Artículo Original

Ranking wild bird species and their settlements for avian influenza virus surveillance in Cuba

Priorización de aves silvestres y sus asentamientos para la vigilancia del virus de la influenza aviar en Cuba

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Pastor Alfonso[1]*, Martin Acosta Cruz[2], Lourdes Mugica Valdez[2,3], Ariam Jiménez[2], Joel Ayala Galindo[1], Magdiel Torres Villar[1,4], Osvaldo Fonseca[1]

[1]Epidemiology Group. National Center for Animal and Plant Health (CENSA). World Organisation for Animal Health (WOAH) Collaborating Center for Disaster Risk Reduction in Animal Health, San José de las Lajas 32700, Mayabeque, Cuba.

[2]Bird Ecology Group, Faculty of Biology, University of Havana, La Habana, Cuba.

[3]Jardín Botánico Nacional, Universidad de La Habana, Cuba.

[4]Department of Preventive Medicine, Agrarian University of Havana (UNAH), P.O. Box 18-19, San José de las Lajas 32700, Mayabeque, Cuba.

ABSTRACT: The aim of this study was to narrow down the number of wild bird species and settlements where avian influenza viruses (AIVs) could be found in Cuba. The species of greatest interest were identified and listed by analyzing the available ornithological information, their behavior and the prevalence reported in the literature. A prevalence-weighted index was developed to rank the wild bird species and their main settlements based on abundance and frequency of the species. Maximum abundance showed large differences among settlements, trending to increase during fall migration, as well as in wetlands with respect to other sampled settlements. The prevalence-weighted approach showed a distribution pattern with very high, high, moderate or low indexes for both species and settlements, which evidenced the distinguishing power of the method developed. A prominent use of Cuban ecosystems was observed during fall migration with respect to spring migration, attributed to the use of alternative migratory routes for return, not including Cuba. Blue-winged teal (*Spatula discors*) was markedly the foremost ranked species, while «Los Palacios» and «La Ciénaga de Zapata» were predicted as the two most appropriate settlements for AIV surveillance during fall and spring migration, respectively. The prospective deduced risk index could provide predictions about AIVs circulation in both species and settlements. In addition, this approach offers a new perspective for understanding the wild bird-poultry interface in Cuba.

Key words: migratory birds, prioritization, prevalence, ecology, wild bird-poultry interface.

RESUMEN: El objetivo de este estudio fue delimitar el número de especies de aves silvestres y sus asentamientos en los cuales se pueden encontrar virus de influenza aviar (VIA) en Cuba. Las especies de mayor interés potencial, se identificaron y listaron mediante el análisis de la información ornitológica disponible, su comportamiento y la prevalencia reportada en la literatura. Se desarrolló un índice ponderado por prevalencia para clasificar las especies y sus principales asentamientos con base en la abundancia y frecuencia de especies. La abundancia máxima mostró grandes diferencias entre asentamientos, con tendencia a aumentar durante la migración otoñal, así como en humedales con respecto a otros asentamientos muestreados. El enfoque ponderado por prevalencia mostró un patrón de distribución con índices muy altos, altos, moderados o bajos tanto para las especies como para los asentamientos, como evidencia del poder discriminante del método desarrollado. Se observó un uso prominente de los ecosistemas cubanos durante la migración otoñal con respecto a la primaveral, atribuido al uso de rutas migratorias alternativas para el retorno, sin incluir a Cuba. El Pato de la Florida (*Spatula discors*) fue notoriamente la especie mejor clasificada, mientras que Los Palacios y Ciénaga de Zapata resultaron los asentamientos más apropiados para la vigilancia del VIA aviar durante la migración otoñal y primaveral, respectivamente. El índice de riesgo deducido prospectivamente podría proporcionar predicciones sobre la circulación del VIA en particulares especies y asentamientos. Además, este enfoque ofrece una nueva perspectiva para comprender la interfaz entre aves silvestres y comerciales en Cuba.

Palabras clave: aves migratorias, priorización, prevalencia, ecología, interfaz aves silvestres y comerciales.

INTRODUCTION

Since 2022, a completely unprecedented highly pathogenic avian influenza (HPAI) situation, in terms of outbreak occurrence and worldwide distribution, has been taking place, affecting domestic and wild birds, and some terrestrial and aquatic mammals. This reflects a change in the epidemiology and ecology of the virus, posing a threat to animal and public health, food security, and biodiversity (1,2). Such dramatic change includes massive infection events in some mammalian species, sometimes with clear evidence of spillover and transmission between congeners (3-6), which reinforces pandemic concerns. In addition, it implies a renewed need to better understand introduction, spread and potential impact of HPAI to improve control and mitigate negative outcomes.

