4. Preparing for an outbreak

Good preparedness planning can be of enormous benefit in successfully preventing or managing an outbreak, and in minimizing its impact when it occurs. This planning should involve a consideration of how best to detect an outbreak rapidly, confirm the diagnosis and implement a rapid and effective control programme. It requires an assessment of veterinary service capabilities and capacity and the legal framework in which these services operate. The structure of national poultry industries should be examined to determine the potential for virus entry. Industry support should be sought in the planning process. Provision for good public awareness programmes should be made, as it is critical to have public support for disease control activities and good public knowledge to minimize the risk of human infection.

4.1 EARLY DETECTION

4.1.1 Disease surveillance: principles

Active surveillance activities should be initiated as soon as a country considers itself at risk for an incursion of HPAI. A syndromic definition of the disease should be developed (i.e. all clinical or pathological conditions that may be confused with HPAI), including case definitions for reporting. In cases of suspected disease, representative samples from all domestic species of birds that die in the area should be investigated, and specimens should be submitted to approved veterinary diagnostic laboratories for diagnosis. In the event of virus isolation, its analysis and, if possible, characterization should be undertaken (See Section 4.4 below). Field surveillance examinations should seek to detect changes in flock health. Trained personnel should be aware of the potential risk to human health and wear protective gear (goggles or face shield, mask, gloves, disposable gowns or coveralls and rubber boots) that can be discarded on site or disinfected before leaving the investigation site.

No evidence of clinical disease describes any farm where the estimated rates are below the threshold defined above. Surveillance for HPAI needs to embrace:

- the development of an appropriate case definition;
- the inclusion of all poultry producers and production sectors, including markets;
- the inclusion of integrated commercial-level poultry producers in larger surveillance (i.e. random) sample survey efforts, even though many of them will carry out their own surveillance and reporting;
- local disease control centre officers carrying out regular telephone surveillance of independent premises; and targeted surveillance of premises in the restricted area and control area, particularly focusing on infected places, suspect places and dangerous contact places, and premises with unusual sickness and/or mortality.

A team consisting of trained personnel should visit the identified possible or highly probable outbreaks immediately (within 24 hours) and proceed to take a history from the

Syndromic surveillance

The following clinical or pathological findings can be confused with infections of a virulent avian influenza virus:

- high mortality in birds (domestic or wildlife);
- ruffled feathers;
- swollen and haemorrhagic areas of the head or shanks;
- diarrhoea;
- neurological signs;
- haemorrhagic internal organs; and
- air sacculitis.

Case definition: The case definition serves to capture clinical cases and should include all cases of the disease *if* it were present (highly sensitive system). The case definition can and should be reviewed if found too broad (e.g. "all cases of chickens with ruffled feathers") or too narrow (e.g. "cases of proventricular mucosal haemorrhage"). Consideration to identify a flock affected by HPAI must encompass basic cumulative morbidity/mortality rates and the known range for the incubation period (1–7 days for chickens and turkeys).

The definition of a "typical" case or suspicious outbreak of HPAI could encompass considerations for the type of production practice and its reporting. The case definition for village- or family-owned scavenging chickens can be different from the one used by big commercial operators. The data collected by the investigating team will be sufficient to identify if a possible outbreak or highly probable outbreak of HPAI is ongoing. A case definition might include the following.

Commercial operator (broilers):

- decrease in water intake by 10 percent over the course of 3 days;
- decrease in feed intake by 5 percent over the course of 3 days; and
- lethargy.

Commercial operator (layers):

- decrease in water intake by 10 percent over the course of 3 days;
- decrease in feed intake by 5 percent over the course of 3 days;
- deformed and soft eggs exceeding >3 percent of expected;
- small production operators in open farms;
- lethargy over the past 7–14 days in >20 percent of the chickens;
- chickens or turkeys with ruffled feathers >10 percent over the past 7 days; and/or
- deaths in chickens, turkeys, quail, etc. in excess of the expected 2 percent over the past 14 days.

Another approach could be to use the following definitions:

Possible outbreak is defined as a cumulative morbidity/mortality rate in last few days up to 1 week between 5 percent and 10 percent; or a cumulative morbidity/mortality rate in a two-week period between 10 percent and 20 percent.

Highly probable outbreak is defined as a situation in which any one of the figures above is exceeded.
Confirmed case/outbreak means that virus isolation was successful and the strain identified is classified as belonging to H5, H7 or H9 by a competent diagnostic or research laboratory; or that clinical diseased birds have tested positive with a reliable rapid field assay.

owner or caretaker, evaluate clinically the affected flock and collect appropriate samples. The team visiting the premises should be equipped with protective equipment and devices for disinfection. If possible, a second specialized team should be ready to undertake culling operations and proper disposal of the carcasses immediately. This second team will be appropriately equipped depending on the size of the operation or the number of households found to have a high probability of infection.

If the team has performed a rapid test on the spot with resulting positive results and clinical data consistent with an HPAI outbreak, the culling of animals should proceed without delay, followed by thorough disinfection of the premises. Samples should also be collected for further confirmation.

Field teams must be aware of the role they could play in disease transmission and outbreak spread if biosecurity principles are breached. Individual team speed is not as critical as good cleaning and disinfection of their equipment, boots, etc. What is critical is having a sufficient number of trained teams to undertake required operations simultaneously.

4.1.2 Domestic poultry surveillance

The identification of poultry at risk should involve poultry flocks located in high-risk agro-ecological systems where migrating birds congregate, as well as in high-risk farming systems and practices such as free-grazing ducks associated with rice production systems (e.g. in Asia) or where mixed species (e.g. chickens, geese, ducks, turkeys) are raised within urban, periurban or rural areas (in Asia, parts of Africa and the Near East, eastern Europe, etc.). At times of particularly high perceived risk, such as the arrival of migrating birds, it might be useful to undertake active serological and virological surveillance of sentinel birds within flocks, especially domestic ducks, which are the most likely to be exposed. It is therefore advisable that countries perform risk assessment studies of the introduction of AI in order to define the most appropriate surveillance strategy.

In any event, each country will have particular priorities and surveillance systems that should be refined to reflect these priorities. For example, HPAI-free countries or those with a lower risk of becoming infected will seek access to detailed, updated information on risks and will focus on the detection of incursions, making early warning and surveillance their priority. For infected countries or those at high risk of infection introduction, surveillance priorities will include the collection of detailed, current information on human health risks, ecological zones and production systems representing the highest risk for the introduction and maintenance of HPAI infection.

The FAO guidelines for HPAI surveillance are particularly relevant to countries exposed to the risk of HPAI introduction and should be read in conjunction with this document (see Annex 1).

The objectives of HPAI surveillance and monitoring are:

- to detect clinical disease and infection;
- to understand the epidemiology and ecology of AI, as well as its socio-economic impact, in order to help to design effective and implementable control programmes for poultry production systems;
- to assess temporal and spatial patterns and thereby improve the effectiveness of control efforts;
- to understand the evolution of AI virus variants by monitoring for antigenic drift or shift of AI viruses through frequent analysis at competent laboratories;
- to help define and control risks to public health;
- to maintain livelihoods and assist in ensuring food security through the implementation of appropriate control measures;
- to demonstrate freedom from clinical disease and absence of infection in a country or compartment and thereby facilitate safe trade; and
- to assess the efficacy of vaccination when used as part of a comprehensive disease control programme.

In addition to formal surveillance and reporting procedures, raising awareness about the risk of highly pathogenic avian influenza is an important means of increasing the likelihood that an outbreak of disease in poultry will be reported rapidly. However, it must also be kept in mind that raising public awareness should not be undertaken in a manner that

Minimum requirements for effective surveillance

(*taken from FAO's Guiding principles for HPAI surveillance and diagnostic networks in Asia*)

The following minimum requirements apply to all countries and compartments:

- HPAI is a notifiable disease (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 and for reporting confirmed cases internationally, in accordance with OIE guidelines.
- The country and/or region must have the technical capability to diagnose HPNAI and LPNAI. See chapter 2.3.4 of the *OIE Manual of diagnostic tests and vaccines for terrestrial animals 2008*.
- The country and/or region must have a system for recording, managing and analysing diagnostic and surveillance data.
- 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.

causes undue concern within the community. Also, veterinary disease control authorities will need to be prepared for an increased workload, because if formal and informal reporting mechanisms work, there will probably be a significant number of investigations that must be made following increased publicity of the risk of disease.

Some HPAI strains (such as Eurasian H5N1) do not affect ducks and geese at the same virulence level as they do chickens, turkeys or quail. Therefore, case definitions and investigations may need to rely more on virological surveillance (identification of carriers).

4.1.3 Wild bird surveillance

It is known that wild birds are a reservoir for numerous avian influenza viruses (particularly waterfowl and waders), but only an occasional healthy bird has been found to be positive for HPAI. Collecting samples from wild birds has thus proved to be an ineffective early warning system, though it is warranted in order to determine if wild birds are potential carriers, transporters or natural reservoirs of HPAI viruses in outbreak settings. One of the most important groups of birds to evaluate are the "bridge species", or those species that may move back and forth from potentially infected farms to wildlife habitats.

Another important factor to consider is that certain specific wild bird species (e.g. geese, swans, grebes, ducks and cormorants) can be highly susceptible to virulent AI chicken viruses as well. This is a unique aspect of the Eurasian H5N1 strain that is not a feature of any other AI viruses for which wild birds are a reservoir. Therefore, if surveillance sampling of healthy wild birds is not possible to test for the presence of AI viruses, it is important to at least set up wild bird monitoring programmes to check for wild bird mortalities, both near farms where outbreaks are occurring and at important wetland sites or other habitats that support large numbers of migratory birds. Bird-watching enthusiasts and clubs, as well as hunters and hunting clubs, could be incorporated into the overall surveillance system, and awareness material could be provided to them by the authorities.

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Figure 10: Sampling of wild birds in Mongolia (August 2005)

In case of an outbreak, although surveillance will begin immediately around the infected place or flock, it will have to be extended very quickly to all other sites where birds, products and contaminated materials might have been moved from the infected area (trace-forward activities). Surveillance of wild birds to determine their potential involvement in the dissemination of the disease may also be considered, but this is likely to have a limited impact on controlling the spread of the disease.

Contingency plans for outbreak investigations at farms could also include identifying the possible role of wild birds in the epidemiology of the current HPAI outbreaks. A wildlife biologist, ornithologist, or wildlife veterinarian with avian expertise could accompany outbreak investigations to collect additional epidemiological information that will lead to a better understanding of the interaction between wildlife, poultry and humans, as well as the role of bird species present or near the outbreak sites in the transmission of the HPAI virus.

