Estimation of the sensitivity of the surveillance system for avian influenza in the western region of Cuba

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Summary
Although avian influenza (AI) virus of H5 and H7 subtypes has the potential to mutate to a highly pathogenic form and cause very high mortalities in some poultry species, most AI infections in poultry are due to low pathogenic AI (LPAI). Hence serological surveys, coupled with passive surveillance activities, are essential to detect sub-clinical infections by LPAI viruses, H5 and H7 subtypes. However the proper planning of an active surveillance system should be based on a careful estimation of its performance. Therefore, the sensitivity of the active surveillance system for AI in the western region of Cuba was assessed by a stochastic model quantifying the probability of revealing at least one animal infected by H5 or H7 subtype. The diagnostic sensitivity of the haemagglutination inhibition assay and different levels of within-flock prevalence (5%, 12% and 30%) were considered. The sensitivity of the surveillance system was then assessed under five different samples size scenarios: testing 20, 30, 40, 50 or 60 animals in each flock. Poultry flock sites in the western region of Cuba with a size ranging from 10,000 to 335,000 birds were included in the study.

Validità del sistema di controllo dell'influenza aviaria nella regione occidentale dell'isola di Cuba

Parole chiave
Cuba,
Influenza aviaria,
LPAI,
Pollame,
Sistema di sorveglianza,
Sottotipo H5,
Sottotipo H7.

Riassunto
I sottotipi H5 e H7 del virus dell'influenza aviaria sono potenzialmente in grado di mutare in forme altamente patogene e causare un'elevata mortalità in alcune specie di volatili. Tuttavia, la maggior parte delle infezioni di influenza aviaria nel pollame sono causate da virus a bassa patogenicità (LPAI). Di conseguenza, controlli sierologici uniti a protocolli di controllo passivi sono essenziali per individuare infezioni sub-cliniche causate dai sottotipi H5 e H7 di virus LPAI. Allo stesso tempo è necessario pianificare appropriatamente il controllo attivo sulla base di una stima accurata della sua efficacia. Questo articolo riporta i risultati di test riguardanti la validità di un sistema di controllo attivo per l'influenza aviaria nella regione occidentale di Cuba. I test sono stati condotti usando un modello stocastico in grado di quantificare la probabilità di individuare almeno un animale infettato dai sottotipi H5 o H7. Sono state considerate la validità diagnostica dei test di inibizione dell'emoagglutinazione e di diverse percentuali (5%, 12% e 30%) di diffusione in uno stesso allevamento di pollame. La validità del sistema di sorveglianza è stata testata ipotizzando cinque diversi campionamenti: 20, 30, 40, 50 e 60 animali provenienti dallo stesso allevamento. Nello studio sono stati inclusi allevamenti di pollame presenti nella parte occidentale dell'isola di Cuba con un numero di animali variabile tra 10.000 e 335.000.

Introduction

Avian influenza (AI) is a highly contagious disease, listed by the World Organisation for Animal Health (*Office International des Épizooties*: OIE), which has attracted much attention due to the public health implications and the effects it has on the poultry industry, given the significant economic losses suffered by countries in which AI is endemic (40). AI is caused by type A strains of the influenza virus that belong to the family *Orthomyxoviridae* (2, 27). The viruses that cause AI are differentiated into two groups, depending on their pathogenicity, namely: highly pathogenic avian influenza virus (HPAIV) and low pathogenic avian influenza virus (LPAIV). LPAIV is mainly responsible for respiratory illnesses and low mortalities in poultry whilst HPAIV causes systemic disease, often resulting in high mortality in turkeys and chickens (33).

The antigenic differences between the two surface glycoprotein haemagglutinins (HA) and neuraminidase (NA) have enabled the identification of 16 HA subtypes (H1-H16) and 9 NA subtypes (N1-N9) of AI viruses. For the purposes of the *Terrestrial Animal Health Code*, avian influenza is a notifiable infection of poultry caused by any avian influenza virus type A belonging to the H5 or H7 subtypes or by any AI virus with an intravenous pathogenicity index (IVPI) greater than 1.2 or causing mortality in at least 75% of cases (40).

