



# Addressing avian influenza A(H7N9)

## *Surveillance guidelines for uninfected countries in Southeast Asia and South Asia*

Issue no. 2



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## EXECUTIVE SUMMARY

A novel avian influenza strain A(H7N9) was detected in eastern China in March 2013. The timely response and effective risk management efforts by the People's Republic of China resulted in a rapid decrease of human cases. Large numbers of animals were tested in China and neighbouring countries with the virus detected in a few animal and environmental samples collected in China, predominantly from live bird markets (LBMs).

A major challenge for devising a surveillance strategy for avian influenza A(H7N9) is its 'silent' infection in birds: a low pathogenic avian influenza (LPAI) virus does not produce clinical signs in infected birds. This increases the risk of undetected incursion and spread of the virus in the animal population as well as potential exposure of humans. Given the possible seasonal pattern of avian influenza viruses and the reopening of LBMs in China, robust, short-term, risk-based surveillance is needed to detect and be able to rapidly respond to the possible resurgence of H7N9 in a timely

manner. Early detection of the virus may prevent its incursion and/or spread into currently uninfected areas as well as prevent its establishment in poultry production systems and human spill-over infections.

This document describes guidelines for a short-term risk-based surveillance strategy over the next 12 months, following current epidemiological knowledge of the virus and the predicted risk of infection for non-infected areas or countries. Survey designs and laboratory protocols include both the surveillance of H7N9 and other zoonotic avian influenza viruses. National animal health authorities may adapt the protocols according to their country's level of incursion risk, the specificities of the area targeted and their surveillance capacities.

The surveillance strategy relies on longitudinal surveillance rounds through repeated sample collection at ports of entry of traded birds from infected areas or countries, in highly connected LBMs and poultry farms, depending on the context of the area of interest. Sampling protocols and the frequency of sampling need to be adapted to country specificities and to evolving information.

## 1. BACKGROUND

On 31 March 2013, the authorities of the People's Republic of China reported three human cases of infection with a novel avian influenza A strain of subtype H7N9 in Shanghai, eastern China. This virus was generated in nature during the mixing (reassortment) of subtypes H9N2, H7N(unknown) and H(unknown)N9 previously identified in wild and domestic birds. Influenza A(H7N9) contains six out of eight genes from H9N2, with H7 and N9 donated by the two other avian influenza virus subtypes.

Information on the exposure history to animals before the onset of symptoms in humans is often missing. Epidemiological data, however, shows that 59 out of 77 confirmed cases (76.6 percent) had a history of contact with poultry (Li, Zhou et al. 2013). In the absence of sustained human-to-human transmission poultry remains the most likely cause of human infection (none of the 339 samples collected from close contacts of 12 patients tested positive (Li, Zhou et al. 2013).

Poultry seems to be the main host of this virus, although the exact reservoir species, production sectors of higher risk and geographical distribution of the virus have not yet been identified. Avian influenza A(H7N9) has been difficult to detect in the animal population of the People’s Republic of China in surveillance conducted after control measures in markets were implemented. Out of a total of 197 389 swabs and 702 369 serum samples, only 53 (0.027 percent) and 35 (0.005 percent) tested positive, respectively. Samples taken in 7 264 locations showed only 18 markets were positive for A(H7N9) detected in animal or environmental swabs with wholesale markets presenting a higher level of risk. The detection of the virus has relied on polymerase chain reaction (PCR) screening at provincial level followed by confirmation via egg-based virus isolation at national level. Specific antibodies were detected via hemagglutinin inhibition (HI) assays at low incidence: out of nearly 330 000 serum samples collected in April from over 26 000 locations, 29 serums tested positive (over 0.01 percent with a cut-off of 4log2). Information on the nature of the farms tested is not available.

During trace back of human cases, high prevalence was also reported in markets: in Zhejiang viral ribonucleic acid (RNA) detection rather than egg isolation was used to identify avian influenza A(H7N9). The virus was detected in all nine poultry markets visited by human cases: 135 samples (faeces, waste and sewage) were obtained and 38 (28.1 percent) samples were positive by real-time RT-PCR. Surveillance was then expanded to seven other live bird markets (LBMs) nearby not visited by human cases: of 75 samples obtained in these markets, 23 (30.6 percent) were positive for A(H7N9) viral RNA (Han, Jin et al. 2013). PCR-based surveillance using environmental samples in LBMs may increase the likelihood of H7N9 detection.

Prospective surveillance with new sample collections and retrospective surveillance, or re-testing of previously collected samples, of domestic birds were carried out in eight countries

presumed uninfected in Southeast and South Asia: the Kingdom of Bhutan, the Kingdom of Cambodia, the Republic of Indonesia, the Lao People’s Democratic Republic, the Republic of the Union of Myanmar, the Federal Democratic Republic of Nepal, the Kingdom of Thailand and the Socialist Republic of Viet Nam. Surveillance results, using PCR methods, showed no evidence of H7N9 circulation in these countries, though sample numbers were low compared to the People’s Republic of China.

Given the possible seasonal patterns of avian influenza viruses in China, robust and short-term, risk-based surveillance is advisable for the coming year both in Southeast Asia and South Asia. Systems for rapid detection and response to the anticipated resurgence of H7N9 are needed in order to: (i) prevent virus incursion into currently H7N9-free areas; and (ii) prevent virus establishment in poultry populations thereby limiting the risk of transmission to humans.

