Ecologic Risk Factor Investigation of Clusters of Avian Influenza A (H5N1) Virus Infection in Thailand

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(See the article by Vong et al. on pages XXX–XXX, the article by Zhou et al. on pages XXX–XXX, and the editorial commentary by Briand and Fukuda on pages XXX–XXX)

This study was conducted to investigate space and time clusters of highly pathogenic avian influenza A (H5N1) virus infection and to determine risk factors at the subdistrict level in Thailand. Highly pathogenic avian influenza A (H5N1) was diagnosed in 1890 poultry flocks located in 953 subdistricts during 2004–2007. The ecologic risk for H5N1 virus infection was assessed on the basis of a spatial-based case-control study involving 824 case subdistricts and 3296 control subdistricts from 6 study periods. Risk factors investigated in clustered areas of H5N1 included human and animal demographic characteristics, poultry production systems, and wild birds and their habitats. Six variables remained statistically significant in the final model: flock density of backyard chickens (odds ratio [OR], 0.98), flock density of fighting cocks (OR, 1.02), low and high human density (OR, 0.60), presence of quail flocks (OR, 1.21), free-grazing duck flocks (OR, 2.17), and a poultry slaughterhouse (OR, 1.33). We observed a strong association between subdistricts with H5N1 virus–infected poultry flocks and evidence of prior and concomitant H5N1 infection in wild birds in the same subdistrict.

Since 2003, outbreaks of highly pathogenic avian influenza (HPAI) H5N1 virus infection have resulted in a high number of affected animals, losses in domestic and international trade of poultry products, socioeconomic impacts, impacts on farmers' livelihoods, and public health consequences. The disease spread widely in >60 countries across Asia, Europe, Africa, and the Middle East [1, 2]. As of 10 November 2008, H5N1 virus transmitted from infected birds had caused 387 human cases, 245 of which were fatal [2–4]. Outbreaks of HPAI H5N1 have emerged and persisted mainly in East and Southeast Asia (southern China [5], central Thailand [6], northern and southern Vietnam [7], and Indonesia [8]). Therefore, it is of interest to study the risk factors associated with disease occurrence in specific locations (cluster areas) and to determine whether they may be present in certain places only at certain times, taking into account bias in H5N1 case detection, control measures, or changes in demographic characteristics in at-risk populations. Risk factors within cluster areas have been investigated for other infectious diseases (e.g., bovine spongiform encephalopathy [9], severe acute respiratory syndrome [10], sleeping sickness [11], and West Nile virus [12]). However, despite the fact that HPAI remains a major threat for animal and public health worldwide [3], the risk factors in H5N1 clusters have remained largely unexplored.

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The HPAI H5N1 epidemics in Thailand during 2004–2007 provided epidemiological data for the study of risk factors associated with the reemergence, spread, and persistence of H5N1 in cluster areas. Although the extent and location of clusters was not well described, field data have allowed for an epidemiological study of H5N1 virus infection in poultry and wild birds. We investigated a number of potential risk factors for H5N1 clusters, including differences and variety in agro-ecology, human and animal demographic characteristics, poultry production systems, and wild birds and their habitats. The latter has not yet been epidemiologically and systematically studied, although it is increasingly acknowledged that the association between infected wild birds and poultry plays a critical role in the maintenance and spread of influenza A virus [13–15].

In this study, we examined the spatial and temporal clusters of outbreaks of HPAI H5N1 at the subdistrict level in Thailand during 2004–2005. We identified risk factors associated with H5N1 virus infection in high-cluster areas with use of a spatial-based case-control study. We also performed a retrospective subdistrict-level analysis of the data from 2004–2005 for the presence of sick or dead wild birds with laboratory evidence of HPAI H5N1 in case and control subdistricts.

