

## GUIDING PRINCIPLES FOR HIGHLY PATHOGENIC AVIAN INFLUENZA SURVEILLANCE AND DIAGNOSTIC NETWORKS IN ASIA

### 1. INTRODUCTION

#### 1.1 Purpose

The purpose of this document is to provide guiding principles and minimum requirements for surveillance and diagnosis of H5N1 highly pathogenic avian influenza (HPAI) that can be applied by countries and regional networks. These principles and minimum requirements are also broadly applicable to other avian influenza (AI) viruses. These guidelines may not be directly applicable to surveillance for low pathogenicity AI (LPAI) viruses. Recommendations of the Office International des Epizooties (OIE, which is the world organization for animal health) ca recommendations on LPAI should be read in conjunction with this document.

The control of H5N1 HPAI in Asia requires an understanding of the behaviour and ecology of influenza viruses generally and of this subtype in particular. It is also important to understand local poultry production and marketing systems, and how these affect the development and course of HPAI. Without a proper understanding of these factors, attempts to control or eradicate the disease will fail.

Much of the current understanding of AI (and the recommendations of international organizations) is based on information from temperate climates, such as Europe and North America. Although there is much that is useful and applicable in this information, poultry production systems differ significantly in Asia, and these differences must be taken into account in designing and implementing surveillance and diagnostic systems.

Although effective surveillance and diagnosis are critical to the control of HPAI, other important measures include:

- Rapid, humane destruction of infected poultry and poultry at high risk of infection.
- Disposal of carcasses and potentially infective material in a biosecure and environmentally sustainable manner.
- Enhanced biosecurity at poultry farms and associated premises, including movement of personnel.
- Control of movement of birds and products that may contain virus.
- Changes to industry practices to reduce risk (e.g. segregation of different poultry species in production and marketing systems).
- The use of vaccination.

All of these measures are risk reduction measures; none implemented singly is sufficient to ensure the control or eradication of HPAI. This document provides minimum requirements for HPAI surveillance and diagnostic networks relevant to the following scenarios in countries or compartments:

- Countries/compartments free from infection.
  - not practising vaccination; wanting to detect early incursions of virus
  - at high risk of infection; practising vaccination.
- Previously infected countries/compartments that wish to demonstrate freedom.
- Infected countries/compartments.
  - not vaccinating.
  - vaccinating.

This paper uses the current draft OIE definition of a *compartment* as an autonomous epidemiological entity defined on the basis of either geography (*zone*) or management (*enterprise*) [for the purpose of international trade].

## 1.2 Background on H5N1 in the region

There are several important differences between HPAI in Asia and the AI situation in other parts of the world, and these must be addressed if countries in the region are to be successful in controlling the disease:

- HP H5N1 viruses have been in the region for more than eight years; they were first found in waterfowl in 1996.
- Their presence has been documented in farmed ducks and live bird markets since 1997.
- Outbreaks of disease reported were relatively limited (Hong Kong in 1997, 2001, 2002 and 2003) before the epidemic in poultry reported during 2003–04.
- Human cases of disease associated with H5N1 viruses occurred in 1997, 2003 and 2004.
- All H5N1 isolates in the region have been highly pathogenic (HPAI) in chickens, including viruses isolated from waterfowl and wild birds.
- There is no evidence that events reported in Europe and North America (where LPAI viruses transfer from domestic waterfowl to chickens and become highly pathogenic) have occurred in Asia.
- HPAI viruses may cause disease in ducks, but infection in ducks frequently occurs without causing any abnormal clinical signs. Due to the highly contagious nature of HPAI, the risk of infection spreading to uninfected areas can only be addressed using regional coordination and a regional network approach.

## 1.3 Poultry Production in Asia

Poultry production is very important in Asia as a source of dietary protein and as a source of income. Poultry production systems in Asia take many forms, from industrialized, highly integrated broiler production systems to village-based production of chicken meat and eggs from 'scavenging' poultry of local breeds. The marketing of chickens and waterfowl (sometimes including wild birds and ornamental species) in live bird markets is a common practice in many countries of the region,

and this presents particular biosecurity challenges and risks to human health. The husbandry of fighting cocks has been associated with human cases of H5N1 infection and the specialized nature of this ‘industry’ presents particular challenges to surveillance and disease control. Variations in the proportion of poultry production in the different sectors must also be taken into account. The quality and capacity of veterinary services and the extent to which the country trades poultry products internationally also vary greatly within the region, and are highly relevant in designing an effective surveillance and diagnostic network. OIE describes ‘quality of veterinary services’ in Chapter 1.3.3 of the OIE *Terrestrial Animal Health Code*. Relevant factors are in Part 1 (notification, principles of certification, etc.) and in the ‘Guidelines for the evaluation of Veterinary Services’ in Chapter 1.3.4.

**Farmed poultry** include all domestic birds raised for food production, including chicken (domestic fowl: *Gallus domesticus*), quail, pigeon, chukar (partridge), pheasant, guinea fowl, silkie chicken, turkey, duck, muscovy and goose. **Domestic waterfowl** include ducks, muscovies and geese farmed for meat and egg production.

This paper draws on the definitions of poultry production sectors in the publication *Types of Farming Practices in Asia* (February 2004). The level of biosecurity practised in each sector is a key consideration in developing an effective approach to surveillance. Production sectors are defined as follows:

*Sector 1:* Industrial integrated system with high level biosecurity and birds/products marketed commercially (e.g. farms that are part of an integrated broiler production enterprise with clearly defined and implemented standard operating procedures for biosecurity).

*Sector 2:* Commercial poultry production system with moderate to high biosecurity and birds/products usually marketed commercially (e.g. farms with birds kept indoors continuously; strictly preventing contact with other poultry or wildlife).

*Sector 3:* Commercial poultry production system with low to minimal biosecurity and birds/products entering live bird markets (e.g. a caged layer farm with birds in open sheds; a farm with poultry spending time outside the shed; a farm producing chickens and waterfowl).

*Sector 4:* Village or backyard production with minimal biosecurity and birds/products consumed locally.

#### **1.4 Objectives of HPAI Surveillance and Diagnostic Networks**

Although the objectives of HPAI surveillance and diagnostic networks may be described in general terms, as outlined below each country has particular priorities, and its surveillance system should be refined to reflect these priorities. For example, HPAI-free countries will seek access to detailed, updated information on risks and will focus on the detection of incursions — making early warning and surveillance at international borders their priority. For infected countries, surveillance priorities will include the collection of detailed, current information on human health risks. Infected

countries that have free compartments that may export to other countries will place emphasis on surveillance within and around these compartments.

OIE guidelines are particularly relevant to countries and compartments involved in international trade and should be read in conjunction with this document.

The objectives of HPAI surveillance and diagnostic networks include:

- To detect clinical disease and infection.
- To understand the epidemiology and ecology of AI, as well as its socioeconomic impact, to help to design effective control programs for poultry production systems.
- To assess the temporal and spatial patterns and thereby to improve the effectiveness of control efforts.
- To understand the evolution in Asia of HPAI virus variants.
- To help define and control risks to public health.
- To monitor for antigenic drift in AI viruses.
- To maintain the viability of subsistence level poultry production and help assure food security.
- To demonstrate freedom from clinical disease and absence of infection in a country or compartment and thereby facilitate trade.
- To assess the efficacy of vaccination (where used).

