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With broader application to other farmed mammals

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With broader application to other farmed mammals

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Summary

Beyond domestic poultry, influenza A(H5N1) of clade 2.3.4.4b has spread to almost all regions, infecting a wide range of wild birds, marine and terrestrial mammals, and recently, cattle in the United States of America. When an influenza virus is circulating in both avian and mammalian populations, the likelihood of spillover to humans and risk to public health may increase.

The reported events of the influenza A(H5N1) virus among terrestrial and marine mammals in several countries, including the recent cases detected in the United States of America, have made it necessary to improve virus detection in cattle and other susceptible mammals and closely monitor virus evolution and adaptation to extraordinary hosts.

These recommendations aim to support countries in planning surveillance for influenza A(H5N1) in cattle to enhance early detection, to generate evidence-based information to mitigate the impacts of spillover from birds to cattle, and to prevent transmission between cattle herds. Additionally, these recommendations aim to assist countries, especially low- and middle-income countries, in optimizing the use of limited resources to achieve their surveillance objectives through leveraging existing surveillance programmes.

The Food and Agriculture Organization of the United Nations (FAO) recommends that all countries maintain passive surveillance for A(H5N1) to rapidly detect spillover events in non-avian species, using an appropriate case definition alongside education and outreach to relevant stakeholders to improve awareness of this emerging disease. Additionally, countries may choose to use other surveillance approaches to leverage routine and opportunistic sampling to evaluate the health of cattle populations. Event-based surveillance may also be a helpful tool in early detection. For at-risk countries,[1] targeted or risk-based surveillance approaches can be used to more closely assess cattle health at the interface with poultry or wild birds, investigate suspected outbreaks in cattle, and demonstrate freedom from infection. These recommendations have a broad application to other susceptible farmed mammals.

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[1] At-risk countries are those that produce cattle but have not reported any cases of influenza A(H5N1) in their cattle populations and meet at least one of the following conditions:

- Highly pathogenic avian influenza (HPAI) cases in wild birds and/or poultry have been reported.
- HPAI has been detected in non-avian species, excluding cattle.
- There is a non-negligible likelihood of HPAI introduction and spillover to livestock.

Background

The highly pathogenic avian influenza (HPAI) H5N1 virus of the goose/Guangdong lineage emerged over 20 years ago and has continually evolved into different clades of viruses. Since 2021, viruses of clade 2.3.4.4b have spread globally, affecting wild-bird populations and leading to spillover into poultry. While HPAI primarily affects poultry and wild birds, avian influenza can occasionally be transmitted to mammals, including humans. Since 2021, an increasing number of cases of influenza A(H5N1) of clade 2.3.4.4b have been reported in terrestrial and aquatic mammalian animals, including foxes, bears, seals and sea lions, and in domesticated animals, including pets such as cats and dogs, farmed fur animals, and more recently, livestock (FAO, 2024a). On 25 March 2024, the United States Department of Agriculture (USDA), announced that influenza A(H5N1) of clade 2.3.4.4b had been identified in dairy cattle for the first time. Six days later, the same virus was detected in a farm worker with presumed exposure to an infected dairy herd. Following this initial detection in cattle, infected dairy herds have been identified in multiple states across the United States of America, with additional cases in farm workers. Currently, the World Health Organization (WHO) assesses the overall public health risk posed by influenza A(H5N1) to be low, though the risk is low–moderate for those exposed to infected birds, animals or contaminated environments (FAO, WHO and WOAH, 2024).

Initial epidemiological investigations suggest that affected cattle may exhibit various nonspecific clinical signs, including:

- decreased milk production;
- thickened, concentrated, colostrum-like milk;
- decrease in food consumption;
- tacky or loose faeces;
- lethargy;
- fever; and
- dehydration.

The incubation period appears to last for between 12 and 21 days, and it is likely influenced by multiple factors, including the route of exposure, the viral dose, the production phase of the animal, and other as yet unknown factors (USDA APHIS, 2024a). There is evidence that lateral transmission among cattle likely occurred, including transmission between subclinical infected cows, although the exact mechanism of spread remains currently unclear. The virus is shed in milk at high concentrations, so it is likely that both fomites contaminated with infected milk and mechanical transmission contribute to virus spread. Evidence from viral sequencing suggests that the virus can spread from affected dairy herds to nearby poultry premises, and several of the affected farms reported concurrent mortality events in wild and peridomestic birds as well as domestic and wild mammals (Caserta *et al.*, 2024), although further investigation is required to confirm the full scope of routes of transmission.

The recent emergence of influenza A(H5N1) in cattle has made it necessary to increase preparedness for outbreaks and to ensure the early detection of and rapid response to spillover events in livestock. Considering the worldwide spread of influenza A(H5N1) of clade 2.3.4.4b, the spillover from birds to cattle (and likely from cattle to humans) in other countries is expected. As a result, it is necessary to provide guidance on surveillance for the detection of influenza A(H5N1) in cattle to inform risk assessment and disease control interventions, to support the evidence-based decision-making process and to closely monitor the evolution of the virus, particularly in relation to viral adaptation to mammals and subsequent pandemic risks.

The Food and Agriculture Organization of the United Nations (FAO) recommends integrated cost-effective surveillance approaches for influenza A(H5N1) virus in cattle herds, utilizing the existing surveillance and control programmes for avian influenza and other cattle diseases. These recommendations have been described in that context and have broader application to other farmed livestock species. These recommendations aim to help countries in designing and implementing surveillance in cattle as part of a larger effort to address the evolving risks of influenza A(H5N1) in mammals.