MATERIALS AND METHODS

Study population. Data on outbreaks of H5N1 in Thailand have been collected since January 2004 by the Department of Livestock Development, Thailand. The virus was confirmed in sick or dead birds and cloacal samples from poultry and wild birds by diagnostic laboratories with use of reverse-transcriptase polymerase chain reaction and virus isolation [16]. The data at the subdistrict level included H5N1 detection date, location and species of dead or sick poultry, animal and human demographic characteristics, evidence of H5N1 virus infection in wild birds, wild bird habitats, and location of poultry establishments (i.e., slaughterhouses, feed mills, and poultry farms). The animal population census data from 2004 were used; the spatial distribution of poultry and flock density in Thailand are shown elsewhere [6]. The outbreak data were based on both the mandatory clinical disease reporting system and the nationwide active surveillance program known as the "X-ray survey," which is described in detail elsewhere [6, 16]; these 2 systems are complementary and ensure disease detection. To detect H5N1 virus infection in wild birds, dead birds that met selection criteria (i.e., the bird died recently and had little apparent decay or trauma) and cloacal samples from live birds were collected for testing for influenza A virus as part of the ongoing wild bird surveillance program. Approximately 10,580 samples were collected during 2004–2005, including either cloacal swab samples or dead wild birds. For the temporal analysis, we used the data aggregated during 2004–2005 for the 6 following study periods: January–February 2004 (period 1), July–August 2004 (period 2), October–November 2004 (period 3), January–February 2005 (period 4), July–August 2005 (period 5), and October-November 2005 (period 6); the last 4 periods were based on the national active surveillance programs. For evidence of H5N1 virus infection in wild birds, we included wild bird infection detected within the month before and the month after the outbreak among poultry in each subdistrict for the 6 study periods. During 2006–2007, H5N1 occurred sporadically in Thailand. This period was excluded from further analysis, because no cluster pattern was detected.

Disease mapping and cluster detection methods. Thailand is administratively divided into 76 provinces (each with its own veterinary services) and is additionally partitioned into 926 districts and 7327 subdistricts (mean area, 70.37 km^2; median area, 45.59 km^2; range, 0.13–2383 km^2); subdistrict data were used in this analysis. To minimize errors in mapping, the locations of H5N1 virus–infected flocks were cross-referenced with the subdistrict names and specific location identification numbers. ArcGIS, version 9.2 (ESRI), was used for mapping and visualization. Incidence (crude rate estimate) was calculated and shown spatially for all subdistricts. An empirical Bayes (EB)–smoothing method was applied to H5N1 outbreak data; this smoothing technique adjusted disease rates, especially in areas with small population estimates, toward the overall mean of the study areas [17]. To examine the spatial clustering of H5N1 outbreaks at a subdistrict level, global and local spatial autocorrelation analyses were performed. First, the spatial pattern of feature values formed by incidence rates and EB-smoothed rates was measured. Global Moran's I spatial autocorrelation statistic (using GeoDa, version 0.9.5-I [18]) was used to quantify spatial dependence [19, 20]. This technique simply reveals whether there is spatial aggregation of the incidence or the EB estimates or whether high or low values of the data are interspersed. It provides a global measure of spatial autocorrelation but can only identify the presence of a cluster, not its specific location. Second, 2 local cluster detection methods were applied to localize the specific clusters as hot spots or cold spots of H5N1 outbreaks. The local spatial autocorrelation analysis was conducted using the GeoDa cluster detection program to generate Anselin's Local Moran test statistics. Local Moran's statistics assess spatial autocorrelation and identify clustering subdistricts with disease rates statistically similar to or dissimilar from their neighbors on the basis of aggregated data [19, 21]. A spatial scan test by SaTScan, version 7.0.3 [22], was then used to fully examine our hypothesis that HPAI would show spatial clustering over subdistricts [23]. The spatial scan statistic test assesses disease distribution with use of centroids and a circular scan. Because different analytical methods may identify different underlying spatial patterns [24], we sought more-robust results with consistent findings. The same cluster subdistricts should be identified with 2 methods.

Case-control selection process and statistical analysis. According to Tobler's first law of geography, everything is related to everything else, but near things are more related than distant things [25]. Case and control subdistricts were selected