Although the focus of this document is on surveillance in avian species, the role of other species (including pigs) in the epidemiology of HPAI is of interest and a topic for future research (see Annex 4). These guidelines thus provide advice on circumstances in which the testing of pigs for HPAI virus may assist HPAI control efforts (see Annex 4).

## **2. MINIMUM REQUIREMENTS FOR EFFECTIVE SURVEILLANCE**

The following minimum requirements apply to all countries and compartments:

- HPAI must be notifiable (i.e. there should be a legal requirement for suspected cases of disease to be reported to the official veterinary services).
- The official veterinary services must have a formal system for detecting and investigating outbreaks of disease (see Annex 1) and for reporting confirmed cases internationally, in accordance with OIE guidelines.
- The country and/or region must have the technical capability to diagnose HPAI (see Annex 2).
- The country and/or region must have a system for recording, managing and analysing diagnostic and surveillance data (see Annex 3).
- The country should participate in the regional surveillance and diagnostic network, including the public health sector, to enable sharing of information to characterize risk, prevent disease spread, and enhance control efforts.
- The frequency of surveillance could be a minimum of every six months within a country or could be less than this if selected 'pilot' areas are targeted for more frequent surveillance.

## **2.1 Surveillance Systems for Countries/Compartments that are Free of Infection**

### **2.1.1 Countries/compartments free of infection wanting to detect early incursions of virus**

Countries/compartments that are free of disease should consider implementing specific surveillance activities in the following higher risk sites or situations to provide early warning of infection (i.e. before disease occurs in terrestrial poultry farms):

- Borders and international entry points (particularly those adjacent to infected countries).
- Domestic waterfowl.
- Unusual mortality in wild birds.
- Live bird markets.

Relevant surveillance strategies are described in Annex 1.

### **2.1.2 Countries/compartments that are free of infection and are practising vaccination**

The country/compartments that is free of infection but practising vaccination must have a defined vaccination policy that is implemented under control of the official veterinary services and meets relevant international guidelines.

Key elements include:

- The type, quality and source of vaccine(s) permitted for use.
- Vaccination protocols employed (species vaccinated, frequency of vaccination, biosecurity measures used to separate vaccinated and unvaccinated populations).
- Systems employed to identify vaccinated places/flocks.
- System for monitoring the implementation of the vaccine policy, including efficacy of vaccination and detection of field virus in vaccinated flocks (see Annex 1).
- Laboratory systems and scientific analysis used to develop and implement vaccination policies (including vaccine development, testing of imported vaccine(s), quality assurance, and molecular characterization of field isolates).
- A system for risk assessment to evaluate, at defined intervals, the need for the continuing use of vaccination.
- Participation in the surveillance and diagnostic network, to enable sharing of information needed to characterize risk, prevent disease spread, and enhance sub-regional and regional control efforts (see Annex 3).

## **2.2 Previously Infected Countries/Compartments that Wish to Demonstrate Freedom**

Veterinary authorities wishing to demonstrate that their country or relevant compartment(s) are free of HPAI would be required to compile a dossier of validated information, based on relevant international guidelines, substantiating their claim to freedom (see Annex 1). Current OIE-endorsed guidelines should also be consulted.

## **2.3 Infected Countries/Compartments**

### **2.3.1 Infected countries/compartments not practising vaccination**

Surveillance should be targeted to the following high risk areas and populations using methods described in Annex 1:

- Domestic waterfowl.
- Unusual mortality in wild birds.
- Live bird markets.
- Sentinel villages.

Molecular characterization of all isolates and molecular epidemiological studies should be performed, drawing on the expertise available in the regional network and in international reference laboratories as required. Isolates of virus must be provided to international reference laboratories and molecular data uploaded to international gene sequence databases (e.g. Genbank)

Detailed epidemiological surveillance and analysis must be performed, drawing on the expertise available in the regional network and in international reference centres, as required.

### **2.3.2 Countries/compartments that are endemically infected and practising vaccination**

The main objectives of surveillance in these countries/compartments are to ensure that vaccinated birds achieve protective levels of immunity and that field viruses in these countries/compartments are detected and fully characterized as per Section 2.3.1 above. Details of the specific requirements for these countries/compartments are outlined in Annex 1.

## **3. ESSENTIAL ELEMENTS OF SURVEILLANCE AND MONITORING OF OTHER ANIMALS**

### **3.1 Fighting cocks**

No additional surveillance is recommended for fighting cocks other than investigation of mortalities, unless vaccination is being used in this compartment. If vaccination is being used, the conditions outlined under Section 2.1.1 (HPAI-free) or Section 2.3.2 (endemically infected), as appropriate, should apply.

### **3.2 Song birds**

No additional surveillance is recommended for song birds other than investigation of unusual mortalities.

### **3.3 Wild birds for consumption**

Wild birds should not be sold in markets with domestic poultry, for the purpose of protecting both animal and public health. It is recommended that authorities prohibit this practice. Where wild birds are sold in markets, targeted surveillance of sick/dead birds and/or cage swabs should be conducted (see Annex 1).

## **4. MEASURES RELEVANT TO RESTOCKING AFTER DEPOPULATING AN INFECTED FLOCK**

For restocking after depopulating an infected flock, the measures to be adopted should be modified depending on whether or not HPAI is considered to be endemic in the country/compartments. In countries/compartments in which HPAI is endemic, consideration should be given to vaccinating replacement stock (particularly for Production Sectors 3 and 4).

### **4.1 Production Sectors 1 and 2**

Restocking may be permitted in accordance with the following measures.

- Cleaning and disinfection has been completed and verified according to official veterinary services' protocols that are consistent with relevant international guidelines.
- Biosecurity has been enhanced to meet official protocols of veterinary services, consistent with relevant international guidelines.
- The premises has been empty of poultry for a minimum period of 3 weeks from the completion of cleaning and disinfection.
- Replacement birds are sourced from flocks for which there is a high degree of confidence that infection is not present (e.g. Production Sector 1 flocks, in areas where infection has not been reported).
- Specific monitoring and veterinary investigation of mortalities should be implemented (see Annex 1).

### **4.2 Production Sectors 3 and 4**

Restocking may be permitted in accordance with the following measures:

- Cleaning and disinfection carried out according to minimum standard appropriate to the conditions at the farm or village (no faeces no feathers, at least in sheds/cages).
- Where possible, enhance biosecurity.
- The premises have been empty of poultry for a minimum of 6 weeks from the completion of cleaning and disinfection.
- Premises should not be restocked if there is evidence of infection in flocks within 3 km, unless other measures (e.g. vaccination) have been implemented.

- Birds of known health status should be used to restock (e.g. at a minimum, from flocks thought to be free of disease or, preferably, from flocks for which there is a high degree of confidence that infection is not present; or vaccinated birds).
- Specific monitoring and veterinary investigation of mortalities should be implemented (see Annex 1).

## **5. ANNEXES**

Annex 1	Surveillance methods
Annex 2	Laboratory procedures and networks
Annex 3	Animal health information systems and networks
Annex 4	Research priorities

## ANNEX 1: SURVEILLANCE METHODS

This annex identifies minimum requirements for data collection, management and analysis to ensure effective surveillance for HPAI in Asia. Countries may choose to adopt more intensive methods, depending on epidemiological considerations, industry characteristics, and available resources. It is important for countries to collaborate and share key information to build and maintain a viable and sustainable regional network. Recommendations of international organizations (OIE/WHO) should also be taken into consideration, as appropriate to the local circumstances.

