

OFFLU Strategy document for surveillance and monitoring of influenzas in animals



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Background

General

Animal influenza threatens animal health and welfare, agricultural productivity, food security, and the livelihoods of farming communities in some of the world's poorest countries. The emergence of H5N1 highly pathogenic avian influenza (HPAI), 1918 pandemic influenza, and pandemic H1N1 2009 (pH1N1) also highlight the potential for animal influenza viruses to evolve into global public health threats. To ensure that the impact and risks for animals and humans are kept to a minimum it is vital that the animal health sector takes the lead in monitoring influenza viruses in animals, in analysing the data, and in sharing this information with the international community particularly with public health partners.

A spectrum of influenza viruses circulates in animals, ranging in its ability to affect animal and human health: HPAIs have a severe impact on animal health, and human infections with H5N1 HPAI have severe consequences; other Notifiable Avian Influenzas are a threat to poultry health; equine influenza has a significant impact on equine health and performance; and swine influenza is often a mild disease of pigs.

The objectives and nature of animal influenza surveillance, and the response to positive findings depend on multiple factors including the implications of the influenza virus for animal and public health; the characteristics of the virus (which may evolve over time); the demographics of the host population; the epidemiology of the infection; geographical factors; involvement of wildlife; the type of control strategy being implemented; whether the disease is OIE listed (notifiable to the international community); and the capacity of the Veterinary Services to undertake surveillance and control. The response to disease detection must be proportionate to the risk and an exit strategy should always be considered when introducing a surveillance or control policy.

Timely sharing of virological and epidemiological information between the animal and the human health sectors and with other key partners is crucial in developing a better understanding of influenza viruses and their risks, and for providing an early warning to emerging threats. On a global level this is underpinned by the level of reporting of important virological and epidemiological data to the relevant international organisations.

Some objectives apply to all animal influenza surveillance, these include:

- Early detection of mutations or reassortments that may alter risks for animal or public health, and inform preparedness and control strategies e.g. for influenza viruses circulating simultaneously in human and animal populations
- To gather information to develop a better understanding of influenza viral characteristics, epidemiology, and risk factors, including in virus reservoirs
- To assess the genetic basis of important viral characteristics such as antiviral resistance, transmissibility, and pathogenicity in different species
- To monitor the performance of diagnostic tools that aim to detect new influenza viruses

Owing to the wide range of characteristics and impacts of different influenza viruses in different animal species, the objectives of surveillance for influenza viruses in these species – and the response to positive findings - will vary accordingly.

Examples of more specific objectives for surveillance include:

- Early detection of animal disease, allowing rapid containment and/or control in affected populations
- To gather antigenic information and biological material for early preparation of animal vaccines e.g. for equine, avian, and swine influenzas; to detect antigenic drift or shift; to match vaccine strains with field virus; and to contribute to preparing vaccines against potential emerging human pandemic viruses
- To assess animal population immune response when vaccination is being implemented for prevention or control in animals
- To detect infected animals in vaccinated populations
- To investigate the animal or environmental source of infections around infections in other species including humans
- To demonstrate freedom from disease

Each module in this document will describe the main objectives of surveillance for influenza in different animal species.

Structure of the document

The document is a dynamic modular document that aims to provide an overview of the objectives and options for surveillance for animal influenza viruses in several different animal species. Each module has been drafted thanks to the generous contributions of experts from OFFLU, the OIE-FAO joint network of expertise on animal influenza.

The materials in this document are relevant to the disease situation and scientific evidence available at the time of writing. Each module is dated according to the time that it was written. If the disease situation or characteristics of an influenza virus change the approach to surveillance and recommended response may also change accordingly.

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Introduction

Influenza A virus infection is endemic in swine populations in North and South America, Europe, and Asia. Typical outbreaks of swine influenza are characterized by fever, anorexia, lethargy, tachypnea, and a labored abdominal breathing. Infection causes a highly contagious respiratory disease that rapidly spreads within a swine herd. Mortality rates are low, but morbidity rates can reach 100 percent. Recovery is usually rapid in the absence of other pathogens; however influenza is often complicated by co-infection with other viral and bacterial pathogens in the field. Reduced reproductive performance, such as abortion, may be an indirect consequence of infection with swine influenza virus (SIV) but is less common. Economic losses from swine influenza result from reduced weight gain resulting in marketing delays and a predisposition to secondary bacterial infection even after the influenza infection is cleared. Many uncomplicated SIV infections have a mild or subclinical course. The clinical outcome of the infection depends on many factors, such as the immune status, age, infection pressure, concurrent infections, housing etc.

Vaccination and enhanced biosecurity practices are utilized to minimise the impacts of swine influenza, but vaccine usage is low in many regions of the world. The performance of SIV vaccines in the field can also be hampered by maternal antibody interference with vaccination of young pigs, a short duration of vaccine-induced immunity, and insufficient antigenic match between vaccine and field strains.

Influenza A viruses of H1N1, H1N2 and H3N2 subtypes commonly circulate in swine populations and are enzootic in many parts of the world. However, the origin and antigenic and genetic characteristics of these subtypes differ between continents and geographic regions. During the last decade, several new and emerging influenza virus reassortants originating from precursor avian, human, and/or swine viruses have emerged in swine-producing regions, and the epidemiology of swine influenza has become very complex. Antigenic drift, although apparent over time, is much less pronounced with swine viruses than with human influenza viruses.

Swine-origin influenza viruses have also been shown to infect other species, including humans, and turkeys. Sporadic, self-limiting human infections with swine-adapted influenza A viruses are generally associated with people in direct contact with infected pigs. Limited serological studies in humans suggest that zoonotic infection of people may be more common in persons with occupational exposure than the general population. However, these studies are difficult to interpret because of possible serologic cross-reactions between human and swine influenza viruses of the same subtype, and the true incidence of zoonotic SIV infections remains unknown.

Human-origin influenza viruses occasionally infect pigs and have added to the genetic composition of numerous multiple reassortant viruses in swine. Since 2009 in several countries, the pandemic H1N1 (2009) variant has been reported to move from humans into some swine populations and is now self-sustaining in many swine populations.

Swine, human, and/or avian-origin influenza viruses (as well as all influenza A viruses) can adapt or swap genes within a number of species, including pigs, potentially resulting in a mammalian-adapted influenza virus of public health concern. Although remotely probable,

this highly consequential zoonotic threat has heightened public health interest in surveillance for influenza viruses in multiple species, including pigs.

Swine influenza is not a food borne disease threat. Influenza viruses are generally restricted to the respiratory tract of pigs, and are not detected in the muscle (meat) of pigs, even during acute illness. The risk of human infection of influenza viruses through the consumption of pork or pork products is negligible.

Main objectives for influenza virus surveillance in pigs

Animal health

- Monitoring for genetic and antigenic evolution of endemic SIV;
- Selection of isolates for the development of more relevant diagnostic reagent and vaccine seed stock products;
- Documentation of endemic and emerging influenza virus ecology in swine;
- Detection of new and emerging influenza viruses in the swine population that may also impact other animal species.

Public health

- Pandemic preparedness, response, and communication planning.
- Timely identification of new and emerging influenza viruses in pigs for human population risk assessment;
- Assessment of viral mutations or reassortment events in pigs and other animals that might signal a public health concern;
- Monitoring of important molecular markers for resistance to antiviral drugs or for increased pathogenicity;
- Procurement of reagent and vaccine seed stock products for pandemic preparedness.

Surveillance approaches

Serological sampling for influenza in swine is of limited value in many countries due to the endemic status of swine influenza virus as well as the use of vaccine in some countries. The exception for the use of serology in a surveillance plan may be in countries that have limited knowledge of the influenza status of the pig population. In these cases, high throughput serology may be a useful screening tool to target more intensive viral surveillance. However, positive serologic findings are nonspecific for detailed genomic analysis. Furthermore, genetic drift and shift can render subtype specific serologic tests (haemagglutination inhibition (HI) or neutralization assays) insensitive to new or emerging strains of influenza. Serologic cross-reactivity with antigenically distinct influenza viruses can also occur, especially in pigs exposed to multiple SIV subtypes and variants or with very high homologous antibody titres. Thus, antigenic testing methods (virus isolation, RT-PCR, and sequencing) are essential to characterize influenza virus infections in swine populations.

Active influenza A infection is most efficiently detected through targeted surveillance activities. Specific implementation choices depend on geographical disease history, swine management practices, and resources available in each country of interest. A customised approach utilizing a combination of some or all of the targeted surveillance options will improve surveillance sensitivity.

Targeted surveillance options

Greater efficiency and cost effectiveness can be achieved by targeting surveillance to swine herds showing compatible clinical symptoms or to those with serologic evidence of previous infection. Case definitions should be developed to target swine populations of interest.

