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**Environmental Factors Influencing the Spread of the Highly Pathogenic
Avian Influenza H5N1 Virus in wild birds in Europe**

[Yali Si](#)^{1,2,3}, [Tiejun Wang](#)¹, [Andrew K. Skidmore](#)¹, [Willem F. de Boer](#)², [Lin Li](#)³, and [Herbert H.T. Prins](#)²

ABSTRACT. A large number of occurrences of the highly pathogenic avian influenza (HPAI) H5N1 virus in wild birds were reported in Europe. The relationship between the occurrence pattern and environmental factors has, however, not yet been explored. This research uses logistic regression to quantify the relationships between anthropogenic or physical environmental factors and HPAI H5N1 occurrences. Our results indicate that HPAI H5N1 occurrences are highly correlated with the following: the increased normalized difference vegetation index (NDVI) in December; intermediate NDVI in March; lower elevations; increased minimum temperatures in January; and reduced precipitation in January. A predictive risk map of HPAI H5N1 occurrences in wild birds in Europe was generated on the basis of five key environmental factors. Independent validation of the risk map showed the predictive model to be of high accuracy (79%). The analysis suggests that HPAI H5N1 occurrences in wild birds are strongly influenced by the availability of food resources and are facilitated by increased temperatures and reduced precipitation. We therefore deduced that HPAI H5N1 occurrences in wild birds in Europe are probably caused by contact with other wild birds and not by contact with domestic poultry. These findings are important considerations for the global surveillance of HPAI H5N1 occurrences in wild birds.

Key Words: *avian influenza; anthropogenic environmental factor; Europe; HPAI H5N1; physical environmental factor; risk mapping; wild birds*

INTRODUCTION

The global spread of the highly pathogenic avian influenza (HPAI) H5N1 in poultry, wild birds, and humans poses a pronounced panzootic threat and a serious public health risk. During the second half of 2005, the HPAI H5N1 virus was detected outside Asia, appeared in Russia, and then arrived in Romania in October (Gilbert et al. 2006a). In 2006, the disease became panzootic around the Black Sea region, the Mediterranean region, Western Europe and Africa (Si et al. 2009). Kilpatrick et al. (2006) suggested that movements of wild birds and trade in domestic poultry and wild birds could act as potential agents for the global dispersion of the HPAI H5N1 virus. An efficient surveillance and disease control system requires a greater understanding of the mechanism responsible for the spread of the HPAI H5N1 virus. Two different hypotheses are currently under investigation. The first argues that human transport of infected

domestic poultry is the underlying mechanism responsible for the spatial pattern in the disease occurrence, whereas the second attests that wild birds (mainly waterfowl) are spreading the disease. These two hypotheses are not mutually exclusive because the two mechanisms can interact.

Because the HPAI H5N1 virus is associated with both migratory and domestic bird populations, it is likely that environmental factors play a significant role in the spread of the disease. A number of efforts have been made to investigate the influence of environmental factors on HPAI H5N1 occurrence. The occurrence of the HPAI H5N1 virus in Nigeria and neighboring countries in West Africa has been linked to differences in plant phenology and land-surface reflectance (Williams et al. 2008). In Southeast Asia, HPAI H5N1 outbreaks were correlated with free-range duck farming and rice-paddy cultivation (Gilbert et al. 2006b, Gilbert et al. 2008). In China, annual precipitation, the minimum

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distance to national highways, and the interaction between minimum distance to the nearest lake and wetland were found to be correlated with the risk of HPAI H5N1 occurrence (Fang et al. 2008). Land-use patterns, the presence of seasonal wetlands, backyard poultry and animal husbandry, as well as the density of the human population, were identified as associated with the HPAI H5N1 virus in the Indian subcontinent (Adhikari et al. 2009). In Bangladesh, HPAI H5N1 outbreaks in backyard chickens were associated with offering slaughter remnants of purchased chickens to backyard chickens, the closeness to water bodies, and contact with pigeons (Biswas et al. 2009). In the Middle East and northeast Africa, HPAI H5N1 cases occurred in areas with large seasonal variation in NDVI values (Williams and Peterson 2009). Inconsistencies in predictions based on HPAI H5N1 occurrences were also reported in different sub-regions, suggesting that certain environmental factors may be of greater importance in some areas than others (Williams and Peterson 2009). Risk factors affecting one specific area may not affect the distribution of the virus elsewhere. The correlation between environmental factors and the HPAI H5N1 virus in Europe has yet to be investigated. The studies referred to above were mainly based on outbreaks in poultry and revealed that both anthropogenic and physical environmental factors have some bearing on the disease outbreaks, suggesting that both domestic poultry and wild birds facilitate the spread of the HPAI H5N1 virus in poultry. The influence of environmental factors on the occurrence of the HPAI H5N1 virus in wild birds, however, is not clearly understood.