Surveillance comprises both active and passive methods. Active surveillance is based on specific targeted investigation of at-risk populations for evidence of infection that may be based on detecting exposure to the agent (antibody detection by serology) or the presence of the agent (virus or antigen detection). The methods used must be modified according to the epidemiology of the disease. The first part of this annex describes the specific requirements for active surveillance in a country/compartiment, as appropriate to the circumstances, for HPAI.

### 1. MINIMUM REQUIREMENTS FOR DETECTION OF DISEASE

Recommended ‘trigger points’ are defined below. These are the criteria that would trigger a disease investigation by official veterinary services. These criteria essentially describe ‘unusual circumstances’, in terms of ‘normal’ poultry production in the four production sectors identified in this paper. The normal or expected fluctuations for food/water consumption and mortality will vary from country to country, and according to the production sector. Official veterinary services, in consultation with industry and poultry production specialists, should confirm the values that are valid for their countries/production sectors and apply them as recommended in this annex. This validation could be done by means of consultation or small surveys. A similar process should be employed to identify norms and trigger points for other poultry species.

If the tolerance for variation (e.g. level of mortality) is too high there will be a loss of sensitivity (i.e. disease may be missed). Conversely, if the tolerance is too small the capacity of veterinary services, including diagnostic laboratories, may be overloaded without yielding useful information on HPAI.

If trigger points are exceeded, the official veterinary services should undertake a field investigation. The data identified in Annex 3 should be collected and analysed. If the history and clinical signs do not rule out HPAI, appropriate samples (see Annex 2) must be sent to the laboratory for exclusion or confirmation of HPAI.

#### Trigger points for chickens in each production sector

Sector	Trigger point for chickens
Production Sector 1	Food and water intake reduced by 20% for one day; or mortality of 1% for 2 days
Production Sector 2	Daily mortality of 1% for 2 days
Production Sector 3	Daily mortality of 1% for 2 days
Production Sector 4	Daily mortality of 5% for 2 days

## **2. SURVEILLANCE IN UNINFECTED COUNTRIES/COMPARTMENTS**

### **2.1 Countries/compartments free from disease wanting to detect early incursions of virus**

#### **Border areas**

Methods that could be used in border areas to detect infection include:

- Inspection of transport vehicles carrying poultry for dead or sick poultry (if dead birds are detected, collect cloacal swab for virus isolation).
- Surveillance of live bird markets.
- Surveillance of slaughterhouses.
- Targeted surveillance on selected farms in Production Sector 3 and 4, especially those near roads or wetlands.

#### **Domestic Waterfowl**

For unvaccinated domestic waterfowl with no evidence of infection, serological testing should be undertaken to assess whether birds may have been exposed to H5 virus (using a screening test).

In a sample of farms in the area, test individual farms at a level to give a 95% probability of detecting at least one seropositive bird if infection is present above 20%. Statistical tables should be consulted to derive the appropriate number of samples for flocks of different sizes (e.g. 14 samples for flocks of 500 birds).

If seropositive, then perform virus isolation on cloacal swabs (pools of 5 swabs per sample bottle) at a level to give a 95% probability of detecting at least one virus positive bird if 2% of ducks are excreting virus (e.g. 100 swabs for flocks of 500 birds). As a supplement, seronegative sentinel ducks could be introduced to the farm and cloacal swabs collected twice weekly for three weeks.

#### **Dead wild birds**

In some countries, H5N1 virus has been detected in dead wild birds. Investigation of unusual mortalities in wild birds can provide an early indication of HPAI infection. Cloacal swabs should be collected as outlined above.

#### **Live bird markets**

Select several live bird markets for sampling based on perceived risk (large throughput, mixed species (including waterfowl), markets near borders. Collect samples (and test as per Section 2.1) from dead birds, according to a protocol such as:

- weekly collection;
- monthly collection;
- all dead birds in one week; or
- dead birds if increase in mortality 50% above normal.

'Cage swabs' (swabs of fresh faecal material from cages use to hold birds in markets) should be collected in selected markets once per month. To enhance the probability of isolating a virus, dirty cages or cages housing mixed species of poultry (especially waterfowl) should be targeted.

A sufficient number of samples should be collected to give a 95% probability of detecting infection if the virus is present in 2% of samples. Samples may be pooled (5–10 per bottle).

## **2.2 Countries/Compartments Free of Disease at High Risk of Infection Practising Vaccination**

### **2.2.1 Monitoring for vaccine efficacy**

Authorities should test a minimum of 14 vaccinated birds per flock of vaccinated poultry (by HI) at least 14 days after vaccination

### **2.2.2 Detecting infection in vaccinated flocks**

Sentinel birds (Production Sectors 1 and 2): In each flock a minimum of 30 poultry should be left unvaccinated and must be permanently identified (e.g. leg-band, wing-band). Any deaths in sentinel poultry must be reported and cloacal samples collected and tested (as per Section 2.1). Sentinels should be bled before slaughter and tested by HI and further investigations undertaken if a positive sample is detected.

Farm surveillance for Production Sector 3: The farmer is required to report dead birds to veterinary authorities who will collect samples for virus isolation from dead birds if mortalities exceed 1% for two days.

Farm surveillance for Production Sector 4: Farmers should be required to report dead birds to veterinary authorities who will collect samples for virus isolation from dead birds if mortalities exceed 5% for two days.

Note that serological tests are being used in some parts of the world to differentiate vaccinated animals from infected animals. It would be desirable that these tests (DIVA, by fluorescent antibody test or neuraminidase inhibition test: see Annex 2) be available at the national network laboratory.

## **3. COUNTRIES/COMPARTMENTS (PREVIOUSLY INFECTED) WISHING TO DEMONSTRATE FREEDOM**

The dossier would be built over a period and would contain a range of information, including:

- Details of biosecurity measures in place to prevent the entry of H5N1 virus.
- Details of surveillance carried out on the population of birds that will give confidence that there is no infection with H5N1 virus in the compartment.
- Surveillance information, including results of investigations of mortalities, serological surveillance of populations, and a defined ongoing surveillance plan.
- Information on the structure and activities of the veterinary services.

- Information on data collection and information management systems.
- Legal provisions for compulsory notification of the disease.

#### **4. SURVEILLANCE IN INFECTED COUNTRIES/COMPARTMENTS**

##### **4.1 Infected countries/compartments not practising vaccination**

Targeted surveillance of high risk areas and populations:

Domestic waterfowl (where these occur): as per Section 2.1.

Dead wild birds: as per Section 2.1.

Live bird markets (where these occur): as per Section 2.1 (except that markets do not need to be near the border)

Poultry in Production Sector 4: Conduct pilot studies using sentinel villages to gather information on village poultry populations and mortalities and to test for presence of virus. Select a small number of sentinel villages and investigate mortalities over each of the four seasons working through the community animal health worker. This may require collection of samples for virus isolation from dead birds over a one week period per season (see Section 2.1).