Type A influenza viruses can infect a wide range of hosts and can be pathogenic to both humans and birds (45). The antigenic characteristics of influenza virus change gradually by accumulating point mutations (antigenic drift) or suddenly by genetic re-assortment (antigenic shift) in the genes primarily encoding HA and NA. The antigenic drift leads to new antigenic variants that require a replacement of the influenza strains used in the human vaccines. The antigenic shift results in the appearance of new strains and is of great importance in the occurrence of seasonal outbreaks of human influenza. The virus strains implicated in the 20th Century's influenza pandemics come from genetic re-assortment between avian and human viruses or transmission of the virus from animals to humans, through adaptation of purely avian strains to humans (35). Occurrences of direct bird-to-human transmission of avian influenza viruses have increasingly been reported in recent years, culminating in the outbreak of H5N1 influenza among poultry in several countries in Asia, and have caused infections in humans (11). In 1997, the first human victims of AI strain H5N1 were documented in Hong Kong (10).

Although several animal species have been shown to be susceptible to influenza virus infections, three animal species besides humans appear to play a more important role in the epidemiology of influenza, namely: birds, pigs and horses (6). In particular, wild birds, ducks and geese are the reservoir of influenza virus. Transmission occurs directly or indirectly through aerosols, water, feed and other materials that have been contaminated by faeces (3). The widespread epidemic of AI in birds increases the likelihood of mutational events and genetic re-assortment. Adequate surveillance, development of vaccines, outbreak preparedness and pandemic influenza planning are important when facing an epidemic (35).

Awareness of AI has increased continuously in recent years. Since 1997, the year that AI caused six deaths among 18 affected patients in Hong Kong, the focus on AI increased. After 2003, with the rapid evolution and spread of subtype H5N1, which affected poultry and wild birds in over 60 countries across 3 continents (4, 41) which resulted in the implementation of strict surveillance by animal and public health agencies both nationally and internationally. The pandemic potential of this situation remains of concern. The emergence of the disease due to HPAIV of subtype H5N1 was associated with laboratory confirmation of 602 human cases, 355 of which were fatal (42). Many control policies for the prevention and response to H5N1 outbreaks have been implemented and surveillance has increased, even in countries with no history of AI (45).

Animal influenza viruses continue to threaten animal and public health, food security and livelihoods, whilst H5N1 AI remains endemic in several regions of the world (36). The increased relevance of AI to animal and human health has highlighted the lack of scientific information on several aspects of the disease, which has consequently hampered the adequate management of some of the recent crises (8). Among these aspects, is epidemiology and surveillance systems have been reinforced to provide an early warning mechanism in the event of an AI incursion and to ensure rapid response in the case of an AI outbreak in poultry.

Although H5 and H7 subtypes of the AI virus have the potential to mutate to the highly pathogenic form to cause very high mortalities in some poultry species, most AI infections in poultry are caused by LPAI viruses (4), which may cause sub-clinical infections and could spread unnoticed to new premises. In such cases, the disease could spread regionally or globally, resulting in serious constraints for control.

Serological surveys are essential to detect subclinical infections of LPAIV H5 and H7 subtypes and are applied to complement the passive surveillance component of programmes (1). The objectives of AI surveillance systems in disease-free regions are not only early detection of all incursions of AI but also to provide evidence of its absence in the population (12). In developing countries, resources

allocated for surveillance and early warning systems are often scarce or inadequate. In these countries, therefore, the implementation of relatively low cost serological surveillance programmes would be beneficial. However, the performance of these systems must be carefully evaluated to enhance the efficacy and efficiency of activities. In particular, when epidemiological data are lacking due to the absence of infection in the territory, risk assessment methodologies may help to model the expected surveillance results under different hypothetical scenarios, thus providing the veterinary authorities and decision-makers with the information to better target the surveillance activities and perform more comprehensive cost-benefit analyses.

The Cuban AI surveillance system, in compliance with the OIE (40) and guidelines of the Food and Agriculture Organization (FAO) (26) is based on serology, viral isolation and nucleic acid detection according to a complex diagnostic algorithm. Serological testing is applied extensively as a screening method in active surveillance. The sensitivity of the active component of surveillance system, based on the haemagglutination inhibition assay (HIA), was studied in the western areas of Cuba where over 70% of the commercial poultry population is located and a greater risk of contact with migratory birds is present.

The objective of this work was to assess the sensitivity of the serological surveillance system for AI in the western region of Cuba. This work was part of a study to re-plan the existing AI surveillance programme in the country, including a spatial analysis of the risk of exposure of Cuban poultry flocks to the AI virus introduction through migratory birds and also taking into account the biosecurity levels of the farm.

