Landscape attributes driving avian influenza virus circulation in the Lake Alaotra region of Madagascar

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Abstract. While the spatial pattern of the highly pathogenic avian influenza H5N1 virus has been studied throughout Southeast Asia, little is known on the spatial risk factors for avian influenza in Africa. In the present paper, we combined serological data from poultry and remotely sensed environmental factors in the Lake Alaotra region of Madagascar to explore for any association between avian influenza and landscape variables. Serological data from cross-sectional surveys carried out on poultry in 2008 and 2009 were examined together with a Landsat 7 satellite image analysed using supervised classification. The dominant landscape features in a 1-km buffer around farmhouses and distance to the closest water body were extracted. A total of 1,038 individual bird blood samples emanating from 241 flocks were analysed, and the association between avian influenza seroprevalence and these landcape variables was quantified using logistic regression models. No evidence of the presence of H5 or H7 avian influenza subtypes was found, suggesting that only low pathogenic avian influenza (LPAI) circulated. Three predominant land cover classes were identified around the poultry farms: grassland savannah, rice paddy fields and wetlands. A significant negative relationship was found between LPAI seroprevalence and distance to the closest body of water. We also found that LPAI seroprevalence was higher in farms characterised by predominant wetlands or rice landscapes than in those surrounded by dry savannah. Results from this study suggest that if highly pathogenic avian influenza H5N1 virus were introduced in Madagascar, the environmental conditions that prevail in Lake Alaotra region may allow the virus to spread and persist.

Keywords: avian influenza, epidemiology, remote sensing, spatial analysis, risk factors, landscape, Madagascar.

Introduction

Madagascar is an island in the Indian Ocean, located 400 km from Mozambique on the south-eastern coast of Africa. Out of the country's 33 million of poultry heads recorded in 2010 (FAOSTAT; http://faostat3.fao.org/home/index.html#HOME), 27 million (82%) are raised in backyard and small-scale commercial farms (Ministry of Livestock; http://www.elevage.gov.mg). Poultry production provides high-quality protein food and represents an important source of income for farmers.

Avian influenza is present in Madagascar and low pathogenic avian influenza (LPAI) viruses are known to circulate in villages and commercial poultry populations (Andriamanivo et al., 2012). The sanitary and economic impacts of LPAI in the Malagasy poultry industry are still largely unknown and surveillance of avian influenza viruses is as much a challenge as it is essential for Madagascar's poultry sector. Early detection of LPAI should help tailoring control measures and limit potential losses. Indeed, LPAI causes mild or no clinical disease experimentally in chickens, but the viruses are important pathogens as they can cause disease and syndromes of economic importance for the poultry industry, including respiratory infection, sinusitis and drop in egg production. In addition, high poultry mortality may be observed in case of co-infection of avian influenza viruses (AIV) with other pathogens, poor sanitary conditions or secondary bacterial infections (Sylte and Suarez, 2012).

Several highly pathogenic avian influenza (HPAI) outbreaks have been reported in Africa: H5N1 virus is now considered endemic in Egypt (EMPRES/FAO-GLEWS, 2012), it hit Nigeria and West African countries in 2008 (OIE, 2008), while H5N2 virus was found in 2011 and 2012 in South Africa (OIE, 2012).

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To date, no outbreaks of HPAI have been detected in Madagascar but, against the background described above, the risk of emergence and spread in the region of a HPAI variant cannot be ruled out. Hypothetically, an HPAI variant could emerge in Madagascar by mutation after the introduction of LPAI viruses of the H5 or H7 subtypes into poultry or, alternatively, an HPAI virus could be introduced from other infected African countries. Hence, detailed studies are necessary to evaluate the risk of introduction of an HPAI strain (H5 or H7 subtype) in Madagascar. There are a series of clues that lead us to believe that this risk is not null. The AIV virus could be introduced in Madagascar by wild birds migrating from Africa (e.g. the pond-heron (*Ardeola idea*) and non-endemic ducks such as *Dendrocygna viduata* and *Anas erythrorhyncha*) (CNPLGA, 2006) or from Asia (Pale-arctic shorebirds, such as *Calidris ferruginea*) (Young et al., 2006). AIV viruses could also be introduced through movements of live poultry. Here also, further studies are needed to quantify this risk, but it is known that day-old chicks are imported through Mauritius (Reshad, 2001) and that fighting cocks are illegally moved for competition from Southeast Asia, where HPAI H5N1 strains circulate, to Madagascar (supported by anonymous field observations).

