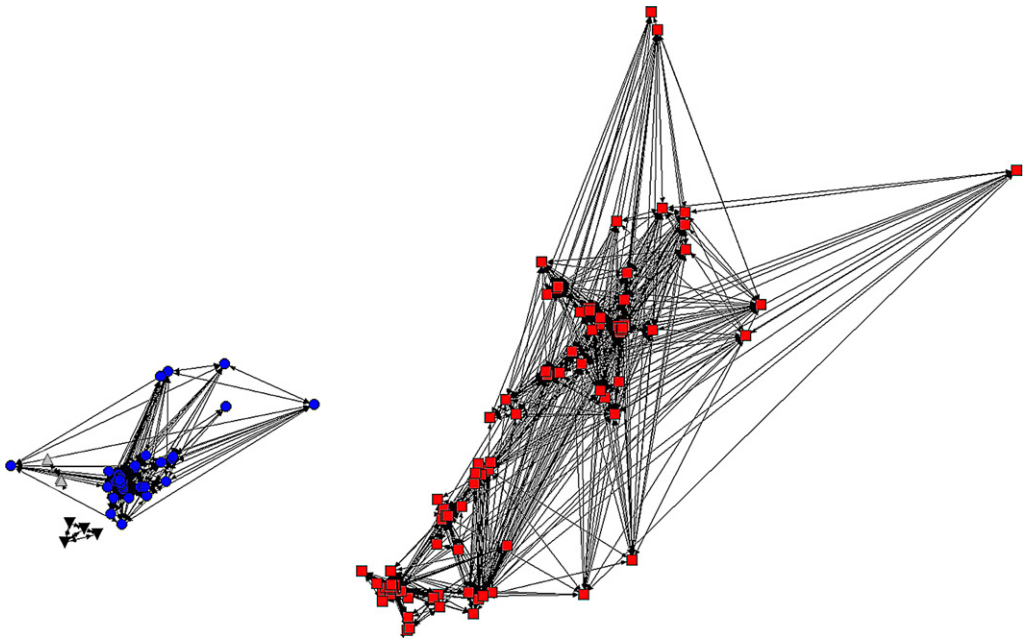

Fig. 4. Representation of Network 2 (1 mode source–source) network and the relative geographical position of its 4 components in southern China, composed of a giant weak component (red squares) and secondary components (blue circles, black down-triangles and grey up-triangles).

Table 3
Difference in mean degree and *k*-core of nodes located in Network 2 according to their Highly Pathogenic Avian Infection H5N1 infection status (represented by the presence or absence of HPAI H5N1 poultry outbreaks or human cases in the same county or the presence of market infection), southern China 2009.

	Mean degree (no. of nodes)		Two-tailed *t*-test probability of the difference of the mean degree	Mean *k*-core (no. of nodes)		Two-tailed *t*-test probability of difference of the mean *k*-core
	Presence	Absence		Presence	Absence	
Poultry outbreaks	20.47 (36)	21.3 (186)	−0.834 (P = 0.73)	17.66 (36)	18.8 (186)	−1.139 (P = 0.53)
Market infection	24.11 (87)	19.27 (135)	4.84* (P = 0.0053)	22.32 (87)	16.24 (135)	6.085* (P = 0.0001)
Human cases	25.2 (15)	20.87 (207)	4.32 (P = 0.22)	21.07 (15)	18.44 (207)	2.62 (P = 0.34)

* Statistically significant.

Table 4
Ratio between observed and expected Type 1, 2 and 3 links within Network 2 and significance test for their association with HPAI H5N1 infection status (represented by HPAI H5N1 poultry outbreaks or human cases in the same county or the presence of market infection).

	Ratio for Type 1 links and two-tailed test probability	Ratio for Type 2 links and two-tailed test probability	Ratio for Type 3 links and two-tailed test probability
	1–1 Infected–infected	0–1 Non infected–infected	0–0 Non infected–non infected
Poultry outbreaks	2.81 (P = 0.002)	0.57 (P = 0.002)	1.1 (P = 0.002)
Market infection	1.23 (P = 0.002)	0.56 (P = 0.002)	1.48 (P = 0.002)
Human cases	1.25 (P = 0.042)	0.39 (P = 0.042)	1.09 (P = 0.042)