Materials and methods

A surveillance system in a disease-free area is implemented with the principal objective of early detection of any incursion of the infection into the territory, thus enabling an assessment of the sensitivity of the system in detecting at least one infected animal. A simulation model was therefore developed to assess the probability that all infected animals tested negative by HIA on the poultry farms of western regions of Cuba under different scenarios of within-flock prevalence and number of tested animals.

The model was based on the following assumptions:

- all infection is detected only by serological investigations, no clinical sign or other evidence of virus presence are considered;
- all flocks have the same probability of being infected;
- the differences of within-flock prevalence are influenced only by the uncertainty of the prevalence estimations, not by animal biological variability;
- the probability of detecting antibodies in an infected animal depends on sensitivity of the test alone;
- the specificity of HIA is considered equal to one and only diagnostic sensitivity is taken into consideration.

The model takes into account the number of poultry farms and sizes of flocks in the western areas of Cuba that are exposed to the possible introduction of AI viruses during the Spring and Autumn migrations of wild birds across the island. In particular, 115 poultry farms housing over 10,000 individuals each have been included in the model. Notifications to the OIE World Animal Health Information System (WAHIS) during 2007-2009 were used to calculate the within flock prevalence levels. In particular, the following three levels were considered in the model: 5.74%, 10% and 31.25% (37-39). The diagnostic sensitivity of HIA reported by Stoyanov was used (32). Table I presents the input variables and the distributions used in the model.

Five scenarios with different numbers of tested animals in each flock (20, 30, 40, 50 or 60) were studied. For each scenario, the model estimates the probability that all animals tested gave negative results under the different prevalence levels.

Table I. *Variables and distribution used in the model.*

Variables	Type of distribution	Distribution parameters	Reference
N = number of susceptible birds in each flock	Cumulative	Actual bird population in the 115 flocks of the region: min = 10,000 max = 335,000	(18)
n = number of animal tested by HIA in each flock		5 scenarios 20, 30, 40, 50, 60	
$Prev$ = within flock prevalence	Beta	α=10; β=174 α=3; β=30 α=15; β=48	(39) (38) (37)
Se = sensitivity of HIA	Beta	α=47; β=5	(32)
P = probability that all tested animals are negative		$(1-Se*Prev)^n$	
I = number of infected animals in each flock		$Prev*N$	

HIA = haemagglutination Inhibition assay

The model was developed using @Risk (Palisade Corporation) (29) and Microsoft® Excel 2007 and the simulation results were obtained after 1 000 iterations with Latin hypercube sampling.

Results

The level of failure of AI serological surveillance is estimated through the probability that all animals tested by HIA gave negative results, considering 5.74%, 10% and 31.25% within-flock prevalence, respectively (Figures 1, 3 and 5) and five different scenarios of animal numbers tested in each flock.

The impact of infection is estimated by the expected number of infected animals in each flock, considering three different within-flock prevalence rates (Figures 2, 4 and 6).

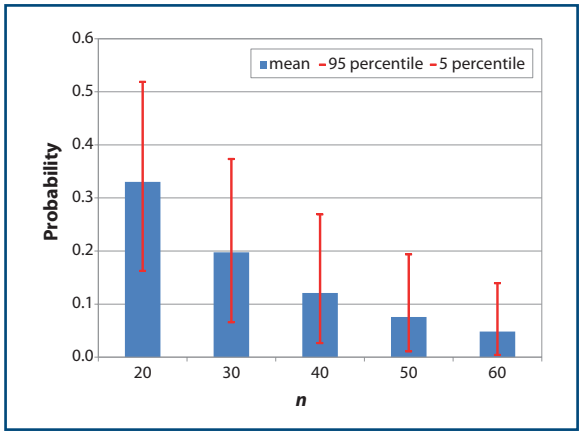

Figure 1. *Probability (mean, 5th and 95th values of the simulated distribution) that all tested animals give a negative results using the haemagglutination inhibition assay (within flock prevalence = 5.7%).*

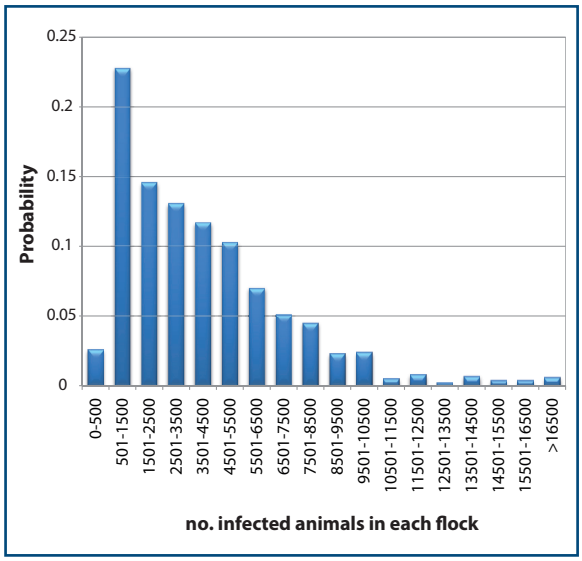

Figure 2. *Expected number of infected animals in each flock (within flock prevalence = 5.7%).*