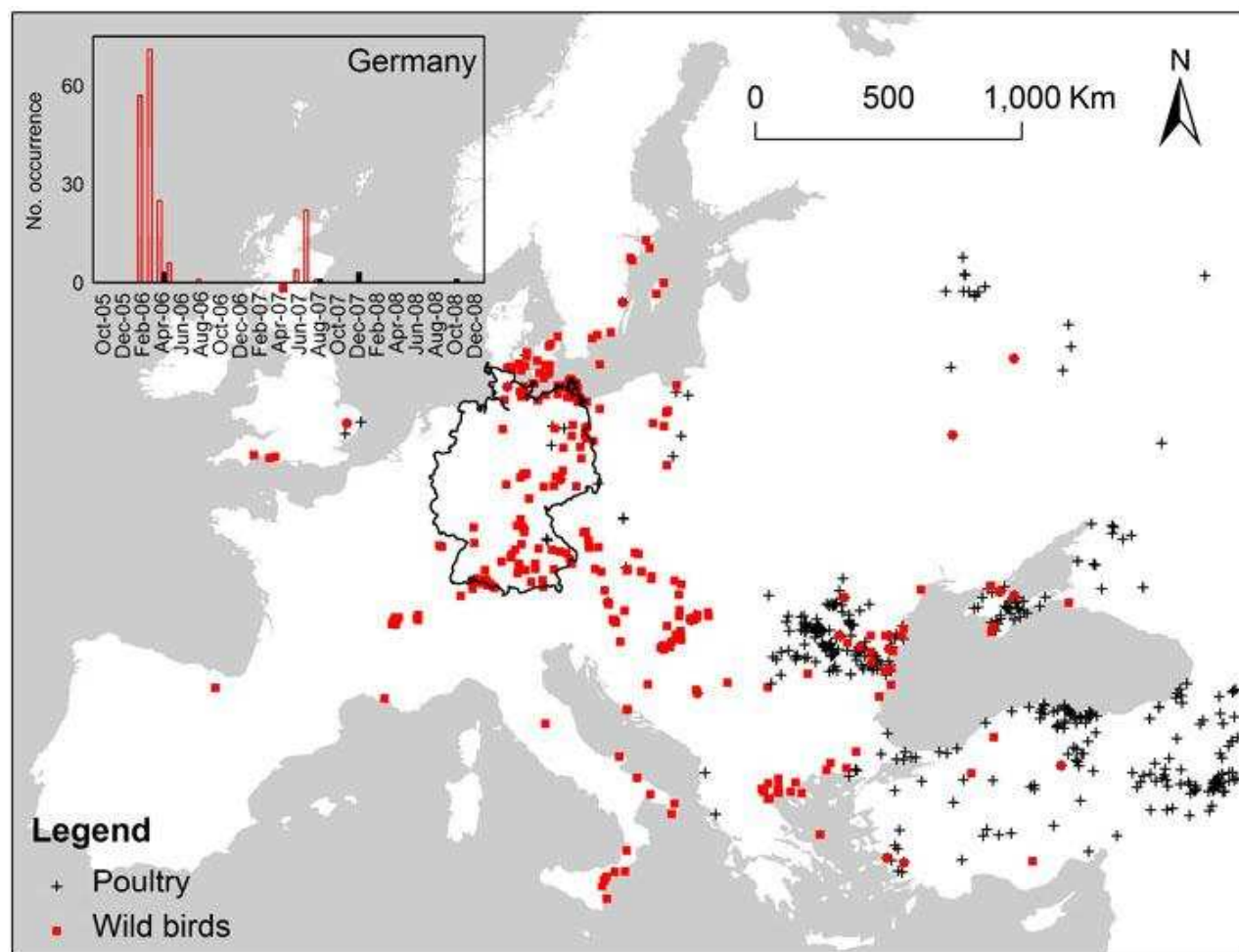
Wild birds are capable of excreting abundant viruses (e.g., in their feces) before and after the onset of clinical signs (Keawcharoen et al. 2008) or even asymptotically (Chen et al. 2006). They are suspected of spreading the virus over either long or short distances (Si et al. 2009). Kilpatrick et al. (2006) even suggested that migratory wild birds were the primary spreading agents of the HPAI H5N1 virus in Europe. Traditional surveillance and control measures may successfully constrain infection by the HPAI H5N1 virus in domestic poultry but not in wild birds. Given the mobility of wild birds and the challenge of tracing different populations, it is of great importance to identify which environmental factors correlate with the occurrence of the HPAI H5N1 virus in wild birds. This knowledge may assist in setting priorities for mitigating actions and in developing the necessary

precautionary measures against future disease outbreaks in wild birds. In this way, the risk of domestic poultry infections through contact with wild birds could be reduced as well.

In contrast to Asia and Africa, Europe has reported large numbers of HPAI H5N1 occurrences in wild birds. Distinct disease patterns have been observed in outbreaks in wild and domestic birds (Fig. 1). In Europe, wild bird infections were mainly found in the northwest, while poultry outbreaks were largely observed in the southeast. Most countries reporting wild bird infections found few or no poultry outbreaks nearby. The highest density of HPAI H5N1 occurrences in wild birds was reported in Germany, where approximately 40% of all occurrences in wild birds in Europe were observed from 2005 to 2008. When considering the temporal distribution of the monthly occurrences of the HPAI H5N1 virus in Germany (Fig. 1), the HPAI H5N1 virus was first reported in wild birds and also mainly circulated among wild birds (with few poultry events reported in between). This finding implies that wild birds may be mainly infected through direct or indirect contact with other wild birds, rather than through contact with domestic poultry. Wild birds, therefore, may play a prominent role in the spread of the HPAI H5N1 virus amongst wild birds in Europe.

If wild birds act as the main spreading agent in the European panzootic in wild birds, then the disease pattern is expected to be strongly influenced by the distribution and movement of wild birds. The distribution and movement of wild birds depends on the availability of natural resources, which can be strongly correlated with physical environmental factors, such as climate, topography, feeding sites, and wetlands (Owen and Black 1990). These factors could, therefore, be used as predictive variables in the analysis of the risk of HPAI H5N1 occurrence in wild birds. In contrast, trade in domestic poultry mainly relies on anthropogenic factors. Thus, these factors may not affect the HPAI H5N1 occurrence pattern in wild birds in Europe. This study aims to examine the key environmental factors associated with the occurrence of the HPAI H5N1 virus in wild birds in Europe, and to map the risk of disease occurrences on the basis of identified explanatory variables.

Fig. 1. Spatial distribution of the highly pathogenic avian influenza (HPAI) H5N1 virus reported in wild birds (red squares) and domestic poultry (black crosses) in Europe from 2005 to 2008. The black boundary indicates Germany and the bar plot illustrates the monthly occurrences of the HPAI H5N1 virus in wild birds (red bar) and domestic poultry (black bar) in Germany.



METHODS AND MATERIALS

Data

Data about the occurrence of the HPAI H5N1 virus in wild birds in Europe consisted of 467 confirmed events reported from 2005 to 2008, provided by EMPRES-I, a global animal health information system of FAO's Emergency Prevention Programme for Transboundary Animal Diseases ([\[i.fao.org/empres-i/home\]\(http://i.fao.org/empres-i/home\)\). Environmental data were categorized into anthropogenic and physical environmental subsets, corresponding to the two disease spreading agents \(i.e., poultry and wild birds\). Table 1 shows the environmental data sets used for this study.](http://empres-</p>
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The anthropogenic environmental data sets contain information about roads, highways, railways, locations of cities and metropolises, as well as

Table 1. Data sets used to generate environmental variables for the analysis of highly pathogenic avian influenza (HPAI) H5N1 occurrences in wild birds in Europe, with data format and source.