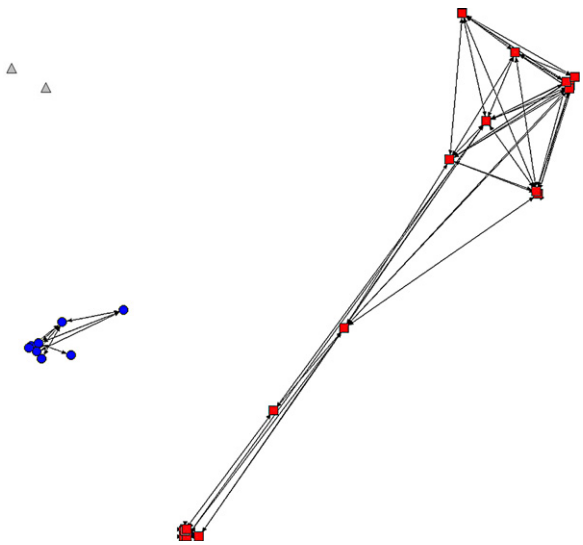

Fig. 5. Representation of Network 3 (1 mode market-market) network and the relative geographical position of its 4 components in southern China, composed of a giant weak component (red squares) and secondary components (blue circles and grey up-triangles).

during 2004–2009 and between the ones located in counties which had HPAIV H5N1 positive results in LBMs or human cases in the same county. Likewise, the test of autocorrelation for Network 3 showed that none of the three types of links (type 1, 2 and 3) were significantly different regarding the three variables.

4. Discussion

In both animal and human populations, the nature of contact between individuals contributes to the incursion and spread of contagious diseases (Meyers et al., 2005; Kiss et al., 2006; Ortiz-Pelaez et al., 2006). In the case of avian influenza, the movement of live birds is a well-known risk factor for the dissemination of the virus to poultry flocks (Kung et al., 2007; Soares Magalhaes et al., 2010). Indeed, once HPAI virus infects poultry, potential risks of exposure and infection both to other poultry and to people are passed on through a chain of economic activities including the purchase and sale of birds and other products and the raising of poultry. Along these chains, LBMs have long been considered important links that lead to persistence and reintroduction of infection. They facilitate the congregation of large populations of animals that originate from a diversity of sources in a fairly large geographical area—in relatively small spaces (Senne et al., 2003; Webster, 2004; Senne, 2007). These markets also serve as a possible mechanism by which infection is maintained for prolonged periods of time, posing additional risk for disease spread and human exposure (Panigrahy et al., 2002; Bulaga et al., 2003; Liu et al., 2003; Choi et al., 2005; Nguyen et al., 2005; Wang et al., 2006).

The findings of our study contribute to a better understanding of poultry trading dynamics in southern China and partially uncover the role played by LBM networks in the presence and possible persistence of HPAIV H5N1, thus offering new prospects for disease control strategy development along market chains. It further supports other studies conducted in Vietnam and Cambodia which have demonstrated the importance of evaluating live poultry movement and trading practices to develop appropriate and targeted surveillance recommendations for active HPAI/H5N1 surveillance program (Soares Magalhaes et al., 2010; Van Kerkhove et al., 2009).

Combining descriptive and network analyses, our study provides evidence that several biosecurity factors such as daily cage cleaning, daily cage disinfection or manure processing contribute to a reduction in HPAIV H5N1 presence in LBMs. Addressing these factors through market