For a long time, little information has been available regarding avian influenza in Madagascar. However a recent study (Andriamanivo et al., 2012) showed that LPAI seroprevalence was significantly higher (25%) around a particular site of Madagascar highlands, the Lake Alaotra region, when compared to the urban region of Grand Antananarivo (3%). The Lake Alaotra site is of primary interest regarding the eco-epidemiology of avian influenza. Indeed, lakesides have large waterfowl populations, including species from the Anatidae family, which are known to harbour the most diverse and also the highest prevalence rates of AIV (Gilbert et al., 2008). Recent reports from Lake Alaotra indicate that avian influenza circulates in *Anas erythrorhyncha*, *Anas melleri* and *Anas hottentota* (Miguel Pedrono, personal communication). These sedentary or nomadic species may thus support the persistence of AIV in Madagascar and represent a high risk for transmission to domestic poultry as wild ducks can easily come into contact with the poultry raised in free-range systems. Previous work (Andriamanivo et al., 2012) also suggests that the high density of domestic palmipeds and the predominance of rice cultivation could favor AIV circulation in Lake Alaotra as it does for H5N1 in Southeast Asia (Gilbert and Pfeiffer, 2012).

In the present paper, we combined serological data on avian influenza infection with landscape classification in order to evaluate the influence of rice cultivation and wetlands on the spatial pattern of avian influenza prevalence in the Madagascar highlands around Lake Alaotra. This study should contribute to identify hotspots associated with circulation of LPAI, which may become high-risk areas in case of introduction or emergence of a highly virulent strain. It is also expected that results of this analysis would provide useful information for tailoring risk-based surveillance of avian influenza in poultry in the Madagascar highlands and similar agro-ecosystems.

Materials and methods

Study site

The study area was the basin of Lake Alaotra in the Madagascar highlands, which is roughly situated between latitudes 17' and 18' S and longitudes 48' and 49' E (Bakoariniaina et al., 2006) (Fig. 1). This region is part of Tamatave province and located about 160 km northeast of the capital Antananarivo (Fig. 1). The altitude is mostly between 750 and 790 m above the mean sea level (MSL), but hills surrounding the basin rise to between 900 and 1,300 m above the MSL (Bakoariniaina et al., 2006). The basin belongs to the sub-humid, bioclimatic domain of Madagascar, where
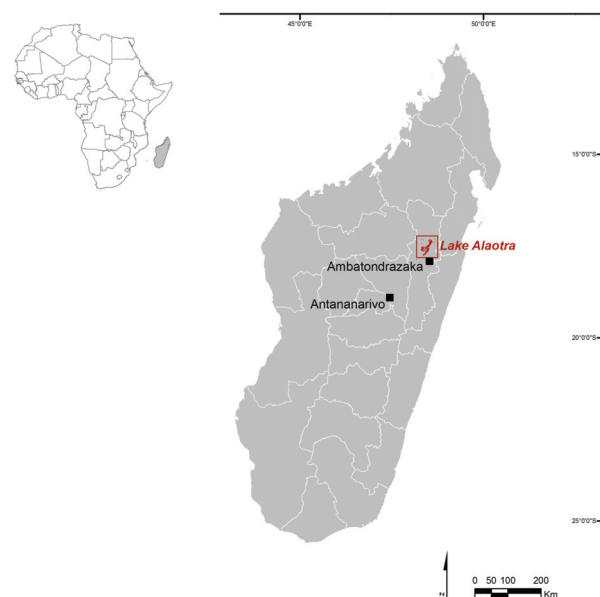

Fig. 1. Location of the study site around Lake Alaotra in the Madagascar highlands.

rainfall ranges between 800 and 1,500 mm annually and the average yearly temperature is around 21 °C (Van Hulst, 2011). The climate is characterised by two alternating seasons: a 5-6 month dry season with moderate temperatures from May to October, and a wet season with elevated temperatures (23.2 °C) from November to April (Pock Tsy et al., 2003; Van Hulst, 2011).

