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Spatial modeling for low pathogenicity avian influenza virus at the interface of wild birds and backyard poultry

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Summary

Low pathogenicity avian influenza virus (LPAIV) is endemic in wild birds and poultry in Argentina, and active surveillance has been in place to prevent any eventual virus mutation into a highly pathogenic avian influenza virus (HPAIV), which is exotic in this country. Risk mapping can contribute effectively to disease surveillance and control systems, but it has proven a very challenging task in the absence of disease data. We used a combination of expert opinion elicitation, multicriteria decision analysis (MCDA), and ecological niche modeling (ENM) to identify the most suitable areas for the occurrence of LPAIV at the interface between backyard domestic poultry and wild birds in Argentina. This was achieved by calculating a spatially-explicit risk index. As evidenced by the validation and sensitivity analyses, our model was successful in identifying high-risk areas for LPAIV occurrence. Also, we show that the risk for virus occurrence is significantly higher in areas closer to commercial poultry farms. Although the active surveillance systems have been successful in detecting LPAIV-positive backyard farms and wild birds in Argentina, our predictions suggest that surveillance efforts in those compartments could be improved by including high-risk areas identified by our model. Our research provides a tool to guide surveillance activities in the future, and presents a mixed methodological approach which could be implemented in areas where the disease is exotic or rare and a knowledge-driven modeling method is necessary.

Keywords

Low pathogenicity avian influenza, backyard poultry, knowledge-driven spatial modeling, risk factors, wild birds.

Introduction

Avian influenza viruses (AIV) can cause disease in domestic and wild birds and are classified into highly pathogenic and low pathogenicity avian influenza viruses (HPAIV and LPAIV, respectively) (Alexander, 2000). Disease caused by H5 and H7 virus subtypes of LPAIV is reportable to the World Organization for Animal Health (OIE). Although it can be associated with no clinical signs, clinical illness can occur thereby leading to production losses and decreased welfare (Spickler et al., 2008).

Despite their low pathogenicity, LPAIVs have the potential to combine into genetic reassortments to generate HPAIVs (Fouchier & Munster, 2009), which can have serious economic consequences. Control measures associated with LPAIV outbreaks, such as stamping out or depopulation of farms, vaccination, restocking bans and movement restrictions, have often resulted in huge economic losses (Busani et al., 2007).

Wild aquatic birds, mainly in the orders Anseriformes and Charadriiformes, are considered major natural reservoirs for LPAIVs (Gaidet et al., 2012). Typically, LPAIV infections in these birds are asymptomatic but can be associated with viral shedding in feces (Webster et al., 1992), and once excreted they can persist in aquatic habitats for extended periods (Brown et al., 2009) thus favoring transmission to other hosts, virus replication and dispersal to new geographic locations through migratory species.

Migratory birds can carry pathogens, particularly those that do not significantly affect the birds' fitness and migration (Olsen et al., 2006). The role of long-distance migration on AIV dispersal to distant geographical regions has been suggested for HPAIV (e.g., Salzberg et al., 2007; Prosser et al., 2009; Gaidet et al., 2010; Takekawa et al., 2010; Pearce et al., 2011; Lycett et al., 2016), but evidence on the rarity of such events also exists (Krauss et al., 2007). Also, studies indicate that long-distance dispersal of LPAIV via migratory birds does occur within North America (Fries et al., 2015) and between North America and Asia (Koehler et al. 2008; Jackwood & Stallknecht, 2007; Fries et al., 2013; Wahlgren et al., 2008; Makarova et al., 1999).

In America, only one species in the order Anseriformes, the blue-winged teal (*Anas discors*), migrates between northern and southern hemispheres (Nearctic migrant) during the non-breeding season, and this species' LPAIV prevalence has been reported to be high during migration (Stallknecht et al., 1990). Regarding the Charadriformes, among the 38 species that migrate between Canada-US and South America (Canevari et al., 2001), most LPAIV isolations have been from the ruddy turnstone (*Arenaria interprets*) (Maxted et al., 2016), which migrates between the Artic and Tierra del Fuego in Argentina every year. Research suggested that the risk for introduction of HPAIV in Argentina via Nearctic migrants is negligible, whereas the risk for LPAIV introduction can be significant (SENASA, 2010).

Generally, the transmission of LPAIV from wild birds to poultry has been supported by phylogenetic studies, which supports the theory that backyard poultry is one of the links that connects LPAIV between wild birds and poultry (Terregino et al., 2005). Then, backyard poultry can play an important role in the epidemiology of the disease (Biswas et al., 2009), and outdoor ranging of the birds represents a major risk factor for the introduction of LPAIV and the potential development of HPAIV subtypes (Koch et al. 2006). Backyard chicken density may also contribute to virus spread (Conan et al. 2013; Walker et al. 2012), as these flocks are usually managed under deficient biosecurity measures and they are at a high risk for exposure to AIV in general, and LPAI in particular, from wild birds.

AIV have been identified in Latin America; however, there is a lack of understanding of these viruses at the regional level. Of the reported cases of AIV in Latin America, 43.7% correspond to migratory birds, 28.1% to local wild birds, and 28.1% to poultry (Afanador-Villamizar et al. 2017). Argentina is considered free from HPAIV and, as major producer and global exporter of chicken meat, the emergence of this disease could have a large economic impact due to mortality and trade restrictions. Contrarily, different LPAIV strains have been reported in wild birds from Argentina (Pereda et al., 2008; Xu et al., 2012) and neighboring countries like Chile (e.g., H7N3: A/CinnamonTeal/Bolivia/4537/01) (Spackman et al., 2006) and Brazil (e.g., H11N9: A/ruddy turnstone/New Jersey/Sg-00564/2008) (de Araujo et al., 2014). Regarding domestic poultry, active surveillance of

LPAIV has been in place in the Argentina since 2014 (SENASA, 2010), with 50 cases reported in the backyard compartment (SENASA, pers. comm.).

Globally, there has been a rapid growth of the poultry sector in recent years, with the most radical shifts in consumption towards poultry meat taking place in Latin American countries, who were the traditional producers and often major exporters of bovine meat (Bruinsma, 2003). As well, the backyard poultry sector has grown considerably in Argentina through the promotion of the activity by government agencies. Despite this regional growth, resources for disease surveillance and control are typically scarce in developing countries, and the use of risk-based methods can be crucial for their optimization. Such methods, however, become less applicable in situations where disease data are unavailable or if a country has not yet detected the incursion of disease. In data-scarce situations like these, knowledge-driven modeling methods such as multicriteria decision analysis (MCDA) appear as a useful alternative approach (Pfeiffer et al., 2008).

The objectives of this study were (1) to generate spatial risk models for LPAIV occurrence at the interface between backyard poultry and wild bird populations in Argentina, which could be used to improve risk-based epidemiological surveillance activities, and (2) to develop a methodological approach that can be implemented under data-scarce scenarios.

