

13 March 2023

Given the increasing detection of outbreaks of highly pathogenic avian influenza (HPAI) in birds registered in 16 countries of the Region of the Americas, the confirmation of the first human infection of influenza A(H5N1) in Latin America and the Caribbean, and the increase of cases in mammals, the Pan American Health Organization/World Health Organization (PAHO/WHO) reiterates its guidelines on surveillance, laboratory diagnostics in human and animal samples and research. PAHO/WHO recommends strengthening surveillance of respiratory disease in animal and human populations, thorough investigation of all zoonotic infections, and pandemic influenza preparedness.

Global context

According to the World Organization for Animal Health (WOAH), the highly pathogenic avian influenza (HPAI) epidemic season continues with outbreaks in poultry, wild birds, and mammals, mainly in the Regions of Europe, America, and Asia. In the current epidemic period, subtype A(H5N1) is predominant and has caused an alarming rate of wild birds killed and an increasing number of cases in mammals, both terrestrial and aquatic, causing morbidity and mortality, raising concerns about the threat it poses to the health of domestic and wild animals, biodiversity and potentially for public health (1,2,3).

In accordance with the seasonal pattern of HPAI¹, the number of outbreaks is expected to increase in the coming months and WOAH recommends that countries maintain and strengthen their surveillance systems, biosecurity measures on farms, and continue with the timely notification of avian influenza outbreaks in both poultry and non-poultry species (domestic and/or wild birds). The quality of surveillance is key for the early detection and timely response to potential threats to animal health with an impact on human public health (1,2,3).

Whenever avian influenza viruses circulate among poultry, there is a risk of sporadic occurrence of infections in humans due to exposure to infected birds or contaminated environments. From 2003 to 26 January 2023, a total of 868 human cases of influenza A(H5N1) infection, including 457 deaths (case fatality rate 53%), were reported worldwide to WHO in 21 countries (4).

¹ Avian influenza viruses are classified into low pathogenic avian influenza viruses (LPAI) and highly pathogenic avian influenza viruses (HPAI) according to their ability to cause disease in birds.

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Situation summary in the Region of the Americas

As of epidemiological week (EW) 9 of 2023, agricultural authorities in Argentina, Bolivia², Canada, Chile, Colombia, Costa Rica, Cuba, Ecuador, The United States of America, Guatemala, Honduras, Mexico, Panama, Peru, Uruguay, and Venezuela³ have detected outbreaks of HPAI A(H5N1) viruses in domestic birds, farm poultry and/or wild birds, and in mammals (**Table 1 and Figure 1**). The detection of HPAI outbreaks in 14 countries in Latin America and the Caribbean is a situation never recorded before. The identified outbreaks are mainly located in areas of the Pacific flyway (**Figure 1**). To date and since the introduction of avian influenza A (H5N1) in the Americas in 2014, two human infections caused by avian influenza A(H5N1) were registered: the first in the United States of America reported on 29 April 2022 (5), and the second in Ecuador, which was reported on 9 January 2023 (6).

Table 1. Avian influenza outbreaks by affected animal type. Region of the Americas, up to week 9 of 2023

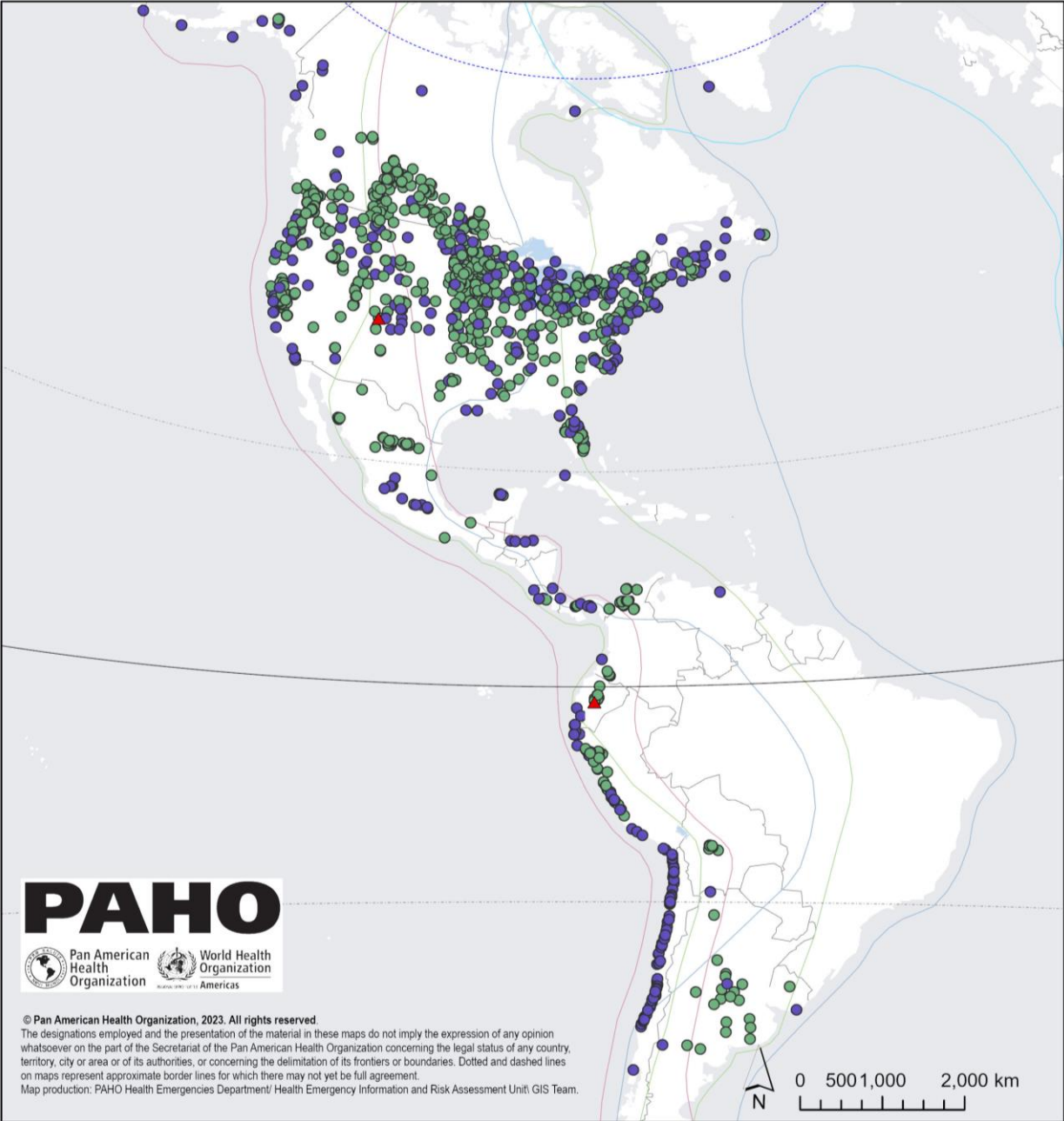
Country	Wild birds	Poultry farm	Backyard poultry	Mammals
Argentina	Yes	Yes	Yes	
Bolivia (Plurinational State of)	Yes	Yes	Yes	
Canada	Yes	Yes	Yes	Yes
Chile	Yes		Yes	Yes
Colombia	Yes	Yes	Yes	
Costa Rica	Yes			
Cuba	Yes			
Ecuador	Yes	Yes		
Guatemala	Yes			
Honduras	Yes			
Mexico	Yes	Yes		
Panama	Yes		Yes	
Peru	Yes	Yes	Yes	Yes
United States	Yes	Yes	Yes	Yes
Uruguay	Yes			
Venezuela (Bolivarian Republic of)	Yes			