Clinical signs in swine, such as fever, anorexia, laboured abdominal breathing, together with suggestive post-mortem findings, can be used to identify potential candidates for surveillance sampling. However, a definitive diagnosis is possible only in the laboratory, and clinical signs do not differentiate between strains known to commonly circulate in pigs. Clinical signs are thus unlikely to effectively identify particular strains of interest in suspected influenza cases. The 2009 pandemic H1N1 demonstrated that influenza viruses of public health concern may show a similar clinical picture as endemic SIVs. Therefore, sampling of any swine showing influenza like illness (ILI) is justifiable to gather a complete picture of strains of interest.

Targeted sampling protocols can include, but are not restricted to:

- Veterinary diagnostic laboratory surveillance - Ancillary testing of swine samples submitted to veterinary diagnostic laboratories from herds exhibiting clinical signs of respiratory illness and/or suggestive pulmonary lesions, focusing on isolation of influenza virus from appropriate samples, followed with molecular and antigenic characterization.
- On-farm surveillance
 - Surveillance in animals with an epidemiological link to known infected animals or caretakers – Sampling herds that have been epidemiologically linked to known infected swine farms, had contact with other species showing ILI, or had contact with caretakers or other humans showing ILI but not associated with a public health investigation.
 - Age-targeted surveillance – Sampling endemically infected swine populations at the time of declining maternal immunity, often at 8-12 weeks of age. Pigs may or may not show obvious clinical signs while shedding virus at this age.
 - Acute outbreaks of respiratory disease in vaccinated herds– Sampling in herds showing clinical signs in the face of vaccine antibodies, at unexpected ages, or within previously recovered populations.
- Slaughterhouse or market place surveillance – Testing animals with signs of respiratory disease consistent with ILI upon ante mortem examination or suggestive pulmonary lesions upon post mortem examination.
- Surveillance at markets, auctions, swine exhibitions – Collection of nasal swabs from animals showing acute ILI at points where animals from multiple sources are concentrated.
- Surveillance associated with public health investigations - Testing animals displaying ILI that are epidemiologically linked to known human cases of swine influenza

Suggested investigative and sampling activities for effective SIV surveillance:

Herds meeting the above targeted characteristics should be promptly visited and assessed by trained veterinarians. If respiratory disease is observed and sample collection is

warranted, samples from multiple animals should be collected in accordance with suggested diagnostic laboratory protocols. Virus shedding and detection is most consistent early in the course of infection and may occur before clinical signs appear, therefore sampling preference should include nasal swabs from acutely ill animals exhibiting respiratory disease and/or a fever as well as unaffected pen-mates or tracheal swabs and lung samples from post-mortem examination of sacrificed pigs or recently deceased acute mortalities. The number of samples collected will depend upon the likely prevalence of infection, results from herd investigations, and financial resources available for virus isolation and/or screening RT-PCR assays.

As with all effective surveillance activities, basic information must be collected to effectively interpret the epidemiology of swine influenza infections. This information includes:

- Location and farm demographics (age range, population, species present, etc.)
- Recent animal movement activities
- Date when signs first started and when samples were collected
- Baseline herd mortality and vaccination status as well as morbidity, mortality, and clinical signs associated with the outbreak
- Association with suspected ILI in other animals or humans

Samples that are collected should be handled to preserve the integrity of the specimen and transported to a diagnostic laboratory that operates to a recognized standard of quality for virus isolation and/or a screening RT-PCR for initial testing.

Diagnostic testing of surveillance samples, reporting of results, and response

Typing of Haemagglutinin (H) and Neuraminidase (N) Genes

A small subset of samples from screening RT-PCR and/or VI-positive specimens should be typed with standardized molecular and/or conventional H1 and H3 and N1 and N2 screening assays. *Non-subtypeable results from these assays are of special concern as they may indicate potential new and emerging swine influenza subtypes and should be at least partially sequenced by reference laboratories to establish their H and N genotypes.*

Antigenic evaluation by HI will provide important information to ensure that existing diagnostic reagents are sensitive to circulating field viruses. Antigenic information linked with epidemiological data will also be used to inform effective vaccine antigen choices against circulating field viruses.

Molecular genome sequencing

Genomic sequencing of multiple genes from a representative subset of isolated viruses is preferred and provides important information about the origins, evolution, and characteristics of isolated viruses, including surface and internal gene reassortment events. Genome sequencing may also assist in determining genetic evidence of antiviral resistance and virulence factors in different species. Molecular sequence data is also important to inform nucleic acid based diagnostic tests and to ensure that these tests are fit for purpose.

Reporting and response

Strains of interest should be defined by the veterinary authority and may range from subtle variations of circulating strains to the appearance of new and emerging gene segments in

the influenza virus genome. Surveillance information should be shared with primary stakeholders such as swine producers, veterinarians, local human health providers (if applicable), and vaccine manufacturers. All relevant findings from influenza surveillance in animals should be reported to animal health and public health authorities (if applicable) at the appropriate level.

Information about the epidemiological and viral characteristics of all typed influenza virus in pigs should be shared with the wider scientific community for analysis. This includes posting genetic sequence data from influenza viruses into publicly available databases and sharing of virus isolates and antisera. Endemic swine influenza is not included in the list of notifiable diseases to the World Organisation for Animal Health (OIE). However, under some circumstances an influenza virus may warrant immediate notification to the OIE as an emerging disease, such as a new and emerging subtype, a variant virus associated with significant increases in morbidity or mortality, or a virus with zoonotic potential (*OIE Terrestrial Animal Health Code*).

Any response to influenza infection in pigs should be proportionate to risk and scientific principles of influenza infection. In particular:

- Culling of infected pigs is not recommended.
- Clinically ill pigs should not be shipped or sent to slaughter until recovered.
- Temporary movement restrictions of pigs between enterprises may be implemented in accordance with good biosecurity practices.
- Healthy or recovered pigs originating from infected farms can be sent directly to slaughter.

Commercial vaccines for swine influenza differ from the vaccines for humans or horses: most vaccines contain potent oil-in-water adjuvants and there is no standardization of vaccine strains, antigenic dose etc. SIV vaccine strain composition differs in Europe and in the US, because of antigenic and genetic differences in the prevailing viruses. Antigenic relevance between vaccine and field strains must be demonstrated, but there are currently no formal recommendations or guidelines for vaccine strain selection. Several findings indicate that factors other than antigenic match, such as the antigenic content and type of adjuvant, may be important for inducing a robust immune response that correlates to the efficacy of SIV vaccines.

Risk communication

Veterinary and public health authorities are encouraged to develop a joint risk communication strategy so that a rapid and coordinated response can be implemented upon finding a strain of concern. This important joint strategy should maintain an appropriate level of awareness among key stakeholders and the general public without creating undue concern or adverse consequences to pork producers.

Role of epidemiological studies and research

Valuable information can be gathered through epidemiological studies and other research to inform the main objectives of surveillance for animal influenza. However, it is beyond the scope of this document to include all of the options under these categories. Countries are recommended to maximise the use of such studies to inform their surveillance programmes, for example through building inter-sectoral partnerships with academia and other actors conducting such research.

Introduction

Influenza A virus infection of poultry results in clinical disease ranging in its presentation from asymptomatic infection to disease characterized by severe morbidity, and mortality rates of up to 100%. The severity of disease is affected by virological characteristics; host factors including the age, species, type and health/nutritional status of the birds; and environmental factors. Avian influenza viruses are a significant threat to poultry health and welfare, production, food security, economics, and trade. Some avian influenza viruses also pose a threat to public health, either through direct zoonotic infection or through infection following reassortment with other influenza viruses.

Avian influenza is a transboundary animal disease which can spread rapidly from continent to continent. The way in which the virus may spread includes through movements of poultry and poultry products; contaminated objects including clothing, machinery and feed; and in some cases wild birds are implicated in its spread.

Avian influenza in poultry is classified according to its pathogenicity in poultry as highly pathogenic avian influenza (HPAI) or low pathogenicity avian influenza (LPAI) based on molecular and pathogenicity criteria. The pathogenicity of a virus in poultry does not necessarily correlate with the pathogenicity of that virus in other species (e.g. humans). Case definitions for HPAI and LPAI are found in the *OIE Terrestrial Animal Health Code*. HPAI viruses can cause severe disease and are often associated with high mortality in poultry. LPAI generally cause milder disease or no disease at all in poultry, but can be associated with severe disease, particularly in the presence of other pathogens or when the health status of infected birds is low. LPAI viruses may become HPAI viruses when genetic mutations result in changes to their HA cleavage site. To date all field infections with HPAI viruses have been of HA subtypes H5 and H7, so LPAI viruses of subtypes H5 and H7 have the potential to become HPAI viruses; this may readily occur under certain conditions e.g. circulation in gallinaceous poultry populations.