For more and specific details on wild bird surveillance techniques and field study methods see the FAO manuals *Wild bird highly pathogenic avian influenza surveillance: sample collection from healthy, sick and dead birds* (ftp://ftp.fao.org/docrep/fao/010/a0960e/a0960e00.pdf) and *Wild birds and avian influenza: an introduction to applied field research and disease sampling techniques* (ftp://ftp.fao.org/docrep/fao/010/a1521e/a1521e.pdf).

4.2. RAPID RESPONSE

4.2.1 Planning for avian influenza control

It is difficult to put in place a rapid and effective response to an outbreak of a new disease if the process has not been planned in advance and the required resources have not been made available. FAO has published guidelines in its *Manual on the preparation of national animal disease emergency preparedness plans* (http://www.fao.org/docrep/004/x2096e/x2096e00.htm), which recommends the development of four sets of complementary technical contingency plans:

1. specific disease contingency plans that document the strategies to be followed in order to detect, contain and eliminate the disease;
2. standard operating procedures that may be common to several or all emergency disease campaigns;
3. enterprise manuals that set out zoosanitary guidelines for enterprises that may be involved in an emergency animal disease outbreak;
4. simple job description cards for all individual officers.

Each national veterinary authority needs to consider its particular needs and be careful not to embark on a programme of emergency manual development that is not sustainable. In any early interaction with other ministries that are important in making financial decisions within government structures and public service in the development of prevention and response plans, it is essential that these ministries understand the additional requirements of the veterinary systems in the event of a disease emergency, such as the incursion of a transboundary animal disease such as HPAI. Emergency funding to carry out disease contingency and response plans must be available on short notice. A critical

element of emergency plans is that they must specifically consider the situation and needs of a particular country (e.g. the structure of the poultry sector, organization of veterinary services, realities of transportation and communication, and state or provincial relationships with central government authorities). In addition, the implementation of contingency plans through simulation exercises is a key element in defining gaps or overlap in responsibilities or resources during an outbreak.

An excellent example is the Australian Veterinary Emergency Plan (AUSVETPLAN), which includes a technical response plan called *Disease strategy: Avian influenza*. This plan can be downloaded from the Internet (see Annex 1). Some of the most important considerations for the contingency planning process are:

1. Consideration of the disease control strategies that are available, the implications of applying them and the most appropriate strategy in different circumstances in a particular country.
2. Financial planning to determine where the necessary funds will come from and to ensure that there is a mechanism and commitment to provide them immediately. A particular issue that needs to be considered is whether adequate compensation will be provided to poultry owners whose birds are destroyed. In the current context of the global avian influenza threat, countries might consider negotiating commitments from international donors to assist in control, should a disease incursion occur.
3. Resource planning, which includes the needs for personnel, equipment and other physical resources. Plans will indicate resource requirements at the time of an outbreak. However, they should also provide a means to determine requirements in advance – and a way to either obtain these resources or make provision to obtain them rapidly when they are needed.
4. The need for appropriate legislation must be considered, because this requires long-term planning systems. Laws, regulations and proclamations are required to give authorized people the power to:
    - proclaim a notifiable disease;
    - enter a poultry enterprise to inspect birds or collect specimens;
    - define infected areas and disease control zones;
    - institute a quarantine of affected or suspect premises;
    - place movement controls on poultry, poultry products and potentially contaminated materials;
    - destroy and dispose of infected or potentially infected birds and contaminated materials;
    - undertake other disease control operations, such as compulsory vaccination; and
    - place controls on the operation of enterprises, such as poultry processing plants.
5. A process of consensus and commitment from all regulatory authorities and industry, as appropriate.
6. The training of personnel so that the appropriate skills are available.
7. Simulation exercises to identify deficiencies and a periodical review of the contingency plan.

4.2.2 Avian influenza control strategies

The primary aim of an early and rapid response to any occurrence of HPAI is to contain the disease before it spreads and eliminate it by stamping out affected premises and establishing proper disposal. Only if this fails should other measures be considered, including vaccination using effective and quality-controlled vaccines. This approach is endorsed by FAO, OIE and WHO. Where it proves impossible to eradicate the disease in the short to medium term, it may be possible to aim for compartment freedom (freedom within the commercial sector in which birds are housed and protected from infection), or zone freedom (freedom in defined geographic areas). In the case of compartmentalization, the poultry industry must take responsibility for its biosecurity (bioexclusion) with the necessary veterinary regulatory oversight in place. In the case of zone freedom, commercial compliance must follow national restrictions to ensure that infection does not enter the free zone. The competent veterinary authorities should be responsible to ensuring the health status at any given time to a country's trading partners.

There is no predescribed road map to control avian influenza outbreaks; the implementation of a strategy needs to be tailored to the environment, culture, marketing system, and country where it occurs. In order to control the disease effectively, countries should have a complete plan of action and the financial and humans resources to implement it under the particular conditions prevailing in the country. A regional approach is also necessary.

4.2.2.1 Culling

The basis of HPAI eradication by stamping out is to:

- immediately impose a quarantine of the affected area (premises or village);
- slaughter all infected and potentially infected birds and dispose of the carcasses;
- decontaminate sheds and other poultry housing areas;
- carry out rapid surveillance of surrounding areas to determine the extent of possible spread;
- close and disinfect markets; and
- keep sick and dead birds out of the human food chain, and not sell them as feed for other animals (e.g. zoo animals).

Quarantine and movement controls

AI is readily transmitted via contaminated objects, so strict control of movement of anything that may have become contaminated with the virus and the immediate imposition of tightly controlled quarantine on all places suspected of being infected are essential to a successful eradication programme. Ideally, quarantine should be imposed on all farms and villages in which infection is either known or suspected, and it should be strictly policed to ensure that no one – including the residents, owners, staff and other visitors – leaves without changing his or her clothes and footwear.

Strict on-farm biosecurity and hygiene are needed to control entry of the disease from outside sources (e.g. feed suppliers, equipment maintenance personnel, wild birds). In areas where poultry are raised in a village environment, particular consideration needs to be given to how quarantine, disposal of carcasses and waste and the decontamination of the environment can be effectively achieved.

CREDIT: V. MARTIN

Figure 11: Restriction zone. Outbreak in Anhui Province, China (July 2004)

Effective quarantine of an area requires around-the-clock security to ensure that only authorized personnel in protective clothing are allowed to enter. It will be necessary to supervise the movements of residents onto and out of the property, and to ensure that all pets are confined. It is also strongly recommended to ban cockfighting, pigeon racing and other avian concentrations in the outbreak area.

Particular attention also needs to be paid to workers on poultry farms who also keep poultry at home. As an additional security measure, commercial enterprises should ensure that their employees do not have poultry of their own.

Slaughter of infected and potentially infected poultry

All susceptible poultry species in infected and dangerous contact premises, or in a large area if this is deemed necessary, must be slaughtered, whether they are obviously diseased or apparently healthy.

Although it is not possible to provide specific and universally applied standards for controlling the disease through zoning for all potential outbreak occurrences, the following definitions and distances should be considered as a guide for rapidly containing HPAI. It must be noted that distances are indicative and subject to changes according to epidemiological characteristics, physical and geographical barriers, poultry density and farming and marketing systems (more details are provided in Annex 6).

Infected area

An area classified as an *infected area* (IA) will be a defined area (e.g. village, farm) in which HPAI has been detected. The IA will be subject to quarantine, and all susceptible animals will be destroyed in this area.

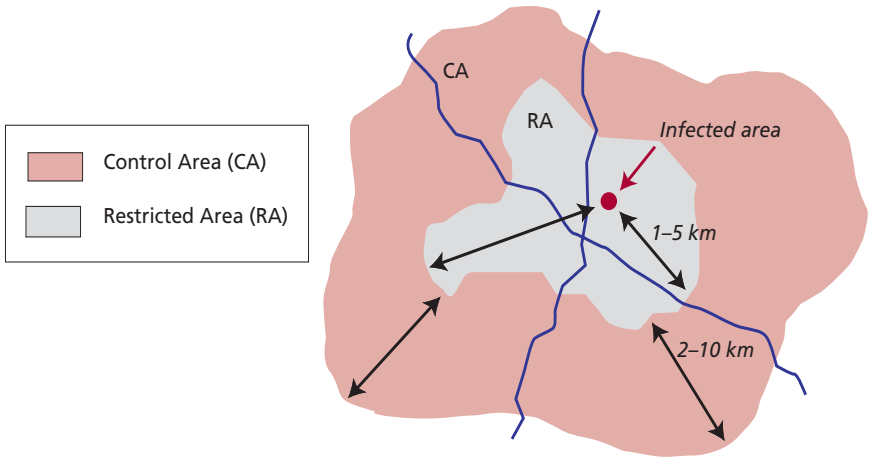
Restricted area

A *restricted area* (RA) will be a relatively small declared area (small compared with the control area – see below) around infected places that is subject to intense surveillance and movement controls. Movement out of the RA will, in general, be prohibited, and movement into the area would be only through regulatory approval. Multiple RAs may exist within one *control area* (CA). The RA does not need to be circular but can have an irregular perimeter, depending on known physical and geographical barriers, markets, poultry density and farming systems. This distance will vary according to the size and nature of the potential source of the virus, but will be approximately 1–5 km around the IA, depending on the density of the poultry premises. The boundary could be the perimeter fence of the IA if the IA is in an isolated location. The boundary in a densely populated area will take into account the distribution of susceptible birds and traffic patterns to markets, service areas, abattoirs and areas that constitute natural barriers to movement. If possible, hatcheries should be kept out of the RA.

Control area

The CA will be a larger declared geographical area around one or several RAs (possibly as large as a province initially) where restrictions will reduce the risk of disease spreading from the RAs. The boundary of the CA will be adjusted as confidence about the extent of the outbreak becomes clearer, but it must remain consistent with the OIE *Terrestrial Animal Health Code* chapter on surveillance and zoning (Chapter 1.4.3). In general, surveillance and movement controls will be less intense and animals and products may be permitted to move, under permit, from the area.

FIGURE 12
Zoning strategy for the control of avian influenza



The declaration of a CA also helps to control the spread of the outbreak from within the RA. The perimeter of the CA is a buffer zone between the RA and the rest of the country. The boundary does not have to be circular or parallel to that of the RA, but it should be 2–10 km from the boundary of the RA. In general, movement of possibly contaminated articles and materials within the CA is allowed, but movement out of the CA is prohibited without approval from the Chief Veterinary Officer (CVO). This type of control area allows reasonable and safe commercial activities to continue.