Purpose and objectives of the recommendations

The recommendations have been developed in consultation with international experts and are for use by veterinary services or research institutions in affected countries and those at risk of influenza A(H5N1) spillover to and transmission among cattle herds. By adopting these recommendations widely, countries can enhance early detection, support decision-making and inform evidence-based risk mitigation measures.

Specifically, the recommendations aim to support countries by:

1. defining surveillance objectives, case definitions, data collection and reporting;
2. selecting surveillance methods to design or enhance a country- or regional-level surveillance strategy;
3. developing guidance on sample types and their prioritization, laboratory diagnosis of HPAI in cattle, and best practices for surveillance feedback and communication; and
4. outlining essential next steps to take if a positive sample is identified.

Defining surveillance objectives, suspected case definition, data collection, and reporting

SURVEILLANCE OBJECTIVES

The surveillance objectives for influenza A(H5N1) in cattle depend on the epidemiological context, such as whether the virus has already been detected in cattle, and would ideally entail multiple objectives to trigger a rapid response, inform risk mitigation measures, and support decision-making and policy. The objectives need to be addressed through relevant surveillance methods or components that do not interrupt HPAI surveillance in birds.

The minimum objective for all countries should be early detection of spillover events from birds to non-avian species, including cattle. Countries may also choose to use surveillance to provide evidence of the absence of infection in cattle populations.

For countries with confirmed cattle infections, the following objectives provide a foundation for surveillance design:

- early detection of infected cattle herds;
- identification and characterization of HPAI viruses circulating in non-avian species, including cattle, to monitor for viral mutations that could suggest mammalian adaptation or antiviral resistance; and
- declaration of freedom from infection in herds, regions or sectors.

SUSPECTED CASE DEFINITION

Countries should develop case definitions for influenza A(H5N1) in cattle that are tailored to the specific country context and regularly updated based on the best available information. The recommended suspected case definition for a country with no previously detected infection of cattle should include at least the following:

- cattle herds directly or indirectly exposed to a human, avian or non-avian[2] case suspected or confirmed to be infected by influenza A(H5N1) in the previous 30 days; and
- cattle exhibiting any signs of disease compatible with influenza A(H5N1) infection.[3]

The above suspected case definition is applicable at the herd and individual animal level. Clinical signs need to be updated when new information becomes available and according to the sensitivity and specificity required.

[2] Including domestic, peridomestic and wild mammals.

[3] Subject to change as more data emerges, but at the time of writing the reported clinical signs are summarized here (FAO, 2024b).

DATA COLLECTION

Collected data should be consistent with the requirements of national and global animal disease reporting systems (e.g. the World Animal Health Information System [WAHIS] [WOAH, n.d.]) and should be designed to serve the planned objectives. It is recommended that data be collected at the herd and individual animal level and include the sampling location, observed clinical signs, samples collected and relevant dates (e.g. disease onset, detection, reporting, sampling, confirmation etc.). Since only a subset of animals from the herd may be sampled, additional herd-level data can be collected to support developing differential diagnoses and disease control activities. This could include recent additions of live animals to the herd, any epidemiologic connections with poultry flocks (e.g. shared ownership or workforce), value-chain-related data including recent sales or purchases of animals or animal products, and any evidence of mortality in cats or birds in the surrounding areas. An example sample collection sheet is provided in Annex 2.

REPORTING MECHANISM

Time of reporting: It is recommended that countries aim to report suspected cases to the appropriate animal health authority within 24 hours of disease suspicion. Due to the current lack of clarity regarding the duration of viral shedding and the virus dynamics within and between cattle herds, suspected cases must be reported immediately to allow prompt sampling of animals or herds.

Reporting form: Standardized field reporting forms are recommended to ensure collection of consistent data and reliable data analysis. The reporting form should aim to collect the data outlined above in a way that ensures consistency while minimizing the burden on livestock producers and data collectors in the field.

Reporting platform: Commonly used electronic and paper-based reporting mechanisms should be checked to make sure that data entry modules (electronic systems) and data entry sheets (paper or excel-based) are adapted to capture data on influenza A(H5N1) in cattle and related data.

Choosing surveillance methods to design or enhance a country- or regional-level surveillance strategy

The choice of surveillance method(s)[4] can be based on many factors, including cost and feasibility. The list below (summarized in Table 1) represents several different surveillance approaches that can be used individually or in combination to meet the surveillance objectives. Effective implementation of any of these methods is contingent on defining the population(s), geographical area(s) of interest, temporal context for the surveillance (e.g. annual, during higher risk seasons etc.), as well as the type of sampling (e.g. animal- or herd-level sampling) and testing methods used. By combining surveillance methods, countries can develop integrated surveillance approaches that take advantage of planned or ongoing work and help reduce surveillance costs.

PASSIVE SURVEILLANCE

Passive surveillance of influenza A(H5N1) in cattle describes the surveillance that is achieved when:

- farmers identify that they have some sick cattle and they contact a veterinarian to seek help (FAO, 2014); and
- veterinary and/or animal health professionals report unexplained mortality, neurological symptoms and/or respiratory symptoms in other animal species on dairy premises to the veterinary services.