## 2. OBJECTIVES

The overall objective of this document is to assist national authorities of uninfected countries in **the early detection of H7N9 along the poultry market chains** in order to limit the impact of the virus on public health, food security, the domestic poultry industry and livelihoods.

Risk-based surveillance strategies are designed according to levels of priority for surveillance, which are related to the likelihood of infection by the virus. Based on existing knowledge of avian influenza epidemiology and the history of highly pathogenic avian influenza (HPAI) H5N1, two main criteria have been identified to determine the level of priority: (i) common borders with infected areas or countries; and (ii) evidence of poultry trade with infected areas or countries. Table 1 presents the criteria used to determine the level of surveillance priority of a given area or country. More detailed country-specific risk assessment guidance can be found in the document “*Addressing the avian influenza A(H7N9) emergency - Emergency risk assessment summary*” (<http://www.fao.org/docrep/018/aq245e/aq245e.pdf>).

In case of incursion, virus isolation and virus sequencing will be used to **monitor viral evolution**, including detection of novel and known mutations such as anti-viral resistance markers. Since the 1990s, influenza virus mixing/reassortment in nature has generated HPAI H5N1, the A(H1N1) pandemic in 2009, and the

**Table 1: Different area/country scenarios according to their H7N9 status**

Scenario 1	
Uninfected area/country with low priority for surveillance	Uninfected areas or countries that: (i) do not share a border with an infected area/country; (ii) have no history of legal or illegal trade in live bird or bird products with an infected area/country; (iii) have no import of live birds or bird products from areas/countries which import live birds or bird products from at least one infected area/country; and (iv) are not connected to an infected area/country through the migration and stopover sites of wild birds, as far as there is no evidence of wild bird infection with H7N9.
Scenario 2	
Uninfected area/country with moderate priority for surveillance	Uninfected areas or countries that: (i) import live birds or bird products from areas or countries which import live birds or bird products from at least one infected area or country, and/or if H7N9 is found in wild birds in the future; (ii) are connected to an infected area or country through the seasonal migration and stopover sites of wild bird species known as the main natural reservoir of low pathogenic avian influenza (LPAI) viruses; and (iii) the cross-border trade of live birds and bird products may include historical or existing legal or illegal trading activities.
Scenario 3	
Uninfected area/country with high priority for surveillance	Uninfected areas or countries that share a land border or have existing or historical legal or illegal trade in live bird or bird products with at least one infected area or country.

**Table 2: Objective of surveillance according to levels of priority**

Scenario 1	
Uninfected area/country with low priority for surveillance	<ul style="list-style-type: none"> <li>• monitor emerging zoonotic avian influenza</li> </ul>
Scenario 2	
Uninfected area/country with moderate priority for surveillance	<ul style="list-style-type: none"> <li>• detect H7N9 incursion as early as possible</li> <li>• monitor emerging zoonotic avian influenza</li> </ul>
Scenario 3	
Uninfected area/country with high priority for surveillance	<ul style="list-style-type: none"> <li>• detect H7N9 incursion as early as possible</li> <li>• monitor emerging zoonotic avian influenza</li> </ul>

recent influenza A(H7N9), among others. A strategic approach to avian influenza virus monitoring is recommended. This approach needs to be based on risk posed to humans and poultry, rather than focusing on one subtype. A minimum panel of H5, H7, H9 avian influenza virus subtypes with the potential of becoming highly pathogenic or with zoonotic potential should be monitored to detect emerging strains with the potential to cause harm to both animals and humans through threats to livelihoods and infections. Furthermore, the strategic approach to avian influenza monitoring will enable the recognition of high-activity areas of emergence, dispersion and points of incursion of the avian influenza virus.

Surveillance efforts will vary according to the levels of risk of virus incursion. Moderate and high-priority countries will be specifically targeted for surveillance of H7N9 as part of early warning for this virus while no specific activities on H7N9 are required in low priority countries. For all areas or countries, the objective of the short-term, risk-based surveillance should therefore include the monitoring of other emerging zoonotic avian influenza viruses. The proposed surveillance strategies will emphasize H7N9, although the sampling efforts will also monitor other influenza viruses including HPAI H5N1 and H9N2 in order to capture overall trends of subtypes and hosts. Time series data (i.e. baseline) will be useful to anticipate prevalence patterns and detect abnormal variations or possible signs of emergence (Pepin, Wang *et al.* 2013).

In addition, the collection of epidemiological data along the market chain (e.g. origin of poultry, animal movements) will help to: (i) identify risk factors and high-risk points along the market chain for H7N9 and avian influenza in general; and (ii) improve preparedness and the understanding of the epidemiology of the H7N9 virus.

In case of virus detection, an emergency procedure will be set up according to the guidelines established in the document “*Addressing the avian influenza A(H7N9) emergency - Guidelines for risk-based surveillance*” (<http://www.fao.org/docrep/018/aq244e/aq244e.pdf>).

### 3. SURVEILLANCE STRATEGY

Due to the absence of clinical signs in poultry infected with influenza A(H7N9) so far, active surveillance is required to determine the presence or absence of this virus. Given the very low detection rates of influenza A(H7N9) in poultry according to official results provided by the Chinese animal health authorities during a wave of infection indicated by human cases, large numbers of virological samples will be required for the detection of H7N9 incursion at the early stage when the expected prevalence is low. Thus it is recommended to conduct targeted virological surveillance on at-risk sites where avian influenza viruses are likely to be spread and amplified, including: (i) market chain sites gathering imported birds from infected areas or countries; (ii) poultry production systems linked to affected countries; (iii) high risk poultry production zones or compartments; and (iv) high risk

poultry species. The detection of any H7N9-positive result should lead to further investigation in the field.