Methods

Expert opinion elicitation and MCDA method

This study was conducted under a data-scarce, highly uncertain scenario because there are only a few reports of LPAIV in the study area. Therefore, a knowledge-driven modeling approach, multicriteria decision analysis (MCDA), was used as an alternative approach to data-driven modeling. Detailed descriptions of the process of spatial MCDA can be found elsewhere (e.g., Malczewski & Rinner, 2015).

Here, experts' opinions were collected in an individual manner using an electronic questionnaire and were later aggregated using a weighted linear combination. Firstly, a survey was submitted to a group of 83 researchers, academics, and staff at non-governmental, governmental and international organizations who have published or actively participated in research on AIV in relation to wild birds worldwide, and they were asked to submit a list of people who they identified as "experts" in this field. A total of 136 researchers were referenced, and each one was cited between one and 13 times. Only those selected >3 times (*n* = 14 researchers) were included in the expert opinion study. An additional sample of 14 highly knowledgeable researchers who were not selected in the first round was added to the expert group. The latter were selected based on their track-record of publications in the field. Although there is no defined guideline on which to base the number of experts to be included in opinion elicitation related to health issues (Knol et al., 2010), the number of experts we consulted is in line with sample sizes previously reported (Krueger et al., 2012).

The selected experts were asked to fill in a pair-wise comparison matrix used in the analytical hierarchy process (AHP), which included four risk factors (see below). Each factor was compared with the others, relative to its importance, on a seven-point scale ranging from 1/4 (“extremely less important”), through 1 (“equal importance”), to 4 (“extremely more important”). Then, weights were generated for each factor using the pairwise comparisons of the AHP (Saaty & Vargas, 2012). The pairwise comparisons were organized into a matrix $C = \left[C_{pk}\right]_{n \times n}$ where C_{pk} is the pairwise comparison rating for the k^{th} and p^{th} criteria. The matrix *C* was reciprocal; that is, $C_{pk} = {C_{kp}}^{-1}$, and all its diagonal elements are unity; that is, $C_{pk} = 1$ for $k = p$. For each pair of risk factors, experts were required firstly to specify whether one of the risk factors (e.g., risk factor A) is more or less important than another risk factor (e.g., risk factor B) with regards to the outcome. Following, they specified the degree of importance on the scale provided. Once the pairwise comparison matrix was obtained, a vector of priorities or criterion weights $[w_1, w_2, \cdots, w_n]$ was derived from each of the individual matrices by solving for the principal eigenvector of each matrix and then normalizing the result (Saaty, 1987). The largest eigenvalue of each matrix was also calculated and used to derive a consistency index (C_i) and a consistency ratio (C_r) for each individual expert, where $C_r < 0.1$ indicates a reasonable level of consistency in the pairwise comparisons and $C_r \geq 0.1$ is indicative of inconsistent judgments, meaning that one should reconsider the answers provided by the expert. The geometric mean was the uniquely appropriate rule for combining judgments (i.e., individual matrices) submitted by all the experts, as it preserves the reciprocal property in the combined pairwise comparison matrix (Aczel & Saaty, 1983). Then, judgements were combined by calculating their geometric mean to obtain the group judgment for each pairwise comparison. C_r and C_i were calculated for the combined matrix.

Risk factors and relationship with LPAIV

The study area included mainland Argentina and Tierra del Fuego province. There is a paucity of studies addressing risk factors for LPAIV in general, and in a spatial context in particular; therefore, the systematic review published by Gilbert & Pfeiffer (2012) was used to identify country-level risk factors associated with the occurrence of HPAIV under the assumption that those risk factors can be used as proxies for the occurrence of LPAIV if they are a priory ecologically meaningful for the system being modeled (wild birds-backyard interface).

Variables were included in the MCDA model provided they met a set of criteria used by Stevens et al. (2013): (i) able to be mapped, (ii) the spatial layers could be sourced from the public domain or could be derived from raw data provided by other researchers, at a sufficiently high resolution to differentiate within-country heterogeneity, (iii) can reflect broad causal relationships at a country-specific scale (i.e. have been identified to be of importance at least in one country), and (iv) have been repeatedly identified to be significantly ($p \leq 0.05$) associated with HPAIV occurrence.

Additionally, an online literature search was done in peer-reviewed journals through PubMed with the term «low pathogenicity avian influenza» or «LPAI» or «avian influenza» combined with the terms «risk» or «risk factor» or «spatial» or «distribution». Based on the results, we performed a selection including all papers with explicit reference to risk factors associated with occurrence of LPAIV. A total of four papers (Gonzales et al., 2012; Tombari et al., 2013; Bouwstra et al., 2017; Belkhiria et al., 2018) were selected, as they provided statistical support to the selection of risk factors according to the above-mentioned criteria.

Spatial data layers with clearly defined attributes (e.g., Euclidean distance to a feature) are known as crisp sets (non-Boolean), as opposed to fuzzy sets, which indicate the hypothesized strength of the association between different values of the risk factor and the outcome (Malczewski, 1999, 2000). Here, because of the lack of sufficient data to rely on a frequentist approach, the underlying uncertainty regarding the association between crisp risk factors and the outcome of interest was modeled using fuzzy membership functions. Thus, instead of associating probabilities to events, we defined degrees of possibility to claims. The choice of the corresponding membership functions was guided by the ontological parsimony principle which prescribes to avoid, in the absence of more relevant information, choosing complex explanations, favoring the simplest ones instead (Quine, 1981). After the fuzzyfication process, different spatial layers were produced which represented the relationship between each risk factor and the outcome while accounting for any underlying uncertainty process. Finally, a risk index (R) was estimated for each spatial unit (raster cell) using a weighted linear combination approach as shown in equation 1:

$$R = \sum_{ij=1}^{n} (W_j \times F_{ij}) \qquad \text{Eq. 1}$$

where R represents the risk index estimate for each raster cell, W is the weight for risk factor j and F is the value of risk factor j for raster cell i.

The following four risk factors were included in the MCDA: 1) proximity to water bodies and wetlands, 2) proximity to areas suitable for rice-growing, 3) presence of wild birds, and 4) presence of backyard poultry.

1. Proximity to wetlands

Proximity to open water (ProxWet) has been shown to be associated with AIV occurrence (Ward et al., 2008; Biswas et al., 2009; Martin et al., 2011), as open water and wetlands can be used by migratory and resident waterbirds. Ward et al., (2008) showed that HPAIV outbreaks in domestic poultry generally occur within 5 km of open water sites. Therefore, this variable was included in models under the assumption that habitat suitability for LPAIV is also highest closest to wetlands and decreases in a sigmoidal, monotonic pattern as distance increases, reaching negligible risk at distances greater than 10 km (Fang et al., 2008). A fuzzy membership function of the form

$$y = \ 1\ /\ (1 + e\char`^(x - 7.5\ ))$$

was used, where y represents a membership value for each cell which follows the shape described above depending on varying values of x (distance from wetland). The proposed mechanism behind the selected function is that backyard poultry close to open water are more likely to come into contact with infected water or wild waterfowl, and that this risk decreases with increasing distance from wetlands.