This method is a baseline requirement for surveillance and should be in place in all affected and at-risk countries.[5] The veterinary services need to ensure the following measures are in place to maintain a functional passive surveillance system:

- Establish a suspected case definition and a reporting mechanism with the suspected case definition included in a list of eligible/required disease notifications.
- Increase epidemiological vigilance among animal health officers by communicating to them the suspected case definition, reporting mechanisms and other follow up actions utilizing a variety of approaches (e.g. training, communication materials, coordination meetings, supervision).
- Consider ways to implement enhanced passive surveillance through efforts to raise awareness among producers and community leaders about the importance of identifying and reporting clinical signs in cattle and other relevant factors such as mortality in other species on the farm or in the surrounding areas.
- Enhance the reporting channels to allow easy and rapid delivery of the producers' notifications to the veterinary services, which may include designated phone numbers for reporting, mobile applications, emails and social media accounts.
- Design appropriate triaging protocols to ensure herds with the highest risk of infection are prioritized for investigation by the veterinary services.
- Secure necessary equipment for cattle sampling, sample preservation and sample transportation.
- Establish a feedback mechanism to ensure information flows to the field as well as to authorities.

ROUTINE INSPECTION

This includes all regular animal inspection procedures conducted by the veterinary services throughout the year, regardless of the epidemiological situation relative to influenza A(H5N1), for example animal and carcass inspections at slaughterhouses, border inspection and quarantine processes, and other similar services where animals matching the suspected case definition could be observed. The veterinary inspectors at such facilities should be aware of the suspected case definition and vigilant in the detection and reporting of suspected cases. It is advised that veterinary services adhere to the recommended measures listed under passive surveillance to maintain an effective approach to influenza A(H5N1) surveillance.

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[4] Additional methods such as wastewater and participatory disease surveillance methods could be useful in improving influenza A(H5N1) detection in cattle but are beyond the scope of these guidelines. Countries interested in utilizing these methods are encouraged to contact FAO to discuss how they might be used in addition to the surveillance methods presented here.

[5] At-risk countries are those that produce cattle but have not reported any cases of influenza A(H5N1) in their cattle populations and meet at least one of the following conditions:

- Highly pathogenic avian influenza (HPAI) cases in wild birds and/or poultry have been reported.
- HPAI has been detected in non-avian species, excluding cattle.
- There is a non-negligible likelihood of HPAI introduction and spillover to livestock.

TABLE 1
Surveillance methods to consider when designing a national or regional surveillance strategy for influenza A(H5N1) in cattle, when to consider using them, and the relative advantages and disadvantages of each

Method	When to consider	Advantages	Disadvantages
Passive surveillance	At any time	Low cost and high coverage	May not be very sensitive due to the lack of sensitization or awareness. May also lack specificity due to vague or unclear clinical signs that mimic endemic diseases (e.g. mastitis)
Routine inspection	At any time, particularly when the country is considered at risk	Takes advantage of routine activities to provide cattle samples for influenza A(H5N1). Can be a cost-effective way to target certain populations.	May require new processes and financial support for sample collection and shipment
Opportunistic surveillance	At any time, particularly when the country is considered at risk	Takes advantage of different animal health related activities to observe or test cattle, may be low cost	May be less representative of the population of concern than other methods and require new processes and financial support for sample collection and shipment
Event-based surveillance	At any time, particularly when the country is considered at risk	Can provide very high levels of coverage of a population at low cost or take advantage of other investments in event-based surveillance	Can be biased towards what is newsworthy rather than what is needed to meet the surveillance objectives
Risk-based surveillance	Affected or at-risk countries	Provides options for targeting surveillance, which can reduce costs while improving early detection	Requires knowledge of the risks, which can be challenging with a new or emerging disease
Outbreak investigation and contact tracing	Affected or at-risk countries	Ensures that cases are investigated and confirmed	Relies on field personnel and the ability to travel to the case location. Must identify and trace connections between poultry and other susceptible species
Surveillance for herd-level freedom from disease	Affected herds	Provides evidence for disease absence in a herd	Can be challenging to conduct follow-up testing in certain husbandry systems, particularly among pastoralist and nomadic communities

OPPORTUNISTIC SURVEILLANCE

Opportunistic surveillance describes an approach that aims to take advantage of different animal health-related activities to detect suspected cases. This could include, but is not limited to:

- campaigns for cattle vaccination or acaricide application;
- procedures for surveillance and post vaccination monitoring of other cattle diseases (e.g. foot and mouth disease, lumpy skin disease etc.); and
- sampling cattle during the response to outbreaks of avian influenza in birds where cattle are found in the same area.

This surveillance method is considered a feasible way to use existing efforts to improve influenza A(H5N1) detection in cattle, optimizing the use of limited resources and minimizing the impact on other activities designed to control transboundary animal diseases. To operationalize this surveillance method, the animal health teams responsible for cattle vaccination, spraying/dipping, the surveillance of transboundary animal diseases, or other similar services should be told to be vigilant to detect and report cattle cases matching the suspected case definition. The veterinary services must adhere to the recommended measures listed under passive surveillance to maintain a functional system.

EVENT-BASED SURVEILLANCE

This type of surveillance includes tracking formal and informal information sources to detect unusual events matching the suspected case definition that might signal an outbreak, and could complement other surveillance activities. This signal then directs the veterinary services and other One Health partners to investigate the potential outbreak. The implementation of this method may require strong collaboration between public and animal health authorities.