The H7 virus has not been detected during current routine surveillance programmes in China nor during other longitudinal studies over the past ten years (Pepin, Wang *et al.* 2013; Zhao, Pan *et al.* 2013) but was occasionally detected in other countries in the region like the Socialist Republic of Viet Nam (Okamatsu, Nishi *et al.* 2013). More recent samplings conducted in the framework of the emergency surveillance for H7N9 in uninfected countries in South Asia and Southeast Asia showed an extremely low prevalence of the H7 subtype. Additionally, none of the countries in this region vaccinate poultry against H7, therefore the detection of H7-seropositive poultry suggests an exposure to the H7N9 virus. For this reason, in addition to virological surveillance, serological screening is highly recommended as part of a scanning type of surveillance. Seroconversion occurs in under two weeks following exposure. This results in the neutralization of the virus; seroconversion can therefore not differentiate between recent or long-standing exposure unless the levels of paired serum antibodies are compared at least three weeks apart in time. As antibodies remain detectable after infection has been cleared, serological tests provide: (i) baseline exposure rates and (ii) historical evidence of previous virus exposure in the population. Serological testing on imported poultry populations can aid in identifying high priority areas, markets and border points of virus incursion. Once a source has been localized through a systematic tracing of poultry movements, the detection of at least one positive result of serological or virological testing should lead to further investigation in the market chain and at the field level.

Due to the possible seasonal pattern of H7N9 in poultry, a longitudinal approach (i.e. repeated sampling rounds over time) is recommended for areas at risk of incursion. Figure 1 gives the general surveillance plan for high and moderate surveillance priority areas and countries. When surveillance strategy frameworks match, samples collected for routine surveillance of HPAI H5N1 are recommended to be tested for H7N9. Where possible surveillance strategies should be combined to avoid collection of duplicate samples. The only difference would be a greater focus on duck samples for H5N1 but if these are also tested for the avian influenza virus it will allow early detection of H7N9 in domestic ducks.

The surveillance plan developed in this document focuses on H7N9 although virological and serological samples will be used to detect the presence of other avian influenza viruses with the goal of monitoring those of concern to animal and human health. More specifically, the sero-bank of serological surveillance samples should be considered as an investment for detection and risk-assessment of emerging avian influenza viruses for every country, including H7N9 low-priority surveillance countries. The establishment of a subtype baseline (of subtype variety and prevalence during the year) will be useful for the early detection of abnormal patterns of serological profiles and for retrospective analytical studies to identify avian influenza risk factors. Syndromic

surveillance based on the monitoring of production parameters (e.g. unusual drop in egg production) is highly recommended.

#### 4. FREQUENCY OF SAMPLE COLLECTION

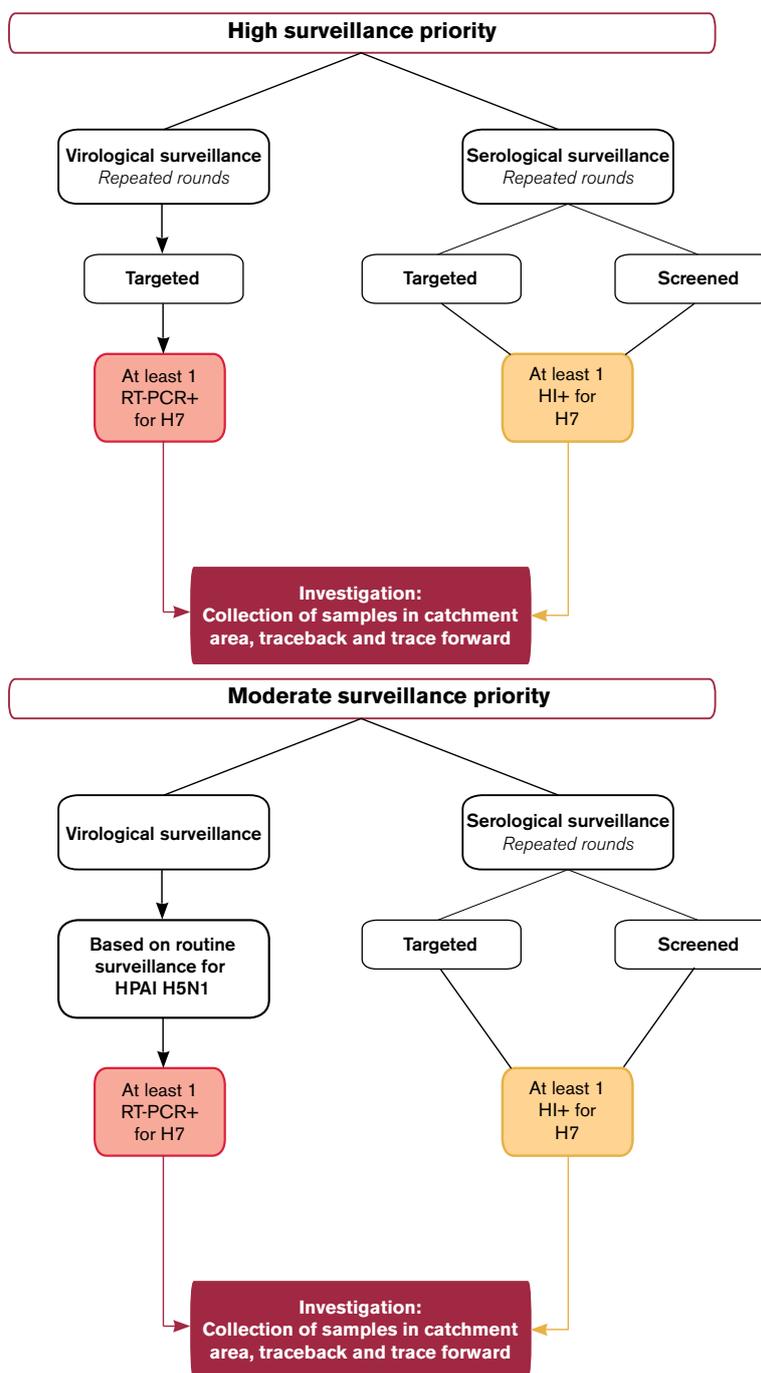
The official incubation period for HPAI given by the World Organisation for Animal Health (OIE) is 21 days. Biologically it can range between 3 and 14 days at individual and flock level, respectively. Sampling frequency will vary according to the epidemiological status of an area or country. The higher the