Briefly, a ProxWet layer was generated using a vector model of wetlands distribution for Argentina (Kandus et al., 2008), which was rasterized and resampled to a resolution of 30 arc-seconds. Following, a new raster was built which showed the Euclidean distance between each cell and the closest cell corresponding to a wetland.

2. Proximity to rice fields

The percentage of land used for rice has been shown to be associated with HPAIV occurrence (Pfeiffer et al., 2007; Gilbert et al., 2008). Here, we assumed that proximity to areas suitable for rice-growing (ProxRice) would have a similar relationship with suitability for HPAIV occurrence as proximity to wetlands. Therefore, we assumed that risk was highest closest to areas suitable for rice-growing (0-5 km) (Ward et al., 2008) and decreased thereafter in a sigmoidal, monotonic fashion with negligible risk after 10 km (Fang et al., 2008). The layer ProxRice was generated using a vector model of rice field distribution provided by the National Institute of Agricultural Technology (INTA) (Perucca com. pers). This information was processed in the same way as ProxWet to generate a raster layer, and the same membership function was applied.

3. Presence of waterbirds

The role of wild birds (WildBirds) as reservoirs of LPAIV and in the geographic distribution of LPAIV and HPAIV has long been acknowledged (Olsen et al., 2006; Beato & Capua, 2011). Here, the potential distribution of 22 species of waterbirds was analyzed using a maximum entropy approach (see below) and then included in the models. Since no information could be found on the possible functional shape of the association between waterbirds presence and LPAIV occurrence, a linear relationship was assumed (Quine, 1981).

4. Presence of backyard poultry

Following Leon et al. (2009), the distribution of domestic birds in Argentina was estimated by using high-resolution population data to obtain the distribution of households, and under the assumption that the probability that a family owns domestic birds decreases as a function of population density. The WorldPop human population dataset (Lloyd et al., 2017) was used with a resolution of 30 arc-seconds. A population density raster was obtained by dividing the population in each cell by the cell area, computed as a function of the cell latitude. To estimate the number of families for each cell, a series of family sizes (number of people) were generated by sampling randomly from a Poisson distribution with a mean household size of 3.3 (INDEC, 2010). Samples were obtained until the sum of individual family sizes reached the total population corresponding to the cell. Finally, the number of families with backyard poultry was estimated by randomly selecting families with a probability that is a function of the population density in the cell (Table 2, Leon et al., 2009).

According to our model, the number of families owning backyard poultry varied between 0 and 4, but in 99.9% of cells with backyard poultry only one or two families had birds. No information could be found on the possible functional shape of the association between backyard poultry and LPAIV; therefore, a linear relationship was assumed between the possible range of families with poultry and the hypothesized relationship with LPAIV occurrence (Quine, 1981).

High-risk waterbird species selection

We used updated information on distribution and migration of waterbird species with a potential role in AIV presence in Argentina. These species were selected following Blanco et al. (2008) and the criteria developed by Delany et al. (2006). Our analyses were restricted to the orders Anseriformes (swans, geese and ducks) and Charadriiformes (waders, gulls and terns) plus the order Pelecaniformes. Initially, we selected 25 waterbird species considered as high risk species (hereafter HRS), for which information was compiled and standardized using the Neotropical Waterbird Census data (NWC) for the period 1990-2006 (Wetlands International, 2008) and other sources of information (literature, unpublished reports, queries to specialists, etc.; see Blanco et al., 2008). The NWC is conducted with the main objective of providing baseline information on the distribution and abundance of waterbirds and wetland habitats within the Neotropics. Standardized waterbird counts are done at the same sites twice a year in February and July.

The list of HRS selected for mapping included 10 Nearctic migrants and 15 Austral migrants, of which 14 are Charadriiformes, 10 Anseriformes, and one Pelecanifomes (Table 3). Neartic migrants are those species that breed in the northern hemisphere and migrate to to the Neotropics, including Argentina, during the non-reproductive season, whereas Austral migrants breed in southern South America and migrate to central and northern South America during the austral winter. For three species, sample sizes were too small which precluded modeling their distribution with adequate confidence; thus, these species were discarded from the analyses, resulting in a final set of 22 species to be considered (Table 3).

Species distribution modeling

The data used for waterbirds distribution originated from the NWC and other sources of information. Here, presence-only data were used for modeling, which helped avoid the disadvantage of resorting to modeling approaches dealing with presence-absence data and the associated biases such as false absences (Peterson et al., 2011).

The potential distribution of each of the 22 remaining waterbird species was modeled using MaxEnt version 3.4.1 (Phillips & Dudík, 2008; Phillips et al., 2006) through the package “dismo” (Hijmans et al., 2017) in the R statistical environment (R Core Team, 2017). Environmental variables in the CliMond dataset version 1.2 (Kriticos et al., 2012) with spatial resolution of 10’ of degree were used as model predictors. Given the large number of MaxEnt models needed in this study, and because our objective was only to map the potential habitat of each species and not to understand the environmental factors which defined it, first five principal components of the 35 CliMond variables were used, which account for over 90% of the variance in the dataset (Kriticos et al., 2014). In addition, given that the distribution of waterbirds is strongly influenced by the presence of wetlands, we generated a raster (10’ of degree resolution) with distance to the closest wetland based on the wetland database developed by Kandus et al. (2008), and included this layer in the MaxEnt models.

The bulk of the waterbird records (n = 3,034; 72.1%) used in this study came from the Neotropical Waterbird Census, in which a representative number (n = 600) of wetlands are visited at least once a year, and all observed species are recorded in a consistent manner. There was noticeable geographical bias in the remaining observations (n = 1,176) that originated from other sources, including literature reviews and museum specimens, among others (Blanco et al. 2008). To avoid incorrect predictions in the MaxEnt models, we accounted for sampling bias by selecting the background data so they reflect the same bias as the occurrence data (Fourcade et al., 2014; Phillips et al., 2009). Because most of the wild bird records came from the NWC, it is reasonable to conclude that the aggregated distribution of all observations is a good proxy for the distribution of sampling effort (Fourcade et al., 2014; Phillips et al., 2009). For sites visited more than once during the year, each observation was included individually adding a small amount of random noise to the site coordinates. The sampling bias layer was computed using a two-dimensional kernel density estimation with an axis-aligned bivariate normal kernel evaluated in a square grid of 120 cells. Values of the kernel were projected to each cell in the model raster, and scaled so their sum equaled to 1. A set of 10,000 background points were selected using the cell values as probabilities, and were used in each of the MaxEnt models.

For the models in this study we used linear and quadratic features to capture unimodal species responses, and hinge features, which are a generalization of linear and threshold features. Threshold features were not included, as their absence generally results in models that are smoother, simpler, and likely to be more realistic. All models used a regularization parameter equal to 1. The selected format for the model output was "cloglog", which under certain assumptions can be considered a probability of presence ranging between 0 and 1 (Phillips et al., 2017).