All HPAI viruses and LPAI viruses of subtypes H5 and H7 in poultry are OIE listed diseases and are notifiable to the World Organisation for Animal Health (OIE).

For the purposes of trade poultry are defined as 'all domesticated birds, including backyard poultry, used for the production of meat or eggs for consumption, for the production of other commercial products, for restocking supplies of game, or for breeding these categories of birds, as well as fighting cocks used for any purpose'

Although factors influencing successful transmission of influenza viruses from one species – in which the virus is well adapted – to a new species are not fully understood, influenza viruses adapted to pigs and humans have been transmitted to birds and vice versa. Opportunities exist for transmission of influenza viruses between pigs, humans, and poultry when they are in close contact. Under such circumstances opportunities also exist for reassortment of genes from these viruses, which may result in a mammalian-adapted influenza virus of public health concern. Although remotely probable, this highly consequential zoonotic threat has heightened public health interest in surveillance for influenza viruses in multiple species.

The variety of ways in which infection can be introduced to poultry flocks and the ease with which some LPAI viruses mutate to virulence after being introduced to a flock undetected, means that outbreaks of LPAI or HPAI may appear unexpectedly and their exact origin may never be determined.

Owing to severity of disease caused by HPAI infection, clinical surveillance is useful for detecting infections of HPAI viruses. Since severe disease and high mortality may also be caused by a number of other infectious and non-infectious causes, diagnostic testing is essential for confirming avian influenza infection. LPAI infection is more difficult to detect through clinical surveillance, and signs may only be obvious for certain influenza subtypes or if co-infection is present. LPAI surveillance is therefore often dependent on diagnostic screening tests, particularly serology, with molecular and virological follow up on positive cases. The sensitivity of clinical surveillance for HPAI may also vary depending on the species of the avian target population; generally the sensitivity of clinical surveillance for HPAI will be greater for gallinaceous poultry than for waterfowl populations.

Newcastle disease (avian paramyxovirus-1) is also an OIE listed disease. Avian influenza and Newcastle disease have a similar clinical picture in poultry and both are caused by haemagglutinating viruses. There are opportunities to combine surveillance for Newcastle disease in poultry with avian influenza surveillance. Depending on the local disease situation, any suspect cases of avian influenza should also be investigated for Newcastle disease and vice versa since clinical signs are not pathognomonic.

In recent years three strains of avian influenza virus have been associated with human mortality. The strain of H7N7 HPAI responsible for a poultry epidemic in the Netherlands in 2003 resulted in infections in over fifty humans exposed to the virus; most infections resulted in mild disease characterized by conjunctivitis, and one death was reported. Human infections with the strain of H5N1 HPAI responsible for the recent poultry panzootic (2003 onwards) have resulted in severe and acute respiratory disease with a high case fatality rate. H7N9 LPAI viruses have also been responsible for morbidity and mortality in humans in China in 2013. Currently there is no evidence that the pathogenicity of avian influenza viruses in poultry correlates with their pathogenicity in humans. In addition, pathogenicity may vary depending on the poultry host species.

Pandemic viruses have resulted from re-assortment of gene segments between influenza viruses that had been circulating in birds, and other animals. Owing to their antigenic novelty and the ability of the viruses to spread well within susceptible human populations, these pandemic viruses have resulted in widespread clinical illness ranging in severity and associated with a varying mortality in the human population.

Avian influenza viruses are considered to be a significant public health risk. Annually the OFFLU network provides WHO with virological and epidemiological data on H5N1 viruses and H9N2 avian influenza viruses - these subtypes are considered to represent a greater risk to public health - to support the selection of candidate viruses that may be used to make pre-pandemic human vaccines.

The exact determinants of severe public health risk from avian influenza viruses remain unclear and are likely to involve a complex interaction of host, virological, epidemiological and environmental factors. More research is needed to better understand the determinants of risk posed by animal influenza viruses to human health. The task of predicting such risks is likely to remain complex and challenging.

The design of any surveillance programme will depend on its objectives. For avian influenza, the objectives of surveillance may vary depending on the relevant stakeholders, poultry

population and local avian influenza situation. The poultry industry may be primarily interested in surveillance for early detection of NAI and to demonstrate freedom of disease from country, zone, or compartment, and public health authorities may be interested in the emergence of any novel influenza virus in birds or other animals which may present a public health threat.

Main objectives for avian influenza virus surveillance in poultry

Animal health

- Early detection of notifiable avian influenza followed by reporting to the international community through OIE and implementation of science-based trade restrictions to prevent further international spread through trade.
- Early detection of notifiable avian influenza to facilitate rapid and effective control aimed at eliminating the virus in the poultry sector.
- Detection of subclinical infections with notifiable avian influenza in poultry populations.
- Detection of LPAI infections in poultry since these may contribute to multifactorial disease, or indicate breaches of on-farm biosecurity measures
- To demonstrate disease freedom from a country, zone, or compartment to self-declare freedom from notifiable avian influenza viruses to facilitate trade in animals and animal products.
- Vaccination – to monitor the effectiveness of vaccination campaigns; select appropriate strains for use in vaccines; and to detect infected birds in vaccinated populations.
- Animal health objectives for surveillance of the influenza virus genome in poultry include:
 - Early detection of infection with an AI virus (leading to follow up investigations)
 - Monitoring for genetic and antigenic evolution of avian influenza;
 - Selection of isolates for the development of more relevant diagnostic reagent and vaccine seed stock products;
 - Documentation of endemic and emerging influenza virus ecology in poultry;
 - Detection of new and emerging influenza viruses in the poultry populations.
 - Describe spatial patterns of molecular subtypes to inform understanding of regional transmission patterns and development of targeted control and prevention policies.

Public health

- Provide information to inform pandemic preparedness, response, and communication planning.
- Early detection of strains with zoonotic risk followed by rapid and effective control in the poultry source e.g. for HPAI H5N1, LPAI H7N9; other LPAI viruses (e.g. H5, H7).
- Source investigation when AI viruses are causing zoonotic disease.
- Timely identification of new and emerging influenza viruses in poultry for animal and human population risk assessment.
- Assessment of viral mutations or reassortment events in poultry and other animals that might signal a public health concern.

- Monitoring of important molecular markers for resistance to antiviral drugs or for increased pathogenicity.
- Facilitate procurement of reagent and vaccine seed stock products for pandemic preparedness.

Surveillance approaches

Clinical surveillance is a valuable tool for detecting avian influenza infection in birds if infection results in clinical signs in infected birds. Any clinical suspicion of avian influenza should lead to a full disease investigation, including sampling and laboratory testing to confirm or rule out the presence of notifiable disease. The investigation should account for other notifiable diseases such as Newcastle disease which may present a similar clinical picture. Clinical signs vary depending on the pathogenicity of the avian influenza virus strain, health, age, type and immune status of the birds, and environmental factors. HPAI is likely to be characterised by dramatic and more obvious signs such as mass mortality and morbidity in poultry. Infection with LPAI viruses may lead to milder signs which may be detected through a decline in production. Clinical diagnosis alone is not enough to confirm or rule out the presence of avian influenza infection or to differentiate subtypes.

Owners and workers must be aware of the range of clinical and production signs that can be seen with different avian influenza viruses under different conditions. A full description of clinical signs which may be seen in avian influenza infection is found in the OIE Diagnostic Manual at http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.03.04_AI.pdf

Virological surveillance is valuable because it allows for confirmation of HA and NA subtype and further characterisation of the virus including through genetic sequencing and analyses. Virus isolation contributes to determination of whether the virus is a notifiable avian influenza virus and whether it is HPAI or LPAI.

Serological surveillance is valuable for indicating prior infection with LPAI in all poultry species and AI in waterfowl, and to target more intensive virological surveillance. Serology allows detection of prior infection in poultry and therefore can be a much more sensitive and informative approach for detecting the presence of avian influenza in a population. However, detection of antibodies alone does not allow detailed genomic analysis of the virus or provide accurate information on when infection occurred or on hosts pathogenicity. Furthermore, genetic drift and shift can render subtype specific serologic tests (haemagglutination inhibition (HI) or neutralization assays) insensitive to new or emerging strains of influenza. Serologic cross-reactivity with antigenically distinct influenza viruses can also occur, and may complicate result interpretation including when poultry may have been exposed to multiple avian influenza subtypes and variants. Antigenic testing methods (virus isolation, RT-PCR, and sequencing) are essential to characterize influenza virus infections in poultry populations. When undertaking serological surveillance full consideration must be given to the potential for exposure to other AI viruses through vaccination or natural infection.