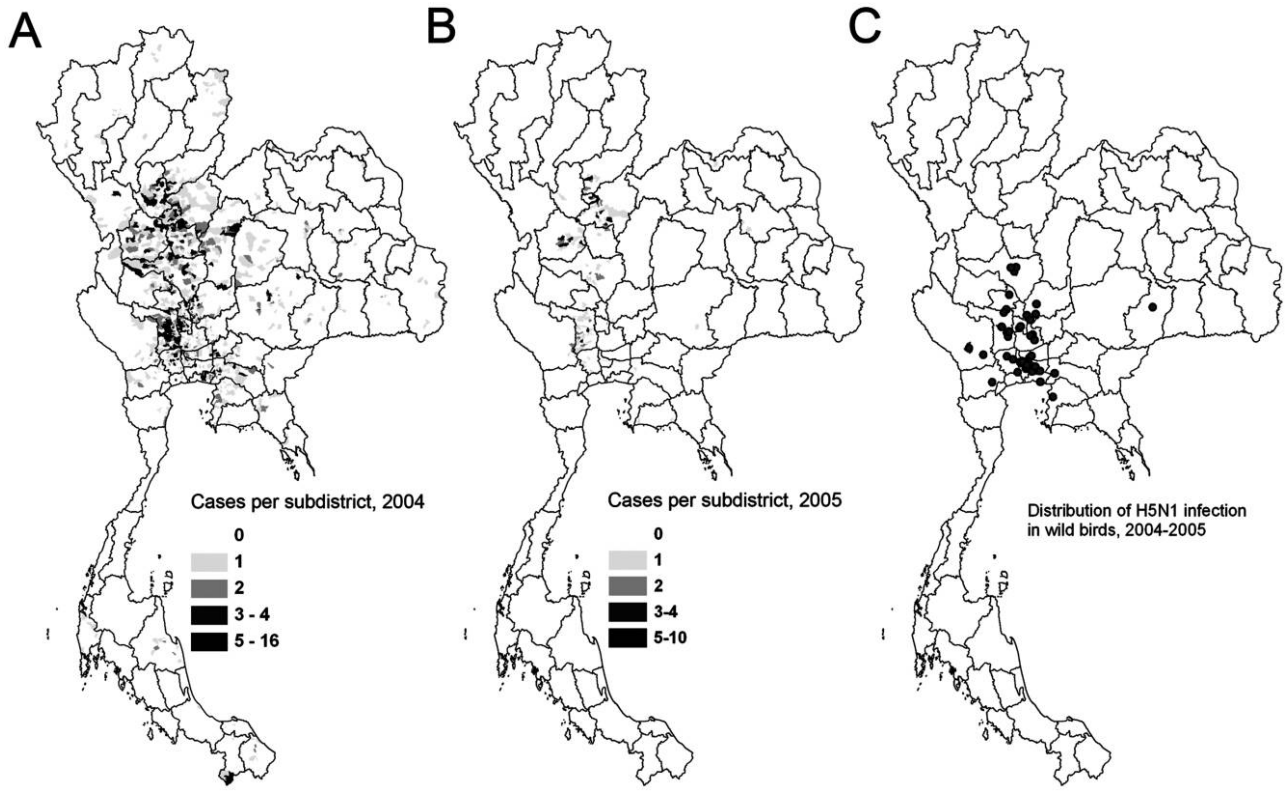

Figure 1. Number of flocks of domestic poultry infected with highly pathogenic avian influenza A (H5N1) virus, by subdistrict in Thailand. *A,* Outbreaks during 2004. *B,* Outbreaks during 2005. *C,* Distribution of H5N1 virus infection among wild birds during 2004–2005 (province boundaries are also shown).

on the basis of the aforementioned spatial cluster analysis. Different buffers (radius, 10–60 km) were created for the case-control selection process. The eligible buffer for selection of case and control subdistricts was defined as a 50-km radius buffer around the centroid of identified cluster subdistricts for the 6 different periods during 2004–2005. Subsequently, all subdistricts within the 50-km radius buffer per period were candidates for case-control selection. The subdistricts with $\geqslant 1$ flock infected with HPAI H5N1 virus during a period were defined as case subdistricts. All remaining subdistricts within the 50-km buffer were candidates for control subdistricts, which were randomly selected with a case-control ratio of 1:4. All variables were individually tested for an association with the case-control status of a subdistrict by univariable logistic regression analysis for each study period and then for all periods combined. Variables that were statistically significant at $P \leqslant .2$ were included for further analysis. To take into account possible nonlinear relations, all continuous variables were categorized using a decile classification scheme. When appropriate, variables were categorized before further analysis [26]. When possible, data were reclassified into plausibly biological appropriate categories. Subsequently, multivariable logistic regression analysis was used to assess an association between the independent variables and the dependent variable (which was either case or control status of a subdistrict). Finally, the 6 study periods were added as fixed variables. A final model was fitted using a backward stepwise procedure. Statistical significance of risk factors was assessed using the likelihood ratio test based on $P \leqslant .05$. Collinearity was assessed by correlation coefficients ($|\rho| < 0.5$) among all covariates to be considered for inclusion in the final model [26]. We then assessed model fit with use of the Hosmer-Lemeshow goodness-of-fit test and the ratio of the deviance to the degree of freedom. Regression coefficients were converted into odds ratios (ORs; $e\beta$) and their 95% confidence intervals (CIs) [27].