##### **4.2 Countries/compartments (endemically infected) practising vaccination**

The following are the minimum requirements for vaccinated poultry in endemically infected countries/compartments. The requirements are as outlined in Section 2.1, with a particular focus on areas where disease has not been seen previously and in vaccinated flocks that develop clinical disease suggestive of HPAI. Testing of wild birds and domestic waterfowl could also be undertaken (as per Section 2.1).

Monitoring for vaccine efficacy: Test a minimum of 14 vaccinated birds per flock by HI at least 14 days after vaccination in randomly selected flocks. It is not necessary to monitor every flock. Veterinary authorities must keep and analyse records of vaccine performance, investigate unsatisfactory results and implement corrective measures.

Detecting infection in vaccinated flocks: For Production Sectors 1 and 2, sentinel chickens can be used to monitor for any infection in vaccinated flocks. In each flock a minimum of 30 poultry should be left unvaccinated and must be permanently identified. Any deaths in sentinel poultry must be reported to local authorities and cloacal samples collected from selected flocks and tested (as per Section 2.1). It may not be feasible to test all dead sentinel birds. Veterinary authorities need to investigate sufficient numbers to ensure that they detect the emergence of variant strains of virus.

Targeted surveillance of live bird markets (where these occur) should be undertaken (as per Section 2.1, except that markets do not need to be near the border).

Surveillance of Production Sector 4 should be undertaken in selected villages as outlined in Section 2.3).

Note that serological tests are being used in some parts of the world to differentiate vaccinated animals from infected animals. It would be desirable that these tests (DIVA, by fluorescent antibody test or neuraminidase inhibition test: see Annex 2) be available at the national network laboratory.

## **ANNEX 2: LABORATORY PROCEDURES AND NETWORKS**

This annex provides guidelines on technical and other matters associated with diagnostic procedures for avian influenza (AI) and the establishment and operation of a regional laboratory network for AI. It does not attempt to define the fine detail, as some issues are to be determined by the individual jurisdictions. Aspects of rapid screening tests for virus, confirmatory tests for virus detection, virus characterization and serological testing are outlined. Table 1 outlines the recommended minimal capabilities and the ideal additional capabilities for sub-national, national, regional network, and OIE/FAO reference laboratories with respect to HPAI testing.

### **1. DIAGNOSTIC PROCEDURES**

#### **1.1 Initial Screening Tests**

##### **1.1.1 Rapid direct antigen detection tests**

- The Directigen® test is at present the most reliable rapid direct antigen detection test, but it is quite expensive (US\$12–\$15 per test).
- It is a group-specific test, and results are available in 20 minutes.
- It is reliable in ill or dead birds and very sensitive if used on lung fluid (not recommended for general screening of clinically normal birds).
- A number of samples should be tested to increase flock sensitivity.
- Other cheaper rapid tests are being developed in the region, but validation is required.
- The SD Bioline® test from Korea, no independent data on sensitivity and specificity.
- Innova® and Vetsma® (new Thai tests) are cheaper. Evaluation done by DLD Thailand for H5N1 samples suggests the tests have similar sensitivity to Directigen, but requires formal validation.
- The need to validate a cheaper rapid antigen detection test is a recognized research issue for the region (see Annex 4).

##### **1.1.2 Immunofluorescence tests**

- Useful on impression smears.
- Detects group antigen.
- H5N1 antigen in comb and spleen in abundance in H5N1 infection.
- Smears need to be prepared at time of necropsy, unless organs can be transported chilled within a few hours to the laboratory.
- Relatively quick and cheap test to perform.
- Requires fluorescence microscope in good condition.
- If experienced with other IFAT (e.g. rabies), staff can quickly adapt.
- IFAT on faecal samples has been introduced in one country, but there is no information on the apparent sensitivity or specificity of the test.

## **1.2 Confirmatory Tests**

### **1.2.1 Virus isolation**

- Isolation is the basic minimum requirement for virus detection.
- Currently, veterinary services in some countries do not have the capacity to do virus isolation.
- Specimens can be pooled to reduce the cost of surveillance (e.g. 5–10 samples can be pooled).
- Tracheal and cloacal swabs, and lung and spleen specimens, are samples of choice for H5N1 (Note: it is best not to pool ‘unlike’ samples).
- For live ducks, cloacal swabs are required; if dead, lung and brain samples are also required.
- Specimen in transport medium (PBS, buffered tissue culture medium, or tryptose broth with antibiotics if possible) on ice to laboratory within 48 hours of collection.
- Inoculation into specific pathogen free (SPF) embryonating eggs, but commercial eggs from known unvaccinated source free of AI can be used as well.
- At least two passages four days apart should be attempted before a test declared negative.
- If positive, the earliest diagnosis is 24 hours; alternatively, a maximum 10 days if negative. Recent reports suggest that some samples from infected, clinically normal birds may prove positive until the second passage.
- Thailand, Vietnam and Indonesia have several provincial laboratories to support diagnosis, with virus isolation facilities available in addition to the facilities available at central diagnostic laboratories.
- Care must be taken by operators to ensure that work practices do not lead to exposure of laboratory staff. Class 2 biosafety cabinets must be used for preparation, egg inoculation and allantoic fluid harvesting during virus isolation work.

### **1.2.2 Gene sequence detection**

- Reverse transcriptase polymerase chain reaction (RT-PCR) and real-time reverse transcriptase PCR (RRT-PCR) are fully validated and can be used for H5, H7 and H9.
- Some prefer to run matrix protein gene detection to detect any type A virus and then do a HA-specific probe to detect quickly any other strains of AI viruses in circulation.
- In some laboratories. RRT PCR has been shown to be as sensitive as egg inoculation for detection of virus.
- PCR-based tests can drop in sensitivity if there is a lot of bacterial contamination and so work more reliably on tracheal and tissue samples.
- Such tests have been used in Hong Kong for routine surveillance as well as on clinical samples.

- Primers are made commercially but are relatively expensive
- RNA extraction kits are also expensive.
- Where RT PCR is used, careful preparation of samples and reagents is required to avoid contamination with traces of amplified DNA.
- This is very important where critical decisions are based on laboratory results of RT PCR
- RRT PCR system has fewer problems with laboratory contamination because a closed tube system can be used.
- Requires relatively sophisticated and expensive equipment.
- The NASBA test developed in Hong Kong is also very sensitive but the kits are expensive. However, NASBA has advantages terms of reducing the risk of laboratory contamination with amplified DNA.

### **1.3 Procedures for Characterization of Isolates**

#### **1.3.1 Haemagglutinin typing**

- Haemagglutinin (HA) typing is carried out on allantoic fluid that shows haemagglutinating activity.
- It requires a panel of reference sera to likely virus subtypes (H5, H9 and NDV as a minimum for this region).
- It is a relatively simple procedure that does not require any sophisticated equipment.

#### **1.3.2 Neuraminidase typing**

- Neuraminidase (N) typing is carried out on allantoic fluid when haemagglutinating activity inhibited by reference H type serum.
- It requires a panel of reference antisera for likely N types.
- It incorporates a biochemical assay that requires specific skill and hence training.

#### **1.3.3 Gene sequence detection and analysis**

- Specific primers for H and N types can be used for RT PCR and RRT PCR, but this does not provide fine detail.
- Further genetic analysis requires access to a DNA sequencer.
- Gene sequence detection and analysis is a relatively expensive process.
- It requires skills in database access and experience with associated analytical software.
- It enables the characterization of viruses as highly pathogenic or potentially highly pathogenic from the genetic sequence of the cleavage site of the HA gene.
- These analyses provide powerful information that enables epidemiological relationships of the virus to be established.