Source: Data provided by IHR National Focal Points or available in Ministries and Institutes of Health of the countries and territories of the Region of the Americas

² Plurinational State of Bolivia

³ Bolivarian Republic of Venezuela

Figure 1. Avian influenza outbreaks and main migratory routes of wild birds. Region of the Americas, up to week 10 of 2023



Avian Influenza Outbreak
November 2021 to March 2023

- Poultry or Domestic Animal
- Wild Animal
- ▲ Human Case

Major Flyways

- Mississippi
- Pacific
- West Atlantic
- East Atlantic

Sources
 - Data: World Organization for Animal Health (WOAH) (2023). Retrieved on 10 March 2023. Data extracted by Pan American Health Organization. Reproduced with permission.
 WOAH bears no responsibility for the integrity or accuracy of the data contained herein, but not limited to, any deletion, manipulation, or reformatting of data that may have occurred beyond its control
 - Cartography: WHO Detailed ADMO Boundaries; Conservation of Arctic Flora and Fauna - Data Service (SHP) Major flyways of Arctic Birds Shapefile Accessed February 2023.

Source: World Organization for Animal Health (WOAH) (2023). Retrieved 10 March 2023. Data extracted and reproduced by PAHO/WHO.

The following is a summary of the situation in countries of the Region of the Americas that reported outbreaks of avian influenza in 2022 and 2023.

In **Argentina**, the National Service of Health and Food Quality (SENASA as per its acronym in Spanish) reported the first detection in the country in wild birds on 15 February 2023, in the Pozuelos lagoon, northwest of the province of Jujuy, near the border with Bolivia (7,8). Until 6 March, 39 outbreaks of avian influenza A(H5) have been confirmed in wild birds, backyard birds and poultry production farms in 9 provinces of the country: Buenos Aires, Chaco, Córdoba, Jujuy, Neuquén, Río Negro, Salta, San Luis and Santa Fe, there have been no human cases of infection with avian influenza A(H5) in relation to the identified outbreak (9,10,11,12).

In **Bolivia**, on 27 January 2023, the National Service of Agricultural Health and Food Safety (SENASAG as per its acronym in Spanish), reported the identification of avian influenza A(H5) in a poultry production farm in the Sacaba municipality, Department of Cochabamba (13). Until 23 February, SENASAG has identified 20 outbreaks of avian influenza A (H5N1) in municipalities of the Cochabamba department: 11 outbreaks in poultry production farms and 9 in backyard birds, so far with more than 218,000 slaughtered birds; no human cases of infection with avian influenza A(H5N1) in relation to the identified outbreaks have been reported (14,15).

In **Canada**, until 28 February 2023, multiple HPAI A(H5N1) outbreaks in poultry and non-poultry birds (including wild birds) were reported in nine of the ten provinces of this country: Alberta, British Columbia, Manitoba, New Brunswick, Newfoundland and Labrador, Nova Scotia, Ontario, Quebec and Saskatchewan. Between epidemiological week (EW) 2 and EW 6 of 2023, HPAI outbreaks occurred in the provinces of British Columbia, New Brunswick, Newfoundland and Labrador, Nova Scotia and Quebec. As of 20 February, an estimated 7,174,000 birds in 90 locations in 9 provinces of the country have been affected by highly pathogenic avian influenza. So far, no human cases of infection with avian influenza have been reported in the identified outbreaks (16,17,18).

In **Chile**, until 28 February 2023, the Ministry of Agriculture together with the Agricultural and Livestock Service (SAG per its acronym in Spanish) of this country, detected the presence of cases of HPAI (H5N1) cases in 21 types of wild aquatic birds distributed in the coastal zone of the territory, in 11 regions of the country: Antofagasta, Arica and Parinacota, Atacama, Biobío, Coquimbo, Los Lagos, Maule, Ñuble, O'Higgins, Tarapacá and Valparaíso (19-25). On 8 February, a positive case of highly pathogenic avian influenza H5N1 was detected in backyard birds in Chañaral de Aceituno area, Atacama Region (26). Additionally, avian influenza A(H5) was detected in four aquatic mammals (3 sea lions and one sea otter) in the regions of Arica and Parinacota, and Tarapacá. So far, poultry production birds have not been affected, and no human cases with avian influenza A(H5N1) infection have been reported in relation to the identified outbreaks (27-30).

In **Colombia**, until 3 March 2023, the Colombian Agricultural Institute (ICA per its acronym in Spanish), identified 48 HPAI A (H5N1) outbreaks, in backyard bird, in 7 departments of the country: Bolívar (5 outbreaks), Cauca (1 outbreak), Chocó (7 outbreaks), Córdoba (23 outbreaks), Magdalena (1 outbreak), Nariño (7 outbreaks) and Sucre (4 outbreaks) (31,32). To date, only the outbreaks presented in February in the departments of Cauca and Nariño remain active. The last outbreak of the disease in backyard birds was identified in a live bird market square in the city of Pasto, which has about 20 stalls selling live birds, with an approximate population of 260 birds of various species (chickens, ducks, turkeys, geese, among others) (33). The last outbreak identified in wild waterfowl (pelicans) occurred in the municipality of Guapi, department of Cauca, on Gorgona Island where an approximate population of 1,000 birds was calculated, of which 300 fell ill and 92 died (34). To date, no human cases of infection with avian influenza A(H5N1) have been identified in the reported outbreaks (32).