RESULTS

Study population. From January 2004 through December 2007, a total of 1890 poultry flocks with laboratory-confirmed H5N1 were detected in 953 (13%) of the 7327 subdistricts; 1323 (70%) of the 1890 H5N1 virus–infected flocks were found mostly in central and northern Thailand. The distribution and number of infected flocks by subdistrict during 2004–2005 are shown in figure 1*A* and 1*B*. The median number of infected flocks per subdistrict was 1 (range, 1–16 flocks); there were 398 subdistricts in which $\geqslant 1$ infected flock was reported. Of the 7327 subdistricts, 48 subdistricts were excluded because of lack of available data; this resulted in a study sample of 7279 subdistricts. On the basis of the cluster analysis, 824 case subdistricts and 3168 control subdistricts were selected for inclusion in this study. Figure 1*C* shows the distribution and

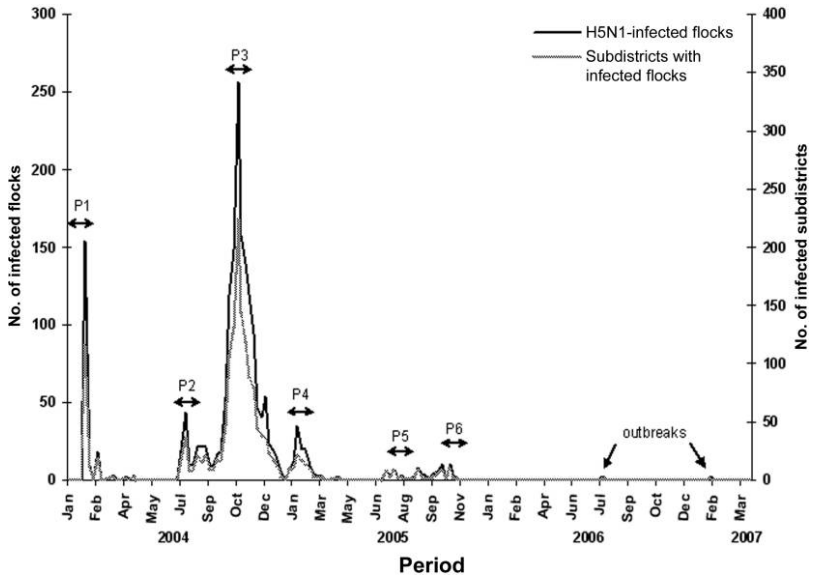

Figure 2. Weekly epidemic curve of the number of flocks infected with highly pathogenic influenza A (H5N1) virus and the number of subdistricts with infected flocks, Thailand, 2004–2007. P1, January–February 2004; P2, July–August 2004; P3, October–November 2004; P4, January–February 2005; P5, July–August 2005; P6, October–November 2005.

location of H5N1 virus–infected wild birds found in 40 subdistricts in Thailand during 2004–2005. The epidemic curve of H5N1 outbreaks from January 2004 through December 2007 and a curve of the total number of subdistricts infected per week are shown in figure 2. The latter curve gives an indication of the spatial extent of HPAI H5N1, and the former curve indicates the magnitude of the epidemic. The dramatic increase in incidence from October and November 2004 through January and February 2005 is related to the effort of the nationwide active surveillance programs. The number of cases and subdistricts with infected flocks decreased in 2005, and outbreaks occurred sporadically during 2006–2007.

Disease mapping and cluster detection. Figure 3 shows maps of EB-smoothed incidence of HPAI per subdistrict in each of the 6 study periods. As suggested in the EB-smoothed rate map and by local cluster detection, EB rates of H5N1 virus infection for clusters at high risk ranged from 1.0 to 73.7 infected flocks per 1000 flocks from July through August 2004 and from 1.7 to 62.4 infected flocks per 1000 flocks from October through November 2004. Global spatial autocorrelation was investigated using Moran's I statistics (table 1); we found that the Z score increased during 2004, which indicates that outbreaks became more geographically fixed. The spatial pattern showed strong clustering during 2 periods: from October through November 2004 and from January through February 2005 ($P < .001$). On the basis of the local Moran statistics test, various multicentered clusters of high HPAI risk were detected from January 2004 through February 2005, and 2 multicentered clusters were detected from July through August 2005 (figure 3). The spatial scan test indicated that the primary multiclusters were located in the central region; the relative risk was 26.9 during July–August 2004 and 38.6 during October–November 2004 ($P < .001$). Secondary clusters were identified in the areas adjacent to the central region; the relative risk was 39.2 during July–August 2004 and 5.5 during October–November 2004 ($P < .001$ for both). The clusters at high risk of HPAI that were detected in GeoDa were either included in or overlapped the clusters identified using SaTScan. The most likely low-risk areas were located in southern and northeastern Thailand ($P < .001$). All low-risk areas were statistically consistent with use of both methods. Although several individual subdistricts appeared to have elevated risk with use of the EB-smoothing method, compared with incidence rate, these were not identified as a statistically significant cluster by either the Local Moran test or the spatial scan test.