### 1.3.4 Live bird challenges

- The intravenous pathogenicity index (IVPI) enables classification of the virus as HPAI or LPAI.
- It requires live bird challenge and hence P3-level animal handling facilities.
- For current H5N1 viruses, it requires operator protection when conducting live bird experiments.

Note the **definition of HPAI** (according to the *OIE Manual of Standards for Diagnostic Tests and Vaccines*):

1. Any influenza virus that is lethal for six or more of eight 46-week-old susceptible chickens with 10 days following intravenous inoculation with 0.2 mL of 1:10 dilution of a bacteria-free infective allantoic fluid, or that has an IVPI of greater than 1.2.
2. Any H5 or H7 virus that does not meet the criteria in item 1 above, but has an amino acid sequence at the haemagglutinin cleavage site that is compatible with HPAI viruses.
3. Any influenza virus that is not an H5 or H7 subtype that kills one to five chickens in the test described above and grows in cell culture in the absence of trypsin.

## 2. SEROLOGICAL ASSAYS

Serological surveillance as part of control programmes to detect circulation of current H5N1 virus is considered to be of limited value because most infected chickens die before they develop a detectable antibody response; most birds surviving outbreaks are likely not to have been infected. However, there is a need for some studies to investigate whether a significant number of native breed chickens survive outbreaks and this has been identified as a research issue (see Annex 4). More information is required about the characteristics of the antibody response of waterfowl to H5N1 infection. Subtype specific serology is required to detect antibody responses to vaccination and is necessary to monitor vaccination programmes. The use of the DIVA approach involves testing for antibody to the neuraminidase of the heterologous vaccine and the field strain. In some instances, for additional quality assurance, breeder flocks and sentinel birds might be checked for specific HI antibody. When an area is considered free from infection, serological surveillance can be used to provide an additional level of certainty that virus is not longer circulating.

### 2.1 Haemagglutination Inhibition Test

- The haemagglutination inhibition (HI) test is the subtype-specific test recommended.
- It is sensitive and specific when an epidemiologically appropriate antigen is used.
- It can be used for monitoring the response to vaccination and, where birds survive infection (e.g. with LPAI or HPAI in ducks), to monitor circulation of virus.

- It can be used to monitor previous infection in waterfowl, although in ducks the sensitivity of the test and the duration of HI antibody responses following H5N1 infection are not known.
- Some problems can occur with non-specific inhibitors of haemagglutination, especially with duck sera, so it is useful to have a second test to confirm positive results.

## **2.2 Agar Gel Immunodiffusion Test**

- The agar gel immunodiffusion test (AGID) is a group-specific test for antibody.
- It is relatively useful on a flock basis for serology for LPAI but of limited use for HPAI strains where mortality is high.
- It is of little use to detect virus infection in waterfowl as the precipitating antibody response to group antigen is generally poor.

## **2.3 Competitive ELISA Using Group Antigen**

- The competitive ELISA is a test system that can be used for all species.
- It is very sensitive and specific for chickens but (as for AGID) considered to be of limited use for sero-surveillance for H5N1.
- It can be used to detect antibodies in ducks, but its use in this species has only limited validation.

## **2.4 DIVA (Differentiating Infected from Vaccinated Animals) System**

### **2.4.1 Antibody detection using immunofluorescence**

This test uses cells infected with a baculovirus vector expressing neuraminidase antigen of interest. Sera are tested by reaction with antigen in fixed cells. The result is read using a fluorescent microscope and thus requires subjective evaluation.

### **2.4.2 Antibody detection using inhibition of neuraminidase activity**

This is essentially a biochemical assay inhibited by antibody. The test uses beta-propiolactone-inactivated antigen. The result is a visible colour change that can be observed by eye. The method has been miniaturised to conduct tests in a 96-well microplate format.

## **3. ESTABLISHMENT OF LABORATORY NETWORKS**

- Figure 1 shows the flow of samples in a laboratory network.
- Standard operating procedures (SOPs) are required to reflect technical aspects of diagnostic testing (see the *OIE Manual of Standards for Diagnostic Tests and Vaccines*), safety procedures for operators at different levels (see WHO guidelines, which provide a useful reference) and contamination control measures for laboratories providing molecular diagnostic services.

- Some level of quality assurance and proficiency testing must be introduced to increase the credibility of results of laboratory tests.
- The laboratory network should coordinate training for operators at different levels.
- The network should strive for sustainability of antigen and antiserum production. Training should be provided by upstream institutions (Regional Network Laboratory and OIE/FAO Reference Laboratory).
- Specific facility at national and sub-national level for specimen receipt and to conduct post mortem examinations of birds.
- Standards for safe transport of specimens and virus isolates around the network are critical. May require training for IATA certification for packaging of dangerous goods.
- Permit system must be in place for samples to be exported and imported
- It should be noted that material transfer agreements (MTAs) are required by some jurisdictions.
- Reporting processes to be agreed and need to accommodate the needs of customers to receive reports before information gets into public arena.
- There is a need to determine the information set required to accompany samples to reference laboratories.
- Procedures are required for sharing information in the network.
- Integration of the laboratory network with national and regional epidemiology network is necessary.
- The national network is the responsibility of the national focal point — regional network laboratory should not interact directly below this level.
- Incorporation of private veterinary laboratories and university laboratories in the national networks to be encouraged.
- Networking with other interested laboratories with specialist expertise (e.g. OIE/FAO and WHO reference laboratories).
- Network meetings are required to build links and increase mutual understanding of the constraints affecting laboratories in different situations. The proposed FAO sub-regional Technical Cooperation Projects will provide support for network meetings and associated activities to strengthen laboratory networks.