The Lake Alaotra basin is the largest wetland area in Madagascar. It is made up of an open water surface of about 200 km², surrounded by a 20 km² marshy zone (Bakoariniaina et al., 2006). The lake constitutes an exceptional site for resident and migratory wild birds, especially waterfowl (BirdLife International; http://www.birdlife.org/datazone/site-factsheet.php?id=6567), and the fertile plains and irrigated lands that surround it yield important crops. This region is the most important rice-growing region in Madagascar with 700 km² of rice paddy fields (Andriamanivo et al., 2012) with production estimated at 200,000 tons per year (Van Hulst, 2011).

Data collection

Serological surveys

The main four municipalities located at the lakeside were selected for the poultry surveys as they represented various types of landscape including rice paddy fields, wetlands and dry vegetation. Two cross-sectional serological surveys were conducted; first in August 2008 and then in May 2009. A two-stage, random sample approach was designed from a farm census database already established for previous studies (Andriamanivo et al., 2012). Villages and farms were the first- and second-degree units respectively. Sample size was first set at 200 birds for 2008 to obtain an absolute accuracy of 5% under the hypothesis of 15% seroprevalence with a 95% confidence level and then was increased to 750 birds in 2009. Blood samples were taken from chickens, ducks and geese that were present on farms. Commercial enzyme - linked immunosorbent assay (ELISA) kits (IDVET© screen test) were used to detect antibodies against AIV type A. All sera testing positive for antibodies against AIV were also checked for subtypes H5 and H7 with other commercial IDVET© kits. In addition, data on farm characteristics were collected through a questionnaire. The geographical coordinates of the farms visited were recorded during fieldwork using a Garmin global positioning system (GPS) instrument.

Landscape classification and extraction of environmental variables

We hypothesised that land cover and presence of water bodies drive the spatial distribution of AIV in Madagascar as has been shown previously for HPAI H5N1 in Southeast Asia (Gilbert and Pfeiffer, 2012). One Landsat 7 enhanced thematic mapper plus (ETM+) satellite image cantered on the lake and marshlands in the South was analysed. The scene was dated 22 March 2007 (Path/Row 158/072); it was cloud-free and had no apparent haze. A supervised classification of land cover was realised with IDRISIsoftware© (http://clarklabs.org/products/idrisi. cfm) based on a maximum likelihood classifier and a three-channel composition (TM3, TM4, TM5). Fifty-one polygons were digitised manually and 351 GPS field observations were used to validate the classification. A categorical variable, corresponding to the predominant type of landscape in the neighbourhood (1 km radius) of farms, was then constructed from vegetation layers. This radius was delimited according to field observation indicating that it was the maximal perimeter for bird ranging around farmhouses. Two alternative buffer radius (500-m and 2-km) were also tested in a sensitivity analysis. The percentage of buffer areas covered by each class of vegetation was calculated using ArcGIS, version 9.3 software (ESRI; Redlands, USA) and the landscape type dominating each farm buffer was identified based on this percentage. The Euclidian distance between each farm and the nearest body of water was calculated from the water polyline layer derived from raster image.

Other explanatory variables

The analysis was also adjusted with regard to other variables that could have contributed to variation of the prevalence at the flock level. These variables were: sampling date (August 2008 *vs*. May 2009), flock species (chicken, duck or geese), flock size and the number of different flocks on each farm.

Statistical analysis

Statistical analyses were carried out using the R 2.14.0 software (The R Foundation for Statistical Computing, 2012). Bird-level AIV seroprevalence and corresponding confidence intervals were estimated for the different poultry species and sampling date using the *epiR* package. The influence of the risk factors on flock-level prevalence was studied using generalised linear models with a logit link function. Clustering of

poultry flocks within farms was accounted for by adding a random effect to the model. Univariate screening revealed that categorization of flock size into a binary variable (1 to 15 birds *vs.* more than 15 birds) and logarithmic transformation of distance to the nearest body of water provided a better fit. A full starting model was first constructed using flock-level prevalence as the output and six potential explanatory variables: year, flock species, flock size, number of different flocks within the farm, dominant landscape type and distance to the nearest water body. Variation in the number of flocks present in each farm was accounted for by adding a weight term to the model. Two-way interactions were added to the multivariate model and their effect was statistically evaluated. The multivariate analysis was carried out using a stepwise, backward elimination process with the final model selected according to the Akaike information criterion (AIC) (Dohoo et al., 2003). Goodness-of-fit of the model was assessed by using the test described by Hosmer and Lemeshow (2000).