When RAs and CAs are declared, the areas must be no larger than necessary, thus restricting the number of properties to be quarantined to only those deemed prudent. If flocks in a quarantine area are not depopulated, then the cost of keeping the birds beyond their normal market age could be substantial.

Birds should be slaughtered by methods that take account of animal welfare concerns and the safety of operations, preferably without moving them from the site.

For small numbers of birds, the preferred method is dislocation of the neck (using burdizzos, bone cutters, secateurs or bare hands). Burdizzos are particularly useful when large numbers of poultry with strong necks (geese, ducks, etc.) are to be destroyed, and they are also helpful because blood contamination of the immediate environment or human exposure is avoided.

For large numbers of birds in commercial poultry units, the preferred method is gassing with carbon dioxide (CO_2). This method involves lining large garbage waste bins (skips) with plastic sheeting that also forms a canopy over the top of the bin. Birds can be caught using teams of labourers (experienced catching teams may be available). Chicks are easily caught under heaters and then transferred to skips in plastic garbage bins. Broilers on the ground are driven, using a movable Hessian wall, to the catching area, where they are caught and placed directly into skips.

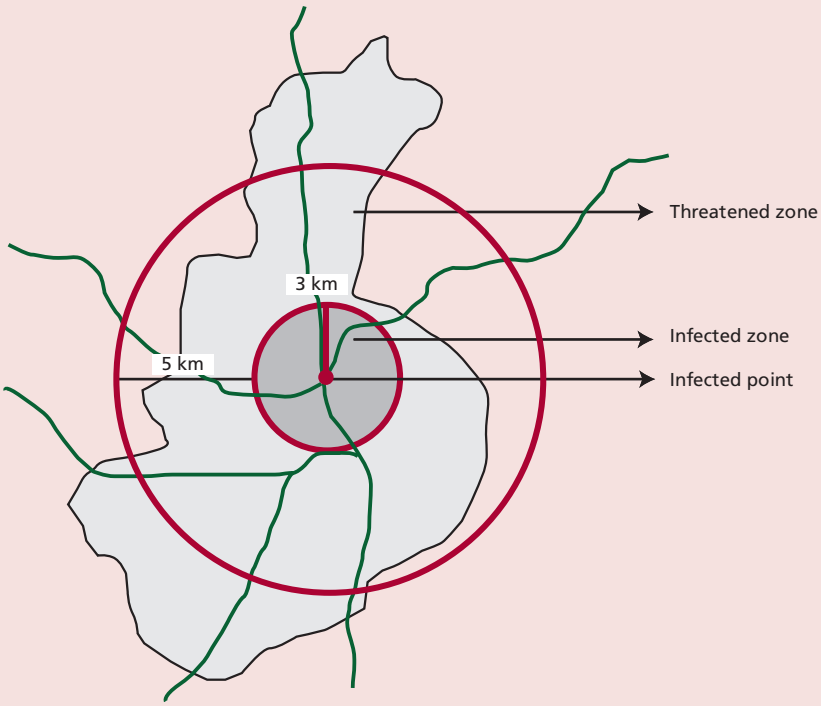
Caged birds are more difficult and progress is slower. Each catcher removes three or four birds from the cages and carries them by the legs to skips. Layers on perches are best caught at night or during low light when they are quiet. Carbon dioxide (CO_2) is transferred to the bottom of the skips through a 2.5-cm garden hose fitted to the top of the cylinders. The carbon dioxide should be decanted in 30–45-second bursts. It is essential not to decant too quickly or the bottles will freeze when they are about half empty. The concentration of CO_2 must be in the range of 60–70 percent in the skip, with the lid tightly closed for a 1–2-minute period to properly stun and kill the birds. On average, half a 45-kg cylinder of carbon dioxide is needed for the 3-cubic-metre skips; three or more cylinders are needed for the 20-cubic-metre skips. Carbon dioxide should be added at a sufficient rate to ensure that birds succumb before other birds are placed on top of them. Skips should be no more than three-quarters (75 percent) filled with birds, sealed and transported to the disposal site. Care must be taken to ensure no bird is still alive when dropped into the burial pit. Should this happen, these birds must be immediately caught and humanely killed.

Safe disposal of carcasses

The disposal of dead birds, poultry litter and other contaminated waste is best done by burial. Sometimes this is not practicable or desirable, because the required equipment is not available

Example of zoning: The case of China*

In this example, as initially used in China, premises with infected poultry or relevant slaughtering houses and other departments were considered *infected points*; areas within the 3-km radius were considered *infected zones*; and areas within 5 km around the infected zones were considered *threatened zones*.



Stamping out. All poultry within infected zones would be stamped out.

Emergency vaccination. All susceptible poultry in the threatened zones were to be compulsorily vaccinated with the vaccines approved by the Ministry of Agriculture. Only healthy birds were to be vaccinated.

Disposal. All poultry carcasses and poultry products in infected points, excretion material, contaminated feed, litter and sewage from the infected points would be subject to biotreatment or disposal.

Cleaning and disinfection. All contaminated items within the infected zones, transportation vehicles, utensils, poultry counters and grounds were to be cleaned and disinfected.

Movement control. Warning signs would be widely posted around the infected zone; disinfection stations would be set up at the transportation entrances of infected zones in order to disinfect vehicles and items entering and exiting zones; movement of all susceptible live birds and their products would be controlled.

Closing the market. All poultry and poultry-product markets in infected zones and live-bird markets within a 10-km radius of infected zones would be closed.

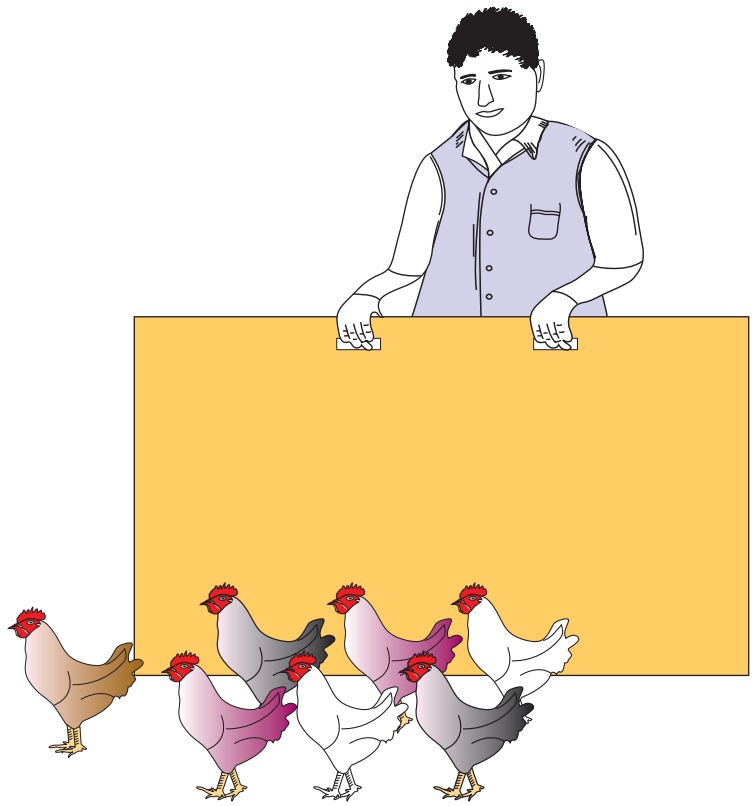
Tracing. If poultry and poultry products were sold during the incubation or clinical manifestation period, or otherwise moved, tracing was to be conducted on the potentially infected or contaminated items to prevent these items from spreading disease.
Financial support. Financial support systems would be established for all poultry destroyed because of HPAI.
Public health. Surveillance of staff of poultry rearing, trade and transportation and processing units, especially of staff in the infected zones, should be intensified, and epidemiological investigation should be conducted. Stringent protective measures must be implemented by staff participating in the destruction of infected birds and cleaning of contaminated premises.
Lifting quarantine. In this example, the conditions for China to lift a quarantine were stipulated as follows: 21 days for the infected point and infected zone after strict treatments were conducted according to the Chinese *National contingency plan for highly pathogenic avian influenza* and standard technical requirements for the treatment of HPAI; over 14 days for the threatened zone where all the susceptible birds were vaccinated with nationally approved vaccines and after no new cases occurred (these areas would be inspected and the findings would have to be accepted by the relevant authorities). When quarantine was to be lifted, live-bird trade markets 10 km around the infection zone would be allowed to reopen.

* This is an example of what one country (the People's Republic of China) uses – other countries or regions would need to develop their own policies and schemes, taking into consideration poultry populations, marketing practices, geographical and ecological environments, veterinary system capacities, and evaluation of current scientific or technical reviews and research.

or because it would have adverse environmental effects, such as the potential contamination of groundwater. In these circumstances, the best alternative might be composting.

Burial. Burial is best undertaken at the infected site. It is best to minimize the distance that infected material needs to be transported. However, a burial place outside infected premises may be the best option in situations where a number of infected foci would have to be depopulated and decontaminated in a given area and where a common burial site would be more efficient. The preferred equipment for digging burial pits is an excavator. This is the most efficient equipment for the construction of long, deep, vertically sided pits. Other advantages include the ability to easily store topsoil separate from subsoil, and the equipment can be used (if required) to fill the pit with carcasses or other materials and close the pit without any disturbance of the carcasses. Loaders, bulldozers, road graders and backhoes (for small jobs) may be used if excavators are unavailable. With the exception of backhoes, all other equipment requires the continual movement of the machine over the site while the pit is being dug. Excavators and backhoes essentially remain in a fixed position during digging; hence they move soil faster, with less cost and less damage to the site surrounding the pit. Most excavators have an attachable hammer for rock work if necessary. The dimensions of the burial pit will

FIGURE 13
Drawing of Hessian wall used to corner poultry for subsequent handling (e.g. transport, sampling, vaccination) with minimal distress



depend on the equipment used, site considerations and the volume of material to be buried. The preferred dimensions are for pits to be as deep as practically possible (reach of machinery, soil type and water-table level being the usual constraints), with vertical sides.