As stated by Balajee *et al.* (2020), the process can be described as the detection of signals or observations that alert the animal health/production community that an event may be occurring in a cattle population. The source of signals can include a variety of information sources such as community informants, educational institutions, public and private animal health officers, dairy industry members, news outlets, and social media. These signals are designed to indicate potential high-priority events of concern.

The signals that will be tracked through the various information sources should be designed to match the desired sensitivity and specificity of a country's surveillance system. For example, an increasing trend in any of the following signals would justify triggering an alert:

- unexplained drop in milk production in cattle;
- changes in milk consistency;
- cattle mastitis not responding to antibiotic treatment;
- flu-like illness, respiratory symptoms or conjunctivitis affecting cattle farm workers; and
- unexplained mortality, neurological symptoms and/or respiratory symptoms in other animals on dairy premises.

FAO supports its Members in tracking informal news on suspected events of transboundary and emerging animal diseases. When an event is identified, FAO contacts the relevant veterinary services to share information and find out more details about an event, including whether it has been confirmed. After event verification, the appropriate level of investigation, response and control measures can be determined.

RISK-BASED SURVEILLANCE

Risk-based surveillance involves looking for disease where and when it is most likely to be found (FAO, 2014). Country-specific risk assessments should be used to prioritize the geographical areas, time, season, value chain nodes, animal species and animal subgroups (including animal-specific factors) associated with influenza A(H5N1) spillover and transmission within or between cattle herds.

The risk factors and derived surveillance targets should be continually updated based on the most reliable and up-to-date information. When countries do not have enough data, expert opinion or the broader scientific literature can be used to inform the risk assessment.[6] In such cases, combining data from different surveillance methods may be beneficial to help address unknown or evolving risks, though it is important to assess the level of uncertainty in the assessment when interpreting the risk

Risk-based surveillance at the interface of cattle and susceptible avian species

For influenza A(H5N1), risk-based surveillance should ideally address the critical nodes in the value chains at the interface of cattle and susceptible avian species. Although this approach should be based on a context-specific risk assessment, the suggested criteria to be considered in this case are:

- relevant geographical level for implementing the surveillance activity (e.g. province, county, district etc.);
- spatial risk factors, which could include high poultry density, high incidence of HPAI in poultry, high density or presence of migratory birds (or proxy factors such as the presence of wetland areas), high density of cattle or high likelihood of cattle–bird exposure;
- high-risk cattle value chain nodes, which could include the production and marketing nodes where influenza A(H5N1) incursion and spillover from poultry to cattle is more likely to happen – this needs to include as a minimum the dairy farming value chain (commercial and/or smallholder farms);
- high-risk cattle subgroups in which the virus is more likely to be detected – this could include as a minimum (but is not limited to) lactating cows and prewean calves being fed whole, unpasteurized milk or colostrum; and
- high-risk periods when incursion and spread is more likely to occur – this may include the season of migratory bird activity and/or the peak season of influenza A(H5N1) outbreaks in poultry.

Risk-based surveillance at critical cattle value chain nodes

Following an initial detection of influenza A(H5N1) in cattle, risk-based surveillance can be applied at critical nodes in the dairy cattle value chains based on a country-specific risk assessment. This surveillance should complement, but not replace, risk-based surveillance at the cattle–bird interface. Given that evidence suggests possible cattle-to-cattle transmission and considering that there is always a chance of having multiple independent spillovers from birds to cattle, affected countries should implement this surveillance method in parallel with the previously described methods. The suggested criteria to be considered in this case may include:

- spatial risk factors, which could include a high density of dairy cattle herds as well as exposures between animals from different herds, e.g. at cattle markets or in common grazing areas;
- high-risk cattle value chain nodes potentially associated with virus amplification and dissemination (FAO, 2011), which may include cattle congregation points such as livestock markets or shows;
- high-risk cattle subgroups in which the virus is more likely to be detected (as a minimum, this would include age categories and sex in relation to movement, marketing or exposure to other herds); and
- high-risk periods (weeks, months or seasons) in which spread among cattle herds is more likely to happen (this may include the seasonal peaks of cattle movement – e.g. open grazing seasons – or times in the production cycle when new cattle are likely to be introduced into a milking herd).

In situations where resources are not adequate to conduct risk-based surveillance at all the highest-risk value

[6] For example: the FAO guidelines (2021).

chain nodes, a more targeted, risk-based approach may be considered. The focus must still be on higher-risk value chain nodes identified through prior risk assessments, but greater consideration may be warranted for targeted populations identified through risk evaluation. This could include husbandry systems in HPAI-infected countries where cattle and susceptible avian species co-exist in close proximity under low biosecurity conditions (e.g. rural backyards or similar community-based systems where HPAI has been identified in wild or domestic birds). Targeted surveillance may also focus on more economically important value chain nodes such as the dairy export sector.

OUTBREAK INVESTIGATION AND TRACING

Epidemiological investigations are triggered by any event where suspected or confirmed transmission from birds to cattle, cattle to human, cattle to cattle, or involving other animal species is reported.