sampling frequency and number of samples tested and the shorter the interval between sampling, the earlier the detection will be. However, countries will need to adapt the frequency of sampling rounds according to their capacities and available resources. Taking into consideration the experience with other LPAI viruses, delay of immune response and available modelling data, the following frequency of sample collection in LBMs is suggested for each level of priority (Pepin, Wang *et al.* 2013).

Scenario 1: Moderate-priority areas or countries should carry out a *scanning strategy*, because these uninfected areas have no

**Figure 1: Surveillance strategy for H7N9 according to levels of surveillance priority**

Both high-priority (A) and moderate-priority (B) surveillance schemes call for virological surveillance and serological surveillance in recurring rounds of sample collection. Sample collection focuses on sampling that is targeted (e.g. LBMs; farms with direct imports from infected areas) or screening (baseline serology screening in an epi-unit such as a province). In a high-priority situation, the virological surveillance is based on market chain and epidemiological information from affected countries or areas. In the moderate-priority situation, the virological surveillance is based on routine surveillance carried out for HPAI H5N1.



**Table 3: Frequency of sampling**

	Virology	Serology
<b>Scenario 1</b> Uninfected moderate priority areas/countries	Depending on H5N1 surveillance programmes	Once a month (resources permitting)
<b>Scenario 2</b> Uninfected high priority areas/countries	Once every 2 weeks	Once every 1-2 weeks or a minimum of once a month (resources permitting)

direct link with infected areas or countries. A monthly sampling frequency for serological surveillance is reasonable as the probability of H7N9 incursion is moderate.

Scenario 2: High-priority areas or countries should implement an *early-warning strategy*, as an incursion into these uninfected areas is anticipated. The frequency of sample collection should be every one or two weeks, depending on the resources available and laboratory capacities.

The sampling frequencies are summarized in Table 3.

After two surveillance rounds the sampling frequency of further surveillance should be adapted according to these initial results as it may improve the early detection of the onset of an avian influenza epidemic. Any abnormal results (e.g. increase of M gene detection; very high serological incidence) should lead to an increased frequency of sample collection in LBMs. Serological findings should be interpreted with vaccination status of sampled birds taken into consideration, especially for H5. To date, no H7 vaccination is being used.

Given the seasonal pattern of avian influenza viruses previously recognized in some areas, adjustment of the sampling during high-activity periods is recommended.

Laboratory results should be produced within one week of sample receipt to be able to adapt the sampling procedure if needed and to detect H7N9 incursion quickly. Sampling does not have to occur at all sites on the same day but should follow a fixed schedule with the same interval for each site.

## 5. TARGETED POPULATION

The targeted population refers to the population of birds that will be sampled. Regarding the detection of incursion within an area or country, it is recommended to target traded poultry originating from infected areas or countries and local poultry, especially those that are mixing with imported poultry.

The following recommendations on target species are based on current knowledge and will be updated as new information emerges.

Species to consider:

- Chickens: data from China show that to date most positive swabs came from chickens (Lam, Wang *et al.* 2013). Therefore, this species will be a priority target.
- Quails: quails are considered as sentinels and should be collected whenever possible, because: (i) they are highly susceptible to all LPAI viruses; (ii) they are highly susceptible to H7N9; and (iii) they shed high titres of LPAI.
- Ducks: ducks should be sampled at a lower priority, except if new information on H7N9 ecology and hosts (e.g. spillover) are discovered. Ducks, however, are very useful for detecting: (i) H5N1 virus, because they show a higher percentage of positives than chickens in routine market swabs; and (ii) other novel influenza viruses, including zoonotic subtypes (H9).

Production type and breeds to target will vary according to country specificities. Production types to consider are:

- Imported poultry (i.e. live spent-hens, spent quails and live commercial broilers; day-old chicks could be tested serologically for evidence of maternal antibodies);

- Local native poultry from the commercial sector (includes layers and broilers).

## 6. SAMPLING FRAME

### 6.1 Selection of administrative unit

The national surveillance plan should focus on those administrative units where the virus is likely to be introduced from an infected area or country. The administrative unit could be a district or province, or its equivalent.

Ideally, the selection of the administrative unit should rely on risks identified through a regional market chain analysis that: (i) identifies international trade routes from infected areas or countries; (ii) describes qualitatively and quantitatively the type of products, including live birds traded; and (iii) identifies points of sale.