In **Costa Rica**, on 24 January 2023, the National Animal Health Service (SENASA as per its acronym in Spanish) reported the detection of four cases of Avian Influenza A(H5) in waterfowl (Brown Pelicans), found on Cocles beach, in Puerto Viejo de Talamanca, Limón province (35). Until 27 February 2023, SENASA of that country detected 13 outbreaks of avian influenza, 12 outbreaks in wild birds, in the provinces of Guanacaste, Limón and Puntarenas, and one outbreak in backyard birds in the Parrita canton, in the province of Puntarenas, in 6 of the detected outbreaks neuraminidase N1 was confirmed (37,38).

In **Cuba**, on 7 February 2023, the National Center for Animal Health of the Ministry of Agriculture of Cuba (CENASA as per its acronym in Spanish), reported the detection of avian influenza A(H5N1) in wild birds of the Zoological Garden of Havana (39). So far, poultry production birds have not been affected, and no human cases with avian influenza A(H5N1) infection have been reported in relation to the identified outbreaks (40,41).

In **Ecuador**, until 28 February 2023, 17 outbreaks of influenza A(H5N1) have been identified in poultry production farms and backyard birds, in 6 provinces of the country: Azuay (1 outbreak), Bolívar (2 outbreaks), Cotopaxi (8 outbreaks), Imbabura (1 outbreak), Pichicha (1 outbreak) and Tungurahua (4 outbreaks) (42-46).

On January 7, 2023, a human infection caused by influenza A(H5N1) was identified in Ecuador in a 9-year-old female living in a rural area of the province of Bolívar, who was in contact with backyard birds, acquired a week before the onset of symptoms and died without apparent cause. This human infection by avian influenza corresponds to the second recorded in the Region of the Americas, and the first in Ecuador and Latin America and the Caribbean. The patient required hospitalization in the pediatric intensive care unit, in isolation and with antiviral treatment and was fully recovered. No additional cases have been identified (6).

In **Guatemala**, on 14 February 2023, the Ministry of Agriculture, Livestock and Food (MAGA as per its acronym in Spanish), reported the identification of avian influenza A(H5N1) in wild aquatic birds (Brown Pelicans), in Puerto Barrios municipality, Izabal department. No cases have been reported among domestic or poultry birds, and no human cases with avian influenza A(H5N1) infection have been identified in relation to these outbreaks (49,50).

In **Honduras**, on 4 January 2023, the National Service of Agrifood Health and Safety (SENASA per its acronym in Spanish), reported the identification of avian influenza A(H5N1) in wild aquatic birds (Brown Pelicans) found in the city of La Ceiba, Atlántida department, and in Puerto Cortés municipality, Cortés department. No cases have been registered among domestic or poultry birds, and no human cases with avian influenza A(H5N1) infection have been reported in relation to identified outbreaks (51,52,53).

In **Mexico**, between October and December 2022, the National Service of Health, Safety and Food Quality (SENASICA per its acronym in Spanish) identified 50 outbreaks of highly pathogenic avian influenza A(H5N1) in backyard birds, poultry farms and wild birds, in 13 states of the country, Aguascalientes, Baja California, Chiapas, Chihuahua, State of Mexico, Jalisco, Michoacán, Nuevo León, Oaxaca, Puebla, Sonora, Tamaulipas and Yucatan, with a total of 5.9 million affected birds (54-56). For the year 2023, on 2 March, SENASICA reported the identification of the avian influenza A(H5N1) virus in three poultry production farms in the municipalities of Asientos and Rincón de Romos, in Aguascalientes state (57). To date, no human cases with avian influenza A(H5N1) infection have been reported in the identified outbreaks (54-57).

In **Panama**, on 20 December 2022, the Ministry of Agricultural Development of Panama (MIDA per its acronym in Spanish) confirmed the detection of HPAI A(H5N1) in a pelican found 32 kilometers from Panama City, on the Coast of the Pearl Archipelago in the Gulf of Panama (58). Until 9 March,

33 cases of highly pathogenic avian influenza (HPAI) A(H5N1) have been identified in wild waterbirds and backyard birds in 4 provinces of the country: Colón, Panama, Panamá Oeste and Veraguas. Poultry production birds have not been affected yet, and no human cases with avian influenza A(H5N1) infection have been reported in the identified outbreaks (59,60).

In **Peru**, until 6 March 2023, 128 cases of avian influenza A(H5) have been confirmed in wild waterfowl (77 cases), backyard birds (39 cases) and mammals (12). Cases in wild birds and backyard birds were identified in 13 departments: Cajamarca (32 cases), Lambayeque (21 cases), Lima (17 cases), La Libertad (13 cases), Ica (9 cases), Piura (7 cases), Ancash (6 cases), Arequipa (3 cases), Tacna (3 cases), Junín (2 cases), Callao (1 case), Moquegua (1 case) and Tumbes (1 case). Cases in mammals have been presented in 5 departments: Lima (3 cases), Piura (1 case), Junín (1 case), Arequipa (1 case), Ica (6 cases). 83% of cases in mammals correspond to sea lions (61-65). Three outbreaks have been recorded in zoos in the departments of Ancash, Cajamarca and Junín. To date, no human cases with avian influenza A(H5N1) infection have been detected in the identified outbreaks (66,67).