The output of the 22 MaxEnt models was combined to produce maps showing the potential distribution of wild bird assemblages by averaging the MaxEnt output in each cell following a "predict first, assemble later" strategy (Barker et al., 2014). Three combined maps were produced, one using all wild bird species and two combining different sets of species according to their migration pattern (Neartic or Austral migrants).

Spatially-explicit risk index

Three different risk index estimates (R) were obtained based on equation 1 and using (i) the combined risk of all 22 bird species, (ii) the risk associated with Neartic migrants only, and (iii) the risk associated with Austral migrants only. The risk index including all the species provides a general model of habitat suitability for LPAIV (Figure 2). The spatial patterns of R computed with Neartic migrants only and Austral migrants only were very similar, which made interpretation of underlying risk patterns difficult to appreciate.

Therefore, the pixel-wise difference in R between the Austral (R_{aus}) and Nearctic (R_{nearc}) models was estimated to produce a map based on their differences (R_{diff}), where $R_{\text{diff}} < 0$ represents areas with higher risk associated to Nearctic migrants, $R_{\text{diff}} \approx 0$ represents areas with nearly equal risk associated with both groups, and $R_{\text{diff}} > 0$ represents areas with higher risk related to Austral migrants (Figure 3). In addition, the relative risk index (RR) was calculated as the pixel-wise quotient, given by $RR_{\text{nearc}} = R_{\text{nearc}}/R_{\text{aus}}$ (Figure 4A). To simplify model interpretation and values of $RR < 1$ (when $R_{\text{nearc}} < R_{\text{aus}}$), the multiplicative inverse was calculated to produce $RR_{\mathrm{aus}} = R_{\mathrm{aus}}/R_{\mathrm{nearc}}$ (Figure 4B). In each model, values of $RR > 0$ indicate how many times higher the risk index is in the baseline group (numerator) compared with the reference group (denominator).

Uncertainty and sensitivity analysis

A sensitivity analysis was conducted to evaluate the sensitivity of the risk estimates to changes in the weights assigned by experts to each risk factor. A total of 100 permutations were performed. In each permutation, the value of each weight was selected at random from a uniform distribution limited by the range of values reported by the experts, and ensuring that the sum of the four weights was always equal to one. For each combination of weights, a new risk map was obtained, and the average risk value was calculated for all cells. The sensitivity was evaluated by fitting a multiple regression model without intercept, using the average risk as dependent variable and the weights as predictors. The estimated coefficients of the regression model were used as measurements of sensitivity, where higher coefficients indicate higher rates of change in the mean risk estimate as a function of the particular weight. In addition, an uncertainty map was produced (Figure 23 in Supplementary Materials) by computing the standard deviation of the risk values obtained in each cell, following Paul et al. (2016).

Map validation

Data on 13 reported cases of LPAIV in wild birds (Pereda et al., 2008; Rimondi et al., 2011; Xu et al., 2012) between 2006 and 2010, and 50 cases in backyard poultry between 2014 and 2018 (SENASA, pers. comm.) were used to evaluate the predictive ability of our general model. Wild bird surveillance was conducted in 21 sites, and positives originated from seven different counties (Figure 1). Among these, two influenza A virus isolations could be obtained by real-time reverse transcriptase PCR (RRT-PCR): an H13N9 (A/Kelp Gull/Argentina/LDC4/06) virus in a kelp gull (*Larus dominicanus*) (Pereda et al., 2008), and an H9N2 virus in a rosy-billed pochard (*Netta peposaca*) (Xu et al., 2012). With regards to the backyard poultry, samples came from ongoing active surveillance in 1,266 flocks. Positives originated from 47 different flocks distributed in 37 counties (Figure 1). These birds had antibody titers (ELISA) against LPAIV subtypes H5 or H7, and their oropharyngeal swabs were subjected to molecular testing (PCR) to rule out HPAIV. The number of cases was too small to be used in the estimation of the receiver operating characteristic curve (ROC AUC). Therefore, three Monte Carlo resampling (permutation-based), one-tailed significance tests (one for all species combined, one for Nearctic migrants, and one for Austral migrants) were done using waterfowl and backyard poultry data combined, under the hypothesis that the mean risk index is higher in counties where LPAIV has been reported than in counties without virus reports. Tests were performed at the county level using 1,000 random permutations.

Risk in commercial poultry farm areas

The association between the general model showing the risk for LPAIV and the distribution of commercial poultry farms was assessed to gain insight into the possible exposure of this compartment to the pathogen. A georeferenced database with the location of 6,185 industrial and semi-industrial farms was used (SENASA, pers. comm.). All cells (*n* = 144,080) within a 5 km buffer of every farm were identified and their mean risk index value was calculated. Following, the same permutation approach describe above was followed (100 permutations) to test the hypothesis that the mean risk index is higher in areas nearer (< 5 km) commercial farms compared with areas at greater distances. The spatial distribution of commercial farms was mapped using kernel density estimation (Figure 5).

Results

Expert opinion and factor weights

After the elimination of missing or incomplete responses, data from 10 experts were retained for analysis. Results from the expert opinion survey and the variable weights are shown as a pairwise comparison matrix in Table 1. Mean consistency ratio among experts was 0.029 (range: 0.004-0.065; *n* = 10). Proximity to wetlands (ProxWet) and the number of families with backyard poultry (BackPoul) were the highest weighted risk factors, and received 27.6% and 25.9% of the total weight, respectively. Proximity to a rice field (ProxRice) received 24.3% of the weight, and the presence of wild birds (WildBirds) received the lowest weighting, with 22.2% of the total weight (Table 1). The correlation among all predictive layers was low (Pearson $R^2 < 0.30$).

Risk index for occurrence of LPAIV

The spatial distribution of the risk index for the occurrence of LPAIV at the interface between backyard poultry and wild birds is displayed on a continuous scale of lowest to highest risk (Figure 2). The risk index computed using all 22 bird species showed that areas with the highest risk were concentrated in Corrientes and eastern Santa Fe and Entre Rios provinces, with the latter having the largest single area with highest risk. Moderately high risk areas were dispersed across most of Buenos Aires, Santa Fe, Entre Rios, Corrientes, Chaco, Formosa, Santiago del Estero, Salta, and eastern Córdoba province.

There were small differences in the risk index computed using Austral and Nearctic migrants (mean = 0.001; range = -0.125-0.107, Figure 3), with the largest positive difference (attributable to larger values of R_{aus}) observed in northwest Chubut and western Río Negro and Neuquén, central La Pampa and San Luis province. The areas with largest negative difference (attributable to larger values of R_{nearc}) were located in Jujuy and Salta province.

In the vast majority of cells, RR_{nearc} values varied between ≈ 0 and 3, with the highest values observed in western Jujuy and northern Catamarca province, where R_{nearc} was over three times higher than R_{aus} (Figure 4A). Similarly, most RR_{aus} values varied between ≈ 0 and 3, with the highest difference observed in northwestern Chubut and western Neuquén province, where R_{aus} was over three times higher than R_{nearc} (Figure 4B).