*Corresponding author: Pastor Alfonso. E-mail: alfonso@censa.edu.cu
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The ability of waterfowl, among which are the main reservoirs of avian influenza viruses (AIVs), to cover large geographic distances when migrating, combined with the substantial prevalence and diversity of AIVs they may carry, provides the opportunity for novel AIVs to emerge through co-infection events, as well as through the introduction of AIVs from different regions into immunologically naïve populations (7). There is growing evidence that HPAIVs are endemic in the avian reservoir, adding complexity to their eradication (8). This may indicate a higher risk of disease occurrence in countries connected with migratory flyways.

Surveillance of AIVs in live wild birds is expensive and difficult, involving substantial labor and costs (9). Given that the prevalence of infection in wild birds is generally low and can vary both temporally and spatially within a species (10,11), it is difficult to make an initial assessment of the most important target species and sites for surveillance. Further, AIV surveillance programs need to be locally adapted to the avian population at each study region (12,13). Additionally, viral strains from different host origin may differ in their affinity for either the digestive or respiratory tract (14,15). Therefore, ideal sampling and monitoring programs involve both cloacal and oropharyngeal samples (preferably not mixed), increasing cost and resources. There are advances in environmental sampling of AIVs (16), but there are no studies in warmer latitudes.

A coordinated annual surveillance system with a global perspective does not necessarily require participation from every country (17). On the other hand, the intensity of surveillance in waterfowl alone does not prevent the occurrence of outbreaks in poultry, as evidenced by some recent epidemics with HPAI H5Nx subtypes, even when there was intensive surveillance of its circulation in wild birds. In this context, resource allocation could be prioritized to provide sustained surveillance in a few targeted locations and in specific seasons that maximize information on viral diversity relevant to potential spread (e.g., high-risk species, species interfaces, major staging and migration stopover sites, and reassortment hotspots) (18). The need of both geographic and species prioritization of sampling efforts to reach a reasonable cost/benefit ratio of wild bird surveillance could be addressed by targeting areas and species most likely to harbor AIVs.

Cuba, within the Caribbean region, could likely be of major importance to conduct virus surveillance of global interest. First, the Cuban archipelago, holding 48 % of the emerged land in the Caribbean, is an important stopover and wintering site for about 193 wild bird species (19). Two of the four North American flyways (the Atlantic and the Mississippi flyways) used by Nearctic migrant birds extensively affects the Cuban territory. Thus, Cuba is not only an important wintering area for migrant birds from North America, but is also a very important staging area for birds moving to other Caribbean islands or birds migrating further south (20). In addition, Cuba holds an important network of 28 Important Bird Areas (IBAs), which is an international designation that highlight critical sites for bird conservation (21). Specifically, 12 of these areas hold globally significant congregations of seabirds and waterbirds (22).

The long and narrow shape of the Cuban archipelago with an insular platform where shallow waters and mangroves abound, facilitate the existence of large coastal wetlands which provide habitat for important breeding and non-breeding waterbird populations (20,23). In summary, wetlands stand for near 15 % of the country area with two thirds of this area designated as Ramsar sites (21).

Habitat availability for waterbirds in Cuba is extended by natural and man-made freshwater bodies, including rice-growing areas, where these birds forage (24). The ecology of the AIVs in rice paddy fields involves an intricate web of drivers for AI occurrence (25). From the perspective of consequences of AIVs incursion, poultry production in Cuba is an important component of livestock economy and represents over 12.8 million individuals, mostly laying hens (56.7%), with their own genetics (26). Consequently, this amount of AIV susceptible population, combined with areas suitable for the main reservoir, may create wild bird-poultry interfaces from which HPAI could occurs and spread. The possibility of AIV incursion in Cuba is not only theoretical, as evidenced by the recent event of infection by HPAI virus H5N1 in zoo birds (27).

Considering the need of strategic allocation of resources for surveillance, the aim of this study was, first, to narrow the number of species and settlements where AIVs could be found in Cuba, by ranking those more probable exposed, in order to prioritize further effort for wild bird surveillance in a cost-effective way. This goal is hypothesized attainable by combining available published data on observed patterns of AIV prevalence in birds in America with local variables of distribution range of main migrating wild birds, abundance and host community composition.

MATERIALS AND METHODS

Study sites

Waterbird surveys (n=194) were conducted in 17 wetlands in Cuba between 2011 and 2015. They included Important Bird Areas or IBA (Sites recognized by Birdlife International for its importance for bird conservation on a global scale), several dams, rice paddies and natural wetlands included in the National System of Protected Areas (table 1)

Table 1. Cuban wetlands under waterbirds monitoring between 2011 and 2015. N: number of surveys made in each wetland. / *Humedales cubanos bajo monitoreo de aves acuáticas entre 2011 y 2015. N: conteos realizados en cada humedal.*