Local knowledge and experience with HPAI H5N1 is required and essential in order to help in the selection of administrative units as well. These units include areas or markets of repeated HPAI H5N1 outbreaks and/or clades of known introduction of new HPAI H5N1.

Administrative units bordering on H7N9-infected areas or countries should also be included in a surveillance plan, especially to capture local legal and illegal trade along the border.

### 6.2 Selection of specific sites for targeted surveillance

#### 6.2.1 Primary sites

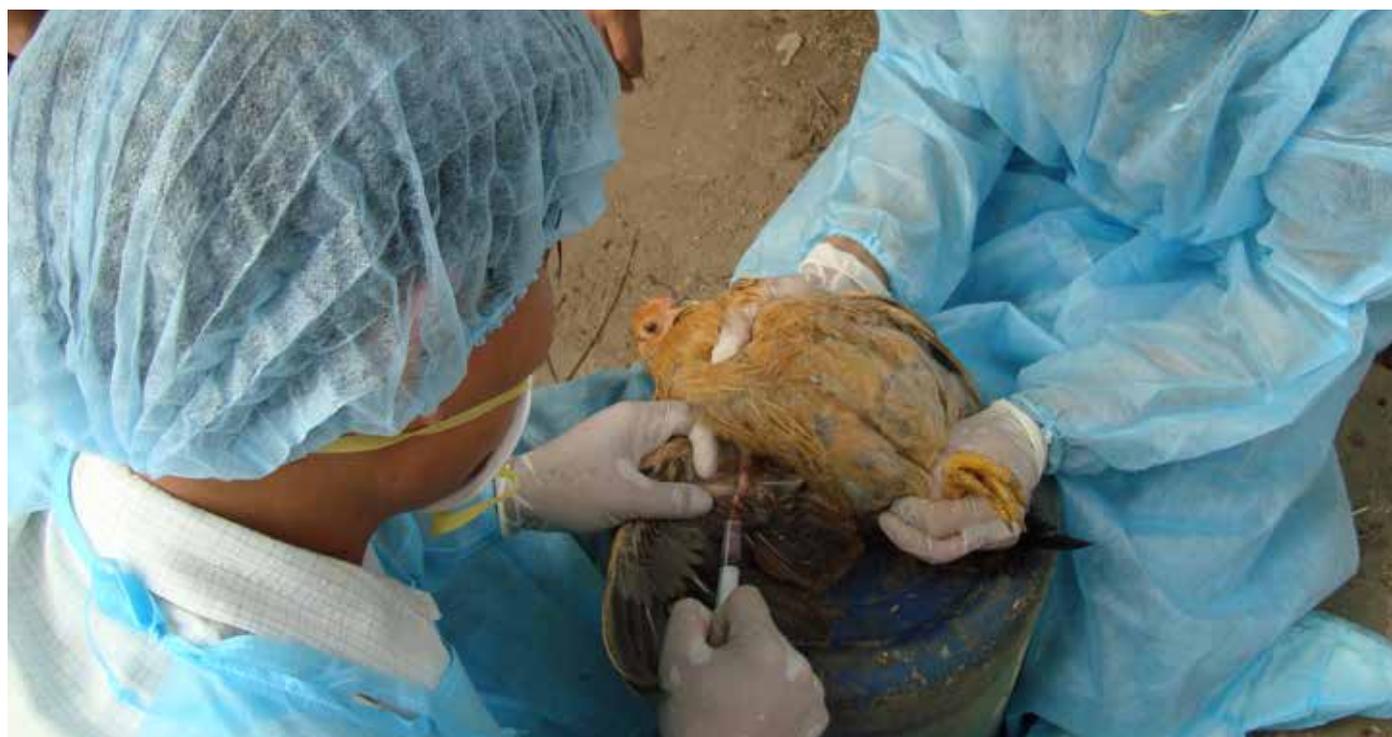
Ports of entry of poultry traded **directly from infected areas or countries** should be included in the surveillance programme in order to detect viruses at their incursion. Depending on country specificities, ports of entry can be LBMs, villages, farms or other types of gathering points with direct imports of poultry from infected areas or countries that are not necessarily along the border. The identification of such sites should be done through a regional market chain analysis. If this is not possible, the selection of ports of entry might also be based on mapping local poultry movement using local knowledge. Sampling of illegally imported poultry should be implemented if possible.

Whenever the port of entry is equipped with slaughter facilities, blood samples should be collected for their serological component in moderate and high-priority countries.

In order to detect an incursion at an early stage, all primary sites of an administrative unit should be visited.

#### 6.2.2 Secondary sites

At a lower priority it is also recommended to investigate the presence of the virus in other LBMs or gathering points where influenza viruses are amplified and maintained (Martin, Zhou *et al.* 2011; Fournie, Guitian *et al.* 2012; Lam, Wang *et al.* 2013; Naysmith 2013). LBMs or gathering points for which the probability of virus infection is the highest should be selected. In order to identify these sites, several criteria can be considered. These criteria have to be adapted according to the level of risk of the area or country and the control measures already in place.