Risk-based surveillance

Greater efficiency and cost effectiveness can be achieved by targeting surveillance based on risk factors.

Specific groups or types of bird kept under certain conditions have a higher risk of being infected with influenza viruses than others, and some species have a greater risk of being

infected without showing clinical signs. Such factors should be considered when developing risk-based surveillance strategies.

The type of surveillance, whether it is clinical, serological, or virological, depends on the exact objectives of the surveillance activity – for example whether it is linked to trade, early detection, research - and the species of the target population.

Risk-based sampling may take into account the following points:

- Suspicious clinical disease (most useful for HPAI) – disease investigations where avian influenza infection is suspected on clinical grounds. Confirmation of avian influenza infection requires laboratory diagnostic testing and characterisation. Other notifiable poultry diseases such as Newcastle disease should be included in the disease investigation.
- Veterinary diagnostic laboratory surveillance - Ancillary testing of poultry samples submitted to veterinary diagnostic laboratories from flocks exhibiting clinical signs of avian influenza and/or suggestive lesions, focusing on isolation of influenza virus from appropriate samples, followed with molecular and antigenic characterization.
- On-farm surveillance - Targeted sampling in poultry can also take into account the location, nature of the holding, and environmental factors which might increase the risk of transmission of avian influenza viruses from migratory water birds to poultry including:
 - Proximity to:
 - Water bodies – wetlands, lakes, sea shores, rivers;
 - Migratory water bird resting and breeding grounds, particularly if these areas are linked, through migratory routes, to areas where NAI is known to be prevalent;
 - Free range poultry premises where wild birds and poultry can be in close contact;
 - Holdings with lower biosecurity levels including the use of surface water as drinking water and method of feed storage i.e. if wild birds can access feed.

Other risk factors which may be used to inform risk-based on-farm surveillance include:

- The presence of more than one poultry species in the same holding particularly domestic ducks and geese together with other poultry species;
- Poultry species and farming systems where surveillance data have shown an increased rate of avian influenza infection;
- Poultry holdings located in areas where there is a high poultry population density;
- Holdings where there is a high level of movement on and off the premises (live birds, products, vehicles etc.);
- Holdings where there are older birds or multi-age groups of birds e.g. egg layers;
- Holdings with an epidemiological link to known infected animals e.g. flocks that have been epidemiologically linked to known infected poultry (or swine farms), live bird markets, or had contact with other species showing ILI.

- Slaughterhouse or market place surveillance – Testing animals with signs of disease consistent with avian influenza upon ante mortem examination or suggestive lesions upon post mortem examination.
- Surveillance at markets, auctions, and exhibitions – Collection of tracheal and cloacal swabs from animals showing signs of avian influenza or randomly sampled birds at points where animals from multiple sources are concentrated. Environmental sampling may also be implemented at such events.
- Surveillance associated with public health investigations - Testing animals displaying suspicious signs that are epidemiologically linked to known human cases of influenza

Some countries undertake statistically robust and representative risk-based serological surveys to demonstrate freedom from LPAI subtypes H5 and H7. This may be used as an opportunity to undertake serological surveillance for other avian influenza subtypes. Positive serology results can be followed up with virological investigation to identify and characterise the virus.

Suggested investigative and sampling activities for effective avian influenza surveillance

In all cases where notifiable avian influenza is suspected, the National Veterinary Authorities must be notified so that they can carry out a thorough investigation to confirm or rule out the presence of notifiable disease. Other notifiable avian diseases such as Newcastle disease should also be included in the disease investigation. Effective investigation of clinical disease relies on the National Veterinary Authorities having a functional reporting mechanism and of stakeholder awareness of clinical signs and the notifiable status of the disease.

Countries should have effective legislation to provide the legal powers necessary to undertake surveillance in the case of routine surveys or disease investigations.

If presence of avian influenza virus is suspected and sample collection is warranted, samples from multiple animals should be collected in accordance with suggested diagnostic laboratory protocols. Virus shedding and detection is most consistent early in the course of infection and may occur before clinical signs appear, therefore sampling preference should include tracheal and cloacal swabs from acutely ill animals and healthy animals and tissue samples (trachea, lungs, air sacs, intestine, spleen, kidney, brain, liver and heart) from post-mortem examination of sacrificed poultry or recently deceased acute mortalities. In the case of surveillance for LPAI viruses where infection does not cause marked clinical disease cloacal and tracheal samples should be taken. The number of samples to be collected for detection of infection will depend upon the likely prevalence of infection, results from flock investigations, and financial resources available for virus isolation and/or screening RT-PCR assays. The statistical basis for determining the sample size should be reported. Diagnostic protocols should comply with the Standards laid down in the *OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*. Various protocols for the pooling of swabs have been investigated for the purpose of reducing resource expenditure.

As with all effective surveillance activities, relevant population demographic and epidemiological information should be collected to provide context and allow meaningful interpretation of the laboratory test results. This information includes:

- Location and farm demographics (age range, population, species present, etc.)
- Recent animal movement activities

- Date when signs first started (if infection causes disease in birds) and when samples were collected
- Baseline flock mortality and vaccination status as well as morbidity, mortality, and clinical signs associated with the outbreak
- Association with suspected influenza in other animals or humans
- Potential for exposure to wild bird populations (specifically waterfowl)

Samples that are collected should be handled such that the integrity of the specimen is preserved and be transported to a diagnostic laboratory that operates to a recognized standard of quality for virus isolation and/or a screening RT-PCR for initial testing.

Diagnostic testing of surveillance samples, reporting of results, and response

Typing of Haemagglutinin (H) and Neuraminidase (N) Genes

A small subset of samples from screening RT-PCR and/or VI-positive specimens should be typed with standardized molecular and/or conventional H5 and H7 screening assays. *Non-subtypeable results from these assays may be of interest as they may indicate potential new and emerging influenza subtypes and should be at least partially characterised (antigenic or genetic methods) by reference laboratories to establish their H and N genotypes.*

Antigenic evaluation by HI will provide important information to ensure that existing diagnostic reagents are sensitive to circulating field viruses. Antigenic information linked with epidemiological data will also be used to inform effective vaccine antigen choices against circulating field viruses.

Molecular genome sequencing

Genomic sequencing of multiple genes from a representative subset of isolated viruses is preferred and provides important information about pathogenicity, the origins, evolution, and characteristics of isolated viruses, including surface and internal gene reassortment events. Genome sequencing may also assist in determining genetic evidence of antiviral resistance and virulence factors in different species. Molecular sequence data is also important to inform nucleic acid based diagnostic tests and to ensure that these tests are fit for purpose. Comparisons of gene sequence data and characterisation of molecular sub-groups (eg. clades) should be used to inform epidemiological investigations, specifically in relation to source of infection.

Reporting and response

Notifiable avian influenza (HPAI virus infection in all birds and infection of LPAI viruses of subtypes H5 and H7 in poultry) must be reported to the National Veterinary Services and by the National Veterinary Services to the OIE. Other subtypes of avian influenza virus may warrant notification to OIE as an emerging disease, such as a new and emerging subtype, a variant virus associated with significant increases in morbidity or mortality, or a virus with zoonotic potential (*OIE Terrestrial Animal Health Code*).

Surveillance information should be shared with primary stakeholders such as poultry producers, veterinarians, local human health providers (if applicable), and vaccine manufacturers. All relevant findings from influenza surveillance in animals should be reported to animal health and public health authorities (if applicable) at the appropriate level.

Information about the epidemiological and viral characteristics of all typed influenza virus in poultry should be shared with the wider scientific community for analysis. This includes posting genetic sequence data from influenza viruses into publicly available databases and sharing of virus isolates and antisera.

Any response to influenza infection in poultry should account for the national notifiable disease control policy and scientific principles of influenza infection and should also account for the International Standards laid out in the *OIE Terrestrial Animal Health Code*. The animal health response to detection of avian influenza will vary depending on whether the virus is demonstrated to be HPAI or LPNAI and on the type of bird (wild vs. domestic poultry) that the virus is detected in. The detection of LPAI (non H5 or H7) should not lead to trade restrictions and the detection of any avian influenza in wild birds should also not have implications for international trade.

Risk communication

Veterinary and public health authorities are encouraged to develop a joint risk communication strategy so that a rapid and coordinated response can be implemented upon finding a strain of concern. This important joint strategy should maintain an appropriate level of awareness among key stakeholders and the general public without creating undue concern or adverse consequences to birds and poultry producers.

