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Transmissibility of the highly pathogenic avian influenza virus, subtype H5N1 in domestic poultry: a spatio-temporal estimation at the global scale

Zhijie Zhang[1,2,3], Dongmei Chen[4], Michael P. Ward[5], Qingwu Jiang[1,2,3]

[1]Department of Epidemiology and Biostatistics, School of Public Health, Fudan University, Shanghai 200032, People's Republic of China; [2]Key Laboratory of Public Health Safety, Ministry of Education, Shanghai 200032, People's Republic of China; [3]Laboratory for Spatial Analysis and Modelling, School of Public Health, Fudan University, Shanghai 200032, People's Republic of China; [4]Laboratory of Geographic Information and Spatial Analysis, Department of Geography, Queen's University, Kingston, Ontario K7L 3N6, Canada; [5]Faculty of Veterinary Science, The University of Sydney NSW 2006, Australia

Abstract. The highly pathogenic avian influenza virus (HPAIV), subtype H5N1 poses a serious threat not only to the poultry industry and wild birds but also to humans. Despite a large number of studies conducted on various aspects of this virus, its transmissibility is still poorly understood. This study quantifies the basic reproductive number (R_0) of the global HPAIV H5N1 spread within domestic poultry during December 2003 to December 2009. Three different approaches were applied to estimate R_0 for HPAIV H5N1: (i) epidemic doubling time; (ii) spatial distance-based nearest neighbour; and (iii) spatio-temporal distance-based nearest neighbour. These three approaches represent temporal (tR_0), spatial (sR_0) and spatio-temporal transmissibility (stR_0), respectively. The joint application of these three approaches provides a more complete profile by characterising the transmissibility traits of infectious diseases from different perspectives. Estimates of tR_0 gradually decreased over the six sequential epidemic waves (EWs) examined, suggesting that the implemented control measures were effective in reducing the number of outbreaks. However, sR_0 and stR_0 increased from EW1, peaked in EW3 and then gradually decreased during EW4-EW6, reflecting different aspects of disease transmissibility compared to tR_0. The application of all three methods in the final EW6 showed R_0 >1, suggesting that the control measures implemented did not completely interrupt the transmission cycle, and hence were insufficient to eliminate HPAIV H5N1. Close monitoring of HPAIV H5N1 outbreaks and enhanced control policies is advised.

Keywords: avian influenza, H5N1, reproductive number, transmissibility, epidemiology, spatial analysis, spatio-temporal analysis.

Introduction

The re-emergence and global spread of the highly pathogenic avian influenza virus (HPAIV), subtype H5N1 since late 2003 has raised concern about an impending influenza pandemic (White and Pagano, 2008). Although there is currently limited evidence of efficient human-to-human transmission of HPAIV H5N1, it would be possible for a new variant to acquire the ability of sustained transmission among humans if it re-assorts with a human virus in co-infected mammalian hosts (human or non-human) (Longini et al., 2005). HPAIV H5N1 has been studied by researchers from various fields, ranging from molecular studies at the micro-scale to spatial analysis at the macro-scale (Li et al., 2004; Carrel et al., 2010; Zhang et al., 2010), but the basic epidemiological characteristics of this virus strain remains poorly understood, especially regarding transmissibility within poultry populations (Bouma et al., 2009; Penny et al., 2010). A better understanding of the transmission dynamics is paramount for assessing the efficacy of implemented control measures and guiding the design of more efficient control policies and surveillance programmes going forward (Stegeman et al., 1999, 2004).