Gas production from decomposition within unopened carcasses may result in considerable expansion in the volume of the buried material, to the extent that the surface of the closed pit may rise and carcasses may be expelled from the pit. Lime may be added to pits to prevent earthworms bringing contaminated material to the surface after pit closure. Covering the carcasses, with 40 cm of soil is suggested, with the addition of an unbroken layer of slaked lime [$Ca(OH)_2$] before filling is completed. Lime should not be placed directly on carcasses because it slows, and may prevent, decomposition.

Inspection of the burial site after closure is recommended so that appropriate action can be taken in the event of seepage or other problems.

CREDIT: V. MARTIN

Figure 14: Burial site in Ahnui Province, China (July 2004)

Composting. Biological decomposition, or composting, is an effective way of dealing with manure and litter waste, and it can be carried out within sheds or otherwise on-site, thus overcoming the risks of disseminating the virus during transport. Composting should be done in a secure area not accessible to susceptible birds or scavenging animals, such as dogs or wildlife. The procedure requires the piling of carcasses with other bulky contaminated or non-contaminated material, such as wood chips or straw bedding, to allow for proper aeration, and that they be covered with a biological filter (i.e. not whole plastic). The pile should not be pressed or otherwise compacted. The site selection is important – away from any residences, with at least 1 metre of ground between the pile and any known water source, with any runoff water from the decomposing material collected and treated. Temperatures for proper composition should reach 55–60 °C within 10 days, and the material should be kept in place for several weeks; subsequently mix but never press the material within the pile. Properly decomposing material at this time should be dark in colour with minimal foul odour.

Burning/Incineration. A burning area outside an infected area may be the best option in situations where a number of infected foci would have to be depopulated and decontaminated and where a common burning site would be more practical. The principle is to place carcasses on top of sufficient combustible material, ensuring that the arrangement of fuel and carcasses allows adequate airflow to enter the pyre from below, and thus achieving the hottest fire and the most complete combustion in the shortest time. Ensuring that the expanding fire will not extend to adjacent vegetation (or overlying trees) and housing is warranted.

When loading of the carcasses is complete and weather conditions are suitable (e.g. no excessive wind), saturate the fire bed and carcasses with diesel or heating oil (NOT PETROL) and prepare ignition points about every 10 metres along the length of the fire bed. These can be made of rags soaked in kerosene. Move all vehicles, personnel and other equipment well away from the fire bed. Start the fire by walking into the wind and lighting the ignition points along the way. The fire must be attended at all times and refuelled as necessary; use a tractor with a front-mounted blade or a front-loader. Ensure any carcasses or parts thereof that fall off the fire are replaced on the fire. A well-constructed fire will burn all the carcasses within 48 hours. The ashes should be buried and the site restored as well as possible.

Rendering. Rendering is a closed system for mechanical and thermal treatment of waste. It is a good method for carcass disposal if the plant has sufficient capacity and if it is possible to effectively decontaminate the rendering plant afterwards. A medium-sized facility could render some 12 tonnes per hour of operation. However, private rendering plants may not be willing to handle infected birds and eggs unless an emergency order is imposed. A disadvantage is that infected material would need to be transported from infected sites to the plant.

Where burial, cremation or rendering is not considered practical or is difficult to carry out in the infected area, permission should be sought to transfer carcasses and/or infectious material to another site for disposal by burial, cremation or rendering. Transport should be in leakproof containers, such as large skips, covered with tough polyethylene covers and sealed at the top. These are secured on the bed of a lorry or another vehicle. It should not be overloaded – half a metre or more (depending on distance to be travelled and temperature) should be left clear for the expansion of carcasses. Vehicles should travel slowly to avoid any splashing of contaminated material, and they should be accompanied by a police vehicle to minimize the chances of accidents and prevent breaches of biosecurity. The escorting officer must carry a supply of an approved disinfectant and basic equipment to deal with minor spills en route. All vehicles must be cleaned and disinfected before leaving the infected place and after unloading.

Decontamination

Soapy water and detergents are the first choice for decontamination. The avian influenza virus is more easily destroyed than many other viruses because it is very sensitive to detergents, which destroy the outer lipid envelope of the virus. Therefore, the washing of contaminated surfaces should always be with detergents (soapy water) or specific disinfectants. Bird droppings are the most difficult material to decontaminate, because the virus can survive in moist environments with high organic content. It is therefore essential to clean and disinfect—thoroughly—items that have been in contact with bird droppings (e.g. cages, shoes, clothes), before working with poultry or entering a place where poultry are kept. Simple hygienic measures can reduce risk, but national authorities are encouraged to prepare and communicate specific guidance for each type of poultry enterprise. More guidance for veterinary services on selection and application of decontamination procedures is given in Table 1 (adapted from AUSVETPLAN). Adaptation to specific country circumstances will be needed.

Rest and restocking period

After slaughter, disposal and decontamination procedures have been completed, the premises must be left without susceptible species for a period of time, determined by the estimated survival time of the pathogen in the particular environment. ***Restocking*** should not take place until at least 21 days after satisfactory cleaning and disinfection have been completed and the outbreak has been brought under control in the area. Restocking should be undertaken by introducing a small number of poultry first, and these should be ***monitored*** daily for signs of disease. Should this occur, notification of the authorities must be immediate, and sampling of the sick or dead birds must be done to determine the cause. If the poultry remain healthy, full repopulation can be carried out. Of course, improvements of biosecurity procedures should be instituted at all stages of production to decrease the likelihood that AI or other diseases will enter the recovered premises. After repopulation, monitoring should be continuous through the sampling of dead birds to determine whether reinfection has occurred.

TABLE 1
Selection and application of decontamination procedures

Item to be disinfected	Disinfectant/chemical/procedures
Live birds	Euthanize (carbon dioxide gas; dislocation of neck)
Carcasses	Bury or burn
Animal housing/equipment	1, 2, 3 (See key below)
Humans	1
Electrical equipment	5
Water	Drain to pasture where possible
Feed	Bury
Effluent, manure	Bury or burn; 4, 3
Human housing	1, 2
Machinery, vehicles	1, 3
Clothing	1, 2, 3

Key

1. Soaps and detergents: leave in contact for 10 minutes.
2. Oxidizing agents:
   - Sodium hypochlorite: liquid, dilute to final 2–3 percent available chlorine; not good for materials with high organic content. 10–30-minute contact time.
   - Calcium hypochlorite: solid or powder, dilute 2–3 percent available chlorine (20 g/litre powder, 30 g/litre solid); not good for organic materials. 10–30-minute contact time.
   - Virkon®: 2 percent (20 g/litre). 10-minute contact time.
   - Virocid® : 0.25 percent (1:400). 10-minute contact time on non-porous surfaces.
   - Use gloves and mask when handling (see manufacturer's instructions).
3. Alkalis (do not use with aluminium and similar alloys):
   - Sodium hydroxide (NaOH): 2 percent (20 g/litre). 10-minute contact time.
   - Sodium carbonate anhydrous ($Na_2CO_3.10H_2O$): 4 percent (40 g/litre from powder, 100 g/litre from crystals); recommended for use in presence of organic materials, as above. 10–30-minute contact time.
   - Use gloves and mask when handling (see manufacturer's instructions).
4. Acids:
   - Hydrochloric acid (HCl): 2 percent (20 ml/litre); corrosive, use only when other chemicals are not available.
   - Citric acid: 0.2 percent (2 g/litre); safe for clothes and body decontamination. 30-minute contact time.
   - Use gloves and mask when handling (see manufacturer's instructions).
5. Formaldehyde gas: toxic, use only if others cannot be used. 15–24-hour exposure time.

4.2.2.2 Vaccination

Vaccination, as a tool to support an overall control strategy, may be considered when the disease has spread to such an extent that it has overwhelmed the resources of disease control authorities, or if the economic cost of a widespread slaughter campaign cannot be borne. Moreover, vaccination may be needed to assist in stopping the spread of an AI virus (such as H5N1, H7N7 or H9N2) at the animal source, and thus decrease the risk of human infections with a zoonotic strain, when detection, reporting and/or implementation of other control measures are delayed. However, a high-quality veterinary service is required to implement and monitor vaccination strategies as well as all other preventive and control measures, including early detection and response. Those countries where veterinary services are weaker would need to rechannel professional resources to prioritize this activity, with inputs from other ministries or even the use of veterinary or animal husbandry educational institutions.

Recommendations emanating from an international conference held in Verona, Italy, in March 2007 on the use of vaccination as a tool for the control of HPAI should be reviewed by the reader (recommendations available at http://www.oie.int/verone/). Recommendations have also been made for the use of OIE-approved AI vaccines, and several such vaccines are commercially available. If used in accordance with FAO/OIE recommendations—*Recommendations on the prevention, control and eradication of highly pathogenic avian influenza (HPAI) in Asia* (available at ftp://ftp.fao.org/docrep/fao/012/ak714e/ak714e00.pdf) and the OIE *Manual of diagnostic tests and vaccines for terrestrial animals* (http://www.oie.int/eng/normes/mmanual/a_summry.htm)—these vaccines provide excellent protection against clinical disease in chickens by reducing mortality, production losses, and environmental contamination through decreased virus shedding. According to current OIE norms, AI-vaccinated poultry are not excluded from international trade, although specific technical guidelines must be followed to ensure that the vaccine is being applied properly and monitored effectively.

Vaccination, when it is applied, must be done in combination with other disease-control measures, including the slaughter of affected flocks and elevating the amount of biosecurity measures undertaken on rearing farms. Efforts to control the disease by vaccination alone, without slaughtering affected birds to reduce the virus load in the environment, will probably not be successful. Depending on the risk or incidence and distribution of outbreaks, vaccination should be strategized as a rationalized and targeted approach where the poultry population is immunized with the highest coverage possible or applied to a specific bird population/sector (focused vaccination) – for example, to species in zoological collections, or parent and grandparent flocks. Ring vaccination, a concept often used to protect susceptible species around outbreak sites, has rarely been successful, unless there is absolute control of poultry movements, their products and their waste; general management of daily human activities; and a means to ensure compliance.