Discussion

The aim of AI surveillance is to provide information on the temporal and spatial variation of circulating influenza viruses, in particular the epidemiology, ecology and evolution of AI viruses, to create an early warning system for the identification of viruses that have the potential to cause human disease.

Surveillance is performed through both active and passive methods. Passive surveillance includes the collection of data in the absence of a formal procedure and is intended to actively collect the information, such as through voluntary submissions of samples to diagnostic laboratories. Active surveillance is aimed at establishing a systematic process for early detection of specific diseases in a population (19).

Active surveillance is based on specific targeted investigation of at-risk populations for evidence of infection that may be based on detecting exposure to the agent (antibody detection by serology) or the presence of the agent (virus or antigen detection). The methods used must be modified according to the epidemiology of the disease (14). LPAIV or its genome can be detected in an individual bird for only few days, depending on several factors, whereas antibodies elicited by LPAIV are often present for the entire production life of the infected poultry (31). The facts that the majority of AI infections are caused by LPAIV (2), reinforces the importance of active surveillance based on antibody detection to H5 or H7 AI subtypes.

The active surveillance system for AI in Cuba is mainly based on antibody detection by HIA to H5 and H7 subtypes (17). HIA is considered the serological gold standard for AI with near-perfect accuracy of sensitivity greater than 98% (9). However, in order to test the active surveillance system under

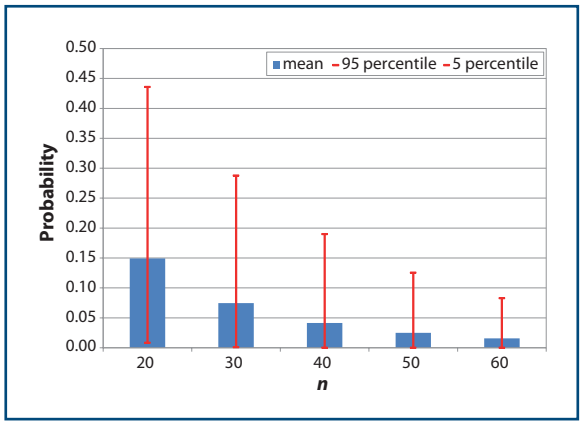

Figure 3. *Probability (mean, 5th and 95th values of the simulated distribution) that all tested animals give a negative results by haemagglutination inhibition assay (within flock prevalence = 10%).*

more exigent conditions we assumed the lowest sensitivity value reported by Stoyanov (32) in spite of differences due to subtype used.

The European Union, together with other authorities from different geographic areas, has proposed additional surveillance studies within their AI surveillance network, with the aim of controlling H5 and H7 influenza viruses. Some of these studies have been focused on wild birds, proposed as a potential early warning system, particularly in those regions where there is a large interface of human-animal contact (15). In Europe, surveillance programmes for wild birds always involves virological surveillance. Anseriformes (water fowl) and charadriiformes (shorebirds and gulls) are the main sampling targets. Active surveillance is conducted on living, clinically healthy and/or clinically diseased, injured or hunted birds. Cloacal swabs, fresh faeces and tracheal or oropharyngeal samples are collected. Passive surveillance is conducted on sick and dead wild birds. Cloacal and tracheal or oropharyngeal swabs and/or tissue (brain, heart, lung, trachea, kidney and intestines) are collected for virus isolation and molecular detection (30).

In recent years several studies have been conducted in other non-European countries to improve AI surveillance. In countries with large domestic duck populations, the control of AI H5N1 infection is considered an important component of the overall control programme (21). Ducks can be asymptomatic carriers of AIV and can play an important role in the transmission of the virus. A study conducted in the Republic of Korea reported that domestic ducks showed no distinctive clinical signs except for a drop in egg production in two of three H5N1 HPAI outbreaks (24). A nation-wide active surveillance of domestic ducks was implemented to control HPAI, including virus isolation to identify infected animals and serological testing for antibody detection. The study was conducted in laying breeder ducks and demonstrated the validity of the egg yolk antibody as alternative source to serum for AI virus antibody (23).