The basic reproductive number (R_0) is a key parameter for understanding disease transmissibility. It is defined as the expected number of secondary cases generated by an infectious case (which can be an individual – a flock or a herd – a village or a region) during its entire infectious period in a fully susceptible population (Anderson and May, 1991; Mills et al., 2004; Massad et al., 2007). If R_0 >1, each infectious

Corresponding author:
Zhijie Zhang
Department of Epidemiology and Biostatistics
School of Public Health, Fudan University
Shanghai 200032, People's Republic of China
Tel./Fax +86 21 5423 7410
E-mail: epistat@gmail.com

case infects >1 susceptible individuals and the epidemic can thus propagate, but if $R_0 \leq 1$ the epidemic will eventually fade out (Garske et al., 2007). Because close contact with sick or dead poultry is a risk factor with respect to human influenza caused by HPAIV H5N1, controlling and preventing the spread of this virus in poultry flocks should effectively reduce the threat of a human HPAIV H5N1 influenza pandemic (Zhou et al., 2009; Yupiana et al., 2010; Zhang et al., 2010). Therefore, quantitatively estimating the R_0 of HPAIV H5N1 in domestic poultry, particularly as a measure of the effectiveness of control, becomes important (Ward et al., 2009). A few studies have reported estimates of R_0 for HPAIV H5N1: Bouma et al. (2009) estimated R_0 in chickens through controlled experimental studies; Tiensin et al. (2007) used mortality data from chicken flocks to estimate R_0 in Thailand; Soares Magalhães et al. (2010) estimated and compared the within-flock R_0 using routine surveillance data in chicken and duck flocks before and during the vaccination period in Vietnam; Penny et al. (2010) investigated the transmissibility of HPAIV H5N1 in wild water birds at Lake Constance in Europe, and Ward et al. (2009) applied different methods to calculate the village-based R_0 of HPAIV H5N1 in Romania. However, to our knowledge, the R_0 of HPAIV H5N1 at the global scale has not previously been reported.

The aim of this study was to estimate and compare the R_0 of HPAIV H5N1 in domestic poultry at the global scale from temporal, spatial and spatio-temporal perspectives based on a worldwide database of reports from December 2003 to December 2009. This work was done with the expectation that the results be used to estimate the efficacy of currently implemented control measures and provide guidance for the further development of control policies.

Materials and methods

Outbreaks of HPAIV H5N1 in domestic poultry

Reported outbreaks of HPAIV H5N1 in domestic poultry were compiled by sub-district on a day-by-day basis from the Food and Agriculture Organization of the United Nations (FAO), the World Organization for Animal Health (OIE) and other sources, covering the period of December 2003 to December 2009. Locations and dates were available for each reported outbreak. Outbreak definition and the database have been previously described (Zhang et al., 2010). Six epidemic waves (EWs) were characterised based on an analysis reported previously (Zhang et al., 2010). Their associated number of outbreaks and proportion were tabulated (Table 1).

Estimation of the reproductive number (R_0)

R_0 measured the overall transmissibility of HPAIV H5N1 among the sub-districts in this study and was defined as the average number of secondary sub-districts (see below) that each (previous) infectious sub-district infected during the entire period of infectiousness in an epidemic wave.

Approach of epidemic doubling time (tR_0)

Assuming HPAIV H5N1 had an exponential epidemic phase and the doubling time of outbreak numbers was constant, tR_0 could be approximated by the formula 3 + *D 1v_d + np4, where D is the infectious duration of an epidemic (the value of 7 days was used for D) (Ward et al., 2009; Iglesias et al., 2011); t_d the time interval in which the number of outbreaks doubles (the doubling time) (Anderson and May, 1991), which were calculated using 10 different starting values (1, 3, 5, 7, 9, 11, 13, 15, 17 and 19) and ln the natural log (e). For each starting value, all possible combinations of t_d were obtained from the ascending (exponential) phase of each epidemic. The average t_d was used as the doubling time for each starting value to compute the tR_0 (Ward et al., 2009). To check the robustness of the results regarding the infectious period (D), we also analysed the case in which the infectious duration was 14 days.

Spatially nearest neighbour method (sR_0)

For each individual outbreak, we defined the closest outbreak as its infection source, while "closeness" was measured using the Euclidean distances among outbreaks in a constrained circular space, where 5-50%

Table 1. Description of epidemic waves used in this study.