biosecurity upgrading and restructuring could have a significant impact on reducing the level of infection in these markets and possibly interrupting the cycle of infection persistence. Of equal importance, we also confirmed through SNA analysis the role of trade and more specifically the key role played by the source or origin of poultry (Network 2: source–source) in supporting HPAIV H5N1 presence in LBMs and possibly its persistence within certain network configurations. Our findings indeed support this hypothesis, as demonstrated by the higher number of links between counties or source nodes having the same status (infected–infected; free–free) while the number of links between free and infected counties was significantly lower than expected. This association was consistently found in Network 2 for the three categories of infection status, namely the presence of poultry outbreaks or human cases in the same county, and HPAIV H5N1 presence in source nodes. This represents knowledge of utmost importance, showing that source nodes connected to LBMs via the same live poultry traders or vendors can act as a network of infection persistence or conversely remain free of infection. We also found that markets where HPAIV H5N1 was isolated were associated with higher degree and *k*-core in Network 2. This finding corroborates the importance of network connectivity and centrality when dealing with infectious disease patterns and further supports the recent results obtained by Kitsak et al. (2010) showing that being located at the core of a network (represented by the *k*-core index) is key for spreading information or diseases. In this case, once the core of a network has been identified, targeted control actions such as increased vaccination of birds traded within this network, behaviour change of LPTs or LBM restructuring can be envisaged and enforced. It is also noteworthy that our study could not establish any correlation between long distance trade, the intensity of this trade or network parameters and LBM infection status in networks 1 or 3. This could be the result of potential bias in LBM selection and the inaccuracy of the answers provided by some LPTs in this study, which was the first of its kind in southern China. As an example, 40% of the network data which aimed at tracing back the origin of birds traded by LPTs could not be geocoded and linked to the county of origin due to inaccurate answers with respect to the geographical source of birds. This represented a limitation in our capacity to capture the entire LBM network characteristics in the three provinces and produce additional inferences regarding network parameters and infection status. In addition, while the LBMs included in the study were a fair representation of the LBM network in each province, their accessibility by CDC provincial staff and willingness to cooperate in the surveillance exercise was also a potential limiting factor, discarding LBM located in remote areas or far from the provincial capital city but of possible interest in terms of HPAIV H5N1 surveillance.

This study further confirms that that current disease control and prevention interventions would benefit from increased knowledge about poultry trading patterns developed on a market-based formal data recording system (Soares Magalhaes et al., 2010). This would allow the capture all possible poultry network configurations for a wider geographical area where poultry production is important, whether for the presence of the traditional free-ranging duck system considered at high risk of virus persistence or the more industrialized husbandry system where the intensity of trade and its geographical extent could contribute to virus dissemination or persistence. Additionally, as opposed to the cross-sectional approach adopted for our study, a longitudinal approach for studying LBM networks would certainly improve the quality of the results obtained by capturing the dynamic nature of these networks, which are sensitive to seasonal effects. Furthermore, based on the combination of identified biosecurity risk factors and network parameters (for example, LBMs having a high *k*-core), LBMs could be categorised into risk classes in order to guide prioritization of market-level restructuring with the final aim of disrupting the network-based virus circulation cycle.

5. Conclusion

China's commitment to HPAI H5N1 control, supported by efficient implementation in the field, has been successful in limiting the number of outbreaks and controlling their spread: no further domestic poultry outbreaks were reported in 2010. As the epidemiological situation evolves and the number of reported outbreak decreases, so will national and provincial plans to answer the new challenges of reducing the level of viral circulation in high-risk traditional ecosystems or specific segments of poultry market chains such as LBMs.

An evolving strategy needs to incorporate new knowledge offered by the combination of epidemiological surveys and socio-economic techniques applied to infectious disease epidemiology. Our results show the relevance of this approach and provide a framework for analyzing the risk of HPAI H5N1 along poultry market chains in Southern China. More specifically, while providing new insights into the role of LBMs in China and HPAIV H5N1 presence, we also demonstrate that network parameters identified through SNA – such as the *k*-core or degree – are highly relevant for better understanding of this risk. Even more importantly, it also offers new perspectives for developing more effective policies that would allow a major leap forward in the control of HPAIV H5N1 and assist in defining geographical areas or poultry production systems free of disease or infection. Ultimately, such methodology could be applied to other diseases of livestock of significant public health or economic importance.

Conflict of interest

No other conflicts of interest exist.

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References

Bingsheng, K., Han, Y., 2008. Poultry Sector in China: Structural Changes in the Past Decade and Future Trend. FAO, Rome.

Bulaga, L.L., Garber, L., Senne, D.A., Myers, T.J., Good, R., Wainwright, S., Trock, S., Suarez, D.L., 2003. Epidemiologic and surveillance studies on avian influenza in live-bird markets in New York and New Jersey, 2001. Avian Dis. 47 (3), 996–1001.

Choi, Y.K., Seo, S.H., Kim, J.A., Webby, R.J., Webster, R.G., 2005. Avian influenza viruses in Korean live poultry markets and their pathogenic potential. J. Virol. 332 (2), 529–537.

Chung, K.S.K., Hossain, L., Davis, J.,2005. Exploring sociocentric and egocentric approaches for social network analysis. In: International Conference on Knowledge Management Asia Pacific, November 27–29. Victoria University Wellington, New Zealand, pp. 1–8.