Name	Description	N	Location	
			Latitude	Longitude
Pretiles	Protected Area	13	22,26	-83,38
Guanahacabibes	Protected Area, IBA	12	21,53	-84,27
San Felipe	Protected Area	12	21,59	-83,38
Los Palacios	IBA, including rice paddies	13	22,36	-83,16
Presa Bacunagua	man-made dam	6	22,66	-83,21
Presa Los Palacios	man-made dam	6	22,63	-83,3
Presa de la Juventud	man-made dam	6	22,59	-83,30
Estación de Alevinaje	man-made dam	6	22,55	-83,31
Presa Niña Bonita	man-made dam	7	23,03	-82,49
Presa Ejército Rebelde	man-made dam	11	23,02	-82,33
Canales del Hanábana	Protected Area	14	22,35	-81,05
Ciénaga de Zapata	Protected Area, IBA, RAMSAR Site	14	22,06	-81,16
Tunas de Zaza	Protected Area, IBA, including rice paddies	14	21,37	-79,32
Monte Cabaniguán Laguna La Zanja	Protected Area	16	20,44	77,19
Monte Cabaniguán Jobabito	Protected Area	16	20,4	-77,16
Delta del Cauto El Mango	Protected Area, IBA, RAMSAR Site	14	20,55	-77,00
Delta del Cauto Leonero	Protected Area, IBA, RAMSAR Site	14	20,65	-77,06

IBA: Important Bird Area

Sampling areas were chosen mainly on the southern coast, where the main wetlands of Cuba are located, taking into account that they harbored the largest number of waterfowl during the migratory period. Besides that, 10 areas belong to the National System of Protected Areas that may contribute to maintain the monitoring efforts. Several dams from the western part of the country were included, taking into account their geographical position more closely related to two main migratory flyways (Atlantic and Mississippi) and because they were located near small towns and human facilities that could contribute to the spread of any pathogenic viruses.

Data sources of and study period

The main data source came from the Waterbird and Seabird Monitoring Program (28), and other unpublished data produced by the Bird Ecology Research Group of the Faculty of Biology of the University of Havana. The study encompassed monitoring of natural wetlands and rice paddies during spring migration (February to March 2012-2013), breeding period (May to June 2011-2013), fall migration (October to November 2011-2013), and monitoring of 5 dams during fall migration between 2013 and 2015 according to the methodology established by Acosta (29).

Analyses for ranking species and settlements

Species of major and potential interest for Cuba were identified by analyzing available ornithological information on Cuban wild birds and their reported prevalence. Thus, both the species of wild birds and the most important sites were deduced through a prevalence-weighted index, combining the analysis of published (28) and unpublished information on abundance and frequency for migratory birds.

For the main waterbird species wintering and staging in Cuba, prevalence is assumed to be the median across the available literature referring to the American continent (7,30-32). Considering that almost no information is available to indicate the prevalence in the Caribbean ecosystem, it is assumed that prevalence maintains similar importance of migratory species in the places of origin. Then the rank of wild bird species in Cuba would mainly depend on the abundance and frequency of these birds weighted by prevalence.

The relative importance of species and sites for AIV surveillance was derived from the equations described below to establish a prevalence-weighted ornithological ranking.

1. Ft: Frequency of the species i within the sampling area j

$$F_t = \frac{n_{ij}}{N_j}$$

Where:
n_{ij}= Counts of the specie i within the area j
N_j= Total counts in the area j

2. N_{ij}: Likely number of specimens of the species to be detected in the sampling area j:

$$N_{ij} = F_t * Am_{ij}$$

Where:

F_t: described in equation 1

Am_{ij}= Maximum number of specimens of the specie i to be detected in the sampling area

3. Frequency of specimens likely infected with AIV:

$$F_{ijp} = P_i * N_{ij}$$

Where:

P_i= Prevalence of infection by AIV according to literature

4. Relative frequency of specimen of the species *i* infected by AIV in the area *j*:

$$FR_{ijp} = \frac{F_{ijp}}{\sum F_{ijp}}$$

The estimated frequencies were obtained for each species and sites. Then, location indexes were overlapped with a poultry density layer using ARGIS to visualize areas in proximity to poultry production and further mapping.

RESULTS

The frequencies of species with high AIV prevalence varied through the locations studied during migration but without a regular pattern (Figure 1). The man-made dams "Presa Bacunagua", "Presa Los Palacios", "Presa de la Juventud", "Estación de Alevinaje", "Presa Niña Bonita", and "Presa Ejército Rebelde" were only sampled during fall migration; Interestingly, they showed frequency values compared to natural wetlands.

The maximum abundance of birds showed large differences among locations. Bird abundance was higher during the fall migration period, except for "Canales del Hanábana", where higher values were observed during spring migration (Figure 2). Los Palacios was the location with the highest number of birds with values twice as high as "Ciénaga de Zapata", the second most important wetland in terms of bird abundance.

The prevalence-weighted approach for species (Figure 3) showed a distribution pattern allocating species as very high, high, moderate or low indexes. According to the rank order index, the top five species belonged to the family *Anatidae*, followed by the American Coot of the family Rallidae and, sequentially, two species, the Laughing Gull of the family *Laridae* and the Ruddy Turnstone of the family *Scolopacidae*, which were of intermediate rank. The differences among species were marked, underlining Blue-Winged Teal (BWTE), followed by two diving duck species Lesser Scaup and Ring-necked Duck. *Anatidae* was the best represented family among the species identified by this index, totalling 10 out of 21 species.