Category	Description of data sets	Format	Data producer
Anthropogenic environmental data	Cities	Polygon	ESRI
	Metropolises	Polygon	ESRI
	Roads	Polyline	ESRI
	Highways	Polyline	ESRI
	Railways	Polyline	ESRI
	Human population density in 2005	Raster	CIESIN, FAO, CIAT
	Poultry density in 2005	Raster	FAO
	Digital elevation model	Raster	WORLDCLIM
Physical environmental data	Global lakes and wetlands database	Raster	WWF, ESRI, CESR
	Ramsar sites	Point	Wetlands International
	Mean annual potential evapotranspiration	Raster	CGIAR-CSI
	Mean annual aridity index	Raster	CGIAR-CSI
	Mean monthly precipitation	Raster	WORLDCLIM
	Mean monthly maximum temperature	Raster	WORLDCLIM
	Mean monthly maximum temperature	Raster	WORLDCLIM
	Monthly NDVI	Raster	NASA

human population and poultry density. The first five factors were selected because the distance to transportation routes and cities was found to be significantly associated with HPAI H5N1 outbreaks in China (Fang et al. 2008). Human population density was included because this may indicate higher trade activity. Human population density was significantly associated with the HPAI H5N1 outbreaks in Southeast Asia and India (Gilbert et al. 2006b, Gilbert et al. 2008, Adhikari et al. 2009). Poultry density was included because larger flocks of birds tend to be at higher risk of disease outbreaks (Gilbert et al. 2006b).

The physical environmental data sets comprise a digital elevation model (DEM) (a digital representation of ground surface topography or terrain), a dataset with the location of lakes and wetlands, Ramsar sites, and information about potential evapotranspiration, aridity, monthly precipitation, monthly minimum and maximum temperatures and the monthly normalized difference vegetation index (NDVI), an index that is closely correlated with photosynthetic mass calculated from the reflectance in the visible and near-infrared domains (Tucker 1979). In this study, the time series NDVI was derived from Moderate

Resolution Imaging Spectroradiometer (MODIS) satellite imagery. The topographic data were included because they were found to be significant risk predictors in Southeast Asia (Gilbert et al. 2008). Because suitable habitats are concentrated in the lowlands, elevation influences the availability of food resources and shelter for waterfowl. The distribution of lakes, wetlands, and Ramsar sites was included because such areas are important for migratory and local waterfowl and provide potential, suitable habitats. Water bodies and wetlands were also found to be significantly associated with HPAI H5N1 outbreaks in China, India and Bangladesh (Fang et al. 2008, Adhikari et al. 2009, Biswas et al. 2009). Potential evapotranspiration, aridity and precipitation were included because lower levels of moisture and precipitation may affect the availability of food resources and thereby influence the distribution of wild birds. Precipitation was found to be an important risk factor affecting the distribution of the HPAI H5N1 virus in China (Fang et al. 2008). Monthly minimum and maximum temperatures were selected because temperature pattern changes may contribute to an increase in disease occurrences and the spread of the HPAI H5N1 virus among live birds (Liu et al. 2007). Killer et al. (2009) also found that cold weather may trigger winter movements of migratory birds and thereby contribute to the spread of bird-transmitted diseases outside the actual migration period. The occurrence of the HPAI H5N1 virus in Africa and the Middle East has been linked to differences in plant phenology using time series NDVI data (Williams et al. 2008, Williams and Peterson 2009), so we also included these data because they correlate strongly with the availability of food resources and, subsequently, with waterfowl distribution and movement. Two additional composite variables (i.e., poultry density associated with the distance to the nearest lake or wetland and poultry density associated with the distance to the nearest Ramsar site) were created to investigate potential linkages between domestic poultry and waterfowl. Poultry distribution that coincides with wetlands and Ramsar sites could be an entry gate for virus exchange between these two disease spreading agents.

Data pre-processing

Layers detailing the distance to the nearest city, metropolis, road, highway, railway, lake or wetland, and Ramsar site were generated in a geographic

information system (GIS) with a spatial resolution of 1 km. Layers depicting the slope aspect (defined as the compass direction of the maximum rate of change) and slope gradient (defined as the maximum rate of change in altitude) were also generated from the DEM. To diminish noise, which was caused mainly by cloud remnants, a clean and smooth 12-month NDVI time series was reconstructed on the basis of four 12-month NDVI time series data sets from 2005 to 2008 by employing an adaptive Savitzky-Golay smoothing filter using the TIMESAT package (Jonsson and Eklundh 2004). Two composite variables were constructed by multiplying poultry density with the distance to the nearest lake or wetland and to the nearest Ramsar site. Table 2 summarizes the anthropogenic, physical, and interaction variables used in this study.

Logistic regressions were carried out to examine the relationship between explanatory factors and the occurrence of the HPAI H5N1 virus in wild birds. Duplicate occurrences of HPAI H5N1 cases in wild birds from the same locality were discarded, resulting in 320 unique geographic coordinates. A total of 296 locations in 2005 and 2006 were assigned with HPAI H5N1 presence data for model training, and an additional 24 locations in 2007 and 2008 were utilized for validation. Because Europe adopted a 10 km surveillance zone policy (Pittman and Laddomada 2008), 10 km radius buffers were generated around the HPAI H5N1 virus presence locations. Absences drawn from too small an area can produce spurious models, while absences drawn from too large an area can lead to artificially inflated test statistics as well as potentially less informative response variables (VanDerWal et al. 2009). The areas within the maximum geographic range of wild bird infections in Europe from 2005 to 2008, except for the HPAI H5N1 presence buffers, were thus defined as non-panzootic areas. To select absence data in a way that is fully representative of disease absence, 9000 absence locations were generated randomly in these non-panzootic areas. The minimum distance between each pair of absence locations was set at 20 km, to avoid overlapping of the surveillance buffers.

The minimum distances were then extracted from the distance layers (i.e., distance to the nearest city, metropolis, road, highway, railway, lake or wetland, and Ramsar site) for all presence and absence locations. Furthermore, using zonal statistics, we calculated mean values of a 10-km buffer zone of

Table 2. Summary of environmental variables used in the analysis of highly pathogenic avian influenza (HPAI) H5N1 occurrences in wild birds in Europe.