Epidemiological datasets often display spatial autocorrelation and if this pattern remains in the residuals of a statistical model based on such data, parameter estimates and standard errors may be biased (Dormann et al., 2007). However, if the spatial dependence in the response variable is completely explained by the spatial pattern of the explanatory variables, no spatial autocorrelation should be present in the residuals and regression should produce correct effect estimates and confidence intervals (Dohoo et al., 2003). Considering this, we analysed the spatial correlogram of the Moran's *I* statistic by computing values at various spatial lags using the R *spdep* package (Trevennec et al., 2011) to identify any not accounted-for spatial correlation in the residuals of the logistic model. The Moran's permutation test was also performed for each km from 0 to 30, the latter value corresponding approximately to half of the maximum distance between pairs of flocks (Bivand et al., 2008).

A prediction map for the risk of avian influenza was produced from landscape rasters covering surroundings of Lake Alaotra. The following equation, based on the model's coefficient, was used to derive a predicted probability (*p*) of AIV infection for small chicken flocks in 2008 (the reference categories):

$$P = \frac{e^{(\alpha + \beta_1 * Dwater + \beta_2 * Wetland + \beta_3 * Rice)}}{1 + e^{(\alpha + \beta_1 * Dwater + \beta_2 * Wetland + \beta_3 * Rice)}}$$

with α being the intercept, β_1, β_2 and β_3 the estimated coefficients, Dwater the log-transformed distance from the farmhouse to the closest water body, *Wetland* and *Rice* the presence of predominant wetlands or rice fields in a 1-km neighbourhood around the poultry farms, respectively.

Results

Landscape classification

Seventeen classes of land cover were identified from the supervised classification (Fig. 2). In a 1-km neighbourhood around the farms considered, the main vegetation types were: grassland savannah, rice paddies, marshes, disturbed marshes (degraded wetlands, where papyrus swamps are regularly burned by villagers during the dry season), marsh savannah, riverine forest, eucalyptus area and euchornia (water hyacinth) areas. Water bodies were also present in the buffers. Three predominant landscapes were identified around the poultry farms:

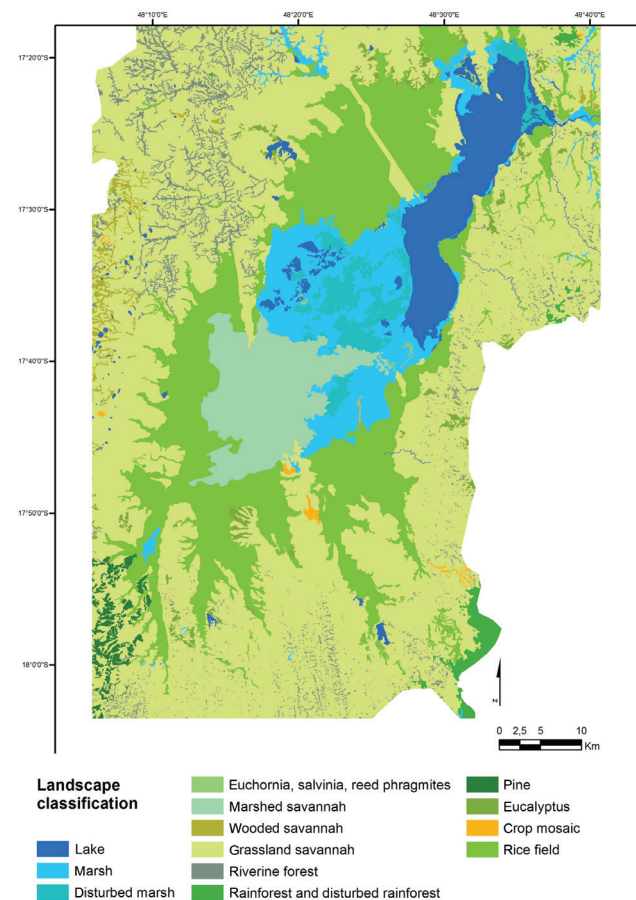(i) dry savannah landscape characterised by more than 60% grassland savannah (43 farms);

Fig. 2. The land cover in the Lake Alaotra region characterised by supervised classification of Landsat imagery dated March 2007.