Epidemic wave	Period	Outbreaks	Proportion (%)
1	10.12.2003-30.06.2004	2,540	30
2	01.06.2004-31.05.2005	1,927	23
3	01.06.2005-31.10.2006	2,338	28
4	01.11.2006-30.09.2007	657	8
5	01.10.2007-31.10.2008	682	8
6	01.11.2008-31.12.2009	283	3

of the maximum distance (M) among outbreaks were used as the circle radii using intervals of 5%, i.e. 5%M, 10%M, 15%M, 20%M, 25%M, 30%M, 35%M, 40%M, 45%M and 50%M, respectively. For each specified space, the process of searching for the spatially nearest neighbour was repeated for all outbreaks, so each outbreak could be assigned a source, excluding the earlier outbreak. The number of outbreaks attributed to each source could be summarised accordingly, and its mean value obtained as the sR_0 (Ward et al., 2009).

Spatio-temporally nearest neighbour approach (stR_0)

The method and procedures of calculating the stR_0 were the same as those for sR_0, except that the definition of "closeness" among outbreaks here was based not only on the Euclidean distance, but also on the time difference. The former was the same as sR_0; the latter required that the date of each studied outbreak should be later than that of the closest outbreak (infection source) and their difference should be ≤7 days (infectious duration). For the sensitivity analysis, the value of 14 days was also used for the duration of infectiousness to investigate the influence of different infectious duration on the estimated stR_0.

Associations between reproductive number (sR_0 and stR_0) and poultry density

The global poultry density was obtained from FAO via the Geonetwork (http://www.fao.org/geonetwork/srv/en /main.home), which provided the values of poultry densities per km^2 (for methodology and sources of the data estimates, see Robinson et al., 2007). The poultry density of each outbreak was first extracted based on the outbreak location, then Spearman rank correlation analysis was used to analyse the associations between the poultry density and sR_0 and stR_0 (both 7 and 14 days were used for infectious duration, respectively). No corrections were made for multiple comparisons.

Results

Spatial distribution of global HPAIV H5N1 outbreaks

Fig. 1 displays the spatial distribution of the global HPAIV H5N1 outbreaks in six sequential EWs. Out of these, EW1 and EW2 were originally contained in East and Southeast Asia. With regard to EW3, which had the widest spatial distribution among all the EWs, the outbreaks eventually spread to Europe and Africa,