#### **4. QUALITY ASSURANCE AND PROFICIENCY TESTING**

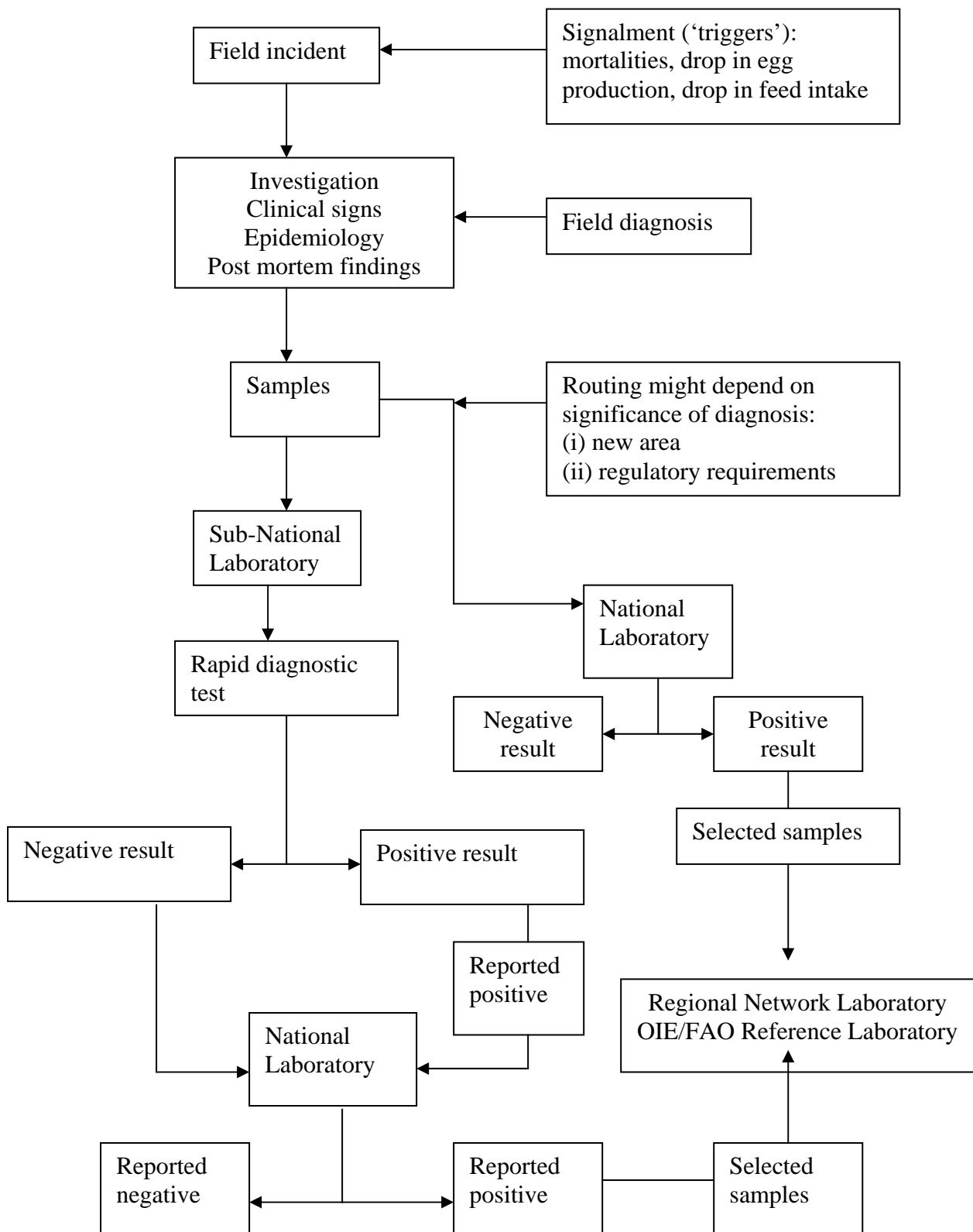
- The regional network laboratory could coordinate, but does require some experience to set up.
- OIE/FAO reference laboratories will assist the regional network laboratory with this.
- Thailand has expressed interest in acting as the regional network centre for Southeast Asia.
- CSIRO AAHL would be interested in participating in this aspect of the network.
- Laboratories must have a quality assurance (QA) programme to monitor the performance of both virus detection and serological tests.
- At this stage, proficiency testing (PT) is likely to be applied to serology only as it is not feasible to send infectious material around the network for virus detection tests.

- It is possible to check operator proficiency for virus detection at training courses or special workshops.
- Funding needs to be considered for both the regional network activity as well as activity within the national networks.

**Table 1: Laboratory capacity requirements for network**

<b>Laboratory Designation</b>	<b>Recommended minimum capability</b>	<b>Ideal additional capability</b>
Sub-national level	Serology by HI if vaccination is used. Rapid antigen detection test (IFAT or commercial antigen detection kit). Facilities for conducting post mortem examination.	Virus isolation. Histopathology. Gene sequence detection by PCR or RRT PCR. C-ELISA for serology on waterfowl.
National level	Virus isolation with HA typing for H5, H7 and H9. Serology for H5 by HI. Facilities for conducting post mortem examination. Rapid antigen detection test (IFAT or commercial antigen detection kit).	Neuraminidase typing. Histopathology and immunohistochemistry. Additional ELISA serology RT PCR for H5, N1, and M specific gene sequences. (RRT PCR for H5, N1, and M specific gene sequences).
Regional network level	System to accept samples from national laboratories in network. Virus isolation with full H and N sub-typing capability. RT and RRT PCR for H5 and N1. HI and C-ELISA for serology. Capacity to produce HI reagents. Access to gene sequence and analysis capability. Training facility.	Capability to conduct QA programme and proficiency testing.
OIE/FAO Reference Laboratory	System to accept samples. Virus isolation with full H and N sub-typing capability. RT and RRT PCR. HI and NI serology and ELISA for serology. Gene sequencing. IVPI testing (P3). Live bird studies (P3). Reagent production. Capability to conduct QA and proficiency testing. Training facility.	

**Figure 1: Representation of sample flow in laboratory network**



# **ANNEX 3: ANIMAL HEALTH INFORMATION SYSTEMS AND NETWORKS**

## **1. INFORMATION SYSTEM**

The information to be collected as part of the surveillance and control strategy for highly pathogenic avian influenza (HPAI) should be stored in a computerized information system. Such a system will be used for data entry as well as production of required outputs. Typical components include a database and geographical information system for entry, management, analysis and presentation of non-spatial and spatially referenced data, respectively. The system should be managed at national or sub-national level, depending on the size of the country.

It may be that the information system can be the responsibility of one national epidemiology unit, or in the case of very large countries such as China epidemiology units may be required in every province. If the latter is the case, the sub-national epidemiology units need to be linked with the national epidemiology unit.

Such a system may already exist in some countries, or needs to be implemented if not currently existing. If the latter is the case, veterinary authorities should look at the FAO-developed and recently updated TADinfo system and assess if it fulfils their requirements. The decision about the suitability of an information system needs to consider the minimum data requirements for effective HPAI surveillance and control.

## **2. DATA**

The usefulness of the information system depends on the relevance of the outputs generated by the system to the stakeholders (i.e. veterinary services, industry etc.). The inputs required for the system are outlined in the Sections 2.1 to 2.2.5; the outputs are described in Section 3.

### **2.1 Population Data**

The population at risk of infection with HPAI needs to be defined, and this will typically be done through a census conducted by most countries on a regular basis. It is desirable for the population data to be available at the highest geographical resolution possible, for example aggregated at the village/commune level. But it may be that in some countries a higher level of aggregation needs to be accepted. The temporal resolution at which the data will be updated depends on the local systems, but if possible they should be updated at least every two years. A livestock census may be conducted by a national department responsible for statistical information and/or by the department responsible for agriculture and/or veterinary services.

The data to be collected should include the number of chickens, ducks and other avian species kept on farms, as well as pigs. It is acknowledged that the accuracy of these data may vary between and within countries, and validation of the data using surveys is encouraged. This could become a regular research activity.

## **2.2 Avian Livestock Population Dynamics**

In addition to the census data outlined in Section 2.1, information on the typical birth and mortality rates in the relevant avian species should be obtained. It is important to consider the likely variation of these data between and within countries in relation to the characteristics of the local production systems. These data could be obtained through review of existing literature, including reports of projects conducted in the region, or through targeted studies.

Ideally, such data could be collected through community animal health workers on a regular basis. Population dynamics of the commercial and integrated production systems can often be obtained direct from the producers.