The process should ideally include:

- Timeliness: It is important to conduct the investigation in a timely manner to be able to collect field observations with minimal recall bias and clinical observations and samples from individual infected animals during the acute phase (before they have recovered, died or been moved off farm) to maximize the likelihood of detecting the virus.
- Event verification and confirmation: Sampling of suspected and exposed animals must be carried out to confirm virus presence.
- Traceback investigation: This aims to identify additional affected herds that may be epidemiologically linked to the affected herd and a potential source of infection. This is achieved through comprehensive traceback of the animals' and animal owners' movements and interactions during the 14 days prior to the date of first suspicion. Several tools are recommended, including field observations, interviews with key informants and data collection, checking the relevant documents from visitor logs, animal movement records, surveillance cameras etc. For information on the One Health principles that are to be applied to the zoonotic disease outbreak investigation, consult chapter 5 of the Tripartite Zoonoses Guide (FAO, WOAH and WHO, 2019).
- Traceforward investigation: This aims to identify probable onward transmission of the virus to other herds or other species roaming within or around the infected herds. This is achieved by identifying epidemiologically linked herds of the same or different species that may have been exposed to the virus from the herd under investigation (e.g. other herds owned by the same owner or served by the same farm workers, or herds exposed to potentially infected animals from the herd under investigation).
- Both the traceback and traceforward investigations would inform the veterinary services about other sites/herds where the virus might be circulating and that therefore need to be targeted by surveillance.

SURVEILLANCE FOR HERD-LEVEL FREEDOM FROM DISEASE

The objective of this surveillance method may be to provide confidence in the absence of the virus within affected herds before the lifting of quarantine and movement control measures, or to demonstrate absence in a population with a desired level of confidence. For an individual herd, the sampling process includes:

- retesting of the individual animals confirmed to be infected, focusing on the same sample types that demonstrated positive molecular results;
- testing of other potentially exposed animals in the same herd, prioritizing those experiencing clinical signs described in the suspected case definition (if no animals are exhibiting the clinical signs, sampling should be carried out based on random or risk-based selection);[7] and
- testing of environmental samples if the country-specific risk assessment indicates that this is necessary.

The timing of follow-up sampling should be based on the expected virus shedding period and animal-level incidence as evidenced by previous surveillance results. This should be done at the earliest opportunity to mitigate the impact on livelihoods when infection affects business continuity. This surveillance must continue until none of the collected samples test positive for the virus ribonucleic acid (RNA) and the desired level of statistical confidence in disease freedom has been met.

Surveillance to support population-level disease freedom usually combines outcomes from several surveillance methods, both passive and active, to provide authorities with a desired level of confidence that a pathogen is absent. Countries may wish to confirm absence in a specific population as part of a larger animal health scheme or in support of trade or animal movement decisions. The details of population and herd-level sampling and analytical approaches are beyond the scope of this document, but previous FAO guidance is available.[8] Leveraging risk assessment approaches and outcomes can help ensure that sampling for disease freedom is appropriately targeted and takes into account the dynamic risks associated with HPAI.

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7 Information on risk-based disease surveillance is available here (FAO, 2014).

8 Available here (FAO, 2014).

Samples, diagnostics and surveillance communication

ELIGIBILITY FOR SAMPLING AND SAMPLE TYPES

The priorities to be considered for herd and animal sampling are summarized in Table 2, where the criteria for herd selection are listed. These criteria are influenced by the specific epidemiological context, previous risk assessments and the surveillance strategy.

When selecting individual animals to sample within the herd, the priority should be any animal exhibiting clinical signs (or that has exhibited clinical signs in the past 15 days) that match the suspected case definition. If the animals are apparently healthy, it is suggested that animal selection be random and from lactating cows.

Ideally, all animals showing signs consistent with the suspected case definition should be sampled. However, if the number is higher than the available sampling equipment, early detection samples must be collected from the animals with an onset of clinical signs in the previous 48 hours to maximize the likelihood of sampling animals that are shedding virus.

When considering the sample types to be collected from an animal with a suspected infection, the following needs to be considered:

- Milk samples have shown the highest success rate for detecting the virus and have the highest virus titres compared to other sample types (Burrough *et al.*, 2024).
- Few nasal swabs have tested positive for the virus (OFFLU, 2024a).
- Serological tests are available for convalescent animals, though these are under validation at the time of writing (IZSVe, 2024).

On this basis and in order of priority, the recommended samples to be collected for detecting virus are:

- unpasteurized milk samples, preferably from individual animals with clinical signs matching the case definition. It is important that each quarter of the udder of an individual animal is sampled and the samples pooled together (IZSVe, 2024);
- deep nasal swabs (targeting the respiratory epithelium of the nasal turbinates) especially for non-lactating cattle, as this tissue has been observed to be the replication site of influenza D viruses in the nasal passage of cattle (Ferguson *et al.*, 2016; Uprety *et al.*, 2021) – note that swabbing the nasal vestibule, nasal septum or nasal meatus as may occur with more superficial swabbing may not achieve the best harvest of the virus genetic material; and
- sera.

If there are any dead animals, tissue specimens from different organs of dead animals, especially mammary tissue, respiratory organs and other organs with apparent lesions, should be taken.

Pooling nasal swabs from different animals is not recommended (IZSVe, 2024) and pooling milk samples from different animals should only be done in the laboratory, as needed (USDA APHIS, 2024b).