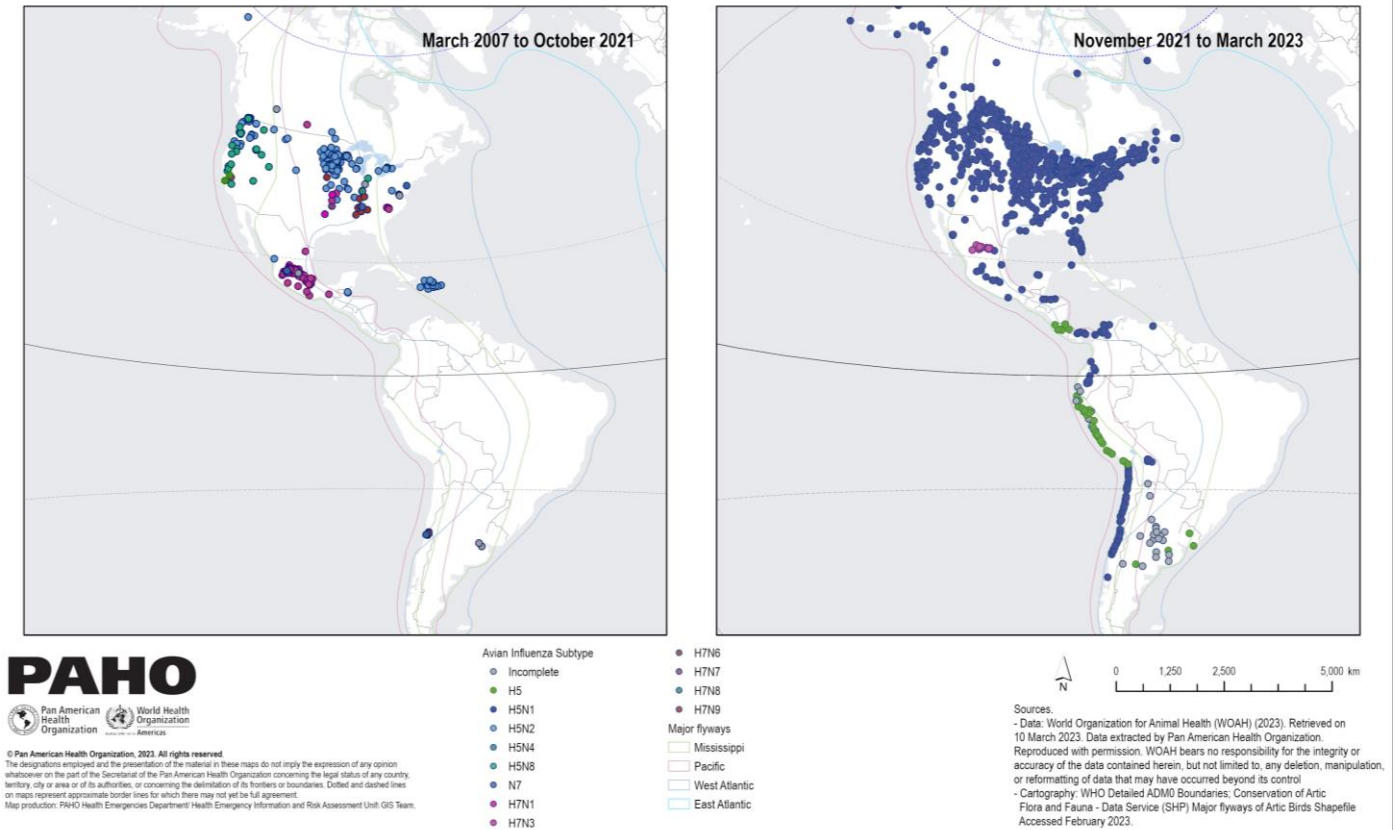
In the **United States**, since late 2021 until 1 March 2023, outbreaks of HPAI A(H5) virus have been reported in wild waterfowl, commercial poultry, backyard birds, and mammals. Preliminary genetic sequencing and RT-PCR tests on some samples identified that these viruses correspond to the HPAI A(H5N1) virus of clade 2.3.4.4. During the same period, HPAI outbreaks have been reported in wild birds in 50 states (968 counties) and poultry in 47 states (392 counties). 6,284 wild birds have been affected and more than 58 million poultry were affected due to identified and controlled outbreaks (47). Regarding the outbreaks identified in mammals until 9 March, 144 cases of highly pathogenic avian influenza (HPAI) A(H5N1) have been identified, in 22 states of the country, among the affected mammals 45.8% correspond to red foxes (*Vulpes vulpes*), 12.21% to port seal (*Phoca vitulina*) and 10.68% to striped skunk (*Mephitis mephitis*) (48).

On 28 April 2022, a human case of influenza A(H5N1) infection was identified in the United States in a person involved in culling birds at a commercial poultry facility in Colorado, where influenza A(H5N1) virus was detected in birds. This was the second human case associated with this specific group of H5 viruses currently predominant, and the first case in the United States. The patient was isolated and treated with antivirals, did not require hospitalization, and was fully recovered. In this event, no additional cases or evidence of human-to-human transmission were identified (5).

In **Uruguay**, on 15 February 2023, the Ministry of Livestock, Agriculture and Fisheries (MGAP as per its acronym in Spanish) detected an outbreak of avian influenza A(H5) in wild birds (Black-necked Swans) in the border area between the departments of Maldonado and Rocha, affecting 100 swans (67,68). Additionally, on 3 March, the MGAP confirmed the detection of an outbreak of avian influenza A(H5) in backyard birds in the city of San Gregorio de Polanco, Tacuarembó department. To date, no human cases with avian influenza A(H5N1) infection have been detected in the identified outbreaks (69,70).