The ranking of the sites (Figure 4) showed indexes that may be considered very high, high, moderate or low. In this case, Los Palacios was of first order (very high) with notable difference from the rest of the sites. Ciénaga de Zapata with second order rank (high) differed widely from the rest of localities, both

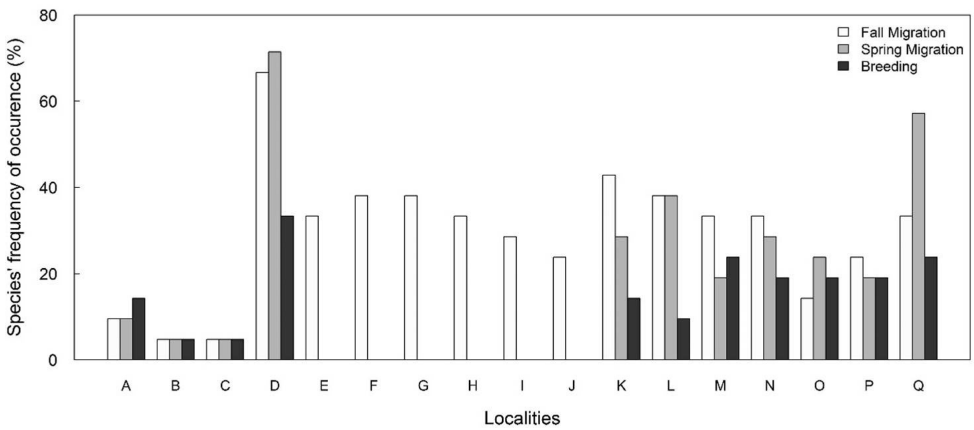

Figure 1. Frequency of waterbirds species in three different periods from 2011 to 2015 in 17 Cuban wetlands. A (Pretiles), B (Guanahacabibes), C (San Felipe), D (Los Palacios), E (Presa Bacunagua), F (Presa Los Palacios), G (Presa de la Juventud), H (Estación de Alevinaje), I (Presa Niña Bonita), J (Presa Ejército Rebelde), K (Canales Hanábana), L (Ciénaga Zapata), M (Tunas de Zaza), N (Monte Cabaniguán Laguna La Zanja), O (Monte Cabaniguán Jobabito), P (Delta del Cauto El Mango), Q (Delta del Cauto Leonero). / *Frecuencia de especies de aves acuáticas en tres periodos diferentes de 2011 a 2015 en 17 humedales cubanos. Los topónimos se mantienen igual al título en inglés.*

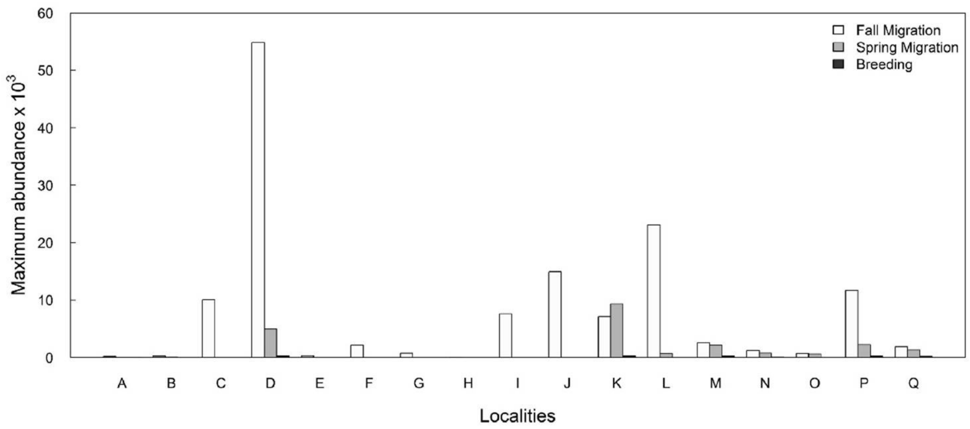

Figure 2. Maximum abundance of waterbirds more likely infected with avian influenza viruses in three different periods from 2011 to 2015 in 17 Cuban wetlands. A (Pretiles), B (Guanahacabibes), C (San Felipe), D (Los Palacios), E (Presa Bacunagua), F (Presa Los Palacios), G (Presa de la Juventud), H (Estación de Alevinaje), I (Presa Niña Bonita), J (Presa Ejército Rebelde), K (Canales Hanábana), L (Ciénaga Zapata), M (Tunas de Zaza), N (Monte Cabaniguán Laguna La Zanja), O (Monte Cabaniguán Jobabito), P (Delta del Cauto El Mango), Q (Delta del Cauto Leonero). / *Abundancia máxima de aves acuáticas con mayores posibilidades de estar infectadas por virus de la influenza aviar en tres periodos diferentes de 2011 a 2015 en 17 humedales cubanos. Los topónimos se mantienen igual al título en inglés.*