Risk factors based on the subdistrict level analysis. A total of 824 case subdistricts with flocks with H5N1 virus infection were identified in a 50-km radius buffer around the centroid of high-cluster subdistricts during the 6 study periods. In addition, a total of 3296 control subdistricts were identified among candidate subdistricts during each study period (table 1). Table 2 shows the variables that yielded a P value $\leq .2$ in the univariable analysis. Eleven of 31 variables were considered for inclusion in the multivariable logistic regression model. In the univariable analysis, the findings reveal that subdistricts with commercial poultry flocks (i.e., broilers; OR, 1.31 [95% CI, 1.10–1.55]), laying chickens (OR, 1.38 [95% CI, 1.12–1.70]), quails (OR, 1.31 [95% CI, 1.10–1.55]), and flock density of meat and laying ducks (OR, 1.07 [95% CI, 1.01–1.15]) were more likely to have infected flocks than were other subdistricts. Subdistricts with free-grazing duck flocks showed an association with H5N1 virus infection (OR, 2.48 [95% CI, 2.12–2.90]). We found a strong association between case subdistricts and evidence of prior

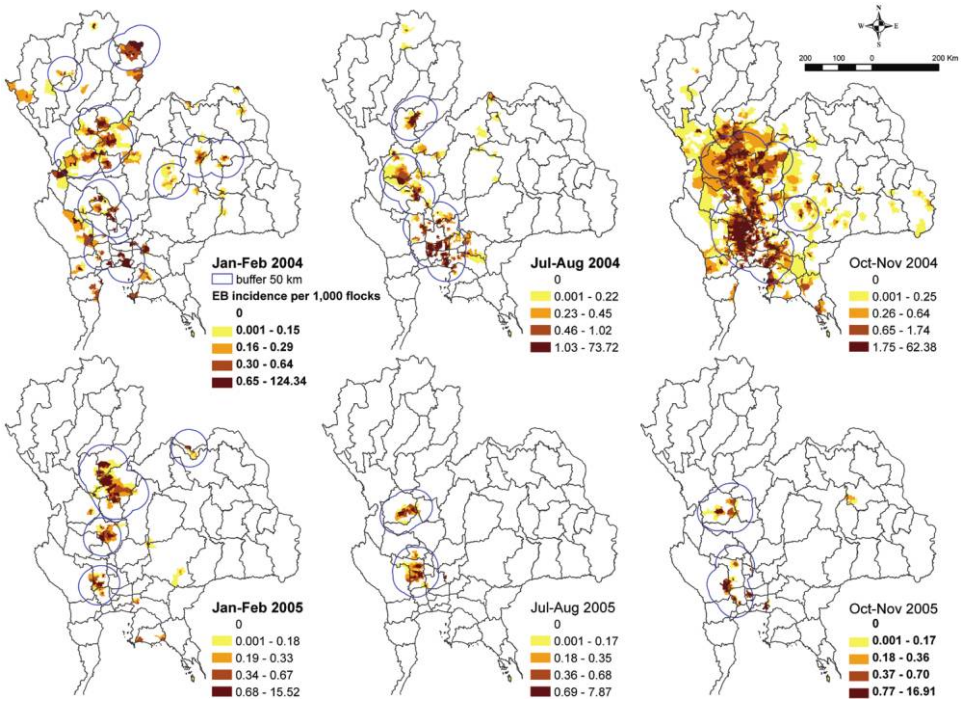

Figure 3. Empirical Bayes (EB)–smoothed incidence of highly pathogenic influenza A (H5N1) virus infection, by subdistrict, for the 6 periods with epidemics during 2004–2005. A 50-km buffer around identified cluster subdistricts is shown. Legend categories vary between maps; province boundaries are also shown.

and concomitant H5N1 virus infection in wild birds in the same subdistrict (OR, 4.00 [95% CI, 1.62–9.85]; $P < .001$). However, only 5 subdistricts had H5N1 detected in wild birds prior to the study periods. Period was forced as a covariate in the model, but it was not statistically significant. We found a statistically significant relationship between the evidence of HPAI in wild birds in subdistricts per period. However, wild birds were not included in the multivariable analysis because of incomplete records (i.e., for many subdistricts, no wild birds were tested). The results of the multivariable logistic regression model, corrected for period, are shown in table 3. Six variables remained in the final model: (1) flock density of backyard chickens (as protective factor), (2) fighting cocks, (3) human density, (4) presence of quail flocks, (5) free-grazing duck flocks, and (6) poultry slaughterhouse. The Hosmer-Lemeshow statistics value for goodness-of-fit of the final model was 8.7 (degree of freedom, 8; $P = .37$), which was considered to be acceptable [27]. No confounding factors were observed during the model-building process.