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- **Highly connected sites** in the network of live bird trade and located in urban areas are congregation points. Congregation points accumulate a large proportion of commercial live bird movements in the trade network within at-risk or already affected areas or countries. They gather a high number of different bird species coming from different production systems and geographic areas that are likely to best reflect the general bird population. They act as “sinks” and are likely to be infected. Ideally, this type of site should be identified using social network analysis<sup>1</sup> following a network survey based on a snowball sampling strategy (Wasserman and Faust 1994). Otherwise the selection should be based on the identification of *markets with the highest number of poultry sellers or poultry sold<sup>2</sup> within a given administrative unit* (Martin, Pfeiffer *et al.* 2011; Fournie, Guitian *et al.* 2013). This information can be collected through discussions (i.e. participatory groups or face-to-face interviews) with local district or province authorities and market stakeholders such as market managers or through using logbooks (see Annex 1).
- If highly connected markets cannot be identified, other characteristics of LBMs can be used to identify markets with a high probability of virus infection: markets that have been repeatedly contaminated by other avian influenza viruses in the past and markets associated with human cases of H5N1 HPAI or H7N9 or any other avian influenza virus.

The low biosecurity level of a site should be taken into consideration for priority inclusion in the surveillance programme, including: (i) markets that do not close; (ii) birds kept for over 24 hours; (iii) no regular rest and cleaning days and (iv) housing of waterfowl together with chicken or quail. Markets that make appropriate changes, like no overnight keeping, may be less risky, but these markets can still be included in the surveillance programme to assess the efficiency of the implemented control measure(s). It is also recommended to include sites gathering birds coming from low biosecurity production systems.

Ideally, to detect an incursion early, all sites that fit the primary and secondary criteria detailed above should be visited. The

sample size required to detect an infection rate of 1 percent is estimated at 545 sites, which may be too large for sustained sampling and laboratory capacity. However, the number of sites decreases when the levels of expected prevalence before detection are increasing: 123 sites for an expected prevalence of 5 percent and 60 sites for an expected prevalence of 10 percent<sup>3</sup>.

### 6.3 Selection of specific sites for screening through serological surveillance

In addition to serum collection at ports of entry (see targeted surveillance above), screening through serological surveillance aims to detect virus activity and spread within the local population. It is highly recommended to keep the same sampling procedure between consecutive rounds in order to ensure the comparativeness of serological profiles in space and time and identify the risk factors of spread. There should be no adjustment to prevalence findings after two rounds. A standard sampling procedure should be developed and endorsed in each country.

Good locations for screening serological surveillance are secondary sites with slaughter facilities (see targeted surveillance above) and poultry slaughterhouses. LBMs, gathering points and slaughterhouses offer the advantage of covering large geographical areas with birds coming from various origins. However, it rarely covers the egg production sector. Layer farms can also be randomly selected. Broiler farms should also be collected whenever slaughter facilities are inaccessible and resources are sufficient.

Broiler and layer farms should belong to the catchment area or the geographical region supplying the traders operating in secondary sites. The identification of the catchment area will

1 A social network analysis is a methodology which provides a mathematical characterization of the relationships between entities, in this case markets and farms, in the network of interest.  
 2 Number of sellers and number of poultry sold can be used as proxy for assessing the market connectivity.  
 3 EpiTools© - Sample size required to achieve target confidence of freedom: Sensitivity = 95 percent; Prior confidence of freedom = 95 percent, Probability of introduction during period = 95 percent; Required confidence of freedom = 95 percent; Population size = unknown; Design prevalence = 1 percent, 5 percent and 10 percent as it appears in the main text.



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require information on the origin of poultry and volumes traded, which can be acquired through market chain analysis and logbooks in each LBM. Interviews with local district or province authorities and market stakeholders, like market managers, may also be helpful.

## 6.4 Biological samples

Early detection of virus incursion in an uninfected area or country is most likely successful through the collection of environmental and oropharyngeal swabs. To reduce cost and work load, environmental swabs can be pooled in the field, while oropharyngeal and cloacal swabs are stored separately. However, RNA from these can be extracted in pools. This allows authorities to: (i) collect various locations within the large space coverage of the LBM; and (ii) decrease the cost of analyses by reducing the number of RNA extractions and RT-PCR reactions (Naysmith 2013).

### 6.4.1 Environmental swabs from primary and secondary sites

Environmental swabs may be pooled together in the field. Swabs are pooled in vials, which may contain up to five environmental swabs each from different areas of the market. The following areas should be targeted: drinking water, cages, waste water/drains, faeces, waste bins, processing or display tables, baskets (with the cut parts) or rags in processing areas (Naysmith 2013).

For a pool size of five swabs, a minimum of seven pools per site should be collected and tested to provide 95 percent probability of detecting a H7N9 prevalence of 10 percent on the site (i.e. 10 percent of places are contaminated). This calculation assumes that the virus is amplified in LBMs or gathering points where a rapid spread is due to high animal densities and low biosecurity. Detection at the very early stages of incursion, when prevalence is expected to be low, requires a higher sample size: 13 pools to detect a prevalence of 5 percent and 63 pools to detect a prevalence of 1 percent<sup>4</sup>.

The sampling details should be finalized by each country<sup>5</sup>. The sampling procedure for one LBM might be:

- drinking water: two pools of five swabs for a total of ten swabs;

- waste water/drains: two pools of five swabs for a total of ten swabs;
- cages: two pools of five swabs for a total of ten swabs; and
- faeces: one pool of five swabs.

### 6.4.2 Oropharyngeal and cloacal swabs

Oropharyngeal and cloacal samples should be taken and stored in separate vials with no pooling of swabs in the field. In case of limited resources, priority should be given to oropharyngeal swabs as H7N9 is shed primarily via this route. Pooling of RNA during laboratory testing may be carried out by swab type, species and site, so RNA from up to five oropharyngeal quail swabs from one LBM may be extracted and tested together. Original swab samples from birds should remain separate at all times.