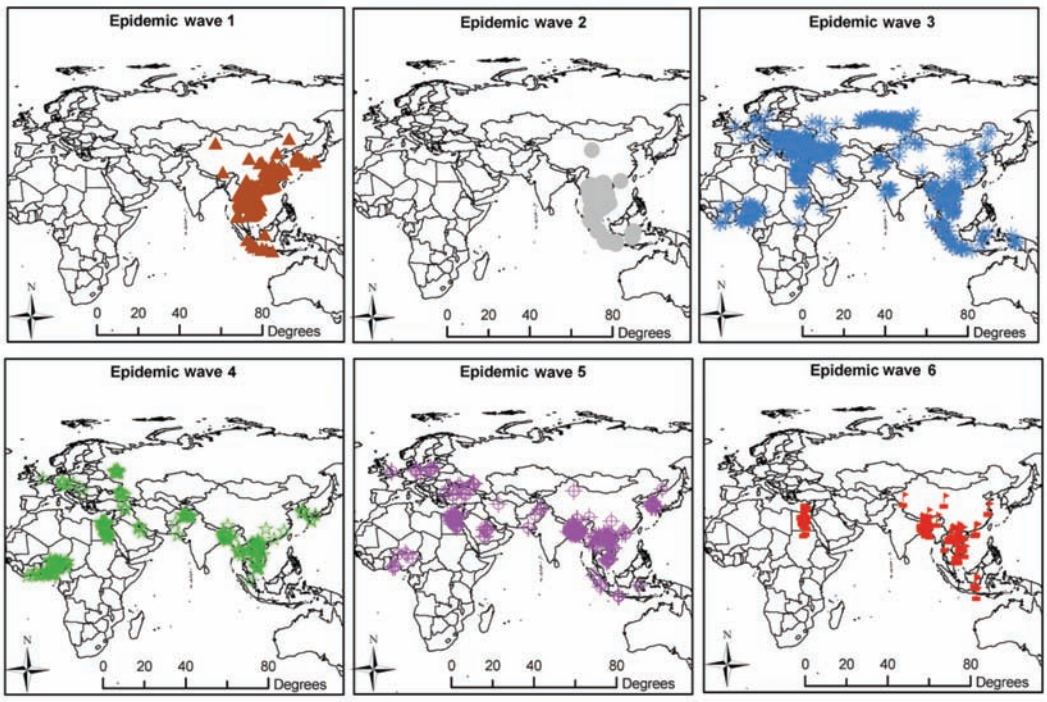

Fig. 1. Spatial distribution of global HPAIV H5N1 outbreaks, categorized as six epidemic waves (EWs). The EW1 and EW2 were originally contained in East and Southeast Asia; in EW3, outbreaks spread to Europe and Africa, which had the widest spatial distribution among all EWs; while in EW4-EW6, outbreaks were gradually mitigated, but the spatial distribution in EW6 was still wider than EW1 and EW2.

while fewer outbreaks were reported for EW4-EW6. However, the spatial distribution in EW6 was still broader than that in EW1 and EW2.

Estimation of tR_0

Table 2 shows the results of tR_0 calculated using epidemic double time with the assumption of infectious duration being 7 days. The results from various start values were only slightly different for each EW, while the overall tendency when comparing different EWs was a decrease of the estimated tR_0 from EW1 to EW3 followed by values, which remained relatively stable at approximately 1.25. The results of tR_0 on the basis of the 14-day infectious duration were similar to that of tR_0 for those with infectious duration of 7 days, except that the values were slightly higher (Table 3).

Estimation of sR_0

The sR_0 obtained from the spatially nearest neighbour method is summarised in Table 4. The sR_0 in EW1 was slightly above the threshold value of 1 but increased substantially in EW2, peaked in EW3 and then decreased in the EW4-EW6 period. All sR_0s from EW2-EW6 were above 2.0.

Estimation of stR_0

Table 5 shows stR_0 with the assumption of the infectious duration being 7 days. The general characteristics of stR_0 across EW1-EW6 were similar to those for sR_0, except that the values of stR_0 were consistently lower than those of sR_0. The results of stR_0, using 14 days for infectious duration, are summarised in Table

Table 2. tR_0 calculated using the approach of epidemic double time (infectious duration = 7 days).

Epidemic wave	Start value										Mean
	1	3	5	7	9	11	13	15	17	19	
1	2.21	1.97	2.39	1.88	1.88	1.97	1.97	1.81	2.21	1.97	2.03
2	2.39	1.44	1.51	1.33	1.54	1.27	1.20	1.24	1.37	1.31	1.46
3	1.35	1.32	1.29	1.22	1.26	1.19	1.14	1.17	1.21	1.22	1.24
4	1.37	1.49	1.24	1.29	1.26	1.23	1.24	1.32	1.26	1.26	1.30
5	1.37	1.29	1.22	1.23	1.22	1.21	1.20	1.20	1.19	1.21	1.24
6	1.44	1.26	1.27	1.22	1.19	1.17	1.16	1.14	1.13	1.12	1.21

Table 3. tR_0 calculated using the approach of epidemic double time (infectious duration = 14 days).

Epidemic wave	Start value										Mean
	1	3	5	7	9	11	13	15	17	19	
1	3.43	2.94	3.77	2.76	2.76	2.94	2.94	2.62	3.43	2.94	3.05
2	3.77	1.88	2.02	1.67	2.08	1.54	1.40	1.49	1.75	1.63	1.92
3	1.69	1.65	1.59	1.43	1.51	1.37	1.29	1.33	1.42	1.43	1.47
4	1.75	1.97	1.49	1.59	1.51	1.46	1.49	1.65	1.51	1.51	1.59
5	1.75	1.57	1.44	1.46	1.44	1.42	1.40	1.40	1.39	1.42	1.47
6	1.88	1.51	1.54	1.44	1.38	1.34	1.32	1.29	1.26	1.24	1.42

Table 4. Summaries of sR_0 from spatially nearest neighbour method.