Table 1. Bird-level, crude seroprevalence and confidence intervals of LPAI by species and sampling date.

Species	August 2008		May 2009		Total	
	Prevalence*	95% CI**	Prevalence*	95% CI**	Prevalence*	95% CI**
Chicken	0.07 (n = 151)	0.03-0.11	0.04 (n = 255)	0.01-0.06	0.05 (n = 406)	0.03-0.07
Geese	0.32 (n = 82)	0.22-0.42	0.12 (n = 317)	0.08-0.15	0.16 (n = 399)	0.12-0.19
Duck	0.56 (n = 62)	0.44-0.69	0.17 (n = 171)	0.11-0.22	0.28 (n = 233)	0.22-0.33
Total	0.24 (n = 295)	0.19-0.30	0.10 (n = 743)	0.08-0.12	0.14 (n = 1,038)	0.12-0.16

*and number (n) of sampled poultry; **95% confidence interval.

(ii) rice landscape characterised by more than 60% rice paddies (51 farms); and

(iii) wetlands with approximately 30% of grassland savannah, 30% of rice paddies and 30% of marsh (61 farms).

Risk factors for avian influenza type A

A total of 1,038 individual bird blood samples distributed among 241 flocks were analysed. These flocks belonged to 155 different farms, 84 of which had one flock, 56 two flocks and 15 three flocks. None of the positive sera for AIV antibodies was also positive for H5 and H7. The crude bird-level AIV seroprevalence and 95% confidence intervals (CIs) are presented in Table 1.

The final model included all the tested variables except the number of different flocks within the farm. According to the likelihood ratio test (P = 0.842), the model was not significantly improved by the addition of this latter variable. None of the interactions tested was retained in the final model. The goodness-of-fit χ^2 test according to Hosmer and Lemeshow (2000) indicated that the model had an adequate fit (P = 0.99). The spatial correlogram of Moran's *I* (Fig. 4) as well as the Moran's permutation test (I = -0.015 and P = 0.64 for the 30-km lag) did not show evidence of unaccounted spatial pattern in the model residuals.

Table 2 shows the results of the final multivariate analysis. Values of the AIC confirmed that the final model corresponding to a 1-km radius provided the better fit. Bird species was the most influential variable, with higher flock-level seroprevalence observed in ducks or geese than in chickens. Flock size had also a significant effect, with flocks of more than 15 birds presenting higher odds of AIV infection than small ones. AIV seroprevalence varied with the sampling date (significantly lower in May 2009 than in August 2008). A significant, negative relationship was found between AIV seroprevalence and distance to the closest body of water. Finally, we found that flocks with predominant wetlands or rice landscapes in their neighbourhood were exposed to greater risk than those surrounded by dry savannah.

Risk mapping for avian influenza type A

The risk of avian influenza type A was found to be influenced by the landscape characteristics and was consequently not homogeneously distributed in the Lake Alaotra region. The main at-risk areas were located in the western and southern part of the lakesides, in areas with predominance of rice fields and in the surroundings of the cities Ambohijanahary, Andromba, Amparafaravola and Ambatondrazaka (Fig. 3).

Table 2. Results of logistic regression of the serological status against LPAI in the Lake Alaotra region, Madagascar.

| Variable	Category	Odds ratio	95% CI*	P-value
Flock species	Chicken	1.00		
	Duck	10.85	5.48-22.75	4.27e-11
	Geese	5.24	2.68-10.83	2.84e-06
Sampling date	August 2008	1.00		
	May 2009	0.40	0.23-0.69	8.55e-04
Flock size	1 to 15 birds	1.00		
	>15 birds	2.04	1.23-3.41	5.81e-03
Distance to water body (m)	Continuous variable	0.71	0.59-0.85	2.24e-04
Predominant landscape	Grassland savannah	1.00		
	Wetland	1.95	1.04-3.80	4.16e-02
	Rice fields	3.38	1.79-7.97	4.25e-03