For a RNA pool size of five, with pooling occurring at the laboratory, a minimum of seven pools per site for a total of 35 birds should be tested to provide 95 percent probability of detecting H7N9 prevalence of 10 percent. This calculation assumes that the virus is amplified in LBMs or gathering points where the spread is rapid due to animal densities and low biosecurity. Detection at the very early stages of incursion, when prevalence is expected to be low, requires a higher sample size: 13 pools for a total of 65 birds to detect a prevalence of 5 percent, and 63 pools for a total of 315 birds to detect a prevalence of 1 percent<sup>4</sup>.

### 6.4.3 Dead birds

Collecting routine mortality is strongly recommended at LBMs or gathering points as this is where the LPAI will be found easily. When available, at least five dead birds per site should be collected weekly. Even if biased, this sampling strategy may be much easier to implement especially to get the market owners' cooperation where other biological samples are difficult to get due to lack of cooperation.

4 Epitools© - Sample size to demonstrate freedom using pooled testing: Size of pools = 5; Test sensitivity = 95 percent; Desired herd-sensitivity = 95 percent; Design prevalence = 10 percent, 5 percent and 1 percent as it appears in the main text.

5 Standard guidelines will be developed



#### 6.4.4 Serum samples

Serological surveillance will be conducted on sites with slaughter facilities and/or directly on farms (see above). Hemagglutinin Inhibition test (HI) for H7 can be performed on both serum and eggs\*, according to the laboratory capacities and preferences. In the framework of establishing a baseline of serological profile for H7 and other subtypes of avian influenza viruses with a zoonotic potential, it is recommended to collect enough samples to compute an estimate of seroprevalence for each sampling group at each sampling round. For an expected seroprevalence of 20 percent, 250 birds per type of production should be collected (e.g. 250 serums from broilers at the slaughterhouse and 250 from layers (blood or eggs\*) from markets or farms)<sup>6</sup>. In the framework of an extended monitoring to other avian influenza subtypes with a zoonotic potential, the same number of duck serums should also be collected and analysed. This sample size is large enough to detect at least one seropositive bird for 2 percent of prevalence within each group (detection of H7 at the early stage of seroconversion of the poultry population).

Considering that LPAI viruses are known to efficiently spread within an infected flock to at least 30 percent of birds, it is important to not collect all the birds from the same trader or seller at the market since the birds might come from the same flock. In order to minimize the selection bias and to guarantee a good representation of the population, it is recommended to collect more than five birds per trader, per cage or per group and more than five birds or eggs\* per barn or farm.

For other zoonotic avian influenza subtypes, the total number of birds and the proportion of each species may be adapted according to the seroprevalence of the previous surveillance round (see Annex 3). Whenever the financial resources and the laboratory capacities allow it, it is recommended to increase the sample size, in order to get a more precise result.

#### 6.5 Epidemiological data

A set of epidemiological data will also be collected for every biological sample (see Annex 2).

<sup>6</sup> EpiTools© - Sample size to estimate a proportion with specified precision: Confidence = 95 percent; Desired precision = 5 percent within an infinite local population.

\* HI tests for eggs in development. Protocols will be supplied if successfully validated.

A logbook of traded poultry should be maintained at every LBM by animal health authorities to record all information related to the number and the type of poultry traded, their origin and their destination (home-consumption or to be traded along the market chain) whenever resources are available. A logbook example is provided in Annex 1.

A summary of the whole surveillance protocol for each level of surveillance priority is presented in Table 4.

## 7. LABORATORY TESTING

Each swab will be tested by RT-PCR or virus isolation for avian influenza and the influenza-positive samples tested for H5 and H7 as per the algorithm described in the Food and Agriculture Organization of the United Nations (FAO) document on laboratory procedures for H7N9 (see link below).

- H5 or H7 positive samples should be reported and handled as laid out in the OIE Terrestrial Animal Health Code, Chapter 10.4;
- non-H5/ non-H7 influenza A samples with threshold cycle values under 30 should be inoculated into fertile eggs and all culture positive samples should be subtyped by HI panel and gene sequences as applicable for the testing laboratory;
- if national laboratories do not have the capacity to perform virus isolation and sequencing, H5- or H7-positive samples should be sent to a OIE/FAO reference centre for confirmation; and
- sequencing procedures for other avian influenza viruses should be discussed directly between the national laboratory and the FAO Regional Office for Asia and the Pacific.

Serums will be tested by ELISA for avian influenza antibodies, and the positive samples tested with an HI panel of H7 and avian influenza viruses with zoonotic potential (H3, H4, H5, H9) and if possible for N9 antibodies for samples that are positive for H7 antibodies.

See guidelines for more details: “Addressing avian influenza A(H7N9) - A(H7N9) - Laboratory protocols and algorithms” (<http://www.fao.org/docrep/018/aq251e/aq251e.pdf>)

**Table 4: Recommended sampling procedure for H7N9 short-term surveillance**

Site	Species	High surveillance priority		Moderate surveillance priority	
		Virology	Serology**	Virology	Serology**
Ports of entry: LBMs, gathering points and farms with direct imports from infected areas/ countries (primary sites)	Chicken	35 OCS*	250	-	250
	Quail (when present)	35 OCS*	-	-	-
	Duck	-	-	-	-
	Environment (not in farms)	7 pools	-	7 pools	-
Highly connected LBMs and gathering points (secondary sites)	Chicken (broilers)	35 OCS*	250	-	250
	Quail (when present)	35 OCS*	250***	-	250***
	Duck	-	250***	-	250***
	Environment	7 pools	-	7 pools	-
Farms	Layer chicken	-	250	-	250
<b>Samples from existing surveillance</b>	Chicken, Quail and Duck	Yes	Yes	Yes	Yes
<b>Epidemiological data</b> (logbook and questionnaires)		Yes		Yes	
<b>Frequency</b>		2 weeks	1 month	-	1 month

\* OCS: oropharyngeal and cloacal swabs (35 birds per site)

\*\* total number of serum samples (or eggs for layers) per administrative unit. Serum samples should be collected in sites with slaughter facilities.

