Highly pathogenic avian influenza (HPAI) represents a threat to poultry industries worldwide and to people’s livelihoods, and a potential threat to human health. The international community has a vested interest in minimizing the spread of this disease. Countries may be under threat of introduction of HPAI through unregulated poultry trade and marketing practices and, on rare occasions, exposure of poultry to wild birds, especially waterfowl.

The Food and Agriculture Organization of the United Nations and the World Organisation for Animal Health prepared the first edition of this manual to help national animal health authorities and other stakeholders prepare for a possible incursion of HPAI, detect disease as soon as possible and respond as rapidly as possible to contain the disease. This second edition reflects lessons learned and provides additional details.

The manual offers practical advice on disease identification, pathology and diagnosis; detection, response and control strategies; and biosecurity measures to prevent outbreaks. It is an invaluable source of useful information for anyone involved in poultry-keeping and animal health practices.
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Preface

The first version of this manual was written in 2004–2005 by FAO while the H5N1 HPAI crisis in Asia and parts of Eurasia was at a high level of occurrence and spread. Subsequently, the H5N1 virus spread to other continents. The second edition takes into account lessons learned from working with and engaging countries and regional bodies in the fight to prevent and better control H5N1 HPAI. Some of the examples used in the first edition were meant only for illustrative purposes but were interpreted by some readers as actions to be applied in their particular setting. Changes have therefore been made for clarity.

In addition, it is evident that the procedures recommended as an immediate response to an H5N1 HPAI incursion are often not sustainable, for logistical or socio-economic reasons, if the disease is entrenched throughout the poultry production sector, markets and the environment. Though the title of this manual refers to preparedness – which alludes to prevention and detection – users of the manual have requested that further guidelines address aspects to be implemented for the progressive control of the virus and its eventual elimination from poultry.

The authors are grateful to our colleagues Astrid Tripodi and Scott Newman for their complimentary additions and suggestions in preparing this edition.

J. Lubroth
Rome, May 2009
# Acronyms

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<tr>
<td>AGID</td>
<td>agar gel immunodiffusion</td>
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<td>AI</td>
<td>avian influenza</td>
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<tr>
<td>AWB</td>
<td>air waybill</td>
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<tr>
<td>BSL2</td>
<td>biosafety level 2</td>
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<tr>
<td>BSL3</td>
<td>biosafety level 3</td>
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<tr>
<td>CA</td>
<td>control area</td>
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<tr>
<td>cDNA</td>
<td>complementary deoxyribonucleic acid</td>
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<td>CO₂</td>
<td>carbon dioxide</td>
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<tr>
<td>CVO</td>
<td>chief veterinary officer</td>
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<tr>
<td>DGD</td>
<td>dangerous goods declaration</td>
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<td>DGR</td>
<td>dangerous goods regulations</td>
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<tr>
<td>DIVA</td>
<td>differentiation of infected from vaccinated animals</td>
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<td>EFSA</td>
<td>European Food Safety Authority</td>
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<tr>
<td>ELISA</td>
<td>enzyme-linked immunosorbent assay</td>
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<td>EMPRES</td>
<td>Emergency Prevention System for Transboundary Animal and Plant Pests and Diseases</td>
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<td>FAO</td>
<td>Food and Agriculture Organization of the United Nations</td>
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<td>GLEWS</td>
<td>Global Early Warning and Response System</td>
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<td>H</td>
<td>haemagglutinin</td>
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<td>HA</td>
<td>haemagglutinin antigen</td>
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<td>HPAI</td>
<td>highly pathogenic avian influenza</td>
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<td>HPNAI</td>
<td>highly pathogenic notifiable avian influenza</td>
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<td>IA</td>
<td>infected area</td>
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<td>IATA</td>
<td>International Air Transport Association</td>
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<td>ICAO</td>
<td>International Civil Aviation Organization</td>
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<td>IEC</td>
<td>information, education and communication</td>
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<td>IVPI</td>
<td>intravenous pathogenicity index</td>
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<td>LPAI</td>
<td>low pathogenic avian influenza</td>
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<td>LPNAI</td>
<td>low pathogenic notifiable avian influenza</td>
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<tr>
<td>M&amp;E</td>
<td>monitoring and evaluation</td>
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<td>N</td>
<td>neuraminidase</td>
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<td>NA</td>
<td>neuraminidase antigen</td>
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<td>NSP</td>
<td>non-structural protein</td>
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<td>OFFLU</td>
<td>OIE-FAO Joint Network of Expertise on Animal Influenza</td>
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<td>OIE</td>
<td>World Organisation for Animal Health</td>
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<td>PBS</td>
<td>phosphate-buffered saline</td>
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<td>PCR</td>
<td>polymerase chain reaction</td>
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<td>PI</td>
<td>packing instruction</td>
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<td>RA</td>
<td>restricted area</td>
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<td>RNA</td>
<td>ribonucleic acid</td>
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<td>RT-PCR</td>
<td>reverse transcription-polymerase chain reaction</td>
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<td>TADinfo</td>
<td>Transboundary Animal Disease Information System</td>
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<td>UN/SCETDG</td>
<td>United Nations Economic and Social Council's Committee of Experts on the Transport of Dangerous Good</td>
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1. Introduction