Category	Description of variables	Abbreviation	Unit
Anthropogenic environmental variables	Distance to the nearest city	City	km
	Distance to the nearest metropolis	Metro	km
	Distance to the nearest road	Road	km
	Distance to the nearest highway	Highway	km
	Distance to the nearest railway	Railway	km
	Human population density in 2005	Hpopden	p/km ²
	Poultry density in 2005	Poultryden	p/km ²
Physical environmental variables	Distance to the nearest lake or wetland	GLWD	km
	Distance to the nearest Ramsar site	Ramsar	km
	Digital elevation model	DEM	m
	Slope aspect	Aspect	°
	Slope gradient	Slope	°
	Mean annual potential evapotranspiration	Mapet	mm/km ² /year
	Mean annual aridity index	Maaridity	No unit
	Mean monthly precipitation	PrecJan to Dec	mm
	Mean monthly minimum temperature	TminJan to Dec	°Cæ*10
	Mean monthly maximum temperature	TmaxJan to Dec	°Cæ*10
	Monthly NDVI	NDVIJan to Dec	No unit
Interaction variables	Poultry density * distance to the lake or wetland	PoultrydenW	No unit
	Poultry density * distance to the Ramsar site	PoultrydenR	No unit

all presence and absence locations for the following variables: human population density, poultry density, elevation, slope aspect, slope gradient, potential evapotranspiration, aridity index, precipitation, minimum temperature, maximum temperature, and NDVI.

Mcpherson et al. (2004) demonstrated that optimal models developed from logistic regressions had intermediate prevalences (50%) and large sample sizes (300-500). We therefore applied a bootstrapping procedure in which, together with the 296 presence locations, 296 absence locations were randomly selected with replacement from the 9000 absence locations. This process was repeated 1000 times to create 1000 subsets for model training. Fig. 2 shows the presence and absence of HPAI H5N1 occurrences in wild birds from 2005 to 2006 in Europe as an example of one training subset.

Statistical analysis

We examined the linear and quadratic effects of each variable on the occurrence of disease separately using logistic regression models. This process was repeated 1000 times using different training subsets, reporting mean values of odds ratios (OR), 95% confidence intervals (CIs) of OR, p-value, pseudo R^2 , Akaike's information criterion (AIC), the area under the Receiver Operating Characteristic curve (AUC), and Kappa for each variable. Odds ratios were used to evaluate the impact of predictor variables. The further below 1.0 the odds ratio is, the greater the effect of that variable in reducing the odds of disease presence, while the higher the odds ratio is above 1.0, the greater the effect of that variable in increasing the odds of disease presence. An odds ratio of 1 corresponds to an explanatory variable that does not affect the dependent variable. Variables yielding non-significant changes in log-likelihood were excluded from further analysis.

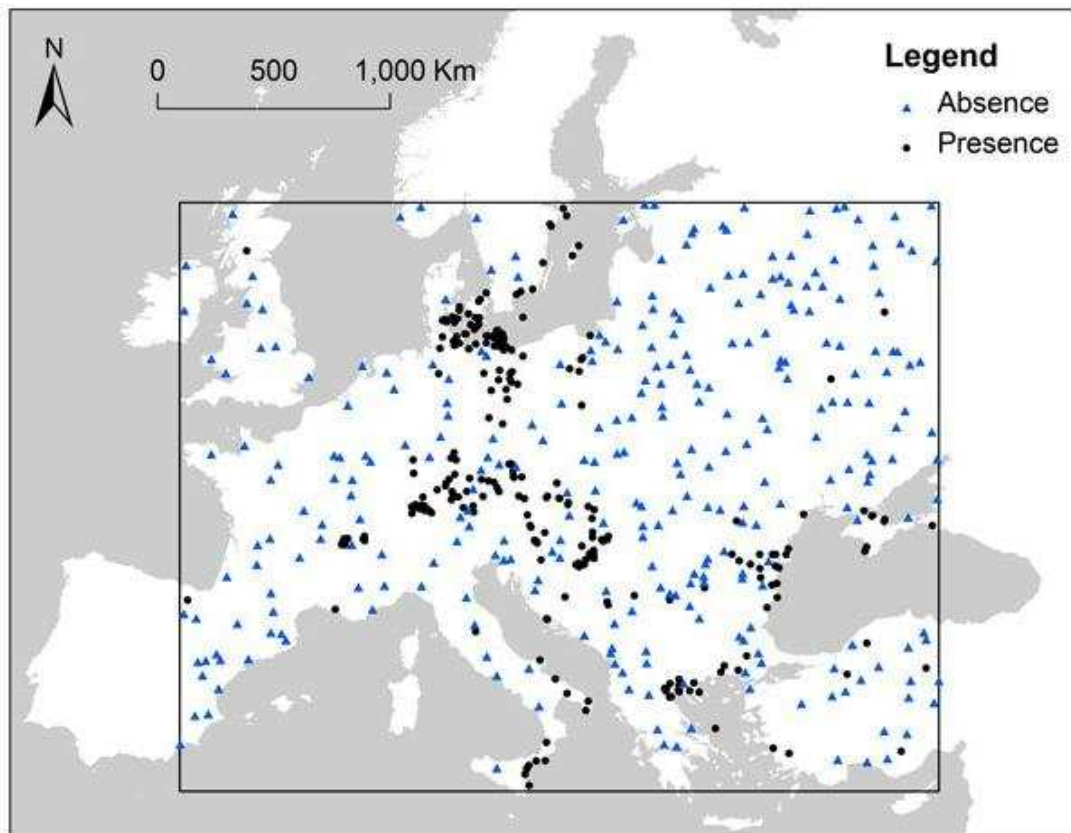
The Akaike's Information Criterion (AIC) (Akaike 1974) is a measure of the goodness of fit of an estimated statistical model. The chosen model should be the one that minimizes the Kullback-Leibler distance between the model and the truth. AUC measures the ability of a model to discriminate between sites where an event is present, versus those where it is absent (Hanley and McNeil 1982). It provides a single measure of the overall accuracy of model fit that is not dependent upon a particular

threshold. It ranges from 0 to 1, with values larger than 0.5 indicating a performance better than what could be achieved randomly. Kappa (Cohen 1960), a chance-corrected measure of agreement, provides an index that considers both omission and commission errors. We calculated a maximum Kappa for each model by calculating Kappa at all possible thresholds and identifying both the maximum Kappa and the threshold at which this occurred.