TABLE 2
Suggested criteria for herd selection for the purpose of sampling

Criteria	Location	Priority
Direct or indirect contact with at least one case of influenza A(H5N1) in humans or mammals	Any area	Very high
Direct or indirect contact with birds infected with influenza A(H5N1)	Any area	Very high
Lactating	High-risk area	High
Non-dairy production purposes	High-risk area	Moderate
Other criteria	Low-risk area	Low

For research purposes, a more comprehensive sampling approach may be considered; for example, as part of a longitudinal study with samples taken to evaluate various shedding routes (nasal, oral, conjunctival, rectal, urine and milk).

Bulk milk samples are likely to be more cost-effective for herd-level diagnosis if applicable. At the time of writing, the use of bulk milk samples has not been validated (USDA APHIS, 2024b), but this is expected to be a useful sample type in the future, given the reported high concentration of virus in the milk of infected animals.

LABORATORY DIAGNOSIS

General considerations

Sample labelling and coding in the field and within the laboratory is critical to show linkage between the herds, individual samples and their respective laboratory results. An archiving system for the positive samples should also be in place.

The reporting of laboratory results should ideally include quantitative values whenever applicable (i.e. cycle threshold [Ct] values for RNA and antibody titres for serology).

In samples with sufficiently high RNA levels, full genome sequencing is recommended on at least one positive sample from each infected herd as a crucial step to monitor for mutations of concern, such as antiviral resistance or mammalian adaptation. Furthermore, virus isolation from initial cases is important to conduct phenotypic studies. If the national capacity in that context is not adequate, this can be done through collaboration with the various regional or international reference centres.

Sharing the results of the genome sequencing and other epidemiological data via the Global Initiative on Sharing All Influenza Data (GISAID) (n.d.) or on other platforms is strongly recommended.

Recommended protocols

IZSVe guidelines for molecular and laboratory assays are available here (2024) and the USDA testing guidance for influenza A in livestock is available here (USDA APHIS, 2024b). As the virus evolution and epidemiological situation are expected to evolve over time, countries should make sure that the laboratory protocols employed are based on the latest recommendations from the WOAH/FAO Network of Expertise on Animal Influenza (OFFLU) (n.d.).

DATA ANALYSIS

Where there is no real-time instant reporting system and interoperability between the laboratory and field surveillance databases, the veterinary services should set a time limit for entering data into a central database. This database should include herd and individual animal metadata (as described in Annex 2) accompanied by the laboratory results (including negative results) and genome sequence data.

In general, data analysis needs to be implemented according to the surveillance objectives. The type of questions that a country wants to answer through data analysis may vary based on the epidemiologic situation and should be defined prior to designing the surveillance strategy in line with the surveillance objectives. Relevant options include:

- What is the spatial and temporal distribution of the infected herds (based on number and proportion of infected herds per area)?
- Which cattle value chain nodes are most likely to be affected and/or at which cattle value chain nodes is the virus most likely to be detected?
- How do attack rates or subpopulation prevalences vary across the population of interest?
- What are the morbidity, mortality and case fatality rates within infected herds?
- What is the relative frequency of clinical signs according to different age groups, sex, physiological status etc.?
- How well is the surveillance system performing?
  - How does sample quality and quantity vary across regions? Is additional support needed?
  - Which surveillance method is the most effective in detecting infected herds (based on the number and proportion of infected herds detected per surveillance method)?
  - Which sample types seem to best detect infection?
  - Which sample types are best for successful virus isolation?
- What are the clade and genotype of the virus detected in infected animals?
  - How closely are the viruses detected in different herds related? Is there evidence of infection spreading between herds or through new introduction from avian populations?
  - Are there any key mutations present that may signal changes in antiviral susceptibility, host adaptation or pathogenesis?
  - Are different influenza A viruses co-circulating in the same herd or area?

Evidence generated from data analysis addressing these questions can be used to **improve the sensitivity and specificity of the risk-based surveillance plans, inform the early warning activities and support target resource allocation** to enable a rapid and effective response.

Surveillance feedback and communication

Effective surveillance systems include timely and accurate sharing of data and test results, as well as robust feedback mechanisms to ensure that results are actionable at all levels. Key considerations for the design of a surveillance feedback and communication strategy include the following:

- Both positive and negative animal test results should be communicated formally by the designated department according to the roles and responsibilities within the national system.
- The veterinary services should define the type of data to be shared and the maximum time between receiving the laboratory results and sharing them with the farm owners and relevant partners.
  - Ideally, results should be shared as soon as possible via means that are easily accessible to the producers and relevant official units (e.g. SMS, email, phone call etc.).
- Surveillance feedback should be shared with the owners of the sampled animals and include the number of animals classified as infected and non-infected according to the laboratory results, as well as the individual identification of infected animals to allow for risk management on the farm.
- Surveillance feedback should be shared among the veterinary services and the relevant One Health partners. It is suggested that this feedback include:
  - as a minimum, the individual animal test results and the herd-level data, shared with the relevant departments within the central, intermediary and field veterinary services, including the members of the team who contributed to data and sample collection; and
  - a situation report of the analysed data at national and sub-national levels.
- Public communication of the surveillance findings through media platforms (if needed) should be accompanied by advice on the food safety issues in a way that avoids a negative impact on value chain operations, the livelihood of the producers and consumption of valuable nutrients.