Role of epidemiological studies and research

Valuable information can be gathered through epidemiological studies and other research to inform the objectives and the design of surveillance strategies for animal influenza. However, it is beyond the scope of this document to include all of the options under these categories. Countries are recommended to maximise the use of such studies to inform their surveillance programmes, for example through building inter-sectoral partnerships with academia and other actors conducting such research.

MODULE 3 | SURVEILLANCE¹ FOR AVIAN INFLUENZA VIRUS IN WILD BIRDS

Wild birds play important roles in the circulation of avian influenza viruses and are reservoirs particularly of subtypes of low pathogenicity. Avian influenza viruses in wild birds can be transmitted to and from poultry, and potentially to and from other domestic animals and people. In order to reduce health risks to wildlife, domestic animals and people, it is important to understand all aspects of the circulation of avian influenza viruses among susceptible populations: wild animals, domestic animals and humans. Thus, surveillance for avian influenza viruses in wild birds can supply important information.

Main objectives for surveillance of avian influenza viruses in wild birds

- To gain a more comprehensive understanding of the epidemiology and ecology of avian influenza viruses
- To detect virus strains highly pathogenic to wild and domestic animals, and to people.
- To detect infection of wild birds with virus subtypes derived from poultry.

Surveillance approaches

General Surveillance (passive surveillance):

Avian influenza virus can be detected, through appropriate laboratory tests, in samples of wild birds received in diagnostic laboratories as part of programs of general disease surveillance in which all causes of morbidity and mortality are under investigation. Most often, general surveillance is carried out on wild birds found dead. General surveillance based on wild birds found dead has proved to be the most effective form of surveillance to detect highly pathogenic virus strains in wild birds.

Targeted Surveillance (active or risk-based surveillance):

Targeted surveillance focuses on sampling according to specified criteria such as species, sex, and age of bird, geographic location, and time of year. Targeted surveillance is a more efficient way to meet the objectives of surveillance and can result in collection of influenza viruses for characterisation in terms of genetic and pathogenic properties. In addition it may contribute to the assessment of the infection status of specific wild bird populations. It may be most efficient to focus surveillance on bird species which use aquatic habitats since influenza viruses have been found most often in aquatic birds species, particularly ducks, geese, and swans. Birds included in targeted surveys are most commonly apparently healthy live wild birds, but survey design may include sick birds, dead birds, and freshly-expelled bird faeces.

¹ In this document, the word “surveillance” is used to include the activities sometimes separated under strict definitions of surveillance, monitoring and disease investigation.”

Sampling

As with domestic species, the main samples to be taken from each bird, whether alive or recently dead, are a sample of oro-pharyngeal fluid and a sample of cloacal content for agent detection and identification. Additional issues associated with sample procurement from wild birds include possession of the necessary legal permits, training and competence to catch, handle, mark and release wild birds in keeping with international standards of animal welfare, and competence to identify correctly the species, and also often the sex and age, of each bird sampled.

Laboratory testing

Appropriate laboratory tests should be used to test samples for the presence of virus. To test for virus, PCR procedures using the currently accepted primers for the matrix protein gene in wild species, or virus isolation carried out by techniques compatible with the *OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*, are the methods of choice. In general, serological studies are only recommended for use in specialist laboratories. In general, serology cannot be used to determine virus strains.

Categories of data needs

- Basic epidemiological information includes:
 - Location (Latitude and Longitude, or UTM coordinates)
 - Date of sample collection
 - Species (*Latin* name), and sex and age where possible
 - Morbidity, mortality and clinical signs, where relevant
 - Co-occurrence of disease in other species, including domestic animals and humans
- Virus Characterisation:
 - Whether an influenza A virus isolate was recovered or whether information is on the basis of sequence analysis
 - HA subtype
 - N subtype
- Molecular genome sequencing

Full genome sequencing provides important information about the origins, evolution, and characteristics of the virus, including genetic reassortment. Full genome sequencing is preferred, and is important in assessing the genetic basis of antiviral resistance and pathogenicity in different species. If full genome sequencing is not possible, partial genome sequencing can provide useful information.

- all sequence data must be clearly linked to the date, location and species from which the sample was taken, particularly when deposited in public-access data banks.
- sequencing should involve determining the inferred pathotype.
- full HA sequencing can determine clade for H5N1 viruses
- molecular sequencing studies are frequently initiated on the basis of HA and N gene analysis.

- Antigen data

Sequencing can provide important information to ensure that diagnostic reagents are compatible with circulating field viruses and therefore is fit for purpose.

Reporting and response

- The OIE should be notified of any infection of wild and domestic birds with highly pathogenic avian influenza. This is a reporting requirement of OIE Members as laid out in the *OIE Terrestrial Animal Health Code*.
- All additional relevant findings from surveillance for avian influenza viruses in wild birds should be reported to wildlife, domestic animal and public health authorities at the appropriate level. It is recommended that countries share information with other interested parties.
- Results of surveillance for low pathogenic avian influenza viruses in wild birds should be included in the annual report on occurrence of non-listed infections in wildlife through the WAHIS-wild reporting system of the OIE.
- The occurrence of avian influenza viruses in wild birds, including H5 and H7 subtypes, does not justify the imposition of trade restrictions.
- In the event of wild bird mortality caused by avian influenza, local poultry farms should be advised to verify or implement appropriate biosecurity measures.

Risk communication

It is important that wildlife, veterinary and public health authorities develop a coordinated risk communication strategy following positive surveillance findings. The risk communication strategy should strive to maintain an appropriate level of awareness among key stakeholders and the general public while not creating undue concern.

Since avian influenza viruses occur regularly in wild birds, it is expected that wild bird surveillance efforts will detect these viruses irrespective of any role wild birds may play in local epidemiological events involving poultry. It is not justified to attribute the source of avian influenza virus infection in poultry to wild birds unless complete investigations have been carried out and the results fully support such attribution. Response actions such as killing wild birds or destroying their habitat are not appropriate.

Outbreak investigation

Under some circumstances, it may be appropriate to remove and properly dispose of the carcasses of wild birds which have died from avian influenza, to prevent or reduce the spread of infection.

In the event of an outbreak of avian influenza in poultry, there may be some value in undertaking surveillance for the causal virus in live and dead wild birds in the vicinity of the affected farm to determine whether or not the causal virus is present in local wild birds. Interpretation of results will not permit determination regarding the direction of transmission of the virus between poultry and wild birds, but may inform biosecurity measures on other premises.

Role of epidemiological studies and research

Valuable information can be gathered through ecological and epidemiologic studies and other research to improve our understanding of the movement, maintenance, transmission and persistence of influenza viruses across the wildlife-domestic animal-human interface. Countries should maximise the use of such studies and research, and share data and results with the wider scientific community to improve local, regional and global understanding.

Introduction

Influenza A virus infection of equids has been reported world-wide with the exception of a small number of island countries including New Zealand and Iceland. Equine influenza (EI) is endemic in Europe and America. Other parts of the world such as Japan, South Africa, India and Hong Kong suffer occasional incursions but the disease is not endemic. Typical outbreaks of EI are characterised by pyrexia, coughing and nasal discharge. Although the mortality rate associated with equine influenza virus (EIV) infection is very low it is considered the most important respiratory virus of horses. This is because it is highly contagious and has the potential to cause significant economic loss due to the disruption of major equestrian events. The equine population is highly mobile and horses travel long distances by road and air for competition and breeding purposes. When an infected horse is introduced into a susceptible population virus spread can be explosive. The incubation period can be less than 24 hours in naïve horses and the continuous coughing which is a major feature of the disease, serves to release large quantities of virus into the environment. The virus is spread by the respiratory route, by personnel, vehicles contaminated with virus, and by fomites. Large outbreaks are often associated with high density stabling, the congregation of horses at equestrian events and their dispersal over a wide geographic area after the event.

The clinical signs are less severe and disease spread is slower in partially immune populations. The majority of outbreaks in endemic populations are contained with limited spread between premises. The severity of the disease depends primarily on the immune status of the horses at the time of exposure, the environment and the stress created by continuing to work or train. EI can be controlled by vaccination and antibodies against the virus haemagglutinin (HA) induced by inactivated and sub unit vaccines correlate with protection. In endemic countries the economic losses due to EI can be minimised by vaccination of highly mobile horses and many racing authorities and equestrian bodies have mandatory vaccination policies that serve as an insurance for business continuity. In well vaccinated race horses or competition horses the predominant sign may be sub-optimal performance and many horses may be subclinically infected. Non-endemic countries rely heavily on vaccination of imported horses to help prevent an incursion. However subclinically infected vaccinated horses can shed virus. Many countries have experienced EI epizootics related to the importation of such horses.