DISCUSSION

Disease mapping and spatial clustering. Our study detected significant spatial clusters of HPAI at a subdistrict level in Thailand during 2004–2005 with use of 2 local cluster detection

Table 1. Results of the global spatial autocorrelation by Moran's I statistics, based on the incidence of highly pathogenic influenza A (H5N1) virus infection, by subdistricts, for the 6 study periods and combined during 2006–2007.

Study period	Moran's I statistic	Z score	*P*	Pattern	No. of case subdistricts	No. of control subdistricts
January–February 2004	0.02	4.58	.006	Clustered	101	404
July–August 2004	0.05	9.71	.002	Clustered	87	348
October–November 2004 (X-ray 1 survey)	0.11	18.42	.001	Clustered	536	2016
January–February 2005 (X-ray 2 survey)	0.06	10.12	.002	Clustered	54	216
July–August 2005 (X-ray 3 survey)	0.05	9.33	.003	Clustered	24	96
October–November 2005 (X-ray 4 survey)	0.01	1.65	.1	Less clustered[a]	22	88
January 2006–October 2007	−0.007	−0.05	.78	Random	. . .	. . .

NOTE. The data from 2006–2007 were excluded from additional case-control study.

[a] Compared with the study periods during January 2004–August 2005.

Table 2. Univariable analysis of potential risk factors for highly pathogenic influenza A (H5N1) virus infection in domestic poultry at the subdistrict level, Thailand, 2004–2005 (with 6 study periods combined).

Variable	No. (%) of case subdistricts (n = 824)	No. (%) of control subdistricts (n = 3168)	OR (95% CI)	P
Backyard chicken flock density (flocks per km^2)	. . .	. . .	0.99 (0.98–1.00)	.1
Fighting cock flock density (flocks per km^2)	. . .	. . .	1.02 (1.01–1.03)	.005
Meat and laying duck flock density (flocks per km^2)	. . .	. . .	1.07 (1.01–1.15)	.032
Broiler flock in subdistrict				
Present	603 (73)	2140 (68)	1.31 (1.10–1.55)	.002
Absent	221 (27)	1028 (32)		
Laying hen flock in subdistrict				
Present	696 (84)	2526 (80)	1.38 (1.12–1.70)	.002
Absent	128 (16)	642 (20)	. . .	
Quail flock in subdistrict				
Present	444 (54)	1496 (47)	1.31 (1.12–1.52)	.001
Absent	380 (46)	1672 (53)	. . .	
Free-grazing duck flock in subdistrict				
Present	496 (60)	1199 (38)	2.48 (2.12–2.90)	<.001
Absent	328 (40)	1969 (62)	. . .	
Human density (persons per km^2)				
<60	48 (6)	306 (10)	0.52	<.001
60–430	655 (79)	2164 (68)	1	<.001
>430	121 (15)	698 (22)	0.57	<.001
Household density (houses per km^2)				
<15	42 (5)	286 (9)	0.49	<.001
15–124	655 (79)	2190 (69)	1	<.001
>124	127 (15)	692 (22)	0.61	<.001
Slaughterhouses in subdistrict				
Yes	164 (20)	484 (15)	1.38 (1.13–1.68)	.001
No	660 (80)	2684 (85)	. . .	
Evidence of H5N1 virus infection in wild birds[a]				
Yes	15 (2)	8 (0.3)	4.00 (1.62–9.85)	.003
No	75 (9)	160 (5)	. . .	
Unknown	734 (89)	3000 (95)	. . .	

NOTE. Variables with $P < .2$ were used in multivariable analysis. CI, confidence interval; OR, odds ratio.

[a] Data are for 258 subdistricts.

methods: Anselin's Local Moran test and the spatial scan statistic test. Both of these cluster detection methods identified approximately the same clusters at high risk and low risk of HPAI, which suggests that our results are robust. Consistent results of both the incidence of HPAI outbreak data and the EB rate–smoothing technique also improved our understanding of the geographic distribution of HPAI by indicating that the disease was likely to occur in areas surrounding hot spots.

Figure 3 shows that H5N1 clusters were scattered all over the country during January–February 2004. This may indicate long-distance spread of the disease through transportation of poultry and poultry products. After control measures had been implemented (i.e., restrictions on transportation of commercial chickens, free-grazing ducks, and fighting cocks and pretesting for H5N1 infection in poultry flocks before movement) [6], the outbreaks mostly occurred in high-cluster areas in central Thailand (figures 1 and 3).