Types of vaccine and their application

There are a number of different avian influenza vaccines available. Conventional vaccines are produced from the allantoic fluid of infected eggs, which is subsequently inactivated and emulsified with an adjuvant. The use of attenuated live influenza virus vaccines in birds is not recommended; because of the risk that the vaccine virus could either mutate

TABLE 2
Vaccine properties

Vaccine type	Advantages	Disadvantages
Conventional Vaccines		
INACTIVATED HOMOLOGOUS VACCINE The same H and N antigens as the strain isolated from the outbreak	• Readily available • Rapid onset of immunity with adjuvants • Inexpensive	• Difficult to differentiate vaccinated from infected birds serologically • Monitoring by using sentinel unvaccinated birds (requires identification, bleeding and swabbing, testing) is time-consuming and requires planning and monitoring • Requires boosters in longer-lived species • Requires percutaneous injection • Implies production of HP influenza strains at a large scale: biosafety issues
INACTIVATED HETEROLOGOUS VACCINE (DIVA Strategy: Differentiation of Infected from Vaccinated Animals) The same HA subtype with a different NA subtype compared with the virus isolated in the outbreak	• NA (neuraminidase antigen): marker of field infection – Positive serology to heterologous *neuraminidase* antigens can determine whether birds in a vaccinated flock have also been infected • NSP (non-structural protein): marker of field infection – Positive serology to *non-structural proteins* can determine whether birds in a vaccinated flock have also been infected • Can be used at hatcheries	• Laboratory capacity to perform the discriminatory test based on the N or other antigens • Serology is expensive, requires additional reagents and requires a complete knowledge of circulating N antigen sub-types • Requires boosters in longer-lived species • Requires percutaneous injection • DIVA testing based on NA antibodies is ineffective in the case of circulation of AI virus with the same NA subtype as the vaccine
Novel Technology Vaccines		
REVERSE GENETIC VACCINES	• Focused vaccination: vaccine strain has HA derived from circulating viruses and can be "changed" with ease as the epidemiological case requires • Derived from a virus with a high replication capacity in eggs • Low virulence in avian and mammalian species • Produced as conventional vaccines • DIVA testing possible if NA gene distinct from circulating N genes	• Reverse genetic capacity required to generate the vaccine strain • As a GMO, could be considered a disadvantage for some regulatory authorities • Percutaneous administration • Licensed technology: royalties to be paid
RECOMBINANT VECTOR VACCINE (e.g. FOWLPOX VIRUS)	• Can be administrated to one-day-old chicks with early protection • Ease in logistics and administration (hatchery level) • Enables the differentiation between infected and vaccinated birds by serologic tests • Specificity of the immune response directed exclusively against HA component • Rapid cellular response is generated • Relative stability at room temperature	• Can be used only to vaccinate chickens without previous fowlpox exposure; therefore, usually applied only to day-old chicks • Interference with anti-H5/anti-fowlpox maternal antibodies • Efficacy not proven for ducks/geese (100 times the dose might be required) • Requires percutaneous injection • Level of antibody response very low with single dose administration – thus post-vaccination monitoring through measurement of antibody levels (individual or flock) is not possible • As a GMO, could be considered a disadvantage for some regulatory authorities

or reassort with other influenza viruses and reversion or emergence of virulence could be established. Furthermore, OIE recommends that for any subtype, only well-characterized influenza A virus of proven low pathogenicity – preferably obtained from an international or national repository – should be used as a master seed for inactivated vaccine production.

Recombinant vaccines have been produced, some of which are already commercially available – in particular, fowlpox virus with the specific influenza haemagglutinin gene inserted. Reverse genetics can be used to create influenza viruses having the H antigen derived from circulating (or epidemiologically relevant) viruses in a genetic background incorporated into a viral backbone that has a high replication capacity in eggs, but that has low virulence in either avian or mammalian species.[3, 4] This combination allows for the generation of a low virulent virus with a high replicative efficiency, ensuring a high production yield of antigen and specific antigenicity. Once the vaccine seed strain is generated, these vaccines are produced as conventional inactivated vaccines and have the same properties as these vaccines.

Conventional inactivated vaccines are widely used, but reverse genetic vaccines are becoming more common, particularly in China and Viet Nam. The main immunogenic component is the haemagglutinin protein, which must be of the same subtype as the field virus. The neuraminidase antigen can be the same as the field virus. However, if differential serology is to be undertaken for monitoring vaccine response or virus activity (the DIVA method), then a different neuraminidase should be used in the vaccine (e.g. H5N2 or H5N9) and heterologous vaccines produced. Specifications for purchasing inactivated vaccines are provided in Annex 2.

The DIVA strategy requires the testing of serum samples for antibody to the neuraminidase or non-structural proteins, to differentiate that of field strain(s) from that of the vaccine strain(s). It assumes that the heterologous N antigen is not circulating in the field, and thus knowledge of circulating AI viruses (virulent or not) is known. Antibodies to non-structural proteins are interpreted as evidence of viral replication, and because the vaccine itself has been inactivated, positive findings represent virus circulation. In circumstances where there may be several strains of avian influenza virus circulating, and in countries where there are vaccines in use with several different neuraminidase antigens, it is difficult to apply this strategy. In making a vaccine selection, the requirements for a DIVA approach involve the use of additional reagents and work for field and laboratory personnel, which add to the overall expense. Therefore, a decision to apply the DIVA strategy needs to be carefully considered against these constraints; it is more appropriate to use DIVA concepts toward the end of a progressive and successful control campaign.

It is also possible to leave a small number of identified sentinel birds unvaccinated; these will aid monitoring for flock infection. If sentinel birds show disease signs or die, virus isolation and serological tests have to be done to confirm flock infection. In the event that results for HPAI virus are positive, the flock or household should be culled, the birds properly disposed of and the premises decontaminated. Field experience has shown that

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[3] D. Middleton, *et al*. 2007. Efficacy of inactivated vaccines against H5N1 avian influenza infection in ducks. *Virology* 359: 66–71.

[4] European Food Safety Authority. 2007. Scientific opinion on vaccination against avian influenza of H5 and H7 subtypes in domestic poultry and captive birds. *EFSA Journal* 489.

the use of sentinel birds is not readily accepted by poultry keepers, who want to have their whole flock protected. If no sentinels can be kept in the flock, detection of virus circulation can be instituted through the detection and reporting of abnormal and unexplained clinical signs, such as a drop in egg production, or by conducting a targeted virological examination (sampling) in vaccinated flocks or holdings. The virological sampling and subsequent testing would require additional investment and the expansion of laboratory diagnostic capabilities.

For inactivated vaccines, two doses of the vaccine must be given, approximately 30 days apart, to achieve adequate protection. The first dose should not be given to chicks before they are 6–7 days old. At the individual level, some vaccinated birds may not be fully protected from infection, but they will have increased resistance to infection, suffer less clinical disease and shed substantially less virus in the event that they become infected. At the flock level, all susceptible species should be vaccinated to minimize the chances for a virus to transmit between individuals (decrease the virus reproductive rate). Longer-lived species (e.g. ducks, geese, yellow chickens) require booster injections of vaccine to maintain protection.

Recombinant fowlpox vaccine can be used for vaccinating day-old chicks (and possibly ducklings and goslings, though immunity in these species is not widely known). The live poxvirus expressing the H antigen is applied by stab inoculation into the wing web; this method is quick (and the training of vaccinators is easy). The use of such a vaccine administration technique facilitates logistics for mass vaccination at the hatchery level. Subsequently, revaccination using a conventional vaccine is recommended to boost immunity levels. This fowlpox-based vaccine cannot be used in older birds, because they are likely to have already been exposed to the fowlpox virus and the effect of vaccination will be neutralized by the natural immune response of the bird.

Vaccination teams

Vaccination teams must be aware of the role they could play in disease transmission and outbreak spread if biosecurity principles are breached, and they must convey to the owners that protection of the birds is not immediate. For targeted approaches to vaccination, it is critical that good coverage of susceptible poultry approaches 100 percent. For this to be successful, having a sufficient number of trained vaccination teams to undertake the required operations simultaneously is essential. Planning for the number of vehicles, cold boxes to maintain the vaccines, ice packs, vaccine administration instruments and other equipment is indispensable. Documentation as to the number and species of birds vaccinated, type of vaccine administered, vaccine vials used, and registration of the owners should be part of the vaccination team's responsibilities.

4.2.2.3 Financial support

The issue of compensation for slaughtered birds, property damaged during decontamination and/or loss of income needs to be carefully considered. In principle, offering compensation encourages owners to report disease. However, it can become a very expensive undertaking and guidelines are usually developed that strictly limit the categories for compensation. It is important to consider what the cost of compensation might be and how it would be funded should a major outbreak of disease occur.

If compensation is paid, it can be controlled as follows:

- have a registration process;
- pay only for animals slaughtered, not those that have died;
- pay promptly and at a level that is close to market value (some countries have used innovative strategies such as paying slightly more than market value for healthy in-contact birds and less for sick birds, a method that allows for quick reporting to authorities);
- do not compensate losses other than livestock (birds);
- ensure that people with very small flocks are also compensated.

There are alternatives to payment of compensation in cash, and experience has sometimes shown them to be acceptable. These methods include the following:

- rather than paying cash, provide replacement birds (this can be difficult sometimes; governments have to think in advance about logistical questions of where to obtain replacements, their transport and the required restocking times);
- provide credit for owners to re-establish their poultry production, including village birds, or facilitate entry into alternative livelihoods;
- provide area assistance to enable market conditions to become re-established without undue delay;
- provide farmers with free technical and veterinary services in re-establishing production schemes.

4.3 MANAGEMENT OF DISEASE CONTROL

The FAO *Manual on the preparation of national animal disease emergency preparedness plans* includes recommendations in regard to the management of disease control operations. The following recommendations should be considered:

1. To manage disease control on a national basis, there needs to be a suitable command structure for veterinary services. It is essential that information flows quickly and efficiently from the field to national headquarters and that, conversely, control mechanisms are continuous from headquarters to the field. In recent years, government veterinary services in many countries have been restructured to include regionalization, rationalization and downsizing, privatization, separation of policy from operational functions, and separation of authority between field and laboratory operations. Countries may should consider whether they should make structural changes or alternative arrangements to adequately deal with animal health emergencies.
2. It is advisable to have a consultative committee that can meet during the period of an animal disease emergency to provide the best technical advice to outbreak management personnel. The committee might include the Chief Veterinary Officer (CVO); private industry; national directors of field and laboratory services; head of the epidemiology unit; AI experts; directors of state, regional or provincial veterinary services; and other key groups and technical experts as required. Gaining the cooperation of police, military and public works offices would be beneficial for the success of any plan.
3. At the time of an emergency, it can be a great advantage to have made arrangements in advance for a national animal disease control centre and local animal disease control centres. The control of diseases in difficult or marginalized areas of some countries may require special consideration.

Lessons learned from the H5N1 epidemics in Asia

As observed in Southeast Asia during the early stages of the H5N1 epidemic in 2004, many countries did not have a plan of action supported by national legislation at the time of HPAI detection.