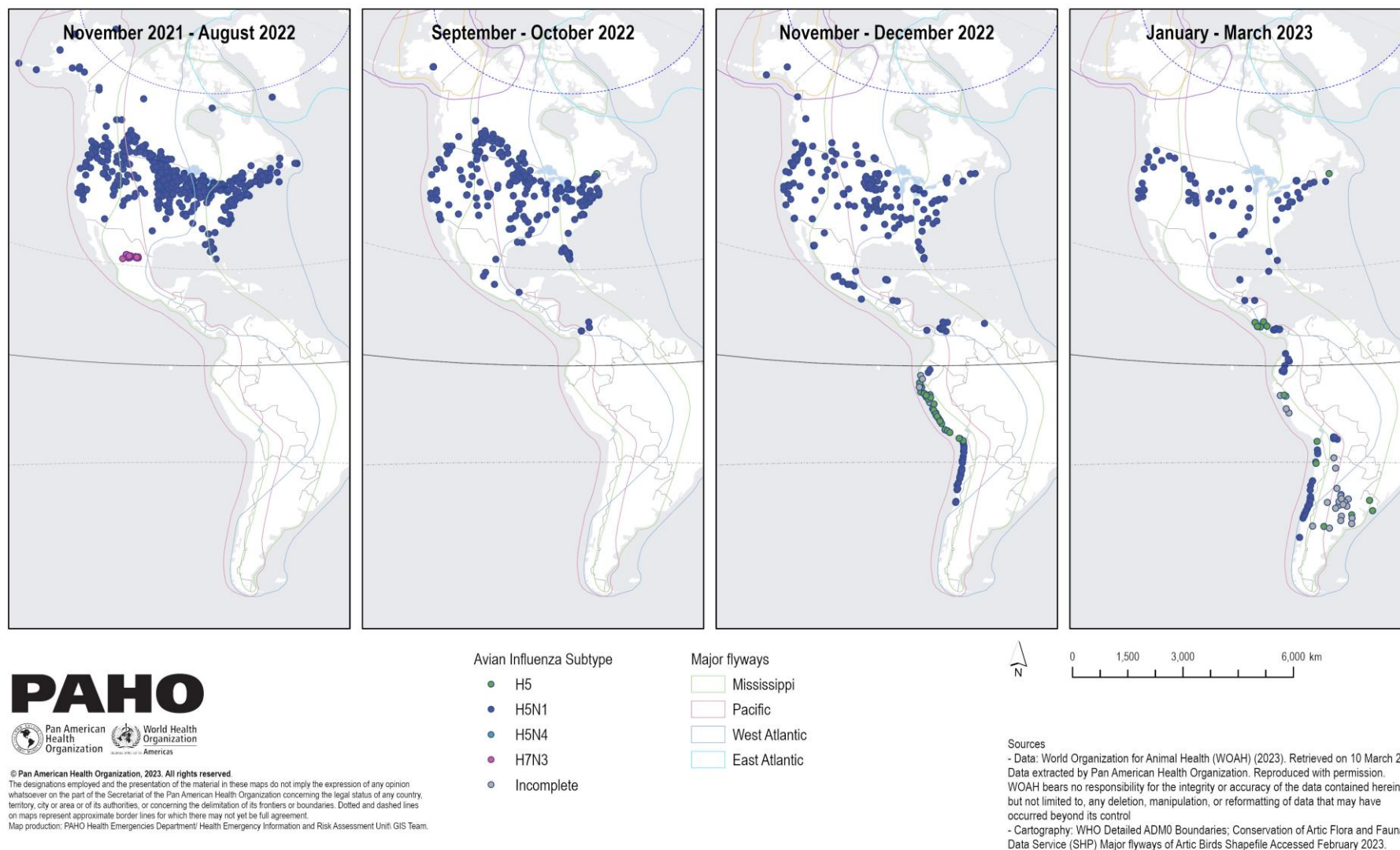
In **Venezuela**, on 29 November 2022, the Ministry of Popular Power for Productive Agriculture and Land, through inspection and epidemiological surveillance activities, identified an HPAI outbreak in pelicans in Puerto Piritu, Anzoátegui state, in which 172 wild aquatic birds were affected. Molecular studies detected Influenza A(H5) virus; this is the first time that HPAI has been detected in Venezuela. Subsequently, the Venezuelan Institute of Scientific Research (IVIC per its acronym in Spanish) confirmed the diagnosis by completing the characterization of the virus as A(H5N1). To date, no confirmed cases of avian influenza A(H5N1) have been identified in humans (71,72,73).

Figure 2. Avian influenza outbreaks and major wild bird migration routes. Region of the Americas, March 2007 to October 2021 and November 2021 to March 2023.



Source: World Organization for Animal Health (WOAH) (2023). Retrieved 10 March 2023. Data extracted and reproduced by PAHO/WHO.

Figure 3. Avian influenza outbreaks and major wild bird migration routes. Region of the Americas, November 2021 to March 2023, until epidemiological week 10 of 2023



Source: World Organization for Animal Health (WOAH) (2023). Retrieved 10 March 2023. Data extracted and reproduced by PAHO/WHO.

Guidance for health authorities in Member States

Both HPAI and LPAI viruses can be rapidly spread among poultry through direct contact with infected waterfowl or other poultry, or through direct contact with fomites or surfaces, or water contaminated with the viruses. Infection of poultry with HPAI viruses can cause severe disease with high mortality. LPAI viruses are more associated with subclinical infection. The terms HPAI and LPAI apply only to the symptoms in birds (chickens in particular), and both types of viruses have the potential to cause severe infections in humans.

While the potential exists for these viruses to cause human infections, infections with avian influenza viruses are punctual and when they have occurred, these viruses have not spread easily from person-to-person. To date, no person-to-person human transmission caused by avian influenza A(H5N8), A(H5N2), or A(H5N1) viruses has been reported either in the Americas or globally.

Intersectoral coordination

Control of the disease in animals is the first measure to reduce the risk to humans. For this reason, it is important that prevention and control actions, both in the animal and human health sectors, are carried out in a coordinated and concerted manner. Agile information exchange mechanisms will have to be established and/or strengthened to facilitate coordinated decision-making.

Implementation of a comprehensive surveillance program, including wild birds and both backyard and commercial poultry, is essential. Targeted risk-based surveillance strategies should be combined with a strengthening of general surveillance. In this regard, sensor awareness tasks are key, particularly in the backyard, to encourage the detection and notification of suspicious events. These programs also provide information that enables spread modeling and more accurate risk analysis.

Comprehensive recommendations for strengthening intersectoral work on surveillance, early detection, and investigation of influenza events at the human-animal interface are available at: <https://bit.ly/3glEUNN>

Risk communication and community engagement

Risk communication is a fundamental component of preparedness and response to health emergencies, especially those emergencies with pandemic or epidemic potential. Early and transparent communication with populations, as well as issuing clear messages about behaviors and preventive measures to be adopted by communities, are vital to reduce transmission. Additionally, adequate risk communication will contribute to reducing rumors, myths and misinformation related to the outbreak and will allow populations to make comprehensive decisions to reduce the risk of spread.

It is worth noting that risk communication in health emergencies is integrated by various aspects and areas and includes, but is not limited to, institutional communication or communication with the media. So, it is important that the leaders or teams in the health authorities that lead in an integral way the technical aspects related to this component, are clearly established.