To-date only two stable subtypes of EI have been reported in horses, H7N7 and H3N8. The first reported outbreak of equine respiratory disease to be confirmed as equine influenza occurred in 1956 in Eastern Europe. The virus isolated was characterised as H7N7. Subsequently H7N7 viruses were identified as the cause of outbreaks in Europe, Asia and the United States. Phylogenetic analysis of these viruses, indicate that they are the most ancient of all mammalian influenza virus lineages. Although H7N7 viruses co-circulated with H3N8 viruses in horses for many years, it is generally accepted that these viruses have not been active for a long period and may be extinct. Phylogenetic analysis of nucleoprotein genes suggest that the H3N8 equine 2 virus genome originated in the late 19th Century but the first isolation of a virus of this subtype took place in Florida in 1963. Since then H3N8 influenza viruses have been responsible for epizootics in all continents. Antigenic drift occurs less frequently in equine influenza viruses than in human viruses but the H3N8 subtype has evolved into two distinct lineages designated the “American-like” lineage and the “European-like” lineage based on the initial geographical distribution of viruses. Three

American sub-lineages subsequently emerged the Argentina, Kentucky and Florida. The Florida sub-lineage has more recently diverged into two Clades; Clade 1 includes the viruses A/equine/South Africa/4/2003, A/equine/Sydney/2007 and A/equine/Ibaraki/2007 responsible for the epizootics in South Africa, Australia and Japan respectively, and Clade 2 includes A/equine/ Newmarket /03 and other viruses that have been circulating in Europe since 2003. Antigenic drift of the H3N8 viruses impacts on vaccine efficacy. This has been demonstrated repeatedly in the field. Since the introduction of mandatory vaccination in the UK in 1981 there have only been two large outbreaks of EI in 1989 and in 2003 and in both instances the vaccine strains had been isolated ten years earlier.

Influenza virus reassortants originating from avian, human and/or swine viruses have not been identified in horses and to-date the epidemiology of EI appears to be somewhat less complex than that of swine or avian viruses. The current EI viruses are believed to be of avian ancestry and more recent transmission of avian viruses to horses and donkeys has been recorded. The sequence analysis of an H3N8 virus isolated in 1989 from horses during an influenza epidemic in North Eastern China established that the virus was more closely related to avian influenza viruses than to equine influenza viruses. It was reported that over 13,000 horses were affected and that the mortality rate was over 20% but the virus did not persist and failed to spread beyond China. More recently avian H5N1 has been associated with respiratory disease in donkeys in Egypt.

EI viruses have the potential to cross species barriers and have been associated with outbreaks of respiratory disease in dogs (primarily but not exclusively, greyhounds and dogs in shelters) in North America, quarry hounds in England and dogs on premises with horses affected by EI in Australia. Interspecies transmission of EIV to dogs maintained in the same stable as experimentally infected horses was demonstrated but there is to-date no evidence of EI transmission from dogs to horses. During 2004-2006 swine influenza surveillance in central China 2 equine H3N8 influenza viruses were isolated from pigs. Despite the successful experimental infection of human volunteers with EIV and the occasional identification of seropositive persons with occupational exposure there is currently little evidence of zoonotic infection of people with EI.

Main objectives for influenza virus surveillance in horses

- To monitor genetic and antigenic evolution of EI.
- To monitor vaccine efficacy.
- To identify the need to update vaccines and select suitable viruses for inclusion in the vaccines.
- To select viruses for use as diagnostic reagents.
- To detect new and emerging influenza viruses in the equine population.
- To serve as an early warning for veterinarians and horse owners, facilitating the implementation of appropriate prophylactic and control measures.
- To reduce the economic impact of EI by maintaining awareness of emergence and international spread of antigenic variants.
- To identify changes in epidemiology and virulence that could impact on equine health and have implications for interspecies transmission.
- To assess viral mutations, reassortment events and changes in ecology that might signal a public health concern.

Current surveillance approach

A formal global Equine Influenza Surveillance Programme was initiated by the OIE Biological Standards Commission and has now been in place since 1995. The programme continues to develop in terms of improving its global coverage. The OIE Reference Laboratories and other laboratories collect data on outbreaks of EI and strain characterisation which is reviewed annually by an Expert Surveillance Panel (ESP) including representatives from OIE and WHO. This panel makes recommendations on the need to update vaccines which are published in the *OIE Bulletin*. The criteria for updating EI vaccines are similar to those for human influenza vaccines and based on analysis of evidence of disease in well vaccinated horses, antigenic changes, genetic changes and when possible, experimental challenge data. The genetic analysis is currently based on the sequencing of the HA gene. The antigenic analysis is based on detection of changes in the HA based on haemagglutination inhibition (HI) tests carried out with ferret and horse antisera. Horse antisera are cross reactive but ferrets mount a more strain-specific response. More recently antigenic cartography has been used to visualise and assist with the analysis of these data.

General surveillance

The extent to which EI is detected through general or passive surveillance in a country depends on the nature of the horse industry, the status of the disease, the laboratory capability and the financial resources available for veterinary intervention and laboratory testing. Many laboratories involved in EI surveillance experience difficulty obtaining sufficient sample numbers as horse owners frequently don't perceive a benefit in obtaining a confirmatory diagnosis for a self-limiting respiratory disease. Thus, they are often unwilling to pay for veterinary assistance and the submission of samples to a diagnostic laboratory.

Furthermore while the introduction of the highly sensitive RT-PCR for EI in many diagnostic laboratories has revolutionised the diagnosis of this disease, there is frequently a failure to submit positive sample material to an OIE reference laboratory for virus characterisation. As a result virus isolation and characterisation may often not be performed although some laboratories retain the sample material for their own investigation. It is essential that diagnostic laboratories submit material to the OIE reference laboratories, or at least liaise with them to obtain any reagents or assistance they may require to characterize their viruses in a timely manner.

Targeted surveillance

EI is most efficiently detected and monitored through a targeted surveillance programme. Several countries have such programmes funded by their Government, the horse industry, vaccine companies or other agencies. However, the current disparity in the level of surveillance and virus collection in different countries results in potentially biased information about the relative prevalence of different viruses. There is a need for increased surveillance on a global level.

Targeted surveillance programmes may include but are not restricted to:

- Sentinel veterinary practices that are supplied with nasal swabs, blood collection tubes, virus transport medium etc. and requested to collect and submit samples from suspect cases. This includes both naïve horses exhibiting acute signs of influenza where it is relatively easy to make a presumptive diagnosis, and vaccinated horses with mild signs or loss of performance. Sentinel practices need to have a wide geographic distribution and a broad based clientele representing all sectors of the

horse industry. EI outbreaks frequently start in one sector and spill over into others with different consequences. The emergence of influenza at a local show with no vaccination requirements for entrants may quickly lead to clinical disease and the temporary closure of establishments with unvaccinated horses. In contrast the circulation of EI in highly mobile vaccinated horses may cause only mild disease but could be a major risk to importing countries with a susceptible population.

- Supplementary testing of all equine samples submitted to a diagnostic laboratory from horses exhibiting clinical signs of respiratory disease or loss of performance.
- The collection and testing of nasal swabs from horses exhibiting clinical signs of respiratory disease at equestrian events such as race meetings, sales and competitions.
- The collection and testing of samples from recently imported horses or new arrivals in a yard. The latter are frequently the index case in an outbreak even though they may not be the source of the virus.
- The collection and testing of samples from animals of other species that are epidemiologically linked to horses suffering from EI.

Categories of data needs

Information must be collected to monitor the extent of the outbreak, the virulence of the virus and the efficacy of the vaccines. Data collected should include but is not restricted to:

- Location and date
- Premises type and demographics
- Morbidity and clinical signs
- Vaccination history
- Recent movement and activities

Molecular and antigenic characterisation of virus isolates is fundamental to influenza control programmes based on vaccination. Vaccine strains must be representative of those in circulation.

Molecular sequence data is also important to inform nucleic acid based diagnostic tests and to ensure that these tests are fit for purpose.

Reporting and response

EI is an OIE listed disease and OIE Members have an obligation to report outbreaks of EI to the OIE. This is a reporting requirement as laid out in the *OIE Terrestrial Animal Health Code*. At a national level any occurrence of EI must be reported to the Veterinary Services, and to enhance transparency also to the relevant stakeholders.

Nasal swabs or viruses should be submitted to an OIE reference laboratory for strain characterisation. Laboratories that characterise their own isolates should do so in a timely manner and report their findings to the ESP. This is essential to ensure that the ESP recommendations relating to updating vaccines are epidemiologically relevant.

Sequence data should be deposited in publicly available databases.