*95% confidence interval.
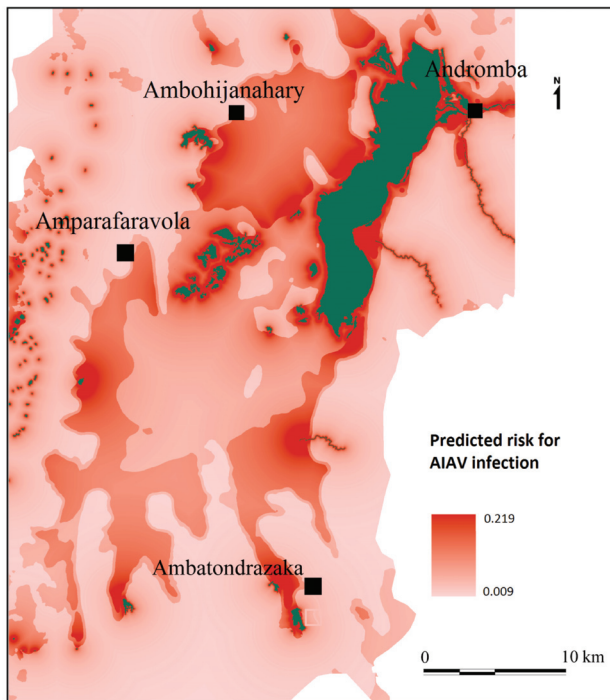
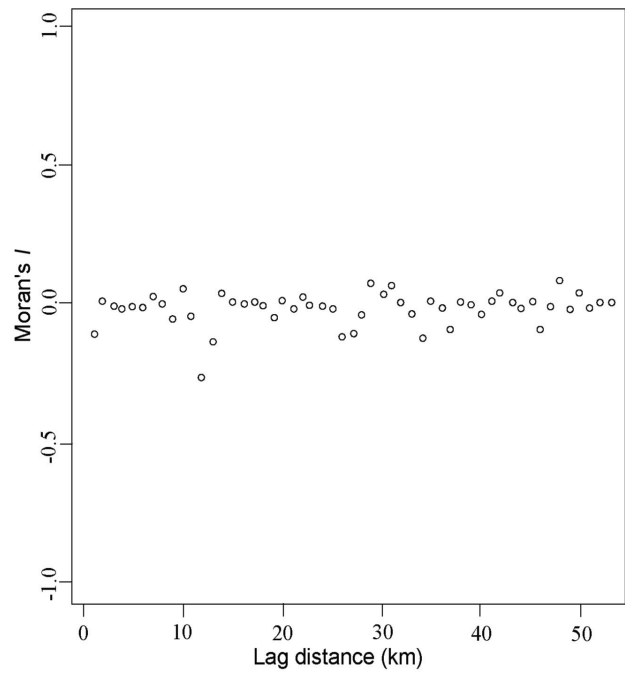Fig. 3. Risk map of 2008 for avian influenza A in small chicken flocks around Lake Alaotra, Madagascar.

Fig. 4. Spatial correlogram of the Moran's *I* for final model residuals.

Discussion

The present study, carried out on a large number of poultry samples, provides new insights into the circulation pattern of LPAI in poultry. It is also the first study on environmental patterns associated with avian influenza in Madagascar. The results clearly demonstrate that, in addition to some flock-level risk factors,

the environmental conditions that prevail around the farms in the Lake Alaotra region actively influence the circulation of AIV in poultry. Having stated this, we feel that some study limitations need to be considered before discussing the results further. First, we derived the epidemiological status of individual poultry from diagnostic tests, which are known to sometimes perform imperfectly. However, the diagnostic errors may

Table 3. Results of logistic regression of the serological status against LPAI, for different buffer size, in the Lake Alaotra region, Madagascar.