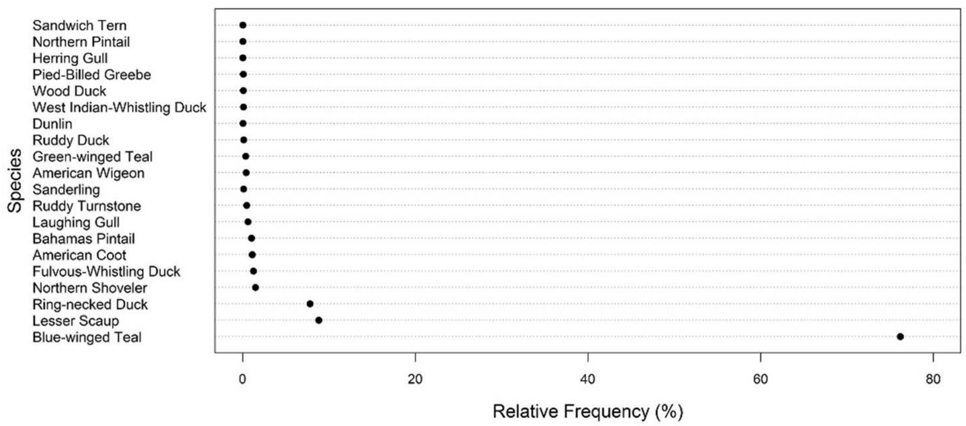

Figure 3. Cuban waterbirds organized according to the relative frequency of the prevalence-weighted index. / *Aves acuáticas cubanas organizadas según la frecuencia relativa del índice ponderado por prevalencia.*

harboring the maximum number of BWTE in the country, species with high prevalence values of AIV during spring migration followed by Delta del Cauto El Mango in Birama Swamp, Canales del Hanábana and San Felipe ranked as moderate. Tunas de Zaza and the rest of the wetlands were considered less important, as they showed the lowest values.

According to local studies and AIVs reported in the literature, 27 species of wild migratory birds may be of interest for avian influenza surveillance in Cuba (Table 2). These species are distributed in six orders and nine families.

Additionally, nine other species (aquatic and terrestrial) were identified as being of potential interest for surveillance but lacking local ecological data (Table 3).

In terms of abundance, BWTE clearly evidenced the wild duck poultry interface. (Figure 5)

DISCUSSION

This study was the first, to the authors' knowledge, to rank the suitability of both species and sites for AIV surveillance within the Caribbean region, by considering prevalence-weighted ornithological data to allocate bird species and, by extension, sites in a suitability index for surveillance effectiveness, risk assessment and potential risk management outcomes. Prosser et al. (33) reveal that adding prevalence to the waterfowl abundance layer is effective in capturing complexity between these two variables at the species level. In addition, poultry density was considered as an ancillary layer, which may bring insights on the Cuban wild birds-poultry interface.

Given that Cuba holds about 48 % of the emerged land in the Caribbean islands and it is an important migration and stopover site in the region, an effective surveillance and risk assessment may have regional implications that benefit other Caribbean islands and countries in South America. Every year, many migratory bird species first pass through the Cuban archipelago during fall migration to continue southward or to countries to the east, and BWTE is one of the best known examples (19). On the other hand, the methodology used in this assessment can be extended to other countries participating in the (Caribbean Waterbird Census), a regional program implemented since 2012 in the Caribbean region that annually updates the ornithological community using most local wetland ecosystems (34).

The prominent use of Cuban ecosystems during fall migration relative to spring migration has been noted and explained by the use of alternative migratory flyways for return not including Cuba (28). Therefore, in general, the probability of AIV incursion and detection would be higher during fall migration. Nevertheless, one of the sites studied (Canales del Hanábana) had a higher population of waterfowl during spring migration.

The marked differences in rank among species could be an effect of combined variables (abundance, frequency and prevalence) to infer importance as a product of probabilities. Therefore, the approach followed may have sufficient differentiating power. Prevalence-weighted waterbird abundance has been used as a proxy for the "effective" waterbird population that may be shedding virus into the environment and potentially exposing susceptible poultry and domestic birds to AIVs (33). However, the rank used in the current study also included frequency (occasions in which a particular species was observed during the study period) to derive significance, which may also indicate the length of the risk of exposure to AIVs.

In the case of Mallard ducks, for instance, the prevalence reported in the literature is very high (35), but the species is very rare in Cuba (36) and it was not reported in any of the wetlands sampled, thus the species does not represent a threat to Cuba.

The predominant presence of the *Anatidae* and *Laridae* families within the highest prevalence-weighted index was consistent with the existing knowledge on Anseriformes and Charadriiformes species as main AIV reservoirs (32). Particularly the highest prevalence-weighted index of BWTE anticipated its use as a sentinel species with a high probability of detecting AIVs, which is consistent with evidence-based studies showing higher prevalence in dabbling ducks (7,37).