Risk Communication

In the event of an increase in EI in an endemic country veterinarians and horse owners should be advised so that vaccines can be administered as appropriate and biosecurity measures can be implemented.

Countries should share information. Results of surveillance should be included in reports to databases for key stakeholders and other interested parties.

Outbreak Investigation

When EI is confirmed an investigation should be carried out promptly to gather all relevant epidemiological and virological information.

Role of epidemiological studies and research

Epidemiological and experimental studies have confirmed the need for surveillance and demonstrated that vaccine strains must be representative of those in circulation. In the field higher levels of antibody were required to protect horses against heterologous strains. The importance of antigenic and genetic drift was supported by experimental challenge studies in ponies confirming that vaccine mismatch reduced protection against infection and virus shedding. Protection against virus shedding has been shown to correlate with the degree of antigenic relatedness of the vaccine strain to the challenge virus. Mathematical modelling studies have also suggested that epidemics are more likely to occur when the vaccines have not been updated.

Sensitive and specific diagnostic techniques are the cornerstone of a surveillance programme. The development, validation and introduction of RT-PCR and ELISA testing as routine diagnostic techniques for EI have made a huge impact on the ability to detect outbreaks promptly. The single radial haemolysis test has proved extremely useful in outbreak investigations in horses vaccinated with whole virus inactivated and subunit vaccines. However other assays for measuring correlates of protection need to be developed for second generation vaccines such as the cold adapted live vaccine that induces protection in the absence of antibodies against HA.

Further research needs to be carried out to optimise vaccination strategies and to identify factors that predispose viruses to increased virulence, transmissibility, persistence and the capacity to cross the species barrier.

MODULE 5 | NEW AND EMERGING INFLUENZA VIRUSES IN ANIMALS – PANDEMIC H1N1 2009

In this document, the influenza A H1N1 virus that caused the human pandemic 2009-2010 will be referred to as pandemic H1N1 2009 (pH1N1). Since its first detection in humans in April 2009 occurrences of pandemic H1N1 2009 have been detected in several species of animals, particularly pigs. In almost all cases this was associated with exposure to infective humans. WHO declared an end to the pandemic of influenza A H1N1 in August 2010.

Published reports and sequence database submissions suggest that, in August 2010, pH1N1 continues to circulate in pig populations and may be endemic in some pig herds. Further surveillance will be needed to monitor this.

Pandemic H1N1 infections have not had an important adverse impact on animal health. It is considered to be a human disease in which animals do not play a significant epidemiological role.

The response to the detection of pH1N1 in animals should be proportionate to the risk posed to humans and animals; and it is therefore recommended that control measures in animals such as culling are not implemented in response to positive findings. It is also strongly recommended that restrictive trade measures are not taken against countries experiencing outbreaks of pH1N1 in animals.

Viruses with reassorted genomes from pH1N1 and endemic swine viruses have also been detected and other variants are likely to occur. Surveillance for pH1N1 should be a component of an overall strategy for surveillance of influenza viruses in animals. Surveillance for pH1N1 in susceptible animal species, in particular pigs and turkeys, has been recommended so that any changes in epidemiology or viral characteristics that might alter the risks to animal or human health are detected early. It will also be useful to establish whether pH1N1 continues to circulate in susceptible animal populations and whether the animal viruses are diverging from the virus circulating in humans.

Main objectives for surveillance for new and emerging influenza viruses in animals

Public health

Timely identification of mutations, or reassortants in animals that might be of public health concern. Monitoring of important molecular markers such as antigenic changes, resistance to antiviral drugs, or for increased pathogenicity. This knowledge is used to inform preparedness, response, and communication plans.

Animal health

Detect influenza infections in animal populations and identify changes in the epidemiology and virulence for pigs and other animals which might have a negative impact on animal health and welfare, productivity, and economics.

There is a need to balance the short term and long term objectives of surveillance for influenza viruses in animals. Surveillance systems for pH1N1 and other new and emerging influenza viruses should be integrated with broader influenza surveillance in animal species.

A - Surveillance for pandemic H1N1 2009 (pH1N1) and other new and emerging influenza viruses in pigs

On 11th June 2009 the WHO declared that an influenza A H1N1 pandemic was underway. The pandemic was declared over on 10th August 2010 in recognition that it was taking on the characteristics of seasonal influenza outbreaks. During 2009 and 2010 sporadic occurrences of pH1N1 infections in pigs have been reported to the OIE. Susceptibility of pigs to the pandemic virus and transmissibility within pig populations was further demonstrated with experimental studies. Initial animal infections most likely resulted from contact with infective humans. In August 2010 there is now evidence that pH1N1 has continued to circulate in pig populations following initial introduction by humans and that it may now be endemic in some herds.

Pigs remain at risk of infection with pH1N1 virus, and other new and emerging influenza viruses or reassortant viruses that are circulating in humans and other animals.

Since the viral properties (gene mutations, gene segment, or combination of genes) of pH1N1 2009 necessary for adaptation to the human host remain unknown, it is important to continue to monitor for reassortant virus in pig and other animal populations that acquire segments from pH1N1. The North American triple reassortant internal gene (TRIG) constellation has demonstrated the ability to acquire a number of surface glycoproteins (HA and NA) since its emergence in 1998 and the potential for the pH1N1 to continue this pattern also remains unknown.

Main objectives for surveillance of pH1N1 2009 and other new and emerging influenza viruses in pigs

Public health

Timely identification of mutations, or reassortants that might be of public health concern. It is important to monitor for molecular markers such as for resistance to antiviral drugs or for increased pathogenicity. This knowledge is used to inform preparedness, response, and communication plans.

Animal health

Detect infections with pH1N1 and other new and emerging influenza viruses in pig populations and identify changes in the epidemiology and virulence for pigs and other animals infected with these viruses which might have a negative impact on animal health and welfare, productivity, and economics.

Surveillance approaches

Detection of pH1N1 and other new and emerging influenza viruses can be achieved using the following components of general and targeted surveillance. The degree to which each component is implemented is dependent upon the disease and the country situation. However, the combination of some or all of these methods will improve the sensitivity of surveillance.

Note: Infections of pigs with the pandemic H1N1 virus or other new and emerging influenzas may lead to sub clinical disease or may cause clinical signs that are indistinguishable from other influenza infections known to commonly circulate in pigs.

General surveillance

Disease detection – When clinical disease suspicious of influenza like illness (ILI) is detected by animal owners, producers, veterinarians or other animal health workers consideration should be given to diagnostic testing for swine influenza viruses, pH1N1 or a new and emerging influenza virus as part of the investigation. Veterinary authorities should be informed in cases where suspicion of pH1N1 or other new and emerging influenza virus is high, including when there is an epidemiological link with ILI in humans or other animal species.

Targeted surveillance

Targeted or risk-based surveillance is the preferred approach over statistically based surveys for early detection of pH1N1 and other new and emerging influenzas. By targeting surveillance to high risk groups in the population greater efficiency and cost effectiveness will be achieved.

Sample targets can include but are not restricted to:

- Laboratory detection – Supplementary testing of samples submitted to laboratories for respiratory syndromes. Laboratory surveillance should focus on virological and molecular detection. All laboratory confirmed infections of pH1N1 or a new and emerging influenza virus should be communicated to animal health authorities for further investigation.
- Slaughterhouse or market place surveillance – Testing of animals with signs of respiratory disease consistent with ILI (including at post mortem in slaughterhouses).
- Animals showing ILI at points of concentrated gathering such as markets, auctions or fairs
- Farms epidemiologically linked to known infected farms
- ILI in animals linked to known human cases
- Pigs in close contact with humans or other animal species showing ILI

Categories of data needs

- Basic epidemiological information:
 - Location and date
 - Farm type and demographics
 - Date when signs first started and when samples were taken
 - Morbidity, mortality, clinical signs
 - Link to suspected human or animal cases
- Molecular genome sequencing

Full genome sequencing provides important information about the origins, evolution, and characteristics of the virus including genetic reassortment. Full genome sequencing is preferred, and is important in assessing the genetic basis of antiviral resistance and pathogenicity in different species. Molecular sequence data is also important to inform nucleic acid based diagnostic tests and to ensure that these tests are fit for purpose. If full genome sequencing is not possible targeted partial genome sequencing can provide useful information.

- Antigenic data

Antigenic data will provide important information to ensure that diagnostic reagents are compatible with circulating field viruses and that diagnostic tests are therefore fit for purpose. It is also important to ensure that vaccine efficacy is optimal in terms of matching vaccine antigen to field viruses.