The following situations existed in most affected countries:

- A structured surveillance programme, including surveillance protocols in suspected or at-risk farms, was lacking.
- Protective equipment was not available for workers and animal health personnel.
- Investigation procedures, including standardized protocols and sets of epidemiological questions for outbreak investigation and mapping, were inadequate. These protocols should also include information on collection of a standard set of samples for disease investigation from infected farms, neighbouring farms and uninfected farms outside the control areas to allow for more detailed pathological examination of dead birds, including questions regarding wildlife.
- Restocking programmes were often incomplete, with no detailed method to control the flock after restocking.
- Vaccination plans were often incomplete and did not have a coherent strategy for post-vaccination surveillance activities. The vaccines used were often of unknown quality and of dubious efficacy.
- Declarations of freedom from disease in areas or zones were made without proper scientific data supporting such claims and were subsequently proved to be false, creating additional uncertainty in the population and loss of consumer confidence.
- There was inadequate legislation, or enforcement was difficult.

4. Arrangements need to be made in advance of an outbreak. They should include negotiation with all government authorities and others who will be involved in assisting in disease control. Because certain strains of avian influenza viruses can infect humans, the respective roles of human health and veterinary services need to be considered in advance.

Field services capacity

Veterinary services must have the capacity to undertake disease surveillance, investigate and respond to disease outbreaks and report to various levels of the official veterinary service structure. In designing a proposed strategy to control avian influenza, careful consideration needs to be given to the capacity of the field services to assume disease management activities in accordance with legislation that enables and empowers decision-makers. Very few national veterinary administrations have the capacity within their own resources to undertake all the measures necessary to combat a major transboundary disease incursion. Lamentably, assistance is often sought from police, military, national disaster and other

emergency organizations late in the process, and then without proper and sufficient training. The immediate and early needs from other public offices and cooperation from private industry must be anticipated to assist field veterinary services in performing their duties. Access to extra budgetary resources should be part of the contingency planning process, and appropriate arrangements for collaboration should be negotiated in advance.

An electronic disease information database can be a great asset in tracking the huge amount of detail that can be accumulated in the course of a disease epidemic. There are many systems available, including the FAO-designed and -supported system, the Transboundary Animal Disease Information System (TAD***info***). FAO, OIE and WHO have collaborated in establishing GLEWS, the Global Early Warning and Response System for Major Animal Diseases, including Zoonoses. GLEWS enables international animal and human health authorities to receive early notice of outbreaks, or of the likelihood that certain diseases will occur, based on disease intelligence and forecasting risks. Data are analysed and information is made available to international and national disease control authorities.

4.4 LABORATORY DIAGNOSIS

Laboratory diagnosis is necessary for any confirmation of avian influenza, its biological characteristics (pathogenicity, virulence, etc.) and its differential diagnosis.

Laboratory services must be able to:

- collect or request the collection of good-quality samples from the field operations and investigations;
- undertake testing of samples collected for surveillance activities;
- test samples collected for diagnosis; and
- test samples collected for monitoring response to vaccination.

Consideration needs to be given to the capacity of veterinary services to collect and deliver specimens to a competent national laboratory. There are benefits in having regional laboratories that might have better access to the field or more advanced methods. In many countries, however, expert personnel are lacking and it may be more effective to have just one central laboratory that is well resourced and properly staffed.

4.4.1 Samples

Taking samples

As with all diagnoses, the quality and interpretation of the diagnosis are directly related to the quality of the samples. Sample selection, collection, and preservation – before and during shipment to the laboratory – are critical points for a good diagnosis of avian influenza. The kinds of samples collected should be appropriate for the intended purpose and adequate in number and amount to provide statistically valid results for the type of analysis required. Whenever one is handling biological material, from either live or dead animals, the risk of contracting a zoonotic infection, such as certain H5 and H7 AI viruses, should be kept in mind, and precautions should be taken to avoid human infection or environmental spread.

Samples, taken in priority from freshly dead and sick birds, should include both oropharyngeal and cloacal swabs because different virus strains may be present in the respiratory tract and in the digestive tract. If cloacal swabbing is difficult to perform, intestinal contents

(faeces) can be collected (at least 1 gram). Samples from the trachea, lungs, air sacs, intestine, spleen, kidney, brain, liver and heart may also be collected, particularly if they show lesions. Brain matter should be collected from any bird with neurological signs. The samples should be kept refrigerated and processed either separately or as a pool of different birds, keeping organs from nervous tissues, the digestive tract and the respiratory tract separate. Organs should be kept in watertight containers to avoid leakage of fluids. Should the investigator not be sufficiently well trained to perform a necropsy (autopsy), whole birds should be bagged twice (one bag inside another), maintained refrigerated at all times and submitted to a laboratory where a proper necropsy and sample collection can be completed.

Samples from live birds should include both oropharyngeal/tracheal and cloacal swabs. Because small, delicate birds may be harmed by swabbing, the collection of fresh faeces may serve as an adequate alternative. Blood samples can also be taken in dry tubes for serology if needed. In a suspicious flock, the carcasses or internal organs of at least 5 sick/dead birds per flock should be sampled, and, ideally, between 20 and 30 swabs and blood samples should be taken from live birds.

The tissue samples should be placed in isotonic phosphate-buffered saline (PBS), pH 7.0–7.4, containing antibiotics. The antibiotics can be varied according to local conditions, but they could be, for example, penicillin (2 000 IU/ml), streptomycin (2 mg/ml), gentamycin (50 µg/ml) and mycostatin (1 000 units/ml) for tissues and tracheal swabs, with fivefold higher concentrations of the antimicrobial cocktail for faeces and cloacal swab samples. It is important to adjust the pH of the PBS solution to pH 7.0–7.4 following the addition of the antibiotics. Faeces and finely minced tissues should be prepared as 10–20 percent (w/v) suspensions in the antibiotic solution. Suspensions should be processed as soon as possible after incubation for 1–2 hours at room temperature (22–25 °C).

All samples should be stored between 2 °C and 8 °C as soon as possible after sampling. Cold boxes should be taken in the field with ice packs to store the samples taken during field investigation or studies. If samples are to be kept for several days before testing/shipment, they should be kept frozen at –80 °C. Freezing any swabs or tissue samples between 0 °C and –20 °C (as in many domestic freezers) should be avoided. Never use alcohol to preserve samples.

Actions before sending samples

Submission of samples to any laboratory outside the country of origin should always be subject to prior agreement between the veterinary authorities of the shipping country and the recipient laboratory (see Annex 4 for details).

Mode of transport

An international courier company specializing in infectious substance transport or air freight[5] is usually used to send diagnostic samples to any laboratory outside the country of origin. If air freight is to be used, a discussion with the recipient laboratory regarding

[5] Air freight is a counter-to-counter package transport system for the shipment of small parcels. These shipments have size, weight, and content restrictions, and usually may be dropped off and picked up at a ticket counter, luggage service or freight office.

the pickup arrangement at the destination airport is necessary, as an airline company will not forward the air freight shipment outside the airport. Some countries have specifically designated airports as the first port of entry for certain types of samples; this practice will limit the possibility of using some airlines or couriers.

Preparation of shipment

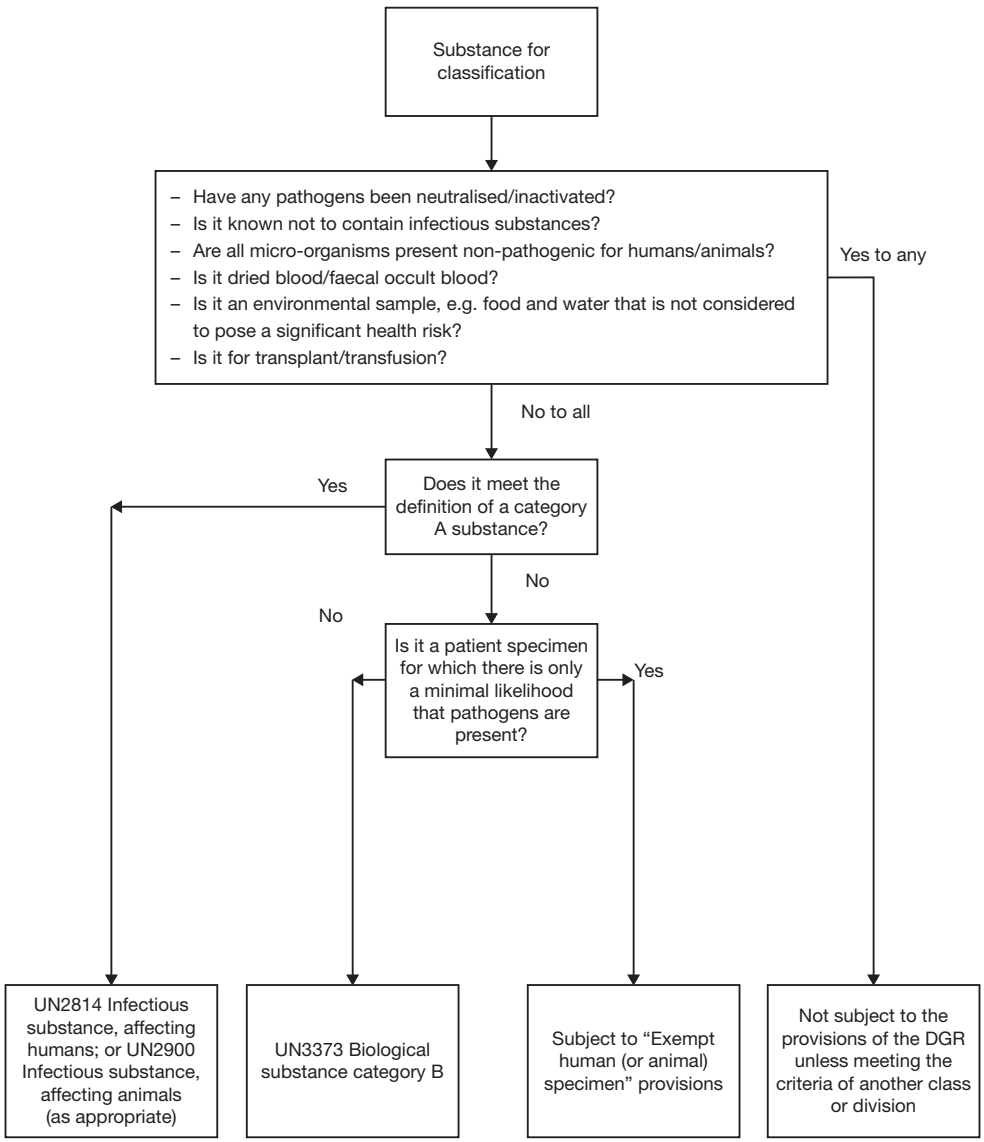
Diagnostic samples and isolates of an animal disease pathogen (packed with or without dry ice) are classified as "Dangerous Goods," and more specifically as "Infectious Substances," under the Dangerous Goods Regulations (DGR) of the International Air Transport Association (IATA). These regulations are based on the rules set by the United Nations Economic and Social Council's Committee of Experts on the Transport of Dangerous Goods (UN/SCETDG) and the International Civil Aviation Organization (ICAO). Submission of samples to any laboratory by air should therefore follow the DGR and be transported in containers that meet the Packing Instruction (PI) described in the DGR. It is required that all personnel who handle the shipment of infectious substances be trained in related regulations in advance. See Figure 15, and Tables 3 and 4, for more information on Dangerous Goods categories.