BWTE is an early migrant that due to its "catchability" would be an accessible hunter-harvested prey. This coincides with information obtained through a survey of Cuban wild bird hunters, who rank BWTE as the most hunted species (38). It is of paramount importance because surveillance sensitivity

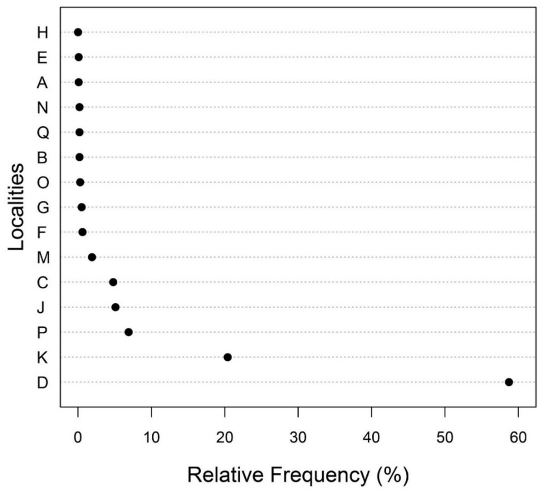

Figure 4. Cuban wetlands organized according to the relative frequency of the prevalence-weighted index. H (Estación de Alevinaje), E (Presa Bacunagua), A (Pretiles), N (MC Laguna La Zanja), Q (Delta del Cauto Leonero), B (Guanahacabibes), O (MC Jobabito), G (Presa de la Juventud), F (Presa Los Palacios), M (Tunas de Zaza), C (San Felipe), J (Presa Ejército Rebelde), P (Delta del Cauto El Mango), K (Canales Hanábana), D (Los Palacios). / *Humedales cubanos organizados según la frecuencia relativa del índice ponderado por prevalencia. Los topónimos se mantienen igual al título en inglés.*

is usually higher in hunter-harvested birds with respect to other sources (39), which contributes to the cost-effectiveness analysis of surveillance.

Another option to improve AIVs detectability is sampling hatch-year or juvenile birds, which are more likely to be naïve to AI virus and more abundant due to the high annual population turnover of these species (40). Despite, overall population prevalence may be biased targeting only juveniles, AIV presence/absence may be confirmed more cost-effectively.

Although prevalence-weighted index of Laughing gull slightly precedes that of Ruddy Turnstone, the latter species is distinguished among Charadriiformes for bringing most of AIV isolates with regard to sympatric shorebirds, but limited to Delaware Bay, noted as an ecological hotspot (41). In other worldwide locations, the prevalence of this shorebird is generally low (32). Nonetheless, the findings of some Charadriiformes species with moderate prevalence-weighted index in the current study, add complexity to the risk of AIV spillover. The seasonal prevalence of influenza viruses in shorebirds is reversed as compared with ducks, with higher virus prevalence (~14%) during spring migration (42).

Unpublished data obtained by the authors in "Ciénaga de Zapata" have shown that shorebird abundance increases in February and March, during spring migration. This period is coincident with the dry season in Cuba and more shallow coastal lagoons are accessible to them. Such pattern, besides favoring virus persistence, could extend the period with risk of transmission in the wild bird-poultry interface.

Table 2. Migratory wild birds of interest for avian influenza virus surveillance in Cuba based on local ornithological data and prevalence from the literature. / *Aves silvestres migratorias de interés para la vigilancia del virus de la influenza aviar en Cuba según datos ornitológicos locales y prevalencia de la literatura.*

Species	Status	Abundance	Common name
PODICIPEDIDAE			
Podilymbus podiceps	PR	C	PIED-BILLED GREBE
ANATIDAE			
Aythya collaris	WM	C	RING-NECKED DUCK
Oxyura jamaicensis	BR	C	RUDDY DUCK
Spatula clypeata	WM	C	NORTHERN SHOVELER
Spatula discors	WM	A	BLUE-WINGED TEAL
Aix sponsa	PR	NC	WOOD DUCK
Mareca americana	WM	C	AMERICAN WIGEON
Aythya affinis	WM	C	LESSER SCAUP
Anas acuta	WM	C	NORTHERN PINTAIL
Anas crecca	WM	NC	GREEN-WINGED TEAL
RALLIDAE			
Fulica americana	BR	A	AMERICAN COOT
SCOLOPACIDAE			
Arenaria interpres	WM	C	RUDDY TURNSTONE
Calidris alba	WN	C	SANDERLING
Calidris alpina	WM	VR	DUNLIN
LARIDAE			
Larus smithsonianus	WM	NC	HERRING GULL
Larus delawarensis	WM	NC	RING-BILLED GULL
Leucophaeus atricilla	BR	A	LAUGHING GULL
Onychoprion fuscatus	SM	C	SOOTY TERN
Thalasseus sandvicensis	PR	C	SANDWICH TERN
COLUMBIDAE			
Streptopelia decaocto	PR	A	EURASIAN COLLARED-DOVE
HIRUNDINIDAE			
Hirundo rustica	WM	C	BARN SWALLOW
PARULIDAE			
Setophaga coronata	WM	C	YELLOW RUMPED WARBLER
Setophaga dominica	WM	C	YELLOW-THROATED WARBLER
Setophaga magnolia	WM	C	MAGNOLIA WARBLER
Setophaga petechia	PR	C	YELLOW WARBLER
Setophaga ruticilla	WM	C	AMERICAN REDSTART
PASSERIDAE			
Passer domesticus	PR	A	HOUSE SPARROW