Variable	Category	500-m buffer			1-km buffer			2-km buffer		
		Coefficient	SE*	P-value	Coefficient	SE*	P-value	Coefficient	SE*	P-value
Intercept		-1.22	0.66	0.064	-1.32	0.65	0.040	-1.03	0.75	0.170
Flock species	Chicken	REF**			REF**			REF**		
	Duck	2.34	0.36	<0.001	2.38	0.36	<0.001	2.22	0.36	<0.001
	Geese	1.64	0.35	<0.001	1.65	0.35	<0.001	1.58	0.35	<0.001
Sampling date	August 2008	REF**			REF**			REF**		
	May 2009	-0.98	0.27	<0.001	-0.92	0.28	<0.001	-0.99	0.27	<0.001
Flock size	1 to 15 birds	REF**			REF**			REF**		
	>15 birds	0.69	0.26	0.007	0.71	0.26	0.006	0.63	0.26	0.014
Distance to water body (m)	Continous variable	-0.29	0.09	0.001	-0.34	0.09	<0.001	-0.35	0.09	<0.001
Predominant landscape	Grassland savannah	REF**			REF**			REF**		
	Wetland	0.25	0.32	0.434	0.67	0.33	0.042	0.37	0.48	0.438
	Rice fields	0.69	0.36	0.060	1.22	0.43	0.004	1.18	0.54	0.029
Akaike information critcriterion (AIC)		257.4			252.0			254.6		

*standard error; **reference category.

have been limited for chickens as sensitivity and specificity of the commercial ELISA test that we used were 98.70% and 98.72%, respectively, according to the manufacturer. On the other hand, this may be more problematic for palmipeds given the lower test performances for these species (89.02% and 88.79%, respectively, according to the manufacturer). False positive and false negative results may thus be present in our dataset. Second, landscape was characterised based on a remote sensing image dating from March 2007, while the serological surveys were carried out in August 2008 and May 2009. This time gap may have resulted in misclassification of environmental variables if the land cover changed over time, a possibility that cannot be excluded, as the expanse of the water surface varies greatly in Lake Alaotra depending on climatic conditions and the dynamism of the marsh and rice cultivation zones (Bakoariniaina et al., 2006). Finally, variations between-farms seroprevalence rates might also have been influenced by poultry farming practices such as biosecurity measures and exchange of live poultry, which were not accounted for in the present analysis (Andriamanivo et al., 2012). In spite of these limitations, the present study made it possible to identify risk factors associated with exposure of poultry to AIV.

The study found higher AIV seroprevalence in palmipeds (ducks and geese) than in chickens, which is consistent with findings by other serological surveys carried out in unvaccinated birds raised in small-scale farms of Indonesia (Santhia et al., 2009) or Vietnam (Henning et al., 2009; Desvaux et al., 2012). The reason why this should be so can be explained. First, ducks and geese serve mainly as egg producers and have a long lifespan and may thus have been exposed to AIV for longer periods than chickens, which are generally slaughtered at the age of 6 to 12 months. Desvaux et al. (2012) have shown that the effect of species on H5 seroprevalence persist after being adjusted for poultry age, and this indicates that age was not the only explanation for the species variation observed. Second, experimental studies suggest that ducks shed virus for longer periods of time and at higher than chickens (Achenbach and Bowen, 2011; Arsnoe et al., 2011; Hénaux and Samuel, 2011) resulting in a greater probability of detection of specific antibodies in ducks. Second, ducks and geese may have had greater exposure to AIV due to differences in flock management. Indeed, while chickens stay in the backyard or in close vicinity to houses, ducks and geese in Madagascar are brought to rice fields where they feed on snails and crop residuals, movements that may enhance their

probability of contact with infected wild or domestic birds (Henning et al., 2013).

We also found that AIV seroprevalence was higher in large flocks (defined as >15 birds) than in small ones. This is consistent with findings from previous studies which showed that the risk of HPAI H5N1 increased with flock size (Desvaux et al., 2012). This may be due to an increased transmission in large flocks, related to higher poultry density and higher degree of contacts between birds. But it should also be noted that flock size could be a surrogate for other unmeasured variables related to avian influenza risk such as contacts with other farms and other poultry chain actors, trading intensity or use of inputs.

At this stage, it is not possible to conclude if our finding that AIV seroprevalence was significantly higher in August 2008 than in May 2009 are related to inter-annual or seasonal effects. They can of course also be a completely unrelated artefact. Temporal variations of seroprevalence should also be considered in regard to the persistence of AIVA antibodies in poultry over time. It has been suggested that AIV prevalence may vary with contacts between poultry and wild birds and virus survival under environmental conditions, including temperature (Molia et al., 2011). Also, seasonality of poultry production and trade may contribute to temporal variations of AIV seroprevalence in Madagascar. Flocks of ducks and geese, which feed with crops residuals, are preferentially brought to rice paddy fields just after the harvest which is carried out in April and May around Lake Alaotra (Van Hulst, 2011). Repeated cross-sectional serological studies are on-going in the same study area to explore the temporal pattern of AIV circulation in more details; peaks of AIV seroprevalence should also be examined in regard to rice harvest calendar and free-ranging of palmipeds.