Autocorrelation and multi-collinearity were assessed by examining Moran's I (Moran 1950) and the variance inflation factor (VIF) (Stine 1995), respectively. A negative value for Moran's I indicates a negative autocorrelation, and a positive value reveals a positive autocorrelation; no autocorrelation is present when the value of Moran's I equals zero. The VIFs were calculated for each predictor as the inverse of the coefficient of non-determination ($1/(1-R^2)$) for a regression of that predictor on all others. VIF is a positive value representing the overall correlation of each predictor with all others in a model. Collinearity is present when the VIF for at least one independent variable is large. All reported Moran's I and VIF values are mean values of 1000 repeated calculations using the 1000 training subsets.

For all significant variables, a pre-selection was carried out to remove those variables with relatively high collinearity and/or relatively high autocorrelation. Highly correlated variables were removed by sequentially dropping the variable with the lowest impact, recalculating the VIFs and repeating this process until all VIFs were smaller than 10. Generally, a VIF greater than 10 indicates "severe" collinearity (Kutner et al. 2004). When two variables had comparable impacts, the one with the lower p-value was preferentially selected. A variable with a Moran's I larger than 0.5 or smaller than -0.5 was not considered for selection. A multiple stepwise logistic regression was carried out by using the pre-selected variables. For each of the pre-selected variables, a quadratic term was included if the quadratic effect was stronger than the linear effect. This stepwise process was repeated 1000 times using the different training subsets. The frequency of each variable being selected was calculated on the basis of applying 1000 best stepwise logistic regression models, ranked by AIC. The mean p-value and its 95% CIs were calculated for each selected variable. Variables yielding non-significant effects in the stepwise logistic regression

Fig. 2. Distribution of presence (black squares) and absence (blue triangles) of highly pathogenic avian influenza (HPAI) H5N1 occurrences in wild birds from 2005 to 2006 in Europe (one training subset). The box indicates the maximum geographic range of HPAI H5N1 occurrences in wild birds from 2005 to 2008.



models were discarded. The remaining variables were identified as key risk factors. A multiple logistic regression was carried out using these key risk factors. This process was repeated 1000 times using the different training subsets. The mean values of coefficients, OR, 95% CIs of OR, p-value, pseudo R^2 , AIC, AUC and Kappa were used as indicators of model performance. A ROC plot was generated by plotting all sensitivity values (true positive fraction) on the y-axis against their equivalent (1-specificity) values (false positive fraction) for all available thresholds on the x-axis. One thousand ROC curves were calculated using the 1000 training subsets.

Based on the model derived from the multiple logistic regressions, a risk map of HPAI H5N1 occurrence in wild birds was generated with a spatial resolution of 1 km. Areas of the risk map were then classified into four levels (i.e., very high, high, medium and low risk) for validation by using independent occurrences (i.e., HPAI H5N1 occurrences in wild birds from 2007 to 2008). A 10-km buffer zone was generated around each validating occurrence, and the mean risk value in each buffer zone was calculated. The percentage of buffer zones occurring in high and very high risk areas was then calculated to evaluate the accuracy of the predictive map.

All statistical analyses were conducted using R statistical software (www.r-project.org) with additional packages for some of the specific analyses.

RESULTS

The linear and quadratic logistic regression analyses demonstrated that physical environmental variables were substantially correlated with the occurrence of HPAI H5N1 in wild birds (Table 3), but no anthropogenic variables selected in this study were significantly associated with HPAI H5N1 occurrences. The composite variables, indicating interactions between poultry and waterfowl, yielded non-significant effects on HPAI H5N1 occurrence in wild birds. Positive linear associations were found between wild bird HPAI H5N1 occurrences and minimum temperature, cold season maximum temperature, and cold season NDVI. Negative linear associations were detected between wild bird HPAI H5N1 occurrence and elevation, slope aspect, slope gradient, and cold season precipitation. Among the linearly associated physical environmental variables for the HPAI H5N1 occurrences in wild birds, the NDVI in December had the strongest effect, with an odds ratio of 30.4 (95% CI 6.6 -141.74). Significant quadratic effects were observed between disease occurrences and minimum temperature, cold season maximum temperature, cold season NDVI, and warm season NDVI. The strongest quadratic effect was observed for the NDVI in March.

Eight out of fourteen pre-selected variables were discarded as they showed non-significant effects (upper 95% CI of p-value > 0.05) in the process of stepwise selection (Table 3). Five key risk factors (i.e., elevation, precipitation in January, minimum temperature in January, NDVI in March (including the square of NDVI in March), and NDVI in December) were selected as inputs for the multiple logistic regressions. Using bootstrapping training subsets, 1000 models were fitted, after which the mean value of each output parameter was calculated (Table 4). The results showed all key risk factors (i.e., lower elevation, reduced precipitation, a higher minimum temperature, an intermediate NDVI in spring, and a higher NDVI in winter) were consistently associated with the occurrence of the HPAI H5N1 virus in wild birds. The predictive power of the fitted models was good; the AUC had a mean value of 0.81 (Fig. 3), and the Kappa had a mean value of 0.52.

A predictive risk map of HPAI H5N1 occurrences in wild birds in Europe was generated based on the model as derived from the multiple logistic regression analysis (Fig. 4). The validation samples of HPAI H5N1 occurrences in wild birds were found for 79% (19 out of the 24 occurrences) in the predicted high or very high risk areas (i.e., predictive risk > 0.4).

DISCUSSION

The results presented in this paper have shown that HPAI H5N1 infections in wild birds in Europe occur under consistent and predictable environmental circumstances. The key environmental factors affecting the occurrence of HPAI H5N1 infections in wild birds in Europe are: an increased NDVI in December; an intermediate NDVI in March; lower elevation, an increased minimum temperature in January; and reduced precipitation in January. We therefore suggest that occurrences of HPAI H5N1 in wild birds in Europe are strongly influenced by the availability of food resources and are facilitated by increased temperatures and reduced precipitation.