In China, Vietnam and Indonesia, vaccination of ducks and other poultry in small commercial farms, villages and households is practised with inactivated H5N1 vaccines (25). In these countries, a surveillance system is designed to determine target levels of post-vaccine antibody response and to ensure that H5N1 virus is circulating in vaccinated duck flocks. Another study, conducted in 2008, investigated an alternative strategy that involved the use of an exogenous positive marker of vaccination in domestic and wild ducks, to provide the relevant authorities with a tool

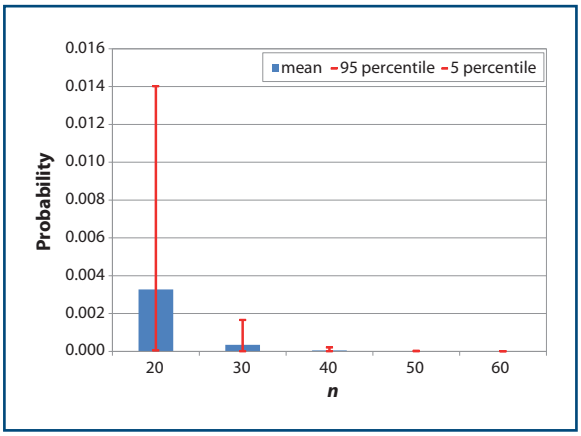

Figure 5. *Probability (mean, 5th and 95th values of the simulated distribution) that all tested animals give a negative result in the haemagglutination inhibition assay (within flock prevalence = 31.25%).*

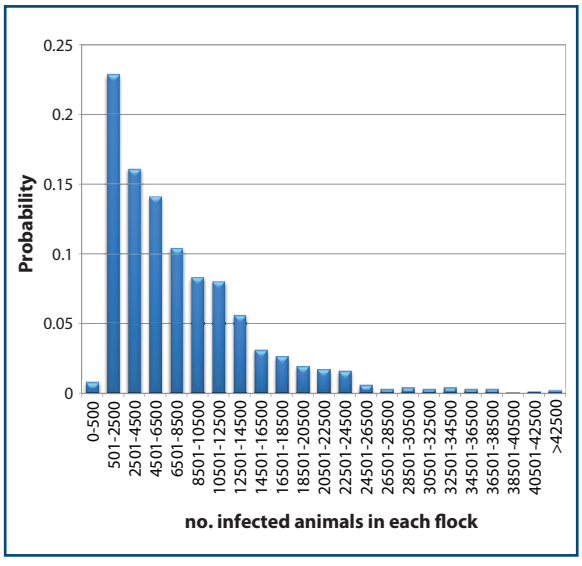

Figure 4. *Expected number of infected animals in each flock (within flock prevalence = 10%).*

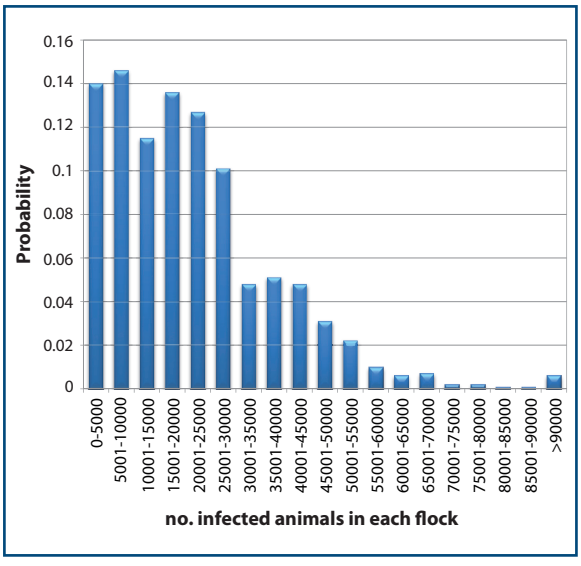

Figure 6. *Expected number of infected animals in each flock (within flock prevalence = 31.25%).*

for post-vaccination surveillance and with more accurate data on the H5 vaccine coverage (22).

Although it will be virtually impossible to prevent new outbreaks of influenza in humans and animals, global animal influenza virus surveillance can play a key role in the early recognition of new threats (6). Public health authorities and international organisations such as the FAO, OIE and World Health Organization (WHO) are tracking and monitoring AI virus circulation and are continuously engaged in the monitoring and characterisation of emerging viruses (3).