Measures to be taken in the event of positive laboratory results

In the event of a sample from a given farm testing positive for influenza A(H5N1), the following actions are recommended:

- Immediately report to the public health authorities to investigate the possibility of the infection of farm workers and other human contacts.
- Conduct an epidemiological investigation to trace other high-risk herds, implement identified risk mitigation measures that should be undertaken on the farm (including the pasteurization/handling/disposal of infected milk or other products and any quarantine measures in line with any national guidelines or policy), and provide clear recommendations for improving biosecurity.
- Sample sick and dead birds and mammals around the affected premises.
- Conduct surveillance on other potentially infected farms, based on prioritized risks.
- Report via regional or global animal health information systems (e.g. the EMPRES-i+ Global Animal Disease Information System [FAO, n.d.] and WAHIS [WOAH, n.d.], with the latter being mandatory for WOAH-listed diseases).
- Consider whether additional research studies should be conducted on the farm in collaboration with academia or other partners based on known knowledge gaps.

In the event that one or more samples from a farm test positive for influenza A(H5N1) antibodies with negative results from polymerase chain reaction (PCR) assays, no actions are recommended other than monitoring the longevity and kinetics of the antibody response (resources permitting) to inform knowledge gaps. Production records may help to indicate the likely time of infection, which may inform epidemiological investigations and tracings to other epidemiological units.

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Annexes

Annex 1
Recommended studies in affected countries to inform risk assessments

LONGITUDINAL STUDIES

- Longitudinal studies involve the repeated inspection and sampling of individual animals confirmed to be infected by influenza A(H5N1), regardless of clinical recovery. Such studies are important when dealing with emerging zoonotic diseases and new spillovers to generate critical information required by the risk assessment at human–animal and animal–animal interfaces.
- Depending on the research needs identified, the main objectives could be to understand the shedding routes, virus dynamics, antibody kinetics and virus evolution in infected cattle as this data is crucial to answering questions related to the risk of spillovers from cattle to human, cattle to cattle, and cattle to other animal species.
- The sampling interval for molecular detection should be fixed (ideally no more than three days) to cope with the potential short duration of infectious viral shedding, whereas it could be once every ten days to monitor the antibody kinetics.
- In the case of a natural suckling system, the calf–dam pair approach is recommended and involves testing the infected dams and their suckling calves to investigate the potential transmission through suckling. In such cases, the sample coding should be designed to allow matching of the infected dams' samples with those of their calves.
- During follow-up sampling, the samples from infected animals should include the various shedding routes (nasal, oral, conjunctival, rectal, urine and milk). This process should be conducted for each route at defined intervals until the laboratory assays reveal negative results.
- This surveillance method is specifically used to answer key epidemiological questions with the aim of informing the risk assessment and disease control measures so should be discontinued once the objectives have been attained. Based on the information generated by the study, veterinary services can decide on the follow-up measures that must be taken before the quarantine measures can be lifted.

Where infected animals cannot be individually identified through ear tags, the surveillance team must uniquely mark them by other means (e.g. spray a number or letter that is unique to each animal on the frontal area, horn or other body areas out of reach of the animal's tongue). Without this process, it is difficult to compare the results of different rounds of surveillance for each animal, meaning that the study objectives cannot be obtained.

Specific considerations for the analysis of the longitudinal data:

- Consider each infected animal enrolled in this study an independent unit: Analyse the data of the different follow-up rounds separately for each animal, then aggregated by group according to appropriate variables.
- Describe the RNA levels over time for each route until the RNA is no longer detected.
- Describe the kinetics and longevity of the antibodies, preferably for each immunoglobulin (IgM, IgG).
- Using the points above, describe the re-infection characteristics (if any) in comparison with the initial infection (if the data are available) and whether similar or different virus genotypes are implicated.
- Compare the findings with the experimental infection studies (when made available).

RETROSPECTIVE SURVEILLANCE

- Whereas influenza A(H5N1) of clade 2.3.4.4b has been circulating globally in domestic and wild birds for more than three years (Wibawa *et al.*, 2024), the implicated genotype B3.13 emerged around September 2023 and has not been detected outside the United States of America (OFFLU, 2024b). It is likely that cattle exposure to clade 2.3.4.4b occurred unnoticed in the past in many regions, so searching for the virus or the antibodies in archived samples may improve understanding of the chronology of the spillover from birds to cattle in different regions and the extent of the infection in different cattle production value chains.
- It is recommended that selection criteria for the archived samples be risk based, and the suggested selection criteria include:

- species: cattle;
- animal profile: dairy animals;
- origin: high-risk areas;
- time frame: collected based on the likely emergence date of the virus in cattle in the particular country;
- sampling date: collected during the high-risk period (peak outbreak among poultry and/or wild birds);
- value chain node: commercial farms, small-scale holders, markets and slaughterhouses; and
- type:
  - sera (for antibody detection);
  - nasal swabs (for molecular detection);
  - respiratory tissues (for molecular detection); and
  - milk (for molecular detection).

- The priority is to select samples with accompanying metadata (i.e. location, farm characteristics etc.).
- Targeting more archived samples from the areas, herds or time frames of influenza A(H5N1) positive detections is recommended.