Elevation consistently showed a negative association with HPAI H5N1 occurrences in wild birds in Europe. Positive associations with a higher NDVI and a higher temperature during the winter were found when a large number of wild birds overwintered and staged in Europe. The increased number of bird populations during winter may also increase the disease risk in Europe. As wetlands, rivers, canals, ponds and irrigated networks are concentrated in lowlands, flat plains, deltas, and coastal areas (Gilbert et al. 2006b, Gilbert et al. 2008), the presence of flat areas in combination with an increased winter NDVI and an increased winter temperature indicate the presence of increased resources for waterfowl in cold seasons.

Waterfowl utilize small plants (e.g., grasses and herbaceous plants) rather than larger plants (e.g., bushes and forest). During spring, the growing season for vegetation, intermediate NDVI values may indicate areas with an ample availability of herbaceous plants, while areas with high NDVI values may be dominated by larger plants. During the maturation stage of vegetation in summer, herbaceous plants may yield relatively low NDVI values compared to other larger plants. This finding may explain the significant quadratic associations observed between the NDVI in warm seasons and

Table 3. Variables significantly associated with highly pathogenic avian influenza (HPAI) H5N1 occurrences in wild birds reported by the linear and quadratic logistic regression analyses using 1000 bootstrapping training datasets. All values in the table are mean values obtained from 1000 runs of the model. Italics indicate quadratic effects. Variables marked in bold were included in the final model.

Linear and quadratic logistic regressions										Moran's I		Stepwise selections		
Variable	OR [†]	95% CI OR [†]		P value	Pseudo R ²	AIC	AUC ± SD	Kappa ± SD	VIF (all)	VIF (selected)	Times P value	95% upper CI		
DEM	0.989	0.984	0.995	<0.001	0.09	784	0.65	0.02	0.3	0.04	0.08	11.65		
Slope	0.8	0.728	0.88	<0.001	0.06	798	0.61	0.02	0.17	0.04	0.13	5.29		
Aspect	0.998	0.997	0.999	0.007	0.03	810	0.58	0.02	0.14	0.04	0.12	1.23		
MpreJan	0.984	0.976	0.991	<0.001	0.05	803	0.55	0.02	0.18	0.03	0.25	46.02		
MpreFeb	0.983	0.974	0.992	0.005	0.04	817	0.54	0.02	0.15	0.03	0.26	42.08		
MpreMar	0.985	0.976	0.995	0.029	0.03	813	0.53	0.02	0.14	0.03	0.31	32.31		
MpreApr	0.985	0.974	0.996	0.046	0.02	816	0.59	0.02	0.21	0.04	0.21	11.37		
MpreOct	0.989	0.982	0.997	0.036	0.02	815	0.54	0.02	0.13	0.04	0.27	13.73		
MpreNov	0.99	0.983	0.997	0.035	0.02	815	0.52	0.02	0.14	0.03	0.24	23.79		
MpreDec	0.985	0.979	0.992	0.001	0.04	804	0.55	0.02	0.15	0.03	0.21	47.98		
TminJan	1.013	1.008	1.018	<0.001	0.08	788	0.63	0.02	0.36	0.03	0.36	467		
TminJan	1.027	1.019	1.035	<0.001	0.23	714	0.71	0.02	0.38	0.04	818	0.017		
TminJan2	0.407	0.318	0.522	<0.001	0.07	791	0.61	0.02	0.35	0.03	0.49	524.7		
TminFeb	1.013	1.008	1.017	<0.001	0.07	719	0.69	0.02	0.37	0.04	-	-		
TminFeb	1.026	1.018	1.034	<0.001	0.22	-	-	-	-	-	-	-		
TminFeb2	0.406	0.314	0.525	<0.001	0.07	790	0.62	0.02	0.35	0.03	0.46	363.9		
TminMar	1.016	1.01	1.022	<0.001	0.21	725	0.66	0.02	0.36	0.04	-	-		
TminMar	1.033	1.023	1.043	<0.001	0.06	810	0.57	0.02	0.21	0.03	0.31	228		
TminMar2	0.441	0.347	0.561	<0.001	0.03	797	0.56	0.02	0.22	0.03	-	-		
TminApr	1.013	1.006	1.021	0.012	0.03	815	0.55	0.02	0.15	0.03	0.32	295.2		
TminApr	1.022	1.012	1.032	<0.001	0.06	797	0.56	0.02	0.22	0.03	0.31	355.3		
TminApr2	0.758	0.652	0.881	0.005	0.05	803	0.57	0.02	0.18	0.03	-	-		
TminMay	1.011	1.004	1.019	0.034	0.02	815	0.55	0.02	0.15	0.03	0.32	295.2		
TminJun	1.014	1.007	1.022	0.006	0.04	809	0.58	0.02	0.17	0.03	0.31	355.3		
TminJun	1.022	1.012	1.033	<0.001	0.05	803	0.57	0.02	0.18	0.03	-	-		
TminJun2	0.818	0.703	0.952	0.041	0.03	810	0.59	0.02	0.19	0.04	0.27	359.6		
TminJul	1.013	1.006	1.02	0.01	0.06	801	0.58	0.02	0.2	0.04	-	-		
TminJul	1.023	1.013	1.033	<0.001	0.06	801	0.58	0.02	0.2	0.04	-	-		
TminJul2	0.799	0.69	0.926	0.019	0.04	805	0.62	0.02	0.27	0.04	0.26	543.9		
TminAug	1.015	1.008	1.022	0.002	0.04	805	0.62	0.02	0.27	0.04	0.26	543.9		
TminAug	1.031	1.019	1.042	<0.001	0.08	788	0.62	0.02	0.27	0.04	-	-		
TminAug2	0.739	0.636	0.859	0.002	0.07	795	0.66	0.02	0.33	0.04	0.25	272.8		
TminSep	1.018	1.011	1.025	<0.001	0.14	760	0.66	0.02	0.33	0.04	-	-		
TminSep	1.04	1.029	1.052	<0.001	0.16	760	0.66	0.02	0.33	0.04	-	-		
TminSep2	0.657	0.567	0.76	<0.001	0.08	789	0.67	0.02	0.32	0.04	0.27	156.17		
TminOct	1.019	1.013	1.026	<0.001	0.16	750	0.67	0.02	0.5	0.04	-	-		
TminOct	1.04	1.029	1.05	<0.001	0.07	795	0.64	0.02	0.34	0.03	0.34	143.6		
TminOct2	0.649	0.563	0.748	<0.001	0.16	752	0.65	0.02	0.47	0.33	-	-		
TminNov	1.016	1.01	1.022	<0.001	0.03	811	0.59	0.02	0.34	0.03	0.42	340.5		
TminNov	1.034	1.024	1.043	<0.001	0.03	811	0.59	0.02	0.34	0.03	0.42	340.5		
TminNov2	0.624	0.533	0.73	<0.001	0.03	811	0.59	0.02	0.34	0.03	0.42	340.5		
TminDec	1.008	1.003	1.012	0.013	0.03	811	0.59	0.02	0.34	0.03	0.42	340.5		