The Pan American Health Organization recommends to member states the following actions among their preparedness measures for risk communication in the event of an outbreak of avian influenza:

- Delegate a person or team responsible for risk communication to review existing risk communication plans or strategies in pandemic or epidemic contexts and make necessary adjustments or updates to strengthen preparedness and respond to an eventual outbreak. Recent WHO guidance on a risk communication plan for respiratory diseases can be consulted at the following link: <https://bit.ly/3GTSKAr>
- Collect existing information and/or conduct qualitative and/or rapid quantitative assessments to know the characteristics of the communities at highest risk, patterns and communication channels, language, religion, influencers. This information is vital to be able to formulate appropriate preparedness and response actions for risk communication.
- Build trust through early, transparent, timely communication, and dissemination across multiple platforms, methods and channels. To maintain the trust of the population, it is also key to communicate even in the midst of uncertainty, clarifying what is known and what is not.
- Identify communities with whom to work on risk communication actions and allow them to participate in their implementation, to ensure that interventions are collaborative, and that the community takes ownership of communication processes. Community involvement will contribute to the adoption of preventive behaviors.
- Issue messages to the public about symptom identification and prevention, particularly to populations with greater potential for exposure to the virus: rural settings, farmers, farm workers. The messages must be broadcast on the channels and through the platforms consulted by each type of audience.
- Activate the social listening of rumors and disinformation through digital platforms and other relevant information exchange channels (telephone hotlines, web portals, etc.), to respond to possible false messages circulating among the public and adapt the messages according to the needs detected by this monitoring.

Surveillance in humans

People at risk of contracting infections are those directly or indirectly exposed to infected birds (domestic, wild, or captive), for example, poultry keepers who maintain close and regular contact with infected birds or during slaughter or cleaning and disinfection of affected farms. For this reason, the use of adequate personal protective equipment (PPE) and other protection measures is recommended to avoid zoonotic transmission in these operators.

Surveillance and monitoring of exposed persons is recommended to early identify cases or events of human-animal interface transmission. Due to the constantly evolving nature of influenza viruses, PAHO/WHO continues to emphasize the importance of strengthening severe acute respiratory infection (SARI) surveillance and influenza syndrome (ILI) surveillance to detect virologic, epidemiologic, and clinical changes associated with circulating influenza viruses that may affect human health. In addition to the active case-finding, identification and contact tracing activities carried out during the epidemiological investigation of zoonotic events, it is

advisable to strengthen existing SARI and ITI surveillance systems in locations where poultry farms are located, where cases reside, where animal outbreaks occur, or where the source of infection is suspected. To complement surveillance for SARI and ILI, PAHO/WHO recommends establishing early warning systems to provide an overview of the situation and to carry out a joint and coordinated risk assessment between the human and animal sectors in a timely manner.

Given the detection of an infection in humans, early notification is essential for an investigation and implementation of adequate measures that include the early isolation and treatment of the case, the active search for other cases associated with the outbreak, as well as the identification of close contacts for management and follow-up (74).

Healthcare personnel in areas where transmission of avian influenza (HPAI or LPAI) in birds is taking place should be alerted about the possibility of infection in people exposed to these viruses.

PAHO/WHO reiterates to Member States the need to maintain and strengthen seasonal and zoonotic influenza virus surveillance, including immediate shipment of human influenza samples caused by avian influenza to the WHO Collaborating Center, the US CDC.

Since information on the circulation of avian influenza A(H5N1) viruses is important for the human zoonotic influenza vaccine composition and for generating data for preparedness and response, countries are encouraged to share animal influenza samples with the WHO Collaborating Center, St. Jude Children's Hospital, which focuses exclusively on the threat to humans from zoonotic influenza viruses.

Case investigation

In the case of a confirmed or suspected human infection caused by an influenza virus with pandemic potential, including avian virus, it is recommended:

- A thorough epidemiologic investigation of history of exposure to animals, travel, and ill contacts should be conducted, even while awaiting confirmatory testing.
- The epidemiologic investigation should include early identification of unusual respiratory events that could signal person-to-person transmission of the novel virus.
- Clinical samples collected from the time and place that the case occurred should be tested and sent to a WHO CC for further characterization within the first week of detection.
- Standard infection prevention and control (IPC) procedures and standard precautions should always be applied, and personal protective equipment (PPE) used according to risk, to protect the health of the investigators. Appropriate PPE (according to the most probable modes of transmission) should be used when in contact with symptomatic persons and in situations where human-to-human transmission is suspected.
- The epidemiological investigation should include information from the official veterinarian services (OVS) and (animal production) private sector about the origin of the animals and the records of movements in and out of the premise. This information will contribute to define the scope (location) of investigations on humans exposed to the infected animals.
- Information from OVS could inform about potential episodes of influenza (both notifiable and non-notifiable) occurring in the area and farms related to the event.

Notification of cases in humans

1. A **confirmed case** of human avian influenza infection should be **reported immediately** via two channels—the WHO International Health Regulations (IHR) Regional Contact Point (ihr@paho.org) via the IHR National Focal Point, and the WHO Global Influenza Surveillance and Response System (GISRS) managed by PAHO and WHO (flu@paho.org). The report should include all available results from the epidemiological case investigation and the virological characteristics of the virus.
2. A **suspected case** of human avian influenza infection should be **reported immediately** to the GISRS (flu@paho.org), and information about the suspected case can be shared with the WHO IHR Regional Contact Point (ihr@paho.org), given it is an unusual event. The report should include all available results from the epidemiological case investigation and the virological characteristics of the virus.

Laboratory diagnosis in humans

Sample collection in humans

Samples should be collected by trained personnel in adherence to all biosafety instructions including the use of appropriate personal protective equipment (PPE) for respiratory viruses.

The recommended samples are the same type(s) of samples used for influenza routine surveillance. A nasopharyngeal swab is the optimal specimen collection method for influenza testing. However, a combined nasal and throat swab specimen or aspirate specimens can be collected. A sterile Dacron/nylon swab should be used for sample collection. Cotton tipped and wooded swabs are not recommended as they interfere in the sample processing and inhibit molecular diagnostic reactions. Swabs should be placed in a viral transport media tube containing 3 mL of sterile viral transport medium and transported in the same tube with viral transport medium (VTM).

Sample collection is recommended within 4 days of symptom onset for the highest influenza virus yield and better detection. Sampling of asymptomatic contacts is not recommended, unless considered necessary according to national guidelines.

Samples should be kept refrigerated (4-8°C) and sent to the laboratory (central, national, or reference laboratory) where they should be processed within the first 24-72 hours after collection. If samples cannot be sent within this period, freezing at -70 °C (or less) is recommended until samples are shipped (ensuring the cold chain is maintained).

Sample flow and laboratory testing algorithm

In the Americas, all national influenza centers (NICs) and national reference laboratories (NRL) for human influenza as part of the WHO Global Influenza Surveillance and Response System (GISRS) use molecular diagnostic protocols and reagents developed and validated by the WHO Collaborating Center at the US CDC.