Epidemic wave	Radius (km)										Mean
	69.3	130.0	256.9	468.0	599.9	668.2	729.9	791.4	859.3	955.6	
1	1.00	1.02	1.04	1.04	1.05	1.05	1.05	1.05	1.05	1.05	1.04
2	1.96	1.99	2.02	2.02	2.02	2.02	2.02	2.02	2.03	2.03	2.01
3	2.80	2.98	3.09	3.19	3.22	3.23	3.23	3.24	3.25	3.25	3.15
4	2.03	2.20	2.31	2.37	2.38	2.38	2.39	2.40	2.40	2.40	2.32
5	1.78	1.95	2.00	2.04	2.06	2.07	2.08	2.08	2.08	2.09	2.02
6	1.76	1.94	2.04	2.09	2.09	2.10	2.11	2.11	2.11	2.12	2.05

6. They are similar to those shown in Table 5, but with slightly higher values.

Relationships between sR_0/stR_0 and poultry density

All the correlation coefficients between sR_0 and poultry density and stR_0 and poultry density were between 0.028 and 0.203 (see Tables 7, 8 and 9). Some associations were statistically significant if 0.05 was set as the significance level (irrespective of multiple comparisons), but the magnitude of the corresponding correlation coefficient was small. No obvious and consistent relationships could be found between sR_0/stR_0 and poultry density.

Discussion

R_0 can be accurately quantified using transmission experiments under controlled indoor conditions (Bouma et al., 2009). However, R_0 values estimated under field conditions differ considerably from controlled experiments, e.g. the numbers of animals within herds or flocks, and the contact structure among animals, cannot be replicated in controlled experiments (van der Goot et al., 2003, 2005; Tiensin et al., 2007). R_0 estimated using actual field data has therefore more practical applicability for disease control. This study is the first attempt to estimate and compare the global transmissibility from sub-district to sub-district of HPAIV H5N1 across a large number of EWs, in this case stretching from December 2003 to December 2009. Such estimates can assist in the design of control programmes for future epidemics (Ward et al., 2009).

Three different R_0 values were estimated based on the attributes of time (tR_0), space (sR_0) and space-time (stR_0). The results of tR_0 from epidemic doubling time showed that tR_0 tended to decrease in magnitude over successive EWs. This suggests that the implemented control measures were effective in reducing transmissibility when using a non-spatial perspective. However, R_0 in EW6 was >1 indicating that the control measures were inadequate to interrupt the transmission cycle and probably insufficient to end the epidemic (Stegeman et al., 2004; Tiensin et al., 2007). The results of sR_0 and stR_0, calculated using spatially and spatio-temporally nearest neighbour approaches, respectively, showed similar features and are consistent with the overall spatial and spatio-temporal characteristics of the epidemic, i.e. the global HPAIV H5N1 epidemic started in EW1, peaked in EW3 and then gradually declined (Zhang et al., 2010, 2012). Because stR_0 showed a pattern similar to sR_0 (but not tR_0), we can infer that spatial transmissibility dominated non-spatial (temporal) transmissibility. This is consistent with previous studies that found that bird migration and transportation of poultry and poultry products are two important risk factors for the spread of HPAIV H5N1 (Gilbert et al.,

Table 5. Summaries of stR_0 from spatio-temporally nearest neighbour method (infectious duration = 7 days).

Epidemic wave	Radius (km)										Mean
	69.3	130.0	256.9	468.0	599.9	668.2	729.9	791.4	859.3	955.6	
1	0.95	0.97	0.99	1.01	1.01	1.02	1.02	1.02	1.02	1.02	1.00
2	1.65	1.75	1.80	1.82	1.84	1.87	1.87	1.88	1.88	1.88	1.82
3	2.13	2.36	2.53	2.70	2.76	2.78	2.79	2.81	2.82	2.85	2.65
4	1.29	1.51	1.68	1.85	1.90	1.92	1.93	1.95	1.96	1.98	1.80
5	1.24	1.48	1.61	1.67	1.71	1.73	1.74	1.74	1.74	1.78	1.64
6	0.67	1.08	1.31	1.60	1.65	1.68	1.69	1.70	1.70	1.70	1.48

Table 6. Summaries of stR_0 from spatio-temporally nearest neighbour method (infectious duration = 14 days).