Winter migrant (WM), Bimodal Resident (BR), Permanent Resident (PR), Summer Migrant (SM), Common (C), Abundant (A), Not Common (NC), Very Rare (VR).

Table 3. Migratory wild birds of potential interest for avian influenza virus surveillance in Cuba based on the prevalence reported in the literature but with few locally produced ornithological data. / *Aves silvestres migratorias de interés potencial para la vigilancia del virus de la influenza aviar en Cuba según la prevalencia reportada en la literatura, pero con escasos datos ornitológicos producidos localmente.*

Species	Status	Abundance	Common name
SCOLOPACIDAE			
Tringa flavipes	WM	*C*	LESSER YELLOWLEGS
Calidris minutilla	WM	*C*	LEAST SANDPIPER
Limnodromus griseus	WM	*C*	SHORT-BILLED DOWITCHER
MIMIDAE			
Dumetella carolinensis	WM	*A*	GRAY CATBIRD
PARULIDAE			
Setophaga palmarum	WM	*A*	PALM WARBLER
CARDINALIDAE			
Passerina cyanea	WM	*A*	INDIGO BUNTING
ICTERIDAE			
Agelaius humeralis	PR	*C*	TAWNY-SHOULDERED BLACKBIRD
Ptiloxena atroviolacea	PR	*A*	CUBAN BLACKBIRD
Quiscalus niger	PR	*A*	GREATER ANTILLEAN GRACKLE
Molothrus bonariensis	PR	*C*	SHINY COWBIRD

Winter migrant (WM), Permanent Resident (PR), Common (C), Abundant (A)

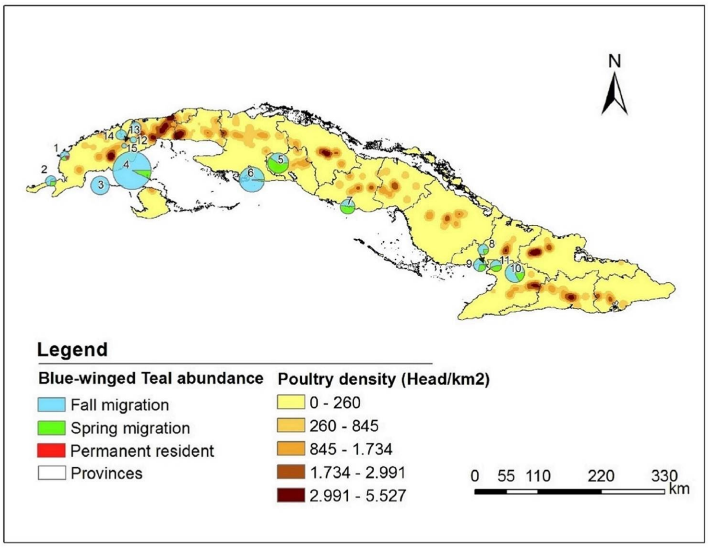

Figure 5. Wild duck poultry interface. The size of the circles corresponds to the Blue-winged Teal (*Spatula discors*) abundance. 1 (Pretiles), 2 (Guanahacabibes), 3 (San Felipe), 4 (Los Palacios), 5 (C. Hanábana), 5 (C. Zapata), 6 (Tunas de Zaza), 7 (Monte Cabaniguán Laguna La Zanja), 8 (Laguna La Zanja), 9 (Monte Cabaniguán Jobabito), 10 (Delta del Cauto El Mango), 11 (Delta del Cauto El Leonero), 12 (Presa Bacunagua), 13 (Presa Los Palacios), 14 (Presa de la Juventud), 15 (Estación de Alevinaje). / *Interfaz patos silvestres aves comerciales. El tamaño de los círculos se corresponde con la abundancia del Pato de la Florida (Spatula discors). Los topónimos se mantienen igual al título en inglés.*

Additionally, several Charadriiformes species nest from May to August in the Caribbean region, with major abundance in Cuba (23).

The relative low prevalence-weighted index of Wood Duck was related to its low abundance (results not showed). However, the resident behavior of this species Ducks may maintain virus into the environment for longer time than migrant species. In fact, Henaux *et al*. (43) demonstrated that resident populations of Wood Ducks, even at low densities and unfavorable environmental conditions, did not prevent low pathogenic avian influenza virus (LPAIV) circulation during summer in California wetlands, while the American Coot is an opposite example.