Category of samples

Specimens collected directly from animals (or humans) that are suspected or confirmed to be infected with the avian influenza A (H5N1) virus, including specimens from the respiratory tract (swabs) and blood specimens, should be shipped as "Biological Substance, Category B" and designated as UN 3373.

Shipping Category A substances requires shippers to have completed specific training. For shipping Category B substances or neutralized/inactivated samples (e.g. nucleic acid preparations—RNA samples—containing no viable virus), no specific training is required. If the shipment also includes other dangerous goods (such as liquid nitrogen or dry ice), shippers must be trained appropriately in the transport of those goods.

FIGURE 15
Classification flowchart



Source: IATA. Guidance Document: Infectious Substances (http://www.iata.org/NR/rdonlyres/9C7E382B-2536-47CE-84B4-9A883ECFA040/0/Guidance_Doc62DGR_50.pdf).

TABLE 3
Sample categories, UN codes, proper shipping names and examples

UN code	Definition	Proper shipping name	Class/ Division	Packing group	Packing Instruction	Susceptible species	Example
UN2814	Infectious substances meeting these criteria that cause disease in humans or in both humans and animals	Infectious Substance, affecting humans	6.2	N/A	PI602	Human; Human and animal	Highly pathogenic avian influenza virus isolates,[1] influenza A H1N1 virus isolates pathogenic to humans, Ebola virus, Nipah virus
UN2900	Infectious substances that cause disease only in animals	Infectious Substance, affecting animals	6.2	N/A	PI602	Animal only	Foot-and-mouth disease virus isolates, ASF virus isolates, other influenza isolates of animal origin
UN3373	Infectious substances that do not meet the criteria for inclusion in Category A (UN2814 or UN2900)	Biological Substance, Category B	6.2	N/A	PI650	Any	Samples for infectious disease diagnosis (e.g. swab, serum samples for avian/ swine influenza diagnosis)
No code (not dangerous goods)	Substances that do not contain infectious substances or that are unlikely to cause disease in humans or animals	N/A	N/A	N/A	N/A	Any	Samples for non-infectious disease diagnosis

[1] See http://www.iata.org/NR/rdonlyres/D9C935A0-7382-4567-B6EB-55B53F757C52/0/dgr50_InfectiousSubstancespdf.pdf
N/A = Not applicable

Temperature conditions during the shipment

If samples (serum, plasma and fresh tissues) can be shipped to arrive at a laboratory within 24–48 hours, they can be packaged with frozen ice packs (which will maintain the samples at around 4 °C during the transfer). Most frequently, samples for virus detection will have to be shipped by air and preserved in dry ice or liquid nitrogen.

TABLE 4
Cooling materials

UN code	Item	Proper shipping name	Class/ Division	Packing group
UN1845	Dry ice	Carbon dioxide, solid (dry ice)	9	III
UN3158	Liquid nitrogen	Gas, refrigerated liquid (liquid nitrogen)	2.2	N/A
No code (not dangerous goods)	Ice pack	N/A	N/A	N/A

Documentation

The following are generally required for shipping:

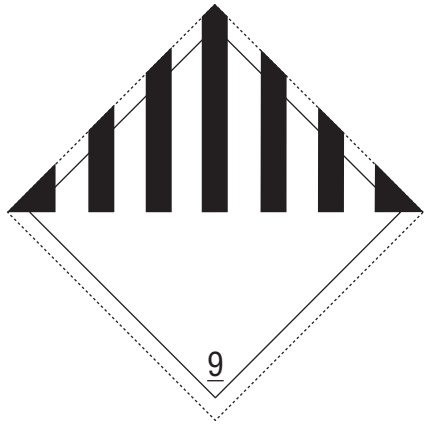
1. *Dangerous Goods Declaration* (DGD, or shipper's declaration). A DGD is required for UN2814 or UN2900 category shipments. The shipper, usually the sending laboratory, is requested to attach the DGD form to such shipments. The shipper must have been trained in specific dangerous goods transport requirements. The DGD must include the name of the shipper and the address, a specification of the shipment with its "UN code" and "Proper shipping name", the "class/division" of the contents (see Tables 3 and 4) and other required information defined in the DGR. The DGD must be signed by the person responsible for the shipment.
   When dry ice is used as a refrigerant for UN2814 or UN2900 samples, the details of the dry ice must be shown on the "Nature and Quality of Dangerous Goods" section of the DGD.
   The DGD is not needed for a UN3373 shipment, as long as the package satisfies PI650 packing instructions.
2. ***Customs invoice*** (pro forma invoice). In most of the cases, a customs invoice prepared by the shipper is required for the sample to undergo customs clearance at the country of destination.
3. ***Import permit***. If contacted prior to the shipment of the sample, the recipient laboratory should be able to give guidance and/or provide an import permit for the sample.
4. ***Export certificate*** (animal health certificate of the exporting country). In some cases, an export certificate is required to import the sample into the country of destination. The recipient laboratory should be able to provide advice on any such requirement.
5. ***Sample details***. A letter addressed to the recipient laboratory should accompany the parcel with as much of the epidemiological history about the samples as possible, including species and age, area/country of sampling, date of sampling, any clinical findings, method and temperature at which samples were stored, etc. If several samples are included, they should have clear and distinct identification numbers. Contact details of the person who was involved in the sampling should also be given to the recipient laboratory.
6. ***Air waybill*** (AWB). An AWB is a receipt issued by an international carrier company of goods and is evidence of the contract of carriage. The shipper must ask for the AWB number and inform the recipient laboratory of the AWB number immediately.

Packing of shipment

All materials should be in leakproof containers (e.g. plastic sample vial). These should then be placed in a leakproof secondary container and transported in an IATA-approved outer container. The required packing type depends on the UN code of the contents (see Table 3). The outer box must be correctly marked and labelled according to the DGR (See Annex 5). A commercially available shipping box satisfying PI602 requirements can be used for UN3373 shipments. In such cases, the Class 6 (Infectious Substance) label (Figure 16) printed on the outer box must be covered or masked (i.e. it must not be visible). When dry ice is used as a refrigerant for UN3373, the net quantity of dry ice must be shown on the

FIGURE 16
Class 6 – Infectious Substances (Division 6.2)

FIGURE 17
Class 9 – Miscellaneous Dangerous Goods



outside of the package. If liquid nitrogen is used for cooling purposes, the plastic primary container and the secondary container must both be capable of withstanding very low temperatures. For more information see the IATA's *Guidance Document: Infectious Substances* (http://www.iata.org/NR/rdonlyres/9C7E382B-2536-47CE-84B4-9A883ECFA040/0/Guidance_Doc62DGR_50.pdf).

It is recommended that photocopies be made of all the above-mentioned documents (in sections 1 through 4 above), and that they be placed in a transparent plastic bag attached to the surface of the shipment package. This is a useful practice that helps avoid delays due to any uncertainty about the contents of the shipment.

Actions prior to, and immediately after, the shipment

As soon as the transport details (shipping date, airline name, flight number) are identified (usually several days before the shipment), the shipper must inform the recipient laboratory in order for it to start the preparations for testing. As soon as the AWB number of the shipment becomes available (usually several hours before the actual shipment) the shipper must inform the recipient laboratory of the AWB number without delay. This AWB number is needed to receive the shipment, especially in the case of air freight.

Contingency planning

- It is recommended to confirm where and how dry ice can be obtained in your country before the actual need arises.
- It is recommended to confirm where and how IATA-approved containers for sample shipment (PI602 and/or PI650) can be obtained in your country before the actual need arises.
- It is recommended to find out which courier or airline operator can transport infectious substances from your country before the actual need arises. If air freight is used, it is strongly recommended to use a direct flight. Because of a lack of requisite training, some airline operators and airports are not able to accept diagnostic samples.

Nor is it easy for a shipper to confirm whether all the relevant personnel at the transit airport(s) or connecting flight(s) are trained in handling infectious substances. It is therefore safer if the shipper can use an international courier company that specializes in transporting infectious substances.

4.4.2 Laboratory tests

Methods for testing are described in the OIE *Manual of diagnostic tests and vaccines for terrestrial animals* (http://www.oie.int/eng/normes/mmanual/2008/pdf/2.03.04_AI.pdf), Chapter 2.3.4. Consideration needs to be given not just to having the appropriate technology but to having the capacity to handle a large number of specimens in the event of a disease control emergency.

Virological methods

Virological characterization must be conducted for confirmation of HPAI suspected cases. Although virus isolation is considered the gold standard, other methods can be used, such as haemagglutination, RT-PCR (reverse transcription-polymerase chain reaction), antigen-capture ELISA (enzyme-linked immunosorbent assay) and certain rapid antigen detection assays.

Virus isolation is done in embryonated chicken eggs and should be conducted in BSL3 (biosafety level 3) facilities to avoid transmission to humans. This method allows the detection of all AI virus subtypes and variants. Haemagglutinin and neuraminidase typing or cDNA sequencing (derived from the RNA virus) are applied on the isolated strains for further virus or genetic characterization.