Epidemic wave	Radius (km)										Mean
	69.3	130.0	256.9	468.0	599.9	668.2	729.9	791.4	859.3	955.6	
1	0.96	0.98	1.01	1.02	1.03	1.03	1.03	1.03	1.03	1.03	1.01
2	1.80	1.85	1.89	1.90	1.92	1.94	1.95	1.95	1.95	1.96	1.91
3	2.41	2.62	2.76	2.87	2.91	2.92	2.94	2.95	2.97	2.99	2.83
4	1.55	1.80	1.94	2.14	2.19	2.21	2.23	2.24	2.25	2.29	2.08
5	1.48	1.68	1.76	1.83	1.86	1.87	1.89	1.89	1.90	1.91	1.81
6	1.01	1.40	1.69	1.85	1.87	1.87	1.89	1.89	1.90	1.90	1.73

2006; Kilpatrick, 2006; Fang et al., 2008).

It is possible that sR_0 and stR_0 results were different from tR_0 because these metrics focus on different aspects of disease transmissibility, suggesting that their joint application can provide a more comprehensive profile of disease transmissibility from the temporal, spatial and spatio-temporal perspective. Previous studies only focused on one dimension of disease transmissibility, which could result in the loss of useful information. It is well known that R_0 is affected by many factors, including epidemiological, demographic and geographical features; density being perhaps the most important influence of HPAIV H5N1 transmission, at least in domestic poultry (Keeling and Eames, 2005; Sharkey et al., 2008; Soares Magalhães et al., 2010). We found that the magnitudes of correlation coefficients between R_0 and poultry density were all very small. If all possible corrections to address the problem of multiple comparisons, e.g., the Bonferroni correction method (Dunn, 1961) are taken into account, then, most of the results were non-significant. Hence, poultry density did not significantly affect the estimated R_0 at the sub-district level in our study. We inferred that other factors were unlikely to have a significant impact on the estimated R_0.

R_0, as estimated in this study, can provide insights regarding the potential risks for future influenza pandemics caused by HPAIV H5N1 variants if we compare it with R_0 from previous influenza pandemics based on the assumption that HPAIV H5N1 has similar transmis-

Table 7. Correlations between poultry density and sR_0 from spatially nearest neighbour method.

EW	Radius (km)									
	69.3	130.0	256.9	468.0	599.9	668.2	729.9	791.4	859.3	955.6
1.00	0.032(0.112)	0.033(0.110)	0.030(0.147)	0.030(0.144)	0.030(0.143)	0.031(0135)	0.031(0135)	0.031(0.134)	0.031(0.133)	0.031(0.133)
2.00	0.099(0.003)	0.097(0.003)	0.097(0.003)	0.096(0.003)	0.097(0.003)	0.097(0003)	0.098(0003)	0.098(0.003)	0.098(0.003)	0.099(0.003)
3.00	0.113(0.003)	0.077(0.040)	0.055(0.144)	0.030(0.431)	0.028(0.454)	0.029(0445)	0.026(0493)	0.026(0.491)	0.027(0.475)	0.026(0.492)
4.00	0.136(0.027)	0.095(0.124)	0.064(0.298)	0.062(0.310)	0.063(0.307)	0.063(0307)	0.063(0307)	0.063(0.310)	0.063(0.310)	0.063(0.304)
5.00	0.129(0.021)	0.084(0.136)	0.072(0.199)	0.049(0.385)	0.041(0.461)	0.035(0538)	0.033(0555)	0.033(0.553)	0.032(0.567)	0.032(0.566)
6.00	0.087(0.328)	0.083(0.345)	0.077(0.382)	0.079(0.374)	0.070(0.432)	0.068(0441)	0.066(0458)	0.066(0.458)	0.066(0.458)	0.053(0.547)

*correlation coefficient (P value).