Model validation, uncertainty and sensitivity analysis

Mean risk index in counties with LPAIV and 95% critical value in counties without virus reports were 0.077 and 0.074 for the model including all 22 species, 0.076 and 0.072 for the Austral migrants' model, and 0.078 and 0.075 for the Nearctic migrants' model. All three permutation tests showed that the mean risk in counties where LPAIV has been isolated is higher ($P < 0.05$) than in counties without reported LPAIV activity.

According to the regression coefficients (β) obtained in the uncertainty and sensitivity analysis, the variation in the risk index was strongly influenced by the predicted distribution of wild birds ($\beta = 0.45$; $p < 0.001$) and the distance to wetlands ($\beta = 0.30$; $p < 0.001$), whereas the presence of backyard poultry ($\beta = 0.01$; $p < 0.01$) and the distance to rice farms ($\beta = 0.07$; $p < 0.05$) showed weaker associations.

Risk in commercial poultry farm areas

The mean value of the risk index in cells located nearer (< 5 km) commercial poultry farms (mean = 0.3525; range: 0.0128-0.8637) was higher ($p < 0.01$) than the mean value for areas located at greater distances (mean = 0.2178; range = 0.217-0.219). Also, there was good, broad visual agreement between high-risk areas in our model and areas with highest density of farms, as shown by the kernel estimation of farms density (Figure 5).

Discussion

As mentioned before, risk analysis conducted for AIV in Argentina suggested that the risk for introduction of HPAIV via Nearctic migrants is negligible, whereas the risk for LPAIV introduction can be significant (SENASA, 2010). However, no study had yet addressed the spatial risk for LPAIV occurrence in the country, and only two studies have attempted to map the risk or suitability of HPAIV occurrence in Argentina (Leon et al., 2007; Baigún & Minotti, 2009). Therefore, the present research is unique in at least two respects: firstly, it focuses on the risk for LPAIV in general, and on this viruses' occurrence at the interface between wild birds and backyard poultry in particular; and secondly, it combines ecological niche modeling theory and a knowledge-driven approach to assess the spatial distribution of risk for this pathogen.

It is important to note that our models are based on assumptions about the relationship between selected risk factors and habitat suitability for LPAIV. Due to lack of information in the study area, the shape of the association between LPAIV and two of the variables (proximity to wetlands and proximity to rice fields) were modeled following Stevens et al. (2013). For the other two variables (presence of waterbirds and presence of backyard poultry), the membership functions were defined based on the simplest possible explanations (linear). These assumptions should be fine-tuned, validated and contrasted with our model outputs as updated information about these relationships is gathered in the future.

Despite the growth of the backyard poultry sector in Argentina, there is currently no survey or census-based information about the spatial distribution of backyard farms, which made simulating this variable challenging. This lack of information can represent one of the main limitations of spatially explicit modeling in general, and spatial MCDA in particular, where fine-scale details of landscapes and of spatially dependent biological processes (e.g., dispersal, invasion, and disease occurrence) or risk factors need to be represented or simulated with acceptable precision. To circumvent this issue, inputs for our backyard poultry model were obtained from expert opinion elicitation done by other researchers (Leon et al., 2009) in combination with human population data from the WorldPop dataset. In this respect, our work also provides a methodological approach to modeling the distribution of backyard poultry in areas where census or survey data are scarce or entirely lacking. Lastly, the model produced will benefit from future refinement and validation using survey data from backyard poultry owners across the country.

Areas identified in our general model as most suitable for LPAIV coincided closely with sites where LPAIV was detected. These high-risk areas were scattered throughout Corrientes (four counties with positive backyard poultry), in eastern Santa Fe (two counties with positive waterbirds), and eastern and northern Entre Ríos (two counties with positive backyard poultry and one county with positive waterbirds). Interestingly, the latter was also identified as one of the regions with highest density of commercial farms. Two other small, high-risk spots were in southeast Formosa and northeast Chaco, where LPAIV was detected in backyard poultry farms.

These observations are supported by our model validation using LPAIV-positive counties, underlying the practical applicability of this model in guiding future risk-based surveillance for this pathogen in the study area, either in wild bird populations or in the backyard poultry sector. Also, our finding of significantly higher risk areas located nearer commercial farms highlights the potential for transmission of these viruses between wild birds, backyard poultry, and commercial farms.

The sensitivity analysis performed shows that the predicted risk areas for LPAIV occurrence according to the risk index are robust, meaning that they remain stable when risk factors weights are varied. Results highlighted a spatial heterogeneity in uncertainty, with higher uncertainty in high LPAIV risk areas.

The difference between the risk indices for Austral and Nearctic species was most notable in western Patagonia, southeast Santa Cruz, central La Pampa and San Luis province, and can be explained by better habitat suitability for Austral migrants than for Neactic ones in those areas, as shown by MaxEnt models of individual species (Figures 1-22 in Supplementary Materials). Regarding our relative risk measures (RR_{nearc} and RR_{aus}), these show the magnitude of the difference in risk attributable to each migration group. These estimates could be useful in guiding active surveillance in wild birds under more specific scenarios; for example if surveillance in areas with highest suitability for one migration group with respect to the other needed to be implemented.

There is overwhelming evidence that LPAIV mutation can take place, producing HPAIV mutants after the first are introduced in poultry (Alexander, 2003). Despite research suggesting that outdoor ranging of poultry represents a major risk factor for the introduction and development of HPAIV (Koch & Elbers, 2006), this phenomenon has so far not been clearly observed in the backyard poultry compartment (Richard et al., 2017). Contrarily, there is strong evidence that HPAIV strains can develop from LPAIV through mutations after the latter are introduced in commercial farms; e.g. in the USA (Bean et al., 1985), Mexico (Garcia et al., 1996), Italy (Capua & Marangon, 2000), Chile (Rojas et al., 2002), the Netherlands (Elbers et al., 2004) and Canada (Bowes et al., 2004). Moreover, in over 79% (19/24) of outbreaks reported worldwide between 1959 and 2006, the virus was introduced from wild fowl and then mutated into an HPAIV variant (Koch & Elbers, 2006). More recently, the outbreaks of the HPAIV H5N8 and H5N2 in wild birds and poultry in North America offer a prime example of the severity of such mutations in avian populations (Kaplan et al., 2016).

In Argentina, the backyard poultry sector is being promoted as a means of poverty alleviation and household food security. However, this growth has not been on a par with increased biosecurity, which still find very little compliance in the backyard or rural environment and represents a potential risk factor for virus transmission from wild birds. Moreover, our finding of higher risk in areas within a 5 km buffer from commercial poultry farms compared with the risk in areas at greater distances, suggests that commercial farms are generally located in high-risk areas. Under certain circumstances, such as breaching of biosecurity measures, contact between wild birds and commercial poultry could be facilitated thus increasing the likelihood of LPAIV outbreaks or virus mutation in commercial farms.