Risk factors. We used a novel approach to select control subdistricts, such that they originated from a high-risk area. The identified risk factors, therefore, would truly differentiate between case and control status. The origin of H5N1 epidemics is complex and multifactorial. Although many of the risk factors in the current study appear to be plausible, an association does not equal causation. As the hypothesized causal pathway in figure 4 shows, multiple factors can cause the disease. This pathway was used to identify potential risk factors in our study at the ecologic subdistrict level. The results show that subdistricts with a high flock density of fighting cocks and with quail and free-grazing duck flocks had higher infection rates than did subdistricts with low flock density of these poultry types. Subdistricts with poultry

Table 3. Results of a multivariable logistic regression model for highly pathogenic influenza A (H5N1) status per subdistrict, Thailand, 2004–2005, corrected for period.

Variables	Adjusted OR (95% CI)	*P*
Backyard chicken flock density (flocks per km^2)	0.98 (0.96–0.99)	.001
Fighting cock flock density (flocks per km^2)	1.02 (1.01–1.04)	.008
Quail flock in subdistrict	1.21 (1.03–1.42)	.02
Free-grazing duck flocks	2.17 (1.84–2.56)	<.001
Human density (persons per km^2)		
<60	0.57 (0.41–0.80)	.009
60–430	1	<.001
>430	0.63 (0.49–0.80)	.001
Slaughterhouse in subdistrict	1.33 (1.08–1.63)	.007

NOTE. H5N1 virus infection in wild birds by subdistrict was excluded from the final model, because the it is a subset of all observations (subdistricts). The Hosmer-Lemeshow statistics value for goodness-of-fit of the final model was 8.7 (degree of freedom, 8; $P = .37$). CI, confidence interval; OR, odds ratio.

slaughterhouses were more frequently infected than were those without slaughterhouses. Subdistricts with poultry slaughterhouses may have increased activities related to transportation of poultry that increase the likelihood that the virus will be brought into these subdistricts. Live-bird markets were not included in this study as a risk factor (although they were included in previous studies [28–30]), because there are very few live-bird markets in Thailand. The sale of live chickens or ducks in the retail market is neither practical nor popular in Thailand, compared with other East and Southeast Asian countries [15, 30, 31]. A poultry slaughterhouse functions as a dissemination point for H5N1 infection, because poultry from many sources (from duck, chicken, and quail farms and free-grazing ducks) are brought there for slaughter, and the movement of cages to and from such slaughterhouses may disseminate virus back to farms. Such mechanisms have been postulated to occur in relation to live poultry markets. Understanding interactions between animals and humans is critical in preventing outbreaks of zoonotic diseases. Our study reveals that the odds of H5N1 virus infection in a subdistrict were significantly higher in the subdistricts with commercial poultry flocks than in subdistricts with backyard flocks. Also, our analysis indicates that subdistricts with lower or higher human population densities had a lower incidence of infection than did those with medium density. This may reflect the fact that both subdistricts with low human density and those with high human density have smaller poultry populations and fewer poultry production–related activities. It seems that certain poultry farming activities (e.g., commercial poultry farming, local slaughterhousing, and cock fighting) increase infection risk at the subdistrict level. Therefore, biosecurity of those activities should be revisited. Understanding the relationship between subdistricts with infected flocks and the presence of free-grazing