RT-PCR is a highly sensitive and specific method that can be conducted on samples that do not require initial virus isolation procedures. RT-PCR is rapid, with results available within 8–36 hours after its initiation, and it provides precise virus characterization (subtype and pathotype). It can be performed in BSL2 facilities because no virus growth is necessary. It allows a pooling of samples (5–10 PCR per reaction). However, it is prone to laboratory contamination, especially conventional PCR, unless strict procedures are followed in every step of the process. Therefore, a competent staff is needed, as well as adequate laboratory facilities (separate rooms to carry out different steps of the PCR procedure, exclusive instruments used only for PCR, specialized supplies, etc.). The high costs of equipment (US$25 000–90 000), equipment maintenance and reagents are likely to be a limitation in certain countries. A first screening of type A positive samples can be performed by conducting the M gene RT-PCR, followed by H5/H7/H9 PCR for specific virus subtyping. Both real-time and conventional PCR can be used. The "rapid antigen detection" assays are included in the OIE *Manual of diagnostic tests and vaccines for terrestrial animals*. They are immunoenzymatic tests for antigen detection (chromatographic or classical ELISA). Several kits are commercially available (e.g. Flu Detect™ by Synbiotics; ANIGEN Rapid AIV Ag Test Kit by Anigen; Directigen Flu A® by Becton Dickinson; BinaxNOW® Avian Influenza Virus Type A Antigen by Binax, Flu OIA® by Biostar Inc.). The main advantages of these assays are their rapid results (obtained within 15–30 minutes) and their simple protocol. As no special facilities are required, they can be used in the field or in laboratories with low biosecurity levels, and they can be stored at room temperature (20–25 °C). However,

because these tests have a lower sensitivity and specificity than PCR or virus isolation,[6] submission to a competent laboratory for confirmation should be systematic for any positive results by rapid antigen detection tests, especially for index cases. In cases of negative results in flocks or individual animals showing clinical signs comparable to those of HPAI, samples should likewise be submitted. False positives or negatives can appear in cases of poor sample quality, low virus excretion (e.g. low pathogenic virus infection strains, vaccinated chickens) or non-specific or interfering reactions. To date, these assays will detect only type A influenza viruses, not specific subtypes. Finally, these assays have not been validated for use on waterfowl and wild birds, and they are relatively costly (between US$2.50 and US$5 per test). These assays, however, can be very valuable for situations where limited laboratory capacities are available and in remote areas far away from laboratories. Their judicious use and a knowledge of their advantages and limitations are considered valuable for taking immediate action to quarantine an area and institute other control measures before final confirmation by a laboratory.

Further virus characterization can be conducted using sequencing and the intravenous pathogenicity index (IVPI) test. Nucleic acid sequencing provides information on the amino acid sequence at the HA cleavage site, and thus allows, for H5 and H7 viruses, a determination of the virus pathotype: HPAI or LPAI. Sequencing is also performed for phylogenetic analysis (genotyping), and the information is ideally deposited in the public domain through a database (see *OFFLU guidance on avian influenza sequence databases,* http://www.offlu.net/OFFLU%20Site/offluwebdatabases.pdf). Other genetic markers can also be studied using sequencing of the NS gene (i.e. resistance to antivirals) or to the PB2 gene (i.e. adaptation to mammalian hosts).

The IVPI test assesses the pathogenicity of AI viruses in chickens isolated from birds for pathotype determination (HPAI or LPAI). This method requires BSL3 animal facilities because live birds will be inoculated with the isolated virus. This test is mainly performed in international reference laboratories. AI isolates that have indices greater than 1.2 are considered HPAI. Sequencing of the HA cleavage site has enabled to avoid systematic *in vivo* tests and has provided quicker and cheaper pathotype determination.

Serological methods

Serological tests can be performed in BSL1 laboratories and require less technology than virological methods. Serological testing is applied in the context of:

- general surveillance programmes;
- differentiation of avian influenza post-infections from other pathogens;
- subtyping post-infections; and
- vaccination efficacy monitoring.

Serological assays include AGID (agar gel immunodiffusion) and ELISA, which allow the detection of type A antibodies and can be used to assess the circulation of AI virus in an avian population. By using HI or subtype-specific ELISA-based assays, H5 and H7 virus

[6] Levels specified by manufacturers in chickens are: 90–100 percent specificity, 60–90 percent individual sensitivity, and 100 percent flock sensitivity.

circulation (notifiable diseases) can be monitored. Serology cannot, however, allow the detection of antibodies against both H and N antigens (e.g. H5N1) .

Countries should also have access to international expertise to assist in confirming the identity and characteristics of isolates and in undertaking further key studies that are of particular importance at the international level. It is important to monitor field viruses for early detection of antigenic/genetic changes, and this can be done through a systematic sampling of birds, both during an epizootic period and in inter-epizootic periods, and sending suspect samples to a reference laboratory for confirmation and further characterization.

A list of the OIE/FAO reference laboratories is in Annex 3. In April 2005, OIE and FAO created and endorsed the OIE-FAO joint Network of Expertise on Animal Influenza (OFFLU). Following the emergence of the novel influenza A/H1N1, the network has expanded its mandate to cover all animal influenzas. The objectives of OFFLU are:

- to exchange scientific data and biological materials (including virus strains) within the network, to analyse such data, and to share such information with the wider scientific community;
- to offer technical advice and veterinary expertise to member countries to assist in the prevention, diagnosis, surveillance and control of animal influenza;
- to collaborate with the WHO influenza network on issues relating to the animal-human interface, including early preparation of human vaccine; and
- to highlight influenza research needs, promote their development and ensure coordination.

Through active and permanent scientific cooperation, the network develops collaborative research proposals, provides experts and multidisciplinary teams to countries requiring assistance and acts as a link between OIE/FAO reference laboratories regional and national laboratories and with WHO and public health laboratories. OFFLU can act as a technical platform for discussions and compilation of information regarding testing protocols, new vaccines, surveillance, etc. OFFLU has two technical projects on AI vaccine efficacy implemented by FAO in Indonesia and Egypt. Sharing permanently updated scientific information and expertise on efficient control methods will provide a proactive approach in helping infected countries to progressively control and eradicate HPAI H5N1 in the poultry sector. It will also help disease-free countries to protect themselves better. For more detailed information, see the OFFLU Web site (http://www.offlu.net).

Field diagnosis

Decisions often need to be made immediately in the field during an emergency. Waiting for a definitive laboratory diagnosis before taking action to quarantine an area can lead to critical delays. Clinical and pathological findings in the field may be sufficient to make a presumptive diagnosis of HPAI, and measured actions can be taken accordingly.

4.5 COMMUNICATION AND PUBLIC AWARENESS

For detection, response and containment measures to be effective, key stakeholders must be in receipt of appropriate, credible and practicable knowledge, awareness and information. Only then can they make appropriate decisions and take necessary action. Thus, com-

munication and public awareness play a significant role in preparing for HPAI or other animal health emergencies. Public awareness campaigns are designed:

- to inform farmers, marketers and intermediaries, traders, animal health workers and consumers about the infection channels and risks related to AI;
- to communicate information (e.g. time and venues for vaccination);
- to promote better farming practices and improved hygiene and biosecurity (though in many village situations it may be unrealistic to expect to be able to improve biosecurity);
- to gain assistance from the community by having poultry owners report unusual sickness and mortalities in poultry and other animals – community residents are encouraged to report through the relevant authorities, animal health workers, community leaders and others, together with the use of hotlines (it is also important that the resources for receiving reports are sufficient);
- to raise awareness about the risk of poultry infection and the means of prevention (see Annex 7: Leaflet example);
- to design appropriate messages and modes of communicating messages for each group (See *Protect poultry, protect people: Basic advice for stopping the spread of avian influenza,* http://www.fao.org/avianflu/documents/ProtectPoultry-ProtectPeople.pdf, for further details on target groups and messages) – messages should be clear, consistent, feasible and achievable;
- to use a combination of communication modes and tools to maximize awareness and behaviour change, including mass media (television, radio, print, etc.), IEC materials (brochures, posters, banners, stickers, etc.), interpersonal communication and community outreach ("face-to-face" meetings involving animal health workers, extension workers, local/community/religious leaders, etc.), and participatory approaches (e.g. utilizing the input of those at risk in developing communication and public awareness plans, activities and materials);
- to mobilize existing networks and structures (e.g. local government authorities, animal health workers, para-vets, extension workers, community leaders) to engage in dialogue with communities, and to ensure that communication training and capacity building are provided to enable this;
- to promote the establishment of networks of local volunteers who will receive training from front-line workers and assist them in providing information on surveillance and in reporting potential outbreaks (or encouraging farmers and other poultry handlers to do so);
- to build and maintain a relationship of trust and credibility with farmers and consumers by communicating information in a timely, accurate, transparent and consistent manner (e.g. announcing outbreak, time and venues for vaccination, communication procedures for obtaining compensation);
- to encourage others to cooperate with animal and human health authorities during disease control activities, should an emergency occur;
- in conjunction with human health authorities, to help raise awareness about the risk of transmission from poultry to humans and about disease in humans;

- to develop a national communication task force and an action plan for addressing HPAI and other transboundary animal diseases; and
- to devise a monitoring and evaluation (M&E) plan before a communication plan, campaign or activity is launched – if an M&E plan is not established at the beginning, it will not be possible to evaluate the impact of communication activities.

4.6 HUMAN HEALTH AND SAFETY CONSIDERATIONS

Certain avian influenza viruses can infect humans, occasionally causing severe disease and sometimes death. The WHO Web site (http://www.who.int) provides updates and recommendations with regard to symptoms, patient care and management, as well as recommendations on how to reduce occupational risks (e.g. using personal protective equipment). Listed below are various factors that should be considered.

General considerations for the human population

- Vaccination with seasonal human influenza vaccine (which contain H1, H3 and B virus antigens) minimizes the risk that multiple influenza infections will cause a new virus to emerge.
- Avoid unnecessary contact with infected or exposed poultry, poultry products and poultry waste.
- In particular, children and people in poor health should avoid all contact with affected birds and their environment.
- Do not purchase or consume poultry that are not healthy or not wholesome.

Specific considerations for all personnel involved in disease control

- In selecting workers, one should avoid those in high-risk categories.
- Workers should wear protective overalls and, where gross contamination is likely, a waterproof apron.
- Disposable or rubber work gloves should be worn.
- A disposable P2 or N97 respirator should be worn.
- Goggles or a visor should be worn to protect against eye splash.
- Disposable footwear or rubber boots should be worn.
- Protective clothing should be disinfected after use.
- Hands should be washed after protective clothing is removed.
- The use of antiviral drugs is recommended, during and for seven days after exposure.
- Workers should monitor their health, watching for signs of fever, respiratory symptoms (e.g. cough) and conjunctivitis (eye inflammation).
- Illness in workers or their close contacts should be reported to public health authorities immediately.