Table 8. Correlations between poultry density and stR_0 from spatio-temporally nearest neighbour method (infectious duration = 7 days).

EW	Radius (km)									
	69.3	130.0	256.9	468.0	599.9	668.2	729.9	791.4	859.3	955.6
1.00	0.072(0.0004)	0.067(0.0009)	0.060(0.003)	0.053(0.008)	0.053(0.008)	0.052(0.010)	0.053(0.009)	0.053(0.009)	0.052(0.010)	0.052(0.011)
2.00	0.116(0.0002)	0.095(0.003)	0.080(0.012)	0.082(0.011)	0.079(0.014)	0.080(0.012)	0.080(0.012)	0.082(0.010)	0.082(0.010)	0.079(0.014)
3.00	0.182(<0.0004)	0.146(<0.0001)	0.104(0.004)	0.067(0.063)	0.063(0.084)	0.070(0.053)	0.074(0.041)	0.081(0.027)	0.081(0.026)	0.080(0.028)
4.00	0.128(0.030)	0.118(0.046)	0.125(0.038)	0.114(0.060)	0.131(0.030)	0.130(0.032)	0.138(0.023)	0.131(0.031)	0.127(0.037)	0.126(0.038)
5.00	0.041(0.444)	0.109(0.045)	0.118(0.030)	0.122(0.026)	0.102(0.063)	0.098(0.073)	0.103(0.061)	0.102(0.062)	0.102(0.062)	0.096(0.080)
6.00	0.189(0.015)	0.151(0.072)	0.174(0.037)	0.161(0.058)	0.161(0.059)	0.174(0.041)	0.167(0.050)	0.169(0.047)	0.169(0.047)	0.170(0.046)

*correlation coefficient (P value).

Table 9. Correlations between poultry density and stR_0 from spatio-temporally nearest neighbour method (infectious duration = 14 days).

EW	Radius (km)									
	69.3	130.0	256.9	468.0	599.9	668.2	729.9	791.4	859.3	955.6
1.00	0.056(0.006)	0.052(0.011)	0.04 1(0.042)	0.038(0.061)	0.035(0.083)	0.035(0.083)	0.035(0.083)	0.035(0.083)	0.035(0.083)	0.035(0.082)
2.00	0.103(0.001)	0.099(0.002)	0.096(0.003)	0.090(0.005)	0.089(0.006)	0.088(0.006)	0.088(0.006)	0.090(0.005)	0.090(0.005)	0.082(0.011)
3.00	0.157(<0.0001)	0.120(0.0001)	0.081(0.027)	0.065(0.079)	0.064(0.083)	0.075(0.043)	0.074(0.043)	0.070(0.059)	0.073(0.049)	0.070(0.059)
4.00	0.120(0.045)	0.102(0.094)	0.105(0.084)	0.107(0.082)	0.110(0.073)	0.107(0.081)	0.115(0.060)	0.112(0.067)	0.113(0.065)	0.115(0.061)
5.00	0.134(0.014)	0.189(0.0006)	0.189(0.0006)	0.175(0.001)	0.166(0.003)	0.164(0.003)	0.173(0.002)	0.173(0.002)	0.173(0.002)	0.173(0.002)
6.00	0.161(0049)	0.145(0086)	0.196(0.022)	0.197(0.024)	0.203(0.019)	0.203(0.019)	0.192(0.027)	0.192(0.027)	0.192(0.027)	0.192(0.027)

*correlation coefficient (P value).