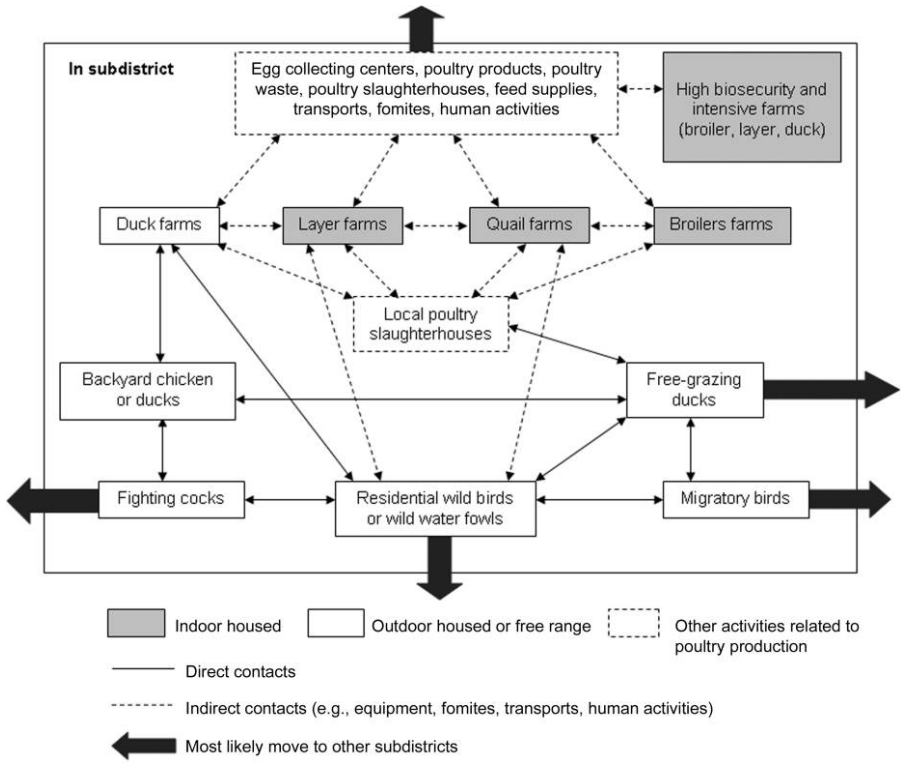

Figure 4. Hypothetical causal pathways of highly pathogenic influenza A (H5N1) virus transmission within and between subdistricts in Thailand.

duck flocks is crucial, and our findings agree with those of previous studies [16, 32]. We observed a significant association between subdistricts with H5N1 virus–infected flocks and evidence of H5N1 virus infection in wild birds in the same subdistrict. Although significantly associated with infection in the univariable analysis, the presence of infected wild birds could not be included in the final model because of missing data on the subdistrict level. Of interest, the presence of infected wild birds within the subdistricts was a significant risk factor, but we did not find an association between wild bird habitats and subdistricts with infected flocks. Therefore, the continued monitoring of influenza A virus infection in wild birds is essential.

Over the 2-year study period, all H5N1 outbreak data were collected systematically across the country, minimizing the chance that unmeasured localized events either temporally or spatially confounded the risk estimates. We found no statistical relationship between H5N1 virus infection in subdistricts and period. Undetected or underreported cases in backyard poultry may have occurred during the early outbreaks in 2004, leading to misclassification of the control status of subdistricts and also influencing incidence estimates in each subdistrict studied [33]. Because of the clustered nature of the epidemics, case subdistricts in one period could be control subdistricts in another period and vice versa. This will have caused nondifferential misclassification in our risk factor analysis [26]. Such misclassification could only bias the results toward the null effect, suggesting even stronger ecologic relations than those reported.

Prevention and control implications. Real-time geographic cluster analysis of H5N1 outbreaks could provide a basis for targeted public education and surveillance activities. Poultry production in Thailand varied from small-scale to large-scale or industrial-scale operations. The larger, integrated commercial chicken farms, operating with modern facilities, tend to be located in the eastern and northeastern regions. Such farms had fewer contacts, combined with enhanced biosecurity, compared with other farms. Of note, these farms were located outside the main affected areas. However, many commercial poultry farms in Thailand operate as small or medium family-run businesses [6, 13], which were associated with subdistricts with a high risk of infection in our study. There is substantial evidence of pathogen movement between and among such farming facilities and release to the external environment [34–36]. These ecologic data suggest that efforts to control H5N1 must consider risk factors related to the long distance movement involved with certain poultry activities (i.e., fighting cocks and free-grazing ducks) and also with commercial poultry production (i.e., transportation of poultry, poultry products, equipment, waste, and by-products). Similarly, our findings reveal that subdistricts with slaughterhouses more frequently had infected flocks than did those without slaughterhouses. Subdistricts with slaughterhouses, therefore, could be targeted as locations needing protective and monitoring measures. These data also suggest that successful strategies to prevent and control HPAI outbreaks must consider risk factors specific to certain types of poultry production. However, the underlying disease dynamics take place at a between-flock level [37] and not at a between-subdistrict level. Therefore, additional study of flock-level risk factors is needed.

Our analysis demonstrated an association between H5N1 infection in wild birds concomitant with the outbreak among poultry in subdistricts. This may reflect spill-over from the infected poultry to affected wild birds, rather than implicate wild birds as the vector introducing the virus to poultry. When H5N1 virus infection is identified in poultry or wild birds, a clustering pattern may provide additional confirmation of an ongoing epizootic and help define the geographic area of increased animal and human risk. The geographic analysis of H5N1 virus–infected dead bird reports may sometimes provide an early warning of viral activity among wild birds and subsequent domestic poultry infection.

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