Corner, L.A.L., Pfeiffer, D.U., Morris, R.S., 2003. Social-network analysis of *Mycobacterium bovis* transmission among captive brushtail possums (*Trichosurus vulpecula*). Prev. Vet. Med. 59 (3), 147–167.

Dohoo, I., Martin, W., Stryhn, H., 2003. Veterinary Epidemiologic Research. AVC Inc., Charlottetown.

Fearnley, L., 2009. The pathology of contact: on the modeling of infection networks in SARS epidemiology. In: International Congress of Anthropological and Ethnological Sciences, Kunming, China.

Gilbert, M., Chaitaweesub, P., Parakarnawongsa, T., Premashthira, S., Tiensin, T., Kalpravidh, W., Wagner, H., Slingenbergg, J., 2006. Free-grazing ducks and highly pathogenic avian influenza, Thailand. Emerg. Infect. Dis. 12, 227–234.

Gilbert, M., Xiao, X., Pfeiffer, D.U., Epprecht, M., Boles, S., Czarnecki, c., Chaitaweesub, P., Kalpravidh, W., Minh, P.Q., Otter, M.J., Martin, V., Slingenbergh, J., 2008. Mapping H5N1 highly pathogenic avian influenza risk in Southeast Asia. Proc. Natl. Acad. Sci. U.S.A. 105, 4769–4774.

Hosmer, D.W., Lemeshow, S., 2000. Applied Logistic Regression. John Wiley & Sons, Inc., New York.

Humphrey, J., Napier, L., 2005. The value chain approach as a tool for assessing distributional impact of standards on livestock markets: guidelines for planning a programme and designing case studies. In: FAO AGA/ESC Initiative on Market Exclusion. Institute of Development Studies, Brighton, UK.

Kaplinsky, R., 2004. Spreading the gains from globalization: what can be learned from value-chain analysis? Probl. Econ. Transit. 47 (2), 74–115.

Kitsak, M., Gallos, L.K., Havlin, S., Liljeros, F., Muchnik, L., Stanley, H.E., Makse, H.A., 2010. Identification of influential spreaders in complex networks. Lett. Nat. Phys. vol. 6.

Kiss, I.Z., Green, D.M., Kao, R.R., 2006. The network of sheep movements within Great Britain: network properties and their implications for infectious disease spread. J. R. Soc. 3 (10), 669–677.

Klovdahl, A.S., 1985. Social networks and the spread of infectious diseases: the AIDS example. Soc. Sci. Med. 21 (11), 1203–1216.

Kobayashi, M., 2007. Poultry Value Chain Study: Consultant Report. FAO, Rome.

Kung, N.Y., Morris, R.S., Perkins, N.R., Sims, L.D., Ellis, T.M., Bissett, L., Chow, M., Shortridge, K.F., Guan, Y., Peiris, M.J., 2007. Risk for infection with highly pathogenic influenza A virus (H5N1) in chickens, Hong Kong, 2002. Emerg. Infect. Dis. 13 (3), 412–418.

Liu, M., He, S., Walker, D., Zhou, N., Perez, D.R., Mo, B., Li, F., Huang, X., Webster, R.G., Webby, R.J., 2003. The influenza virus gene pool in a poultry market in South central China. Virology 305 (2), 267–275.

Martin, V., Pfeiffer, D.U., Zhou, X., Xiao, X., Prosser, D.J., Guo, F., Gilbert, M., 2011. Spatial distribution and risk factors of highly pathogenic avian influenza (HPAI) H5N1 in China. PLoS Pathog. 7 (3), e1001308.

Meyers, L.A., Pourbohloul, B., Newman, M.E., Skowronski, D.M., Brunham, R.C., 2005. Network theory and SARS: predicting outbreak diversity. J. Theor. Biol. 232 (1), 71–81.

Mounts, A.W., Kwong, H., Izurieta, H.S., Ho, Y., Au, T., Lee, M., Buxton Bridges, C., Williams, S.W., Mak, K.H., Katz, J.M., Thompson, W.W., Cox, N.J., Fukuda, K., 1999. Case-control study of risk factors for avian influenza A (H5N1) disease, Hong Kong, 1997. J. Infect. Dis. 180 (2), 505–508.