Table 4. Summary of the multiple logistic regression models for the occurrence of the highly pathogenic avian influenza (HPAI) H5N1 virus in wild birds in Europe from 2005 to 2006. All values in the table are mean values obtained from 1000 runs of the model.

	B	OR [†]	95% CI OR [†]	P value	AIC ± SD	Pseudo R ² ± SD	AUC ± SD	Kappa ± SD
Model				<0.001	638 ± 25	0.38 ± 0.04	0.81 ± 0.02	0.52 ± 0.03
Intercept	-10.86			<0.001				
Dem	-0.001	0.999	0.998	0.999	0.016			
PrecJan	-0.037	0.963	0.950	0.976	<0.001			
TminJan	0.025	1.026	1.018	1.034	<0.001			
NDVIMar	50.6	1.170	0.059	23.630	<0.001			
SNDVIMar	-56.36	0.408	0.307	0.543	<0.001			
NDVIDec	6.102	1318.596	100.248	17685.59	<0.001			

[†] Calculated using z-score standardized variables to avoid too small or too large OR values

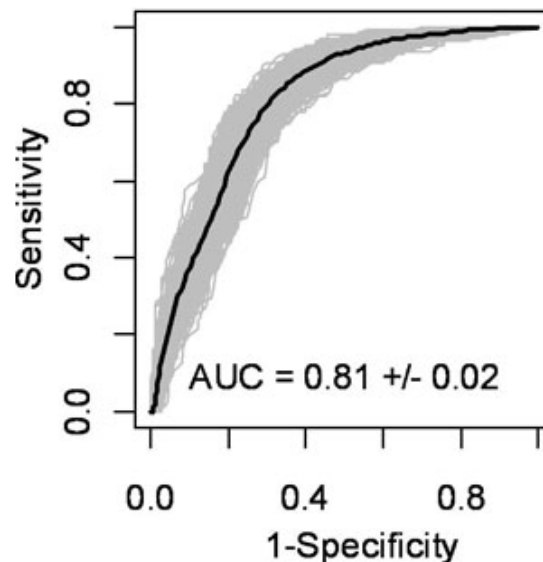
the occurrence of HPAI H5N1 in wild birds. The risk of HPAI H5N1 infections, however, is not elevated in areas close to wetlands (identified as a non-significant variable), probably because wetlands are so extensive in Europe, both in panzootic and non-panzootic areas.

This study highlights that climatic factors substantially contribute to HPAI H5N1 occurrences in wild birds. In agreement with recent findings from China (Fang et al. 2008), we also found that precipitation is negatively associated with the risk of HPAI H5N1 occurrences in wild birds in Europe, possibly because less precipitation leads to a higher concentration of birds in the limited suitable habitats and, therefore, to increased opportunities for contact. Areas with higher minimum temperatures, especially during the cold seasons, consistently show higher risks of disease occurrence, because higher temperatures can stimulate viral activity. A confounding factor is that wild birds seek refuge from cold weather and congregate in warmer areas as temperatures drop, resulting in a higher probability of disease occurrence. Previous studies have suggested that the western European panzootic was caused by unusual waterfowl movements, due to extreme cold weather in the Black Sea area, where

the virus was already established (Gilbert et al. 2006a, Keller et al. 2009). Hence, dramatic drops in temperature may trigger the spread of HPAI H5N1 viruses (Liu et al. 2007).

The analyses in this study have demonstrated that the risk of HPAI H5N1 infections in wild birds is affected only by selected physical environmental factors, indicating that the rate of HPAI H5N1 occurrence in wild birds in Europe may be influenced mainly by direct or indirect contact with infected wild birds. The surveillance and control measures in Europe (Pittman and Laddomada 2008, World Organization for Animal Health (OIE) 2009) may affect the occurrence pattern of HPAI H5N1 in wild birds because our results indicate that HPAI H5N1 occurrences in wild birds in Europe are not related to any of the anthropogenic environmental factors selected in this study. Composite variables linking poultry density and the location of wetlands yielded non-significant effects, suggesting a general paucity of interactions between domestic poultry and waterfowl. One reason for this general lack of interaction is that biosecurity measures (e.g., the quarantine of free ranging poultry) has successfully limited contact between domestic poultry and wild birds in poultry areas (Sinclair et al. 2006). This

Fig. 3. ROC curves of the predictive power of the multiple logistic regression models on the presence/absence of the highly pathogenic avian influenza (HPAI) H5N1 virus in wild birds in Europe.