Highly pathogenic avian influenza (HPAI) has seriously affected poultry farmers wherever and wherever it has appeared. Historically, outbreaks of HPAI have occurred on all continents. The current avian influenza epidemic, caused principally by the H5N1 strain, has been continuing since it was first recognized in the Republic of Korea in December 2003. Thailand, Cambodia, the Lao People’s Democratic Republic, India and Malaysia have been able to eliminate disease occurrence, but recrudescence, or reintroduction of disease, has been observed. Viet Nam and China have controlled extensive outbreaks through the widespread application of vaccine, while Indonesia and Bangladesh report widespread occurrence with and without vaccination.

Two circumstances have increased international concern about the behaviour and spread of this disease. The first is that 433 cases of transmission of the virus to humans had been recorded as of 2 June 2009, with 262 deaths reported.1 There is increasing concern that in the future the virus will adapt to enable human-to-human transmission with ease and result in a global human influenza pandemic. Second, the disease has now spread over a much greater geographical area. Between August and December 2005, the disease was reported in the Russian Federation, Turkey, Croatia, Romania and Ukraine. In February 2006, it spread to the African continent, where the first notification of the H5N1 HPAI strain occurred in Nigeria. The occurrence of the disease in Africa is of major concern, putting at immediate risk the livelihood of millions of people relying on poultry production for income generation and sources of protein.2 If this situation runs out of control, it will have a devastating impact on the poultry population in the region and increase the exposure of humans to the virus.

It is difficult to predict the severity of either of these threats. The virus has been present in China since at least 1996, and it probably disseminated to Southeast Asian countries at least some months before it developed into the epidemic that began in 2003. There has been enormous opportunity for the virus to infect humans, and such infection has probably occurred much more than has been identified, yet adaptation for human-to-human transmission has not yet occurred. However, this does not imply that it will not occur, and the greater the shedding of virus from infected poultry, the greater the risk of adaptation leading to a human pandemic. Similarly, despite opportunities for virus introduction by wild birds to poultry or from poultry to wild birds, by far the most important aspects of

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2 In this document, poultry is defined as “all birds reared or kept in captivity for the production of meat or eggs for consumption, for the production of other commercial products, for restocking supplies of game, or for breeding these categories of birds”. This definition was adopted by the OIE in the 2005 edition of the Terrestrial Animal Health Code (see chapter on avian influenza).
the spread and maintenance of the disease are the various approaches used around the world in poultry husbandry practices, marketing and trade. At the time of this writing, the areas of great concern are China, the countries that border the Bay of Bengal (Bangladesh, Myanmar and India), Indonesia, Pakistan, Egypt and Nigeria. The H5N1 virus in its highly virulent form has not been reported in the Americas or in Oceania.

This manual is intended to assist national animal health authorities and other stakeholders in preparing for a possible incursion of HPAI, detecting disease at the earliest opportunity and responding as rapidly as possible to contain the disease after it is detected.

The international community has a vested interest in minimizing the spread of this disease. FAO, the World Organisation for Animal Health (OIE) and the World Health Organization (WHO) are the key agencies for coordinating an international response to the threat. This manual also assists countries in determining the means of obtaining outside assistance to improve their preparedness for highly pathogenic avian influenza and its detection.
2. Avian influenza and the viruses that cause it

Avian influenza is caused by influenza viruses that are common in wild birds and occasionally infect poultry. When poultry are infected, they may have no disease, mild disease or very severe disease. Chickens, quail and turkeys are especially susceptible, while ducks more commonly show no disease but act as a reservoir for the virus. Other poultry species, including guinea fowl and pheasants, and also ostriches, can become affected. While wild birds are generally not affected by the AI viruses that they carry, they can occasionally suffer disease. This has been observed as a result of infection with the H5N1 virus in Asia and parts of Europe and may be a result of the virus’s first becoming highly virulent in domestic birds.

Influenza viruses have two main surface antigens: haemagglutinin (H) and neuraminidase (N). There are many H and N subtypes, but highly pathogenic avian influenza viruses have historically been H5 and H7. In addition, H9AI viruses are widespread around the world and known to cause sufficient morbidity to warrant their monitoring to see if they develop into more virulent forms. The avian influenza virus currently causing the major epidemic in Asia is H5N1, with some occurrences of H5N2 being reported as well. The viruses that have caused disease in Pakistan in recent years are H7N3 and H9N2, but H5N1 has also become a problem of great concern there. AI viruses are also classified by pathotype – highly pathogenic (HPAI) and low pathogenic (LPAI) – a biological characteristic of the virus’s virulence in chickens. Currently, the pathotype definition has been expanded to include the genetic sequence coding for basic amino acids