Despite H5 and H7 subtypes are those of major concern for poultry, the inference of the prevalence-weighted index was based on overall prevalence of wild bird species to influenza A virus. Nonetheless, it is thought the approach followed could capture the importance of AIVs circulation in wild birds no limited to H5 and H7 subtypes. In fact, several avian influenza virus subtypes other than H5Nx and H7Nx have the ability to infect mammals, including humans (44-46) with zoonotic/pandemic concern. In addition, positive findings for H5 and H7 subtype viruses are commonly reported without denominator data, and the particular relative risk for spillover is difficult to assess.

Site selection criteria revealed a high importance for the prioritization of wild bird surveillance. The highest rank of Los Palacios depends on foremost levels of waterbird abundance observed in this location where there is a close relationship between wetlands and rice fields (28). The proximity of rice cultivation to roosting, resting and refuge areas in Cuban coastal wetlands as in the case of "Los Palacios", is an important factor that allows birds to use both ecosystems to provide their daily needs with relatively low energetic cost (20). In addition, this western location, due of its geographical position and the size of the wetland, should be an important starting setting for waterbird arrival to Cuba during fall migration according to the dynamic of waterbird abundance thus, facilitating early warning.

Indeed, two Cuban rice plantations ("Humedal Sur de Pinar del Río" & "Humedal del Sur de Sancti Spíritus"), both included in this study, are declared as IBAs (21). Ninety-seven bird species, most of them (74 %) totally or partially migratory, have been reported using rice fields and surrounding areas in Cuba (20).

In terms of virus incursion consequences, factors associated with the occurrence of HPAI such as poultry population, human and road density (47), are scarce in "Ciénaga de Zapata". Consequently, its priority for surveillance as a method of early warning is reduced. On the contrary, "Los Palacios" could be further prioritized, because poultry production areas and rice fields neighbor it. For similar reason, "Tunas de Zaza" ranked sixth, requires additional considerations in the prioritization process. Active surveillance for avian influenza in Cuba is carried out under considerations of risk of disease occurrence that take into account the potential magnitude of losses (48,49).

Cuba has not initiated active surveillance on AIV in wild birds, but the current work could be a starting point considering the existence of laboratory infrastructure. Given that most of countries are typically able to allocate a limited number of resources for sample collection, the question of how those limited resources may be most efficiently applied to maximize the probability of detection of an infectious agent could be critical to further improving prevention programs. In this regard, the prospective targeting of species and locations of foremost importance may allow a more effective planning of resources.

The remarkable distance of BWTE prevalence-weighted index from other species places it as the most important for deciphering the wild duck-poultry interface, including locations with the greatest potential for AIV spillover to poultry and domestic birds. Other studies in Cuba model the transmission from waterbird to poultry (50), but in a general way, without discriminating the importance of different wild bird species as a reservoirs.

Transmission mechanisms at the wild bird-poultry interface can be complex, as both wild waterfowl and terrestrial birds can be involved. (51,52). Given the complexity of avian influenza control (53), countries free of the disease, as is the case of Cuba, need to strengthen resilience capacities, for which it is appropriate to anticipate the risk of occurrence of the disease. Those locations that may form a wild bird-poultry interface must be prioritized in a broad sense (risk management through enhanced biosecurity and surveillance for early alert). Therefore, current outputs add knowledge to previous studies aimed at capacity building for early warning and resilience in Cuba against this global hazard (38,48,50,54).

Shortcomings of the analysis and areas for future research

Other locations that may have importance for waterbirds could not be included in the analysis due to the lack of updated ornithological data. Among these locations are the coastal lagoons in southern "Sancti Spíritus" province and in the north coast "Río Máximo" wetland (Camagüey Province) and "Gran Humedal del Norte de Ciego de Ávila" wetland, both of which may harbor important populations of ducks and shorebirds in the winter season. Predictions have not yet been tested, but the findings found in this study justify further research to test the hypothesis of more suitable species and areas as contribution of the coordinated efforts within the Caribbean. Since most of IBAs were ranked and population data of poultry are accessible, further research characterizing the wild bird-poultry interface may be a worthwhile opportunity.

CONCLUSIONS

These current results provide a novel contribution to early planning of AIV surveillance in migratory wild birds based on the influence of seasonal fluctuations of prevalence-weighted abundance and frequency that may strengthen detectability of AIVs targeting most suitable species and locations. Blue-Winged Teal was markedly the foremost ranked species, for Cuba, while "Los Palacios" and "Ciénaga de Zapata" were predicted as most appropriate locations during fall migration. The prospectively deduced risk index could provide predictions about AIV circulation in each species and location, but would also offers a novel insight for understanding the wild bird-poultry interface in Cuba. The prospect of poultry production management avoiding its growth in proximity to identified risky areas was an ancillary benefit even from a conservationist perspective, considering the bidirectional transfer of pathogens between the wild bird-poultry interface.

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