Early detection and early warning, rapid confirmation of suspects, rapid and transparent notification, rapid response (including containment, management of poultry movement, zoning and compartmentalization, stamping out and vaccination) are key activities when faced with an outbreak of AI (30). For this reason, the OIE has adopted new standards for the quality of national veterinary services in which disease notification systems and information systems are improved so as to ensure early and accurate epidemiological information on a worldwide basis, in particular through its early warning system.

According to the OIE *Terrestrial Animal Health Code*, surveillance strategies must take into account many variables such as the poultry species at risk, different biosecurity levels and production systems and the frequency of contact of domestic poultry with wild birds (40). The target population for surveillance aimed at identifying the infection should cover all susceptible poultry species within a country, zone or compartment. Surveillance should include random and targeted approaches using molecular, virological, serological and clinical methods (40).

In addition, the FAO indicates five points that need to be taken into account when a surveillance programme for AI is planned, which are (14):

- correctly identify the population at risk;
- accurately identify the susceptible animal units (intensive flocks, markets, backyard farms, individual animals, etc.);
- select the target prevalence level for the sampling scheme;
- identify the size of the target population;
- define the confidence level (95%, 99%).

In compliance with European Union regulations, a sampling scheme for detecting infection in the poultry category (excluding for ducks, geese and turkeys) must be able to detect an infected flock with a confidence level of 95%, when the between-flock prevalence is equal to 5% and the within-flock prevalence is approximately 30% (1).

According to the surveillance programme for AI implemented in Cuba, 30 birds in each flock are tested annually by HIA. The results of the simulation model clearly demonstrate that 30 birds tested in each flock would detect the infection when the within-flock prevalence of infection is approximately 30%. In this case, the probability of having at least one positive result exceeds 99%. It has been reported with populations of 10,000-∞, that a prevalence of 30% and a confidence level of 95% is sufficient to detect infection in one out of nine animals (7). According to the guidelines of the European Union, the samples within each category of poultry (except ducks, geese and turkeys) must be designed to detect at least one infected bird with a 95% confidence if the seroprevalence within each shed of the holding is 30% (1, 43).

When lower levels of within-flock prevalence are considered, the probability of failing to detect the infection with 30 tested birds raises levels to 7.5% and 19.8% with 10% and 5.7% within-flock prevalence, respectively. In these circumstances, the AI surveillance system clearly shows lower sensitivity, which can be restored by increasing the number of birds tested in each flock (5). The active surveillance program of the IA in the Republic of Argentina, aims to detect 15% prevalence with 95% confidence, with 20 samples per farm (13). However, these considerations are valid when a purely random sampling scheme is considered (2) and no samples are collected on the basis of clinical signs, which will increase the probability of AI detection.

In addition, not all animals will be infected at the same time during an epidemic episode and, therefore, the choice of the level of prevalence is strictly related to the rapidity of recognition of infection targeted by the surveillance system (18). Increasing the sensitivity of the surveillance system, selecting lower prevalence levels for detection and, therefore, increasing the number of samples to be taken, also means that there will be a higher probability of more rapid recognition of any introduction of infection in the target population (34).

The choice must be balanced, taking into account the epidemiological characteristics of the infection, the severity of the consequences of delayed diagnosis of infection and the resources available. In this regard, the limited resources usually available mean that a careful evaluation must be made to maximise the efficiency and the efficacy of surveillance activities (18).

A risk-based approach is generally the best option but requires correct and scientifically valid risk assessments. The selection of the areas to be monitored, the time and frequency of sampling and the number and distribution of samples to be collected, are all aspects that need to be evaluated

scientifically and objectively (44). This paper describes an attempt to scientifically evaluate the impact of different choices in the number of samples to be taken within the current AI surveillance plan in Cuba. Further aspects should also be also taken into account to increase the sensitivity of the system, such as the existence of an effective passive surveillance and the appropriate timing of investigations during the period of time of increased risk of AI introduction, represented by the migratory birds that transit in Cuba during the spring and autumn.

The correct choice of the target population is as important as the other variables. Geographic areas and populations most at risk to exposure of infection must be evaluated very carefully. Settlements of migratory birds must be defined geographically, as should their proximity to poultry farms. The possible exposure of the latter, considering existing biosecurity measures in place and the consequences of such exposure, in terms of animal densities and the existence of the flocks greater economic importance (genetic centres, laying hens intensive farms, etc.), must also be carefully evaluated to better target the surveillance system.

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