sibility in poultry and humans. For the 1918-1919 influenza A (H1N1) pandemic, the R_0 estimates were between 1.3 and 3.0, depending on different models and model assumptions (Mills et al., 2004; Germann et al., 2006; Massad et al., 2007; Chowell et al., 2008; Zhang et al., 2010); R_0 was estimated to be 1.68 for the 1957-1958 influenza (H2N2) pandemic (Longini et al., 2004) and 1.89 for that of 1968-1969 (H3N2) (Rvachev and Longini, 1985). The fact that the R_0 estimates for HPAIV H5N1 in poultry, including our current estimates, were located in the same range as those of previous influenza pandemics suggests that HPAIV H5N1 constitutes a risk for a potentially pandemic influenza in humans based on transmissibility. However, more reasonable control strategies can be designed based on our knowledge of R_0 for the HPAIV H5N1 strain (Longini et al., 2005). The vaccination strategy is considered to be one of the most effective measures for controlling influenza, but it is important to determine the critical proportion of susceptible sub-districts that need to be immunised in order to interrupt an epidemic. This target proportion can be obtained using the formula $1\text{-}1/R_0$ (Anderson and May, 1991; Ferguson et al., 2005; Tiensin et al., 2007). Taking the R_0 in EW6 as an example, tR_0, sR_0, and stR_0 were 1.26, 2.05 and 1.48, respectively. Thus, the minimum vaccination coverage needed in the sub-district poultry population would be 21%, 51% and 32%, respectively, and the maximum value (51%) should be used as target to completely bring the epidemic under control. It should be acknowledged that vaccine efficacy is never 100% (van der Goot et al., 2005; Swayne et al., 2006; Webster et al., 2006), so an extra amount of vaccination coverage should always be added, e.g., 10% (van der Goot et al., 2005; Savill et al., 2006; Tiensin et al., 2007).

Two issues must be stressed. The first of these regards the assumption of the approaches used in this study to estimate R_0. It was supposed, according to tR_0, that the epidemic phase follows an exponential distribution. With regard to sR_0, the Euclidean distance-based nearest neighbouring sub-district was presumed to be the infection source for each outbreak of interest, which assumes that spatial "closeness" is the strongest factor influencing the spread of HPAIV H5N1. The assumption of the stR_0 method was that the Euclidean distance-based nearest neighbouring sub-district within the infectious duration window was the infection source for each following outbreak of interest, which assumed that proximity in both time and space is the strongest factor driving the spread of the HPAIV H5N1 virus (Ward et al., 2009). The infectious duration is an important parameter for estimating tR_0 and stR_0 (Ferguson et al., 2006; Chowell et al., 2007, 2008; Andreasen et al., 2008; Zhang et al., 2010), but accurate estimates of the duration of infectiousness at the sub-district level are unavailable (Garske et al., 2007) so different researchers have assumed different values (Mannelli et al., 2007; Tiensin et al., 2007). In this study, we assumed that sub-district-based infectious duration was 7 days, which remained constant during the whole epidemic similar to the village-based study conducted by Ward et al. (2009). To investigate the robustness of the results, we also assumed a value of 14 days. Both values of tR_0 and stR_0 assuming a 14-day infectious duration were slightly higher than those assuming a 7-day infectious duration, but their results were robust and use of either estimate arrive at the same conclusions. The second issue is that the potential underreporting and large numbers of asymptomatic infections can result in an incomplete outbreak database (Cauchemez et al., 2006; Chowell et al., 2007; Glass et al., 2007), which may bias the results. However, outbreaks in our study were re-defined as the confirmed presence of HPAIV H5N1 (clinically expressed or not) in at least one poultry flock in a specified sub-district during a certain period of time (Zhang et al., 2010). In our study, this definition probably reduced the impact of underreporting at the flock-level to some extent. However, the magnitude of the influence due to underreporting cannot be evaluated, exactly because the true numbers of outbreaks is an unknown parameter.

Conclusion

The joint application of these R_0 estimation methods applied in this first study to quantify the R_0 of HPAIV H5N1 transmission within domestic poultry by sub-district at the global level considering temporal, spatial and spatio-temporal perspectives, provides a more complete profile of the transmissibility of this virus. The results indicate that currently implemented control measures are effective to reduce the number of HPAIV H5N1 outbreaks, but not to prevent the spatial spread of the infection. Since it is inadequate to interrupt the transmission cycle, close monitoring of HPAIV H5N1 and an enhanced control policy are strongly needed.

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