Nguyen, D.C., Uyeki, T.M., Jadhao, S., Maines, T., Shaw, M., Matsuoka, Y., Smith, C., Rowe, T., Lu, X., Hall, H., Xu, X., Balish, A., Klimov, A., Tumpey, T.M., Swayne, D.E., Huynh, L.P., Nghiem, H.K., Nguyen, H.H., Hoang, L.T., Cox, N.J., Katz, J.M., 2005. Isolation and characterization of avian influenza viruses, including highly pathogenic H5N1, from poultry in live bird markets in Hanoi, Vietnam, in 2001. J. Virol. 79 (7), 4201–4212.

Ortiz-Pelaez, A., Pfeiffer, D.U., Soares-Magalhaes, R.J., Guitian, F.J., 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.

Panigrahy, B., Senne, D.A., Pedersen, J.C., 2002. Avian influenza virus subtypes inside and outside the live bird markets, 1993–2000: a spatial and temporal relationship. Avian Dis. 46 (2), 298–307.

Pfeiffer, D.U., Minh, P.Q., Martin, V., Epprecht, M., Otte, M.J., 2007. An analysis of the spatial and temporal patterns of highly pathogenic avian influenza occurrence in Vietnam using national surveillance data. Vet. J. 174, 302–309.

Reardon, T., 2006. The rapid rise of supermarkets and the use of private standards in their food product procurement systems in developing countries. In: Ruben, R., Slingerland, M., Nijhoff, H. (Eds.), Agro-food Chains and Networks for Development. , pp. 79–105, Chapter 8.

Senne, D.A., Suarez, D.L., Pedersen, J.C., Panigrahy, B., 2003. Molecular and biological characteristics of H5 and H7 avian influenza viruses in live-bird markets of the northeastern United States, 1994–2001. Avian Dis. 47 (3), 898–904.

Senne, D.A., 2007. Avian influenza in North and South America, 2002–2005. Avian Dis. 51 (1), 167–173.

Shortridge, K.F., 1995. The next pandemic influenza virus? Lancet 346, 1210–1212.

Soares Magalhaes, R.J., Ortiz-Pelaez, A., Thi, K.L., Dinh, Q.H., Otte, J., Pfeiffer, D.U., 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.

Van Kerkhove, M.D., Vong, S., Guitian, J., Holl, D., Mangtani, P., San, S., Ghani, A.C., 2009. Poultry movement networks in Cambodia: implications for surveillance and control of highly pathogenic avian influenza (HPAI/H5N1). Vaccine 27 (45), 6345–6352.

Van Kerkhove, M.D., Mumford, E., Mounts, A.W., Bresee, J., Ly, S., Bridges, C.B., Otte, J., 2010. Highly pathogenic avian influenza (H5N1): pathways of exposure at the animal-human interface, a systematic review. PLoS One 6 (1), e14582.

Wang, M., Di, B., Zhou, D.H., Zheng, B.J., Jing, H., Lin, Y.P., Liu, Y.F., Wu, X.W., Qin, P.Z., Wang, Y.L., Jian, L.Y., Li, X.Z., Xu, J.X., Lu, E.J., Li, T.G., Xu, J., 2006. Food markets with live birds as source of avian influenza. Emerg. Infect. Dis. 12 (11), 1773–1775.

Webster, R.G., 2004. Wet markets—a continuing source of severe acute respiratory syndrome and influenza? Lancet 363 (9404), 234–236.

Yi-Da, C., Chunju, T., Chwan-Chuen, K., Tsung-Shu, J., Hsinchun, W.C., 2007. Incorporating geographical contacts into social network analysis for contact tracing in epidemiology: a study of Taiwan SARS data. In: Zeng, D., et al. (Eds.), Biosurveillance 2007, 4506. LNCS, pp. 23–36.

Zheng, X., Zeng, D., Sun, A., Luo, Y., Wang, Q., Wang, F., 2008. Network-based analysis of Beijing SARS data. In: Zeng, D., et al. (Eds.), BioSecure 2008, 5354. LNCS, pp. 64–73.

Zhou, L., Liao, Q., Dong, L., Huai, Y., Bai, T., Xiang, N., Shu, Y., Liu, W., Wang, S., Qin, P., Wang, M., Xing, X., Lv, J., Chen, R.Y., Feng, Z., Yang, W., Uyeki, T.M., Yu, H., 2009. Risk factors for human illness with avian influenza A (H5N1) virus infection in China. J. Infect. Dis. 199 (12), 1726–1734.