Annex 2

Example of a standard sampling sheet

Date	County	District	Village/township	Latitude	Event ID
........./........./..........	....	....	....	.... Longitude	....
Owner name .... Owner tel.		**Surveillance method** ☐ Risk based ☐ Passive ☐ Follow-up ☐ Other			
Animal species present					
Date of disease onset					
Number of animals on date of disease onset					
Number of dead animals since disease onset					
Number of sick animals on date of investigation					

Animal ID	Species	Sex *M/F*	Age *(Months)*	Farming purpose *Dairy* *Fattening* *Other*	Physiological status *Lactating* *Pregnant* *Dry* *Suckling* *Other*	Clinical signs *(Type the corresponding NUMBER)* *1. No signs* *2. Lethargy* *3. Off food* *4. Drop in milk production* *5. Changes in milk consistency* *6. Changes in faecal consistency* *7. Other (please specify)*	Sample type[1]				
							N	O	R	M	S	Other *(Please specify)*

[1] N: nasal swab; O: oral swab; R: rectal swab; M: milk; S: serum.

Notes: ....

....

....

Reporting officer(s): ....

Tel.: ....

Annex 3
Sample coding

The suggested sample coding is for countries where there is neither an animal identification system nor an electronic sample coding system.

LOCATION AND DATE CODES

- unique farm ID (if applicable), otherwise use other coding such as area telephone code; and
- date of sampling (DD.MM.YY): example 25.09.24.

ANIMAL CODES

- species: cattle lactating (L), cattle pregnant (P), suckling calves (C), fattening cattle (F) (this could be replaced by the ear tag number if applicable); and
- serial number: 1, 2, 3 etc.

SAMPLE TYPE CODES

- animal sample type: nasal swab (N), oral swab (O), rectal swab (R), milk (M), serum (S).

For longitudinal follow-up surveillance: Consider including the unique ID of each animal.

Example of sample code of **nasal swab** collected from lactating cattle number 1 on 30 May 2024 in the area with telephone code 551: 551.L1.**N**.30.05.24

Annex 4

Sampling equipment needed for the surveillance visits

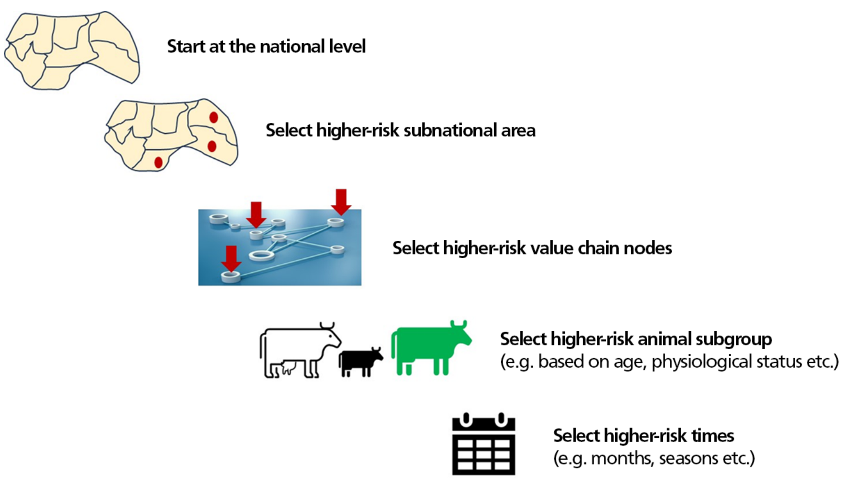
- nasopharyngeal swabs, preferably flocked swabs commonly used for virology research (note: If tipped swabs are used, avoid those with wooden handles and/or cotton tips);
- virus transport medium (VTM);
- tissue-stabilizing solution (RNAlater® or similar);
- tubes (for keeping the collected swabs in VTM);
- specimen container (for keeping the collected tissue specimen in VTM or tissue-stabilizing solution);
- scissors (for cutting the swab handle);
- alcohol (minimum concentration: 70 percent);
- cotton or absorbent tissue paper;
- vacutainer for serum separation;
- tubes for milk samples;
- portable cooler with dry ice;
- sharps disposal bin;
- personal protective equipment – see WHO guidance (n.d.);
- bin bag;
- permanent marker pens; and
- data collection sheets – either printed on paper, on a portable computer or as an app on a smartphone or tablet.

Annex 5

A logical framework for risk-based surveillance planning

Framework for applying risk-based surveillance to detect influenza A(H5N1) virus in cattle. Note that within each sampling site (e.g. commercial farm or small-scale holder), individual animals to be sampled should be selected either based on the presence of suspected clinical signs or, if none of the cattle are showing signs of the disease, at random.

FIGURE A.1
Sample selection process



Source: Authors' own elaboration.

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1. Collection of entomological baseline data for tsetse area-wide integrated pest management programmes, 2009 (En)
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As influenza A(H5N1) of clade 2.3.4.4b continues to spread from wild birds to poultry and to both terrestrial and marine mammals, the recent cases in cattle highlight the critical importance of being prepared for and responding rapidly to spillover events and of planning for early detection and response at the country level, especially in countries of low and middle income.

These recommendations from the Food and Agriculture Organization of the United Nations (FAO) aim to support countries in enhancing influenza A(H5N1) surveillance in cattle populations, with broader application to other farmed mammals, to inform risk assessment and evidence-based disease control measures. Integrated surveillance strategies can leverage existing programmes for avian influenza and other cattle diseases, enabling countries to enhance monitoring capabilities while maintaining cost efficiency.

With regard to preparing effectively, FAO recommends a combination of different surveillance methods including risk-based surveillance strategies tailored to individual country contexts. Adopting these recommendations will strengthen early detection efforts, support evidence-based decision-making and help implement targeted risk mitigation measures to protect both livestock and public health.

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