With this background, we suggest that the implementation of a risk-based method would be expected to increase the sensitivity of surveillance systems already in place (Cameron, 2012). Also, the available resources could be optimized by prioritizing surveillance across all three compartments (i.e., wild birds, backyard and commercial poultry) in highest-risk areas, and through the concentration of surveillance during the period at risk for virus introduction, such as during waterbird migration and concentration of multiple species in large numbers on feeding grounds.

In conclusion, our research provides a cost-effective tool to guide surveillance activities in the future in Argentina, and also presents a methodological approach which could be implemented in regions where the disease is exotic, or where it is present but a knowledge-driven modeling approach is warranted due to lack of sufficient information.

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Legends for figures and tables

Figure 1. Argentina main administrative divisions (provinces) are shown. Letters represent provinces: Jujuy (JY), Salta (SA), Formosa (FM), Chaco (CC), Catamarca (CT), Tucumán (TM), Santiago del Estero (SE), Catamarca (CT), Santa Fe (SF), Corrientes (CN), Entre Ríos (ER), Córdoba (CB), San Luis (SL), San Juan (SJ), Mendoza (MZ), Buenos Aires (BA), La Pampa (LP), Neuquén (NQ), Río Negro (RN), Chubut (CH), Santa Cruz (SC), Tierra del Fuego (TF). Blue and green polygons represent counties where LPAIV was isolated in wild birds and backyard poultry, respectively.

Figure 2. Map derived from model 1, which represents the risk for occurrence of LPAIV in Argentina. Risk is displayed on a continuous scale from lowest to highest, as defined by the multicriteria decision analysis.

Figure 3. Map derived from model 2, showing the pixel-wise absolute difference in risk index between the Austral (Raus) and Nearctic (Rnearc) models for the occurrence of LPAIV in Argentina.

Figure 4. Maps showing relative risk indices RRnearc (A) and RRaus (B) for the occurrence of avian influenza virus in Argentina, displayed on a continuous scale, where values of RR > 0 indicate how many times higher the risk is in the baseline group (numerator) compared with the reference group (denominator).

Figure 5. Kernel density estimation of commercial poultry farms in Argentina is presented as yellow areas for higher density, light-blue areas medium density, and dark-blue areas for lower density.

Table 1. Pairwise comparison matrix of the analytical hierarchy process (AHP) for risk factors associated with LPAIV. Figures represent the geometric mean of judgments provided by the 10 experts.

Table 2. Probability of backyard poultry presence as a function of population density in Argentina (Leon et al. 2009).

Table 3. Results from MaxEnt models for individual waterbird species: first five principal components of the 35 CliMond variables (PCA1 through PCA5) and proximity to wetlands (ProxWet). The permutation importance values for each variable are obtained by randomly permuting the values of that variable on presence and background locations, reevaluating the model and normalizing the resulting drop in the area under the curve (AUC). Sample sizes (n) for each species are presented.

Table 1

	WildBirds	BackPoul	ProxWet	ProxRice	Weight
WildBirds	1.00	0.90	0.84	0.84	0.222
BackPoul	1.12	1.00	0.91	1.15	0.259
ProxWet	1.20	1.10	1.00	1.15	0.276
ProxRice	1.20	0.87	0.87	1.00	0.243

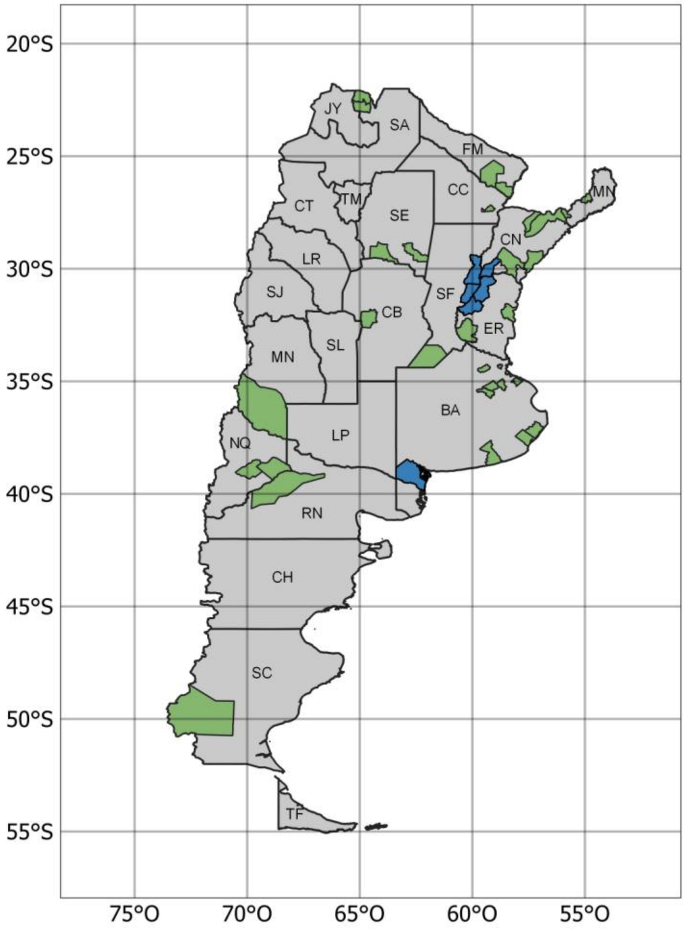
Table 2

Human population density (people/km^2)	Probability
<1.5	0.9
1.5-4.2	0.5
4.2-6.0	0.2
6.0-10.0	0.1
>10.0	0.0

Table 3

					Permutation importance					
Species	Taxonomic order	Migration	n	AUC	PCA1	PCA2	PCA3	PCA4	PCA5	ProxWet
Anas flavirostris	Anseriformes	Austral	224	0.708	17.73	30.43	24.75	6.08	15.8	5.22
Anas georgica	Anseriformes	Austral	267	0.672	18.77	17.41	11.11	18.44	18.9	15.37
Anas platalea	Anseriformes	Austral	186	0.693	33.28	20.76	7.01	13.31	12.33	13.32
Anas sibilatrix	Anseriformes	Austral	109	0.753	30.96	13.55	6.33	21.9	20.64	6.62
Anas versicolor	Anseriformes	Austral	199	0.683	16.69	30.33	33.95	7.56	4.06	7.4
Calidris fuscicollis	Charadriformes	Neartic	84	0.779	10.98	23.8	17.47	35.95	7.04	4.75
Calidris melanotos	Charadriformes	Neartic	92	0.702	0.53	21	40.56	0.72	2.5	34.69
Charadrius falklandicus	Charadriformes	Austral	35	0.838	23.31	13.12	29.31	27.99	2.86	3.41
Chloephaga picta	Anseriformes	Austral	67	0.952	23.83	8.91	11.02	39	16.7	0.53
Cygnus melanocorypha	Anseriformes	Austral	127	0.758	16.09	22.44	41.71	6.59	8.55	4.61
Dendrocygna bicolor	Anseriformes	Austral	111	0.722	4.03	37.64	40.1	0	0	18.23
Dendrocygna viduata	Anseriformes	Austral	222	0.751	7.93	15.08	43.64	23.94	2.36	7.05
Larus cirrocephalus	Charadriformes	Austral	121	0.742	26.54	20.83	24.28	12.33	8.47	7.55
Larus maculipennis	Charadriformes	Austral	149	0.743	20.97	13.73	29.11	22.31	0	13.88