Reporting and response

All relevant findings from influenza surveillance in animals including positive results from laboratory testing should be reported to animal health and public health authorities at the appropriate level. It is recommended that animal health authorities share information with other relevant stakeholders including local public health authorities. The occurrence of an emerging disease (a new pathogen or a known pathogen that was not OIE listed because it did not meet the criteria and that is now behaving differently) determined by a significant increase in the morbidity, or mortality in animals or having a zoonotic impact should be notified immediately by national veterinary authorities to the OIE.

Information about the epidemiological and viral characteristics of pH1N1 and other new and emerging influenza viruses in pigs should be shared with the wider scientific community. This includes depositing genetic sequence data into publicly available databases.

Under the current epidemiological situation, the response to infections of pH1N1 and other new and emerging influenza viruses in pigs should be proportionate. In particular:

- Culling of infected pigs is not recommended.
- Clinically ill pigs should not be shipped or sent to slaughter.
- Temporary movement restrictions of pigs between enterprises may be implemented.
- Veterinary authorities may consider licensing the movement of live pigs from infected premises.
- Healthy pigs from infected farms can be sent directly to slaughter.

Risk communication

It is important that veterinary and public health authorities develop a coordinated risk communication strategy following positive surveillance findings. The risk communication strategy should strive to maintain an appropriate level of awareness amongst key stakeholders and the general public whilst not creating undue concern.

Outbreak investigation

Further to a positive surveillance finding, an outbreak investigation should aim to gather all relevant and useful epidemiological and virological information, and should be conducted without undue delay.

Role of epidemiological studies and research

Valuable information can be gathered through epidemiological studies and other research to inform the main objectives of surveillance for animal influenza. However, it is beyond the scope of this document to include all of the options under these categories. Countries are recommended to maximise the use of such studies to inform their surveillance programmes, for example through building inter-sectoral partnerships with academia and other actors conducting such research.

B - Surveillance for pandemic H1N1 2009 (pH1N1) and other new and emerging influenza viruses in poultry

During the influenza A H1N1 pandemic of 2009-2010 occasional cases of natural pandemic H1N1 2009 (pH1N1) infection were reported in turkeys. Initial animal infections most likely resulted from contact with infective humans. To date, attempts to experimentally infect poultry including turkeys, through the respiratory route, with the pH1N1 virus isolated from people have not been successful, except in the case of quail that were infected but no onwards transmission was demonstrated. Unlike pigs, there is no surveillance evidence to suggest that pH1N1 is continuing to circulate or is endemic in any turkey flocks.

Highly pathogenic avian influenza (HPAI) viruses and low pathogenic avian influenza (LPAI) viruses of subtypes H5 and H7 in poultry are OIE listed diseases because they are a serious threat to poultry health; pH1N1 in poultry is not an OIE listed disease and is not, at this time, a significant threat to poultry health.

Main objectives for surveillance of pH1N1 and other new and emerging influenza viruses in poultry

Public health

Timely identification of mutations, or reassortants that might be of public health concern. It is important to monitor for molecular markers such as for resistance to antiviral drugs or for increased pathogenicity. This knowledge is used to inform preparedness, response, and communications plans.

Animal health

Detect infections with pH1N1 or other novel influenza viruses in poultry populations and identify changes in the epidemiology and virulence which might impact on animal health and welfare, productivity, and economics.

Surveillance approaches

An increase in the proportion of influenza like illness (ILI) in animals should be investigated. Clinical suspicion of avian influenza should be investigated by competent Veterinary Services since Notifiable Avian Influenza is an OIE listed disease.

Note: ILI in birds may vary considerably in clinical presentation; there is limited information available about signs of disease associated with pH1N1 in poultry. In turkey breeders, a drop in egg production has been reported to be a sign consistent with pH1N1 infection.

Detection of pH1N1 and other novel influenza viruses can be achieved using the following components. The degree to which each component is implemented in a country is dependent upon the country situation. However, the combination of some or all of these methods will improve the sensitivity of the surveillance system.

General surveillance

- Disease detection – When clinical disease suspicious of influenza like illness (ILI) is detected by animal owners, producers, veterinarians or other animal health workers; consideration should be given first to Notifiable Avian Influenza and then to other

subtypes including pH1N1. Veterinary authorities should be informed in cases where suspicion of pH1N1 or other new and emerging influenza virus is high, including when there is an epidemiological link with ILI in humans or other animal species. Poultry keepers should monitor production parameters in order to detect the presence of influenza viruses (e.g. through egg drop).

- Testing of a subset of positive influenza A samples from routine avian influenza surveillance programmes for pH1N1 and other novel influenza viruses when samples are negative for H5 and H7.

Targeted surveillance

Targeted or risk-based surveillance is the preferred approach over statistically based surveys for early detection of pH1N1 or other new and emerging influenzas. By targeting surveillance to high risk groups in the population greater detection efficiency and cost effectiveness will be achieved.

Sample targets can include but are not restricted to:

- Laboratory detection – Investigation of positive detections of influenza A virus for the presence of HPAI and subtypes H5, H7. In addition samples can be tested for pH1N1 (in particular turkeys) and other subtypes when positive for influenza A and negative for H5 and H7. All laboratory confirmed pH1N1 or new and emerging influenza virus infections should be communicated to animal health authorities for further investigation.
- Slaughterhouse/processing plant or market place surveillance – Testing of animals with signs of respiratory disease consistent with ILI (including at post mortem in slaughterhouses).
- Poultry species on farms where pH1N1 or other influenzas have been detected in humans or other animals, particularly pigs.
- Animals showing ILI at points of concentrated gathering such as markets, auctions or fairs
- Farms epidemiologically linked to known infected farms
- ILI in animals linked to known human cases
- Pigs in close contact with humans or other animal species showing ILI
- Supplementary testing of samples submitted to laboratories for avian influenza investigation or any other signs that may be consistent with infection with pH1N1 or other new and emerging influenza in poultry.
- A subset of samples taken from routine statistical surveys can be tested for pH1N1 or other subtypes of influenza – these may be targeted to higher risk birds based on the current scientific evidence (e.g. turkeys for H1N1).

Categories of data needs

Basic epidemiological information includes:

- Location and date
- Farm type and demographics
- Date when signs first started and when samples were taken

- Morbidity, mortality, clinical signs
- Link to suspected human cases

Molecular genome sequencing

Full genome sequencing provides important information about the origins, evolution, and characteristics of the virus including genetic reassortment. Full genome sequencing is preferred, and is important in assessing the genetic basis of antiviral resistance and pathogenicity in different species. Molecular sequence data is also important to inform nucleic acid based diagnostic tests and to ensure that these tests are fit for purpose. If full genome sequencing is not possible, targeted partial genome sequencing can provide useful information.

Antigenic data

Antigenic data will provide important information to ensure that diagnostic reagents are compatible with circulating field viruses and that diagnostic tests are therefore fit for purpose. It is also important to ensure that vaccine efficacy is optimal in terms of matching vaccine antigen to field viruses.

Reporting and response

All relevant findings from influenza surveillance in animals including positive results from laboratory testing should be reported to animal health and public health authorities at the appropriate level. It is recommended that animal health authorities share information with other relevant stake holders including local public health authorities.

The occurrence of an emerging disease (a new pathogen or a known pathogen that was not OIE listed because it did not meet the criteria and that is now behaving differently) determined by a significant increase in the morbidity, or mortality in animals or having a zoonotic impact should be notified immediately by national veterinary authorities to the OIE.

Information about the epidemiological and viral characteristics of pH1N1 and other novel influenza viruses in poultry should be shared with the wider scientific community in a timely manner. This includes depositing genetic sequence data into publicly available databases.

The response to pH1N1 infection in poultry should be similar to non notifiable avian influenza virus infection. In particular:

- Culling of infected poultry is not necessarily recommended.
- Clinically ill poultry should not be shipped or sent to slaughter.
- Temporary movement restrictions of poultry between enterprises may be implemented.
- Healthy poultry from uninfected units on infected farms can be sent to directly to slaughter.
- Vaccination for pH1N1 in poultry is not currently available or recommended.

Risk communication

It is important that veterinary and public health authorities develop a coordinated risk communication strategy following positive surveillance findings. The risk communication strategy should strive to maintain an appropriate level of awareness amongst key stakeholders and the general public whilst not creating undue concern.

Outbreak investigation

Further to a positive surveillance finding an outbreak investigation should aim to gather all relevant epidemiological and virological information, and should be conducted without undue delay.

Role of epidemiological studies and research

Valuable information can be gathered through epidemiological studies and other research to inform the main objectives of surveillance for animal influenza. However, it is beyond the scope of this document to include all of the options under these categories. Countries are recommended to maximise the use of such studies to inform their surveillance programmes, for example through building inter-sectoral partnerships with academia and other actors conducting such research.