## **2.3 Disease Data**

The surveillance activities will involve collection of data on disease frequency as part of the screening and confirmatory laboratory diagnostic activities. The method of diagnosis needs to be recorded. These data will also be included as part of outbreak investigations. The geographical location of any such events has to be recorded at the highest resolution possible, ideally as point location coordinates of the affected farms. The format of the data collected should follow a standardized format defining the minimal required data items. The minimum data fields required are listed in Table 1.

## **2.4 Movement Data**

The dynamics of AI infection will be strongly influenced by movements of birds and people. Although detailed data will be difficult to obtain, even basic data can be useful (for example, surveys at a bird market will allow a crude description of the market's catchment area and provide useful information when considering the spatial coverage of surveillance activities). District veterinary officers may also have semi-quantitative information about movement patterns of avian livestock. Countries with movement licensing systems should consider including this information in the animal health information system. It needs to be recognized that it will not be possible to obtain data on illegal movements. Access to data on human movement patterns should also be explored.

## **2.5 Risk Factor and Other Data**

Different types of risk factor information should be included in the animal health information system. This information should be geographically referenced so that it is suitable for incorporation into a geographical information system. Most of these data do not change frequently and are likely to exist already within a country. Some of these data sources can also be obtained from international organisations such as FAO. The geographical resolution of the data should be as high as possible. The types of data required are listed in Table 2.

## **3. OUTPUTS**

The sustainability of the animal health information system for avian influenza (AI) depends on the usefulness to stakeholders of the outputs generated. It is important to

recognize that these outputs need to be considered as being useful by data providers at all levels in the data collection process. As part of the strategy to maintain and improve data quality, relevant outputs need to be provided to at least the district level of veterinary services and to selected private sector industry groups.

Examples of outputs that could be provided include maps of outbreak locations, tabulated or mapped descriptive statistics, summary data on movements, mapped surveillance intensity, and risk maps (qualitative or quantitative). The communication mechanisms available to disseminate such outputs include websites, e-mail, fax and postal mail.

#### **4. ANIMAL HEALTH INFORMATION NETWORKS**

Networks for the purpose of sharing of expertise and data should be established within countries as well as at the regional level. These will be important for ensuring the effectiveness and the sustainability of the information systems, and functional networks at the national level will be of key importance in this context.

##### **4.1 National Networks**

The purpose of each country's national network is to facilitate information flows within the country, and also to regional and international systems. An established network will allow a faster response during emergency outbreak situations, as well as result in improved general communication. It will lead to enhanced technical expertise in data collection, management and analysis, as well as improve the use of existing expertise as it allows network members to learn from each other.

The network should include surveillance information from official veterinary services, laboratories, universities, non-governmental organizations, private sector industry and national public health organizations. It may also be useful to include links to other government departments, such as those responsible for the collection and collation of national statistics.

It is recommended that authorities in each country identify a national coordinator who will be responsible for the management of the national network and its interfacing with the regional network. The activities of this person related to the network need to be given high priority. National veterinary services need to make a commitment towards regular budget allocation towards the maintenance of the network.

##### **4.2 Regional Network**

The purpose of the regional network is to coordinate the disease control efforts in the region, and to improve transparency and communication between countries. It will also strengthen technical capacity in the region. The data generated by the network will become the basis for risk analyses in the context of targeted surveillance and trade with countries within and outside the region.

The concept of a regional network recognises the fact that an infectious disease such as AI can only with great difficulty be managed by a national effort, due to the possible continuing risk of introduction from neighbouring countries.

The regional component of the network will receive data from each country at a 'below national-level' of aggregation (e.g. data aggregated at the level of provinces). It needs to be emphasized that provision of data only at national level aggregation will severely limit the usefulness of the system. The data flows will have to occur on a regular basis, ideally at least at monthly intervals. Links with public health organizations should be established.

The sustainability of the regional network will depend on the benefits it provides for its members. These therefore need to be clearly identified and communicated. The funding of the system may have to be a mixture of external donor and government sources, but could also include private sector industry sources. An example of such a network is the APHISA (Animal Production and Health Information System for ASEAN) project.

**Table 1: Minimum data collection requirements in a disease investigation**

<b>Items</b>	<b>Content</b>
General	Type of observation: initial/followup Date of observation Date of reporting Name of entry officer Reporting officer/institution Sensitivity: high/low Source of information Reference Date of first case Date of end of outbreak Public and private comments
Locality (with coordinates)	Country Administrative level 1 (e.g. province) Administrative level 2 (e.g. district) Locality Farm Farming System
Animals affected	Species Number of cases/death/at risk/examined Age/sex Vaccine used
Clinical signs and lesions	Species/signs Species/lesions
Samples	Species Sample type Sample identification Type of test Date sample sent Date result received Laboratory results Comments
Diagnosis	Tentative diagnosis (differential diagnosis) Final diagnosis (diagnosing officer and date of diagnosis)
Epidemiology	Source Comments
Actions and treatment	List of actions (destruction, vaccination, quarantine, stamping-out) and number List of treatments and number [trace-back and trace-forward activities]
Validation	Validation: date of initial data entry and date last modified

**Table 2: Risk factor and other data**

<b>Data category</b>	<b>Data</b>	<b>Type</b>	<b>Comments</b>
General	Surface water	Geographical: vector	rivers and lakes
	Road network	Geographical: vector	major roads
	Populated places	Geographical: vector	
	Administrative boundaries	Geographical: vector	smallest level possible
Agriculture and disease	Land use	Geographical: vector	
	Farming systems	Geographical: vector	
	Husbandry systems	Geographical: vector	
	Market locations	Geographical; vector	
	Slaughterhouse locations	Geographical: vector	
	Poultry species density	Geographical: raster	can be obtained from FAO
	Vaccine usage		
Natural environment	Rainfall, temperature, humidity etc.	Geographical: raster	
	Elevation	Geographical: raster	
	Vegetation	Geographical: vector	
	NDVI	Geographical: raster	
	Wild birds	Geographical: vector	Maps of migration patterns
Human population	Socioeconomic data	Geographical: vector	poverty maps
	Festivals	Temporal and geographical	
	Cultural factors	Geographical	consumer habits

## **ANNEX 4: RESEARCH PRIORITIES**

At present, there is a serious lack of information about H5N1 avian influenza (AI) viral infection in domestic ducks, other domestic waterfowl, and wild waterfowl. In East Asia, these viruses are believed to be maintained in and spread by domestic ducks (see recent publications: Chen et al. 2004; Li et al. 2004). Wild waterfowl are also suspected to be harbouring and spreading infection in some areas where they are prevalent.

The July 2004 FAO consultation of technical experts in Bangkok was unable to give unqualified support to the use of vaccines to control H5 AI viruses in domestic waterfowl because there are no data to confirm that vaccination of ducks will lead to the development of protective immunity or reduction in excretion of H5N1 HPAI viruses.

Given this background, the research priorities identified are dominated by the urgent need to gain more information on the disease in ducks and the potential capacity to vaccinate them as a measure to control the disease.

### **1. DOMESTIC WATERFOWL**

The extent of infection in domestic waterfowl in the region is not known and there is limited information available on issues relating to infection in ducks and geese including:

- The length of time that infected waterfowl shed virus.
- The level and duration of immunity following vaccination and the impact of vaccination on excretion of viruses by these birds.
- The reasons why some infected ducks develop severe disease.
- The genotypes of H5N1 (and other viruses) present in waterfowl.
- Alterations in duck-origin viruses after passage in chickens.
- The impact of vaccine on meat quality (vaccine granulomas) in ducks and geese.