Limosa haemastica	Charadriformes	Neartic	51	0.787	13.45	23.01	8.16	15.22	5.11	35.05
Netta peposaca	Anseriformes	Austral	263	0.711	13.95	40.31	37.44	0	1.18	7.12
Phalacrocorax olivaceus	Pelecaniformes	Austral	200	0.636	8.34	28.44	21.46	14.15	15.21	12.4
Steganopus tricolor	Charadriformes	Neartic	85	0.73	18.49	30.99	13.76	23.22	0.38	13.16
Pluvialis dominica	Charadriformes	Neartic	91	0.761	29.2	2.85	12.8	31.84	0	23.31
Rynchops niger	Charadriformes	Austral	54	0.825	33.65	8.47	28.84	16.8	7.29	4.95
Tringa flavipes	Charadriformes	Neartic	174	0.657	11.58	23.39	29.24	14.32	10.22	11.25
Tryngites subruficollis	Charadriformes	Neartic	43	0.85	6.83	18.32	3.47	18.19	0	53.2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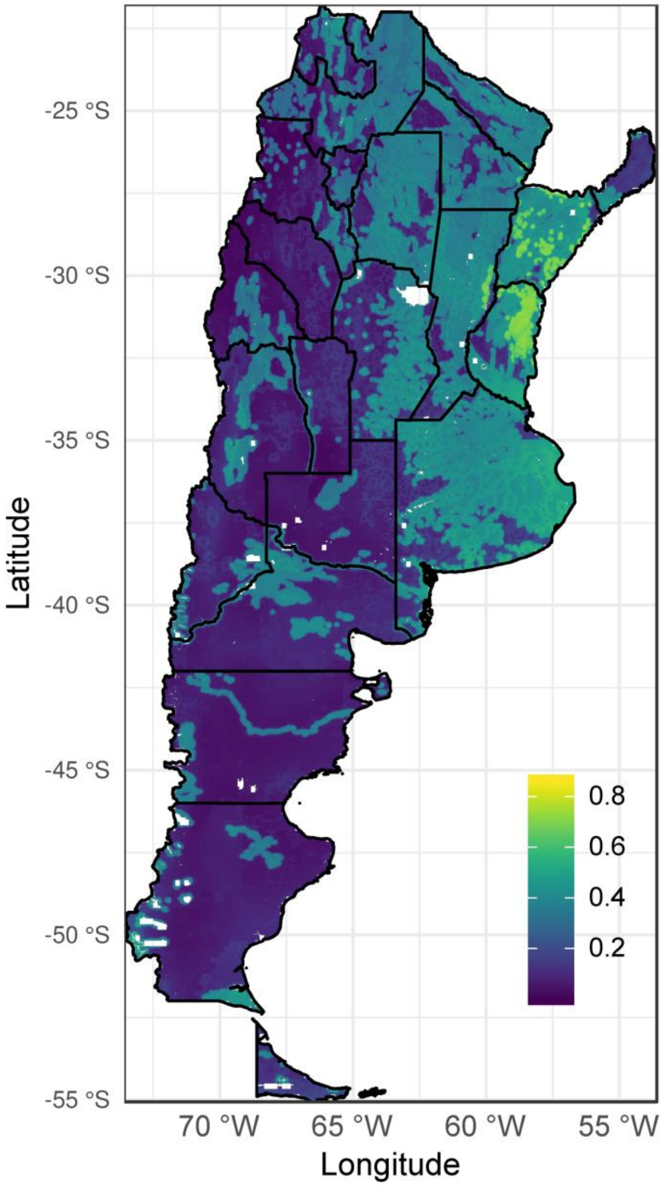
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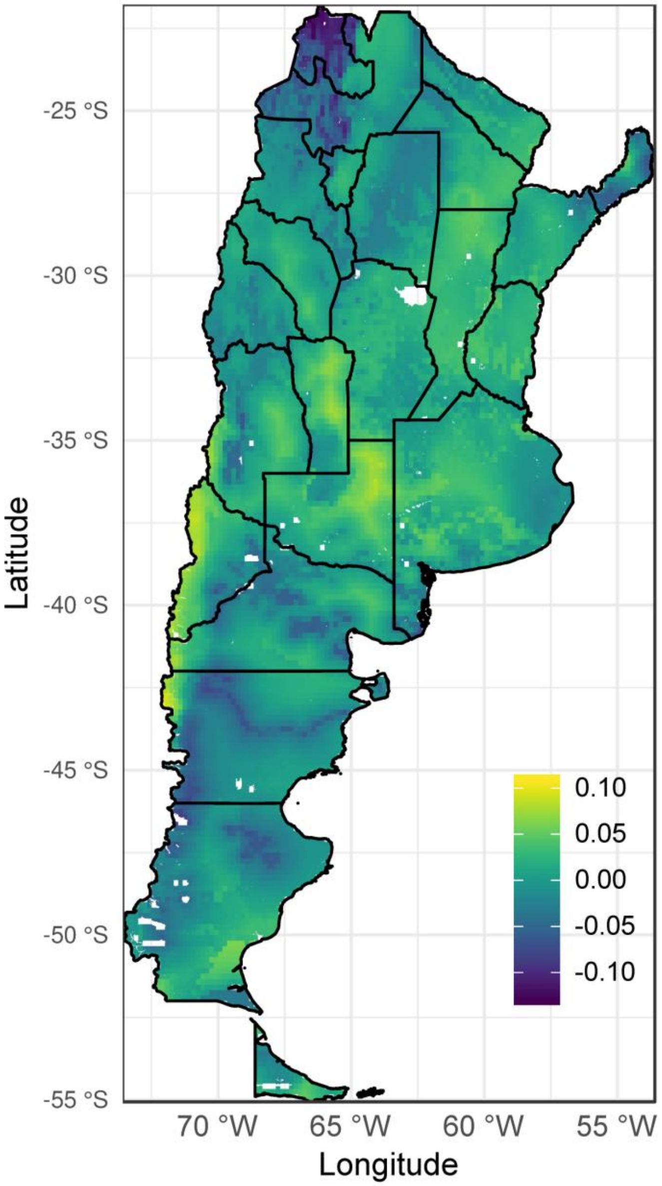
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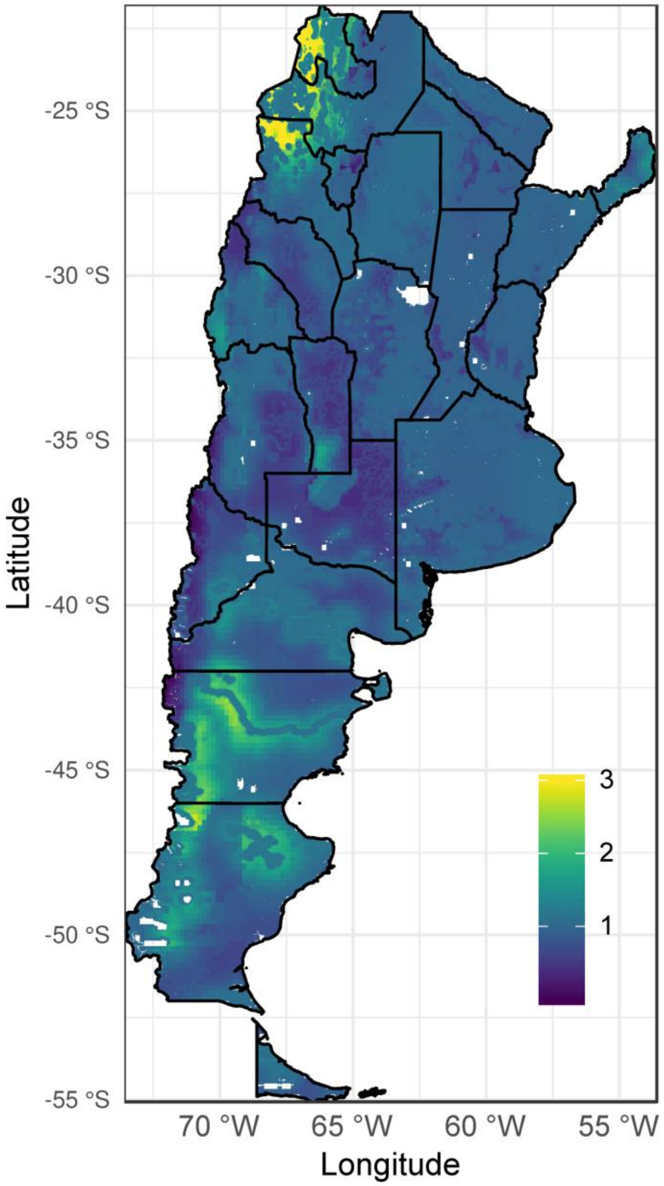
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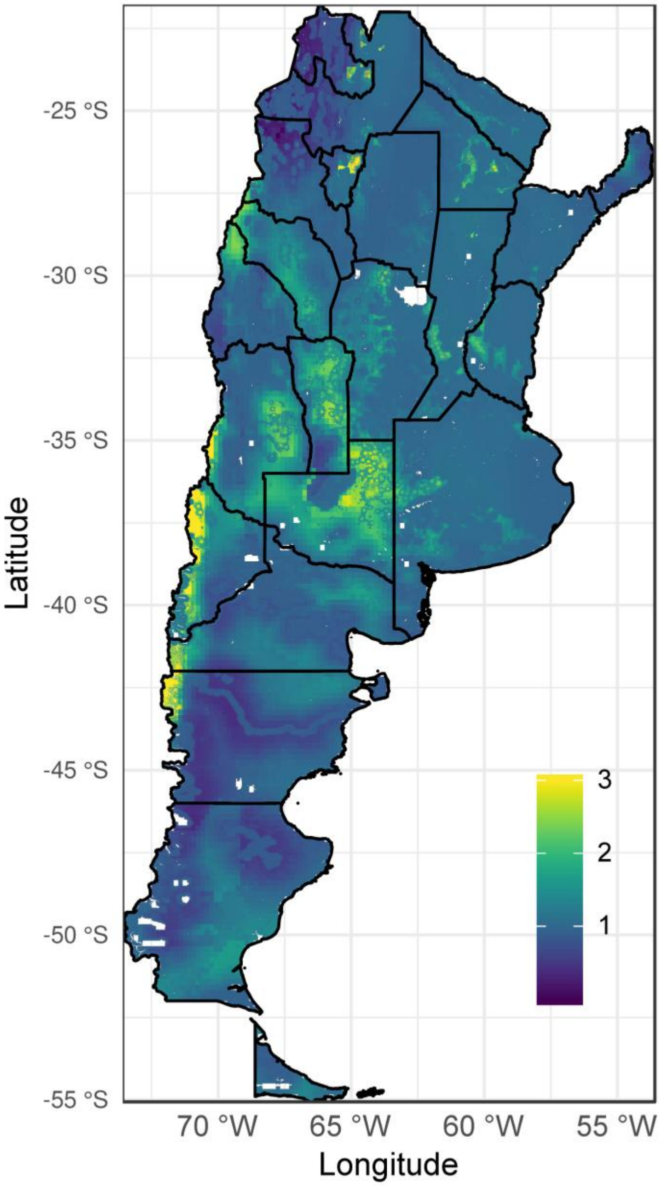
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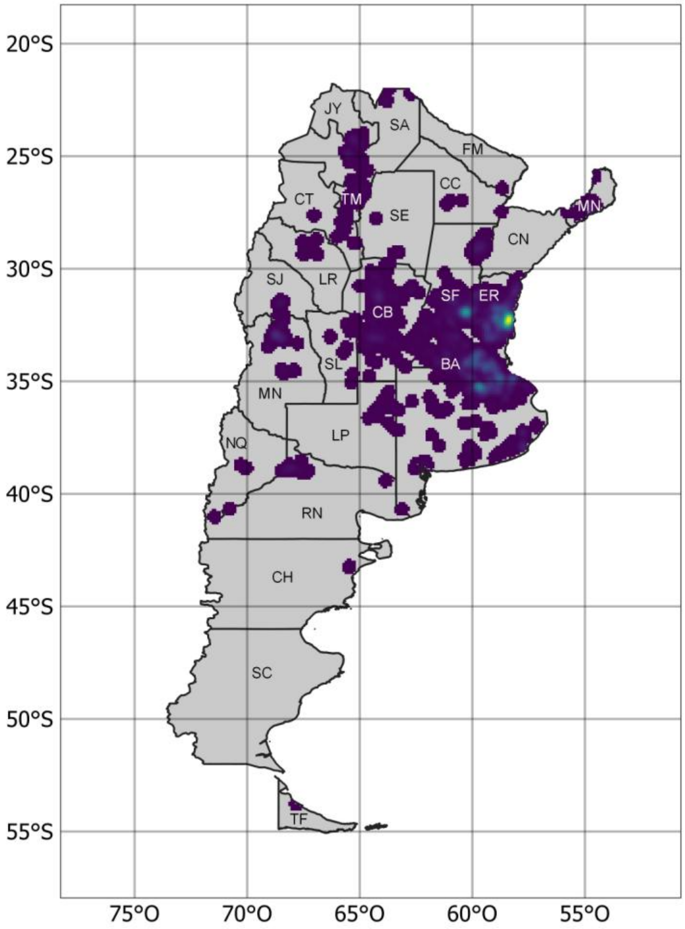
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