\*\*\* Sampling required for monitoring of other avian influenza subtypes

	Highly recommended
	Strongly recommended
	Recommended when resources are available
	Suggested
	Not necessary
	Recommended for other avian influenza subtypes with zoonotic potential

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## ANNEX 1

### Example of Avian Influenza Surveillance Form: Collector Yards

Collector Yard ID:         Collector yard Name: \_\_\_\_\_

Market ID:         Market Name:City: \_\_\_\_\_ Admin Unit: \_\_\_\_\_

Date (dd/mm/year):

GPS coordinates: N         / E         (unit decimal degree)

For each trader, one line should be filled for each farm visited.

Truck ID	Truck Plate number (write down)	How many farms visited during the trip?	Origin				Duration How long have the birds been on the truck?	Number of birds by type					Sample Team ID and Signature	
			Type of place	District	Sub district	Village		Quails	Broiler	Layer	Java ducks	Muscovy ducks		Other
			Farm 1/ CY				Hours							
			Farm 2/ CY				Hours							

## ANNEX 2

### Information sheet for biological samples

**Date:** date that sample was collected: DAY/MONTH/YEAR

**Field ID:** ID number of location where the sample has been collected

**Central lab sample ID#:** Country-specific sequential ID# assigned by testing lab when receiving the field sample (e.g.: Myanmar sample 453 = M-453)

**multiple samples from same animal?** If more than 1 sample was collected from one host (bird/animal) than identify by using a number and a letter

**Collector:** name of individual collecting the sample

**Host scientific name:** as much specific information as possible

**Host common name:** as much specific information as possible

**Bird Type:** WM/ WN/ D/ WU= Wild Migrating/ Wild Non-migrating/ Domestic/ Wild Unknown

**Vending type:** retail live bird market /wholesale live bird market / farm/ brooder hens/ etc.

**Collection site:** City/ State (Province)/ Country/ (latitude & longitude if possible)

**Production system (of origin):** layer/broiler/dual-purpose/breeder/local village poultry

**Age:** H/ A/ U= Hatch year/After hatch year/Undetermined

**Sex:** Male/ Female/ Undetermined

**Sampling situation:** A/ K/ O/ U= Active surveillance/ Killed/ Outbreak/ Undetermined

**Vaccination status regarding H5 and H7:** date of last shot

**Health status:** H/ S/ D/ U= Healthy/ Sick/ Dead/ Undetermined

**Sample Type:** C/ OP/T/ B/ F/ E(C/W/D/P/B/T/R) = Cloacal/ Oral-pharyngeal (tracheal- oropharyngeal)/ Tissue\*\*/ Blood/ Fecal/ Environmental(Cages/Water/Display table/Processing table/Basket (with cut parts)/trash bin/rags in processing)

**Results from central lab:** FluA/H7/H5/unclear/negative

\*\*Be specific as to where the tissue was collected i.e. brain, lungs, etc.  
only for outbreak situation

Responsibility of COLLECTION TEAMS													Responsibility of CENTRAL TESTING LAB				
Date collected (D/M/Y)	Field Vial #	multiple samples from same animal?	Collector	Host Scientific Name	Host Common Name	Bird Type	Vending type	Collection site	Age	Sex	Sampling situation	Health Status	Sample Type	central lab sample ID #	Date received (D/M/Y)	Results from central lab (FluA/H7/H5/un)	Date results (D/M/Y)
11/05/2013	mtk-1	/	Ms. Smith	gallus gallus	domestic chicken	D	spent hen	Yangoon market A	A	F	A	H	B	M-13-1	11/07/2013	nonH5, nonH7	11/11/2013
11/05/2013	mtk-2	/	Ms. Smith		quail	D	retail	Yangoon market A	U	F	A	H	B	M-13-2	11/07/2013	H5, nonH7	11/11/2013





## CONTACT :))

The Emergency Prevention System (EMPRES) is an FAO programme, founded in 1994, with the goal of enhancing world food security, fighting transboundary animal and plant pests and diseases and reducing the adverse impact of food safety threats. EMPRES-Animal Health is the component dealing with the prevention and control of transboundary animal diseases (TADs).

To subscribe or to ask for information about EMPRES-Animal Health send an e-mail to:

**empres-animal-health@fao.org** or a fax to **(+39) 06 57053023**

For more information visit us at <http://www.fao.org/ag/empres.html>

EMPRES-Animal Health can assist countries in the shipment of samples for TAD diagnostic testing at a FAO reference laboratory and reference centre. Please contact **Empres-Shipping-Service@fao.org** for information prior to sampling or shipment. Please note that sending samples out of a country requires an export permit from the Chief Veterinarian's Office of the country and an import permit from the receiving country.



These guidelines are based on the information available to date and will be reviewed as new information becomes available.

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