Our study made it possible to provide evidence of environmental variables associated with increased AIV circulation in Madagascar, and we showed that AIV circulation was influenced by the type of landscape close to poultry farms (≤1 km). In particular, the present study not only showed that neighbourhood landscapes with predominant rice production were associated with higher seroprevalence level, which is consistent with previous studies on eco-epidemiology of HPAI H5N1 in Southeast Asia (Gilbert et al., 2008), but was also the first to demonstrate this effect for LPAI in a non-Asian setting. It has been suggested that rice paddy fields may favour HPAI H5N1 persistence because they constitute an interface between wild birds feeding there, and domestic poultry. The fre-

quent flooding of rice fields would also allow a greater viral persistence in this environment compared to dry soils (Van Boeckel et al., 2012). Reports from Thailand, Vietnam and Indonesia (Gilbert et al., 2008; Loth et al., 2011) found that HPAI H5N1 outbreaks occurred mainly in areas where rice is cultivated through an intensive, multiple crop system. The authors suggested that this may be related to the fact that the highest numbers of free-grazing ducks are raised in these areas, where the irrigation network is the densest and flooding the most frequent. It is hence noteworthy that we highlighted the role of rice cultivation on AIV circulation in Lake Alaotra region, where rice cultivation is extensive and based on only one cycle per year. Further studies are now needed to examine the comparative dynamics of rice cropping, flooding, field irrigation and AIV circulation.

The finding that AIV circulated more intensely in wetland areas or at short distance from a body of water (lake, river or canal) is consistent with previous studies on HPAI H5N1 (Gilbert and Pfeiffer, 2012) and indicate that water media probably also played a role in persistence and spread of low pathogenic avian influenza. Wetlands and vicinities of water bodies are critical habitat for waterfowl, which are recognised as the natural reservoir of AIV (Olsen et al., 2006): wetlands of Lake Alaotra region may thus be favourable to AIV transmission through direct contact between waterfowl and poultry. These contacts may have been particularly frequent in Lake Alaotra, given the fact that most poultry are raised in the backyards or by free-range systems. Research to date also suggests that AIV could be transmitted through indirect faecal-oral route involving contaminated water, as supported by findings showing that AIV infectivity can persist over days at warm temperature (Brown et al., 2009). The high density of wild waterfowl present on the lakesides (BirdLife International; http//www.birdlife.org/datazone/sitefactsheet.php?id=6567) may as well have enhanced this route of transmission.

Hotspots identified on the present risk map could inform veterinary authorities for strengthening surveillance protocols of avian influenza in poultry in a risk-based perspective. The risk map (Fig. 3) suggests that lakesides may constitute high risk areas and would thus deserve long-term monitoring for AIV circulation in domestic and wild birds. However, field observation indicate that implementation of such a programme might be challenging. Indeed, due to their remote location and the scarcity of animal health workers network in this area, high-risk villages located on the lakesides have low accessibility to veterinary services.

The risk map also suggests that, based on landscape characteristics, the risk for AIV infection is high in vicinities of cities such as Ambohijanahary, Andromba, Amparafaravola and Ambatondrazaka. This also raises attention as these cities may constitute a risk node for AIV, given the presence of live bird markets and flows of poultry transported in their surrounding areas through road networks.

The highlighted environmental pattern of AIV circulating more intensely in areas where wetlands and rice landscapes are predominant in the highland region of Madagascar shares similarities with Southeast Asian agro-ecosystems, which have been previously found associated with an increased risk of HPAI outbreaks. Results from the present study thus suggest that if the H5N1 virus was introduced in Madagascar through wild birds or poultry products movements, the environmental conditions that prevail in Lake Alaotra region may allow the virus to spread and persist. However, this risk might be mitigated by the low poultry and human population densities that are encountered in Madagascar when compared with Southeast Asia.

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