In case of identification of suspected cases of human infection caused by avian influenza A/H5, a respiratory specimen should be taken and refer to the NIC or NRL for testing (**Figure 4**) (75).

Samples collected from suspected human cases exposed to birds or humans infected with avian influenza A/H5 should be tested for influenza; influenza A-positive samples should be subsequently subtyped for H5 (**Figure 5**)

Figure 4. Sample flow for samples of influenza A/H5 suspected cases at sentinel sites and/or decentralized laboratories.

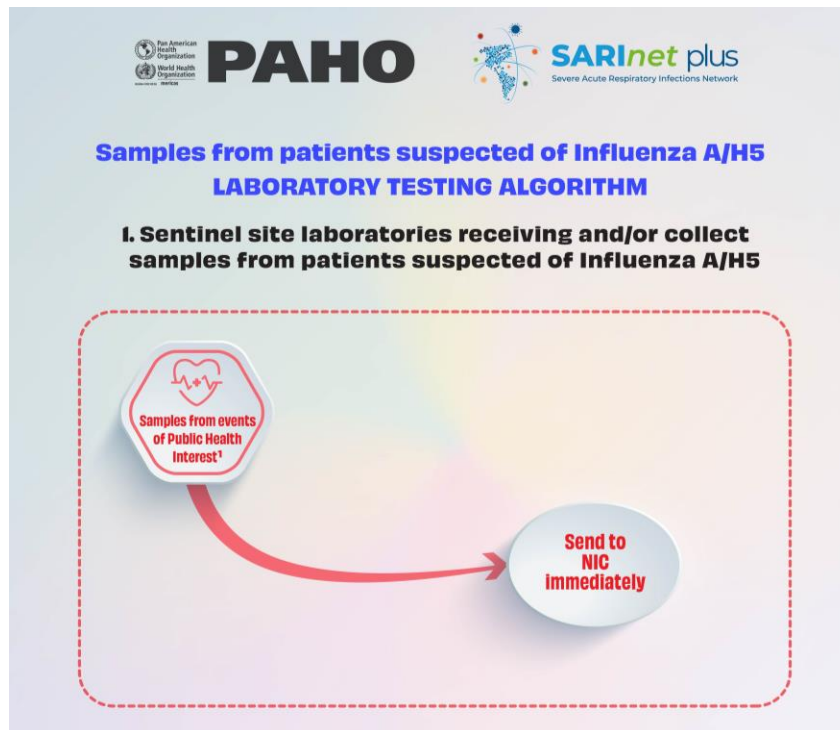
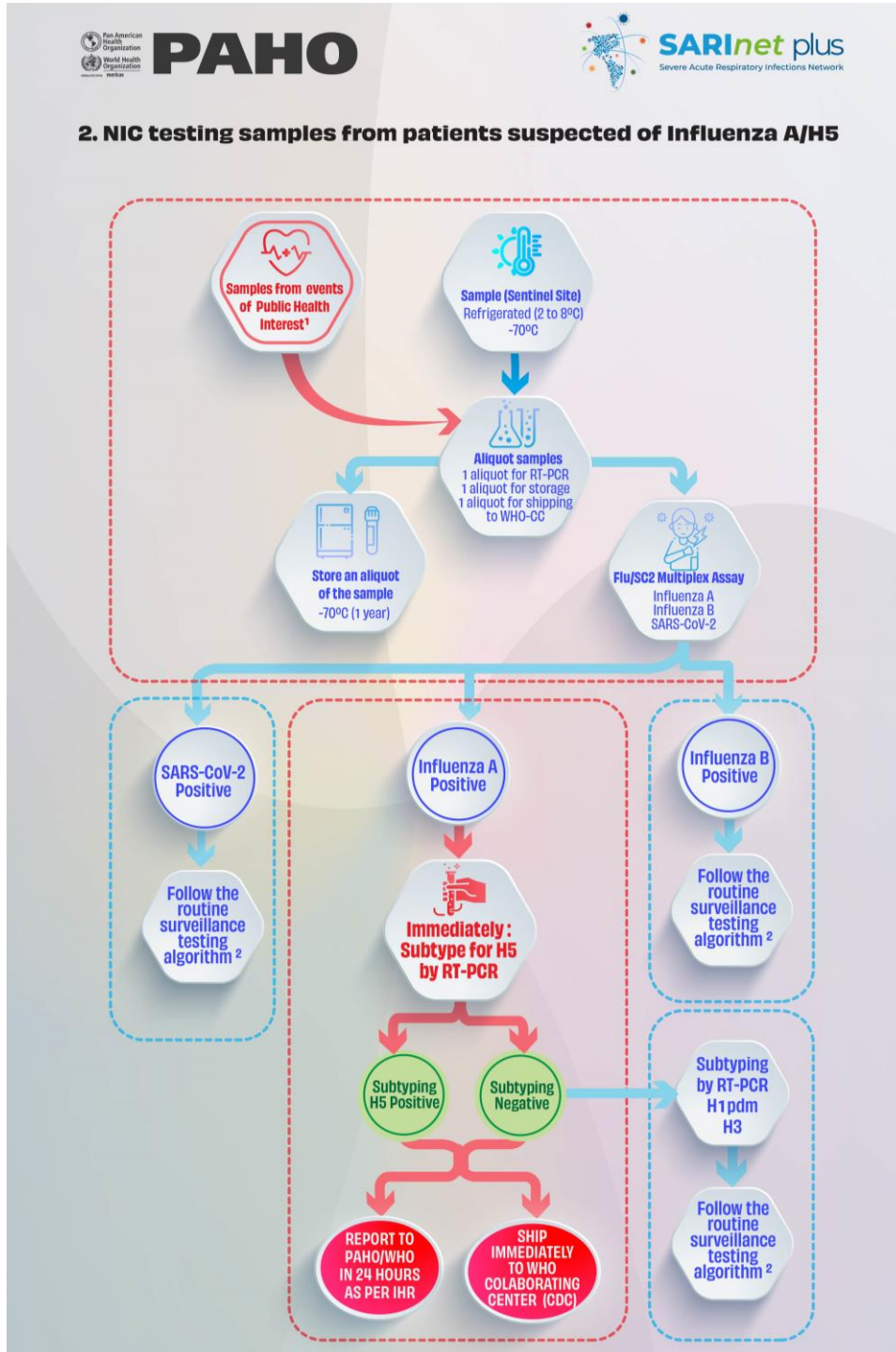


Figure 5. NIC testing samples from suspected cases of influenza A/H5 (76,77)



Laboratory reagents

US CDC kits for real-time reverse transcription polymerase chain reaction (qRT-PCR) detection of influenza viruses are available through the International Reagent Resource (IRR).