The following is a list of possible research projects, in approximate descending order of importance, that would help to provide key information to assist with the control of AI infections in domestic waterfowl.

#### **1.1 Variation in Virulence of H5N1 Strains in Waterfowl**

There is anecdotal evidence of variations in virulence of H5N1 viruses in ducks in the region. It is therefore important to understand if the virulence observed is related to the H5N1 virus alone or to interactions with other infectious (e.g. concurrent infections) or environmental factors. Pathogenesis studies should be carried out by two or three laboratories to develop the best model for answering these and other questions concerning HPAI infection in waterfowl (e.g. strain variation in virulence, and level and duration of excretion; preferred replication sites). In collaboration with other international reference laboratories it might be possible to correlate pathogenetic characteristics in waterfowl more closely with viral genetic markers.

## **1.2 Efficacy of Vaccination in Ducks**

Vaccination of ducks can achieve two outcomes — to protect them from fatal systemic infection and to prevent gastrointestinal replication and significant shedding of virus. At present, it is not clear whether vaccination of ducks will prevent systemic disease with H5N1, but it seems likely this is the case. Studies should involve investigation of the effect of vaccination on the replication of AI viruses on the gastrointestinal tract of domestic waterfowl. The studies should involve at least two (or three) laboratories, undertaking work independently, with a range of selected HPAI viruses from the region. In addition, appropriate institutions in the eastern Asian region should be approached to obtain results of any vaccination and challenge studies that have been carried out in ducks.

## **1.3 Development of New Vaccine Strategies for Ducks**

There is a standing population of about 1000 million domestic waterfowl in the region and they fill important economic, social and environmental roles. Thus reduction of H5N1 circulation in this reservoir will be critical to control of the disease in chickens and to reduce the public health threats. Ducks generally require three doses or more of conventional inactivated vaccines to reduce shedding of virus and such a vaccination regimen is not likely to be appropriate for ducks in this region. New vaccination strategies should be explored and could involve the development of new vaccines for ducks (see comments on new vaccines).

## **1.4 Validation of Serological Testing of Waterfowl to Detect Infected Flocks**

At present, there is no good information in the scientific literature about the serological response of ducks or other waterfowl following H5N1 infection and therefore there is no validated diagnostic test for detection of antibodies in waterfowl (i.e. sensitivity and specificity values for HI or C-ELISA are not available for these species). It is clear these technical gaps must be filled as quickly as possible to improve the efficiency of surveillance activities.

## **2. WILD BIRDS**

Research is required to define more clearly the movement patterns of wild birds, including migratory pathways, to assess the risk of HPAI outbreaks in poultry arising from these birds. It should be noted that determining the extent of exposure of wild birds to AI viruses through serological and virological examination of wild birds caught for population and banding studies will provide some scientific information but may not be very rewarding in terms of disease control.

At some point in the future, when H5N1 viruses have been characterised for virulence in domestic waterfowl, it would be useful to obtain some information about their pathogenesis in wild waterfowl prevalent along key migratory bird flight ways in Asia. This might give some insights into the epidemiology of AI in the region.

### **3. MARKET FLOWS**

There is a need for studies to document and analyse the market flows of poultry and other avian species in Asia to help understand the movement of birds/products and their associated viruses.

### **4. NATIVE OR VILLAGE CHICKENS**

There is a need to undertake studies on the susceptibility of native or village chickens to HPAI. The need arises from reports that some village chickens apparently survived outbreaks, but it is not clear if they actually infected with or without clinical signs and then recovered, or whether the survivors were escaped infection). This question could be answered by some targeted sero-surveillance and has implications for sero-surveillance for H5N1 infections in Production Sector 4.

### **5. LABORATORY STUDIES**

#### **5.1 Monitoring Antigenic Drift in Avian H5N1 Viruses**

Ongoing monitoring should be undertaken to determine the degree of antigenic drift that may be occurring in H5N1 viruses across the region. Comparison of viruses obtained from countries that are and are not carrying out vaccination would help to determine the likelihood that vaccination is causing more drift than occurs in unvaccinated populations. Viruses should be obtained from both chickens and ducks to enable comparisons to be made. This work would need to be undertaken at least at a Regional Network Laboratory and preferably at an OIE/FAO Reference Laboratory.

#### **5.2 Validation of Immunofluorescence on Faeces to Detect Virus**

An immunofluorescence test has been used to detect virus in fresh faecal samples from poultry. If the test is to continue to be used as a front-line test for HPAI control programs, then it should be fully validated.

#### **5.3 Validation of New Rapid Tests for Antigen Detection**

New commercial tests are appearing in the market place and proper validation is required before they are accepted as routine diagnostic tools.

### **6. EPIDEMIOLOGICAL STUDIES**

Once good quality data are available from enhanced national and regional surveillance networks, epidemiological analyses can be conducted to provide trend analyses, tabulated or mapped descriptive statistics, summary data on movements, maps of surveillance intensity, and risk maps. There will also be a need to conduct more sophisticated epidemiological analyses using geographical information systems and disease modelling to help inform decision-makers.

Epidemiological modelling of the spread of H5N1 infection on individual chicken farms (cage- and floor-raised) and in populations (with and without vaccination) would also assist decision-making. However, such modelling could not be validated

without more data from research trials and surveillance activities than are currently available.

Periodically, the quality of data provided to the animal health information system need to be validated. Such validation should be conducted using surveys and might be efficiently conducted in selected pilot areas as an ongoing research project.

## **7. VACCINES**

### **7.1 Marker Vaccines to Distinguish Vaccinated from Infected Birds**

If inactivated vaccines are to be used in ducks then it could be useful to consider incorporation of a non-viral marker would allow vaccinated birds to be readily identified by a simple test to detect antibody to the marker antigen. This would not be a DIVA approach but would allow surveillance programs to identify birds vaccinated with the marker vaccine from those that have not been vaccinated.

### **7.2 Viral Vector Vaccines**

A further method to identify vaccinated chickens is to incorporate just the protective HA antigen into a viral vaccine vector, so that vaccinated, uninfected birds do not have antibodies to either the neuraminidase antigen or the Group A antigen. Antibodies to group antigen can be readily detected by ELISA if virus was circulating in vaccinated birds. A disadvantage of the approach is that co-circulation of other group A viruses would also result in a positive result in the group A test. Detection of antibody to the neuraminidase of the field strain could then be used in these cases to determine if there was HPAI virus infection in the vaccinated birds.

### **7.3 Improved Methods of Vaccine Delivery**

Current vaccines need to be delivered by injection, which is time-consuming, labour intensive, and can cause downgrading of carcasses from residual granulomas at the injection sites. Research leading to the development of vaccines that could be delivered more efficiently (e.g. by the oral route, eye drop or spray) would be very beneficial.

## **8. INFECTIONS IN PIGS**

There is a need for ongoing assessment of the possible role of pigs in the epidemiology of H5N1 HPAI. If pigs are kept on premises in which HPAI is confirmed in poultry, samples should be taken for virus isolation from the pigs. Within 3 km of known infected sites, pigs on premises where there is close contact with poultry should be investigated if there is excess mortality or respiratory disease in the pigs (serology and/or virology).