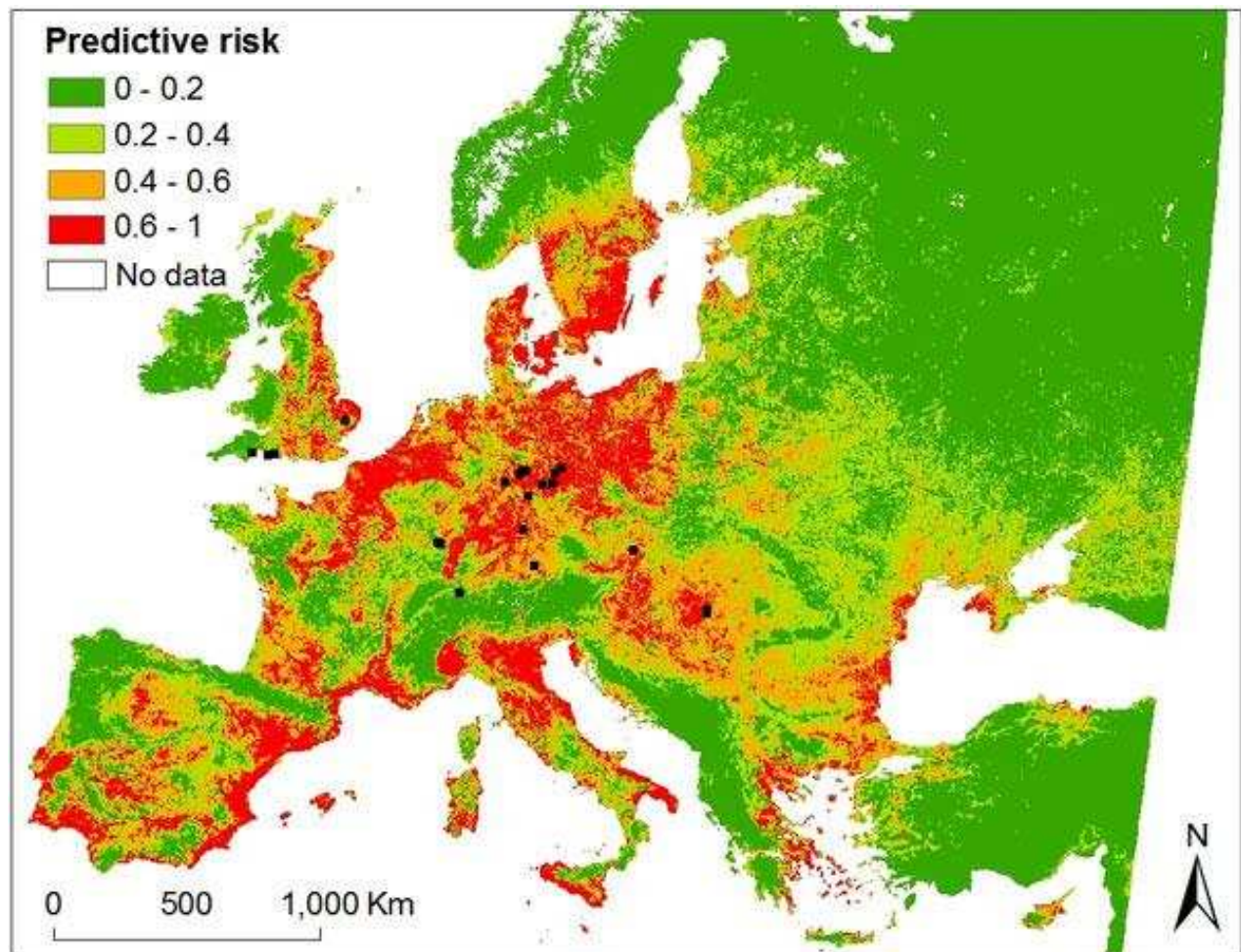
finding suggests that the role of domestic poultry as a spreading agent may be effectively minimized in the case of wild bird infections in most European regions. Wild birds themselves may therefore play a prominent role in the occurrence of HPAI H5N1 in wild birds in Europe.

The heterogeneity in surveillance and control measures across Europe may lead to a bias in reporting and differences in sensitivity to risk factors. For example, the interaction effect between domestic poultry and waterfowl appears to be more pronounced in some countries (e.g., Romania and Ukraine) than other countries (e.g., France and Switzerland). This interaction is dependent upon whether the occurrences of HPAI H5N1 in domestic poultry and wild birds overlap (Fig. 1). A previous study has suggested that the Danube River Delta (an area inhabited by both waterfowl and domestic poultry) played a critical role in the introduction and initial spread of HPAI H5N1 in Romania (Ward et al. 2008). The same interaction may potentially occur in other countries, but stricter quarantine measures may reduce the chance of contact between domestic and wild birds.

In line with previous studies (Fang et al. 2008, Gilbert et al. 2008), we demonstrated that the logistic regression model was quite robust in identifying the environmental factors influencing the spread of HPAI H5N1 and in predicting the risk of HPAI H5N1 virus occurrence in Europe. Compared with one previous study (Fang et al. 2008) that reported the quadratic effect did not perform significantly better than a linear association for any of the selected environmental factors in the logistic regression model, we found that only the NDVI in March yielded consistent quadratic effects. A possible reason is that the quadratic effect of the NDVI might be stronger in areas with HPAI H5N1 occurrences in wild birds than in those with domestic poultry. To gain more insight into non-linear responses of the HPAI H5N1 virus to environmental factors, other analytic tools (e.g., neural network, GARP or Maxent) should be considered.

By simply focusing on the spatial position and environmental characteristics of sites where disease occurs, any occurrence can create a non-random distribution that appears predictive, and independent testing and repeated challenging are needed to

Fig. 4. Predictive risk map of highly pathogenic avian influenza (HPAI) H5N1 occurrences in wild birds in Europe. Black dots indicate HPAI H5N1 occurrences in wild birds in 2007 and 2008.



evaluate models (Peterson and Williams 2008). We therefore investigated the relationship between environmental factors and HPAI H5N1 occurrences using statistically independent occurrences (i.e., space-time clusters of disease occurrence identified by space-time scan statistics). The results indicated that only cold season temperature (minimum and maximum) and cold season NDVI yielded significant effects. The occurrence risk was largely underestimated by the model fitted using space-time clusters, and the map of predictive risks was characterized by low accuracy (25%). By discarding

sporadic H5N1 occurrences and aggregating individual occurrences into space-time clusters, a large part of the potentially important information was lost. Small sample sizes may also influence the model's predictive power and the sensitivity of risk factors. Models fitted using original disease occurrences and incorporating maximum information showed high predictive accuracy (79%) after independent testing, suggesting that the identified key environmental factors are consistently affecting the occurrence of HPAI H5N1 in wild birds.

Responses to this article can be read online at:
<http://www.ecologyandsociety.org/vol15/iss3/art26/responses/>

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