For influenza detection and Influenza A/H5 subtyping, the following kits and controls for molecular detection are available:

- Influenza SARS-CoV-2 Multiplex Assay (RUO) (500 reactions) (Catalog No. FluSC2PPB-RUO), dried primers and probes
- Influenza SARS-CoV-2 Multiplex Assay Positive Controls Kit (RUO) (500 reactions) (Catalog No. FluSC2PC-RUO)
- CDC Real-Time RT-PCR Influenza Virus A/H5 (Asian Lineage) Subtyping Panel (VER 4) (RUO) (Catalog No. FluRUO-13)
- CDC Influenza A/H5N1 (Asian Lineage) Real-Time RT-PCR Positive Control with Human Cell Material (RUO) (Catalog No. VA2715)

Interpretation of results

The markers (targets) of the US CDC kits for influenza A/H5 subtype detection are as follows: INFA (M), H5a (HA), H5b (HA), and RP.

When using the US CDC influenza A/H5 subtyping kit:

- Samples positive for INFA, H5a, and H5b markers are considered **positive for influenza A/H5**.
- Samples positives for only one H5 marker are considered **presumptive for influenza A/H5**.

In both cases, samples should be referred to a WHO Collaborating Center for further characterization or for confirmation (in the case of presumptive results). Nevertheless, a positive sample for Influenza A/H5 (both markers positive) should be reported immediately.

Currently, PAHO is working to support Member States on preparedness and response to Influenza A/H5. For additional support, please contact flu@paho.org.

Shipment of samples

The US CDC is the designated WHO Collaborating Center in the Americas Region for receiving human samples positive for avian Influenza A/H5. Shipment of human samples to the US CDC WHO Collaborating Center internationally and by air must be in compliance with all international standards according to the International Air Transport Association (IATA), being necessary special documents for transportation to the United States other than documents for routine shipment of seasonal influenza sample. It is important to note that the samples should **not** be sent as routine influenza samples to US CDC.

Laboratory surveillance and diagnosis in animals

Veterinary laboratories in countries generally have the ability to detect and to some extent type the virus in both serological and molecular samples. The most recent round of proficiency developed by the WOAHA regional reference laboratory in Campinas, São Paulo,

Brazil carried out in 2021 with the support of PANAFTOSA-PAHO/WHO, which verified the capacity of the participating laboratories to perform serological diagnostic tests (ELISA, HI and AGID) and molecular (RT-qPCR) in order to reach a final diagnosis of avian influenza. This round included Argentina, Bolivia, Chile, Colombia, Costa Rica, the Dominican Republic, Ecuador, Paraguay, Peru, and Uruguay, as well as Brazil as the coordinating laboratory.

Surveillance strategies combine the use of serological and molecular techniques to rule out not only previous exposure to the virus but also the current presence of the virus. This last point is very relevant to achieve early detection. The sub-typing of the virus in birds mainly seeks to rule out/check for the presence of influenza A, H5 or H7 viruses. Of these subtypes, laboratories usually do not have the necessary reagents to continue diagnosis. However, these analyses to differentiate the presence of HPAI are sufficient for support and field actions.

The regional reference laboratory in Campinas, Brazil, is supporting the confirmation of country diagnoses and sub-typing. Total virus sequencing is being carried out with support from other laboratories including the USDA WOA reference laboratory in Ames, Iowa, USA.

Countries have some demand for reagents for molecular techniques to be able to follow up on the surveillance activities required during the control of outbreaks found in birds, particularly in the perifocal zone.

Shipment of samples

Animal samples should be sent to the WHO Collaborating Center at St. Jude Children's Hospital. Special documents are necessary for transportation to the United States and must be compliant with all international standards.

For further information regarding logistical and shipment of human or avian Influenza A/H5 samples, PAHO/WHO should be contacted at flu@paho.org.

Genomic sequencing and surveillance

Sequencing

Submission of a positive sample for influenza A/H5, animal or human, to the appropriate WHO Collaborating Centre should be prioritized for antigenic and genomic characterization of the sample.

For laboratories that have sequencing capacity, in addition to sending the positive sample to the Collaborating Center, it is encouraged to sequence the sample to generate genomic sequencing data and to upload the sequences in a timely manner to the GISAID global platform.

The publication of sequences in GISAID requires the use of the nomenclature recommended by the WHO (33):

- The format for humans is:
[influenza type]/[region]/[internal reference number]/[year of collection]
Ex: A/Wisconsin/2145/2001
- For all other animal hosts:
[Influenza Type]/[Host]/[Region]/[Internal Reference Number]/[Year of Collection]
Ex: A/chicken/Rostov/864/2007

Genomic Surveillance

Human influenza A/H5 viruses: Since the beginning of 2020, influenza A/H5 viruses reported to WHO detected infecting humans are of genetic group 2.3.4.4b. The virus sequences of these human cases, when available, showed no markers of adaptation in mammals or resistance to antivirals, including oseltamivir and baloxavir (79).

Influenza A/H5 viruses, animal: Avian influenza A(H5N1) viruses, especially those of genetic group 2.3.4.4b, continue to diversify genetically and spread geographically. In addition, infection in wild and migratory birds has led to multiple separate incursions into domestic species. This circulation of the virus has led to opportunities to generate multiple genotypes with varied clinical signs. Through routine monitoring and viral sequencing, few sequences with markers of adaptation to mammals were found. These mutations probably occurred after transmission to the mammalian host and do not appear to be transmitted forward. The sequences available for genetic group 2.3.4.4b of viruses of avian and mammalian origin indicate that markers associated with reduced susceptibility to antivirals are rare (79).

Zoonotic influenza vaccine candidate viruses: WHO's Global Influenza Surveillance and Response System (GISRS), in collaboration with veterinary and animal health colleagues, regularly evaluates vaccine candidate viruses. Candidate influenza A/H5 vaccine viruses of genetic group 2.3.4.4b are determined. This includes a candidate A/H5N8 virus, in fact, A/Astrakhan/3212/2020, as well as an A/H5N1 virus, A/chicken/Ghana/AVL-76321VIR7050-39/2021. Vaccine virus A/Astrakhan/3212/2020 is closely related to recently detected circulating influenza A/H5 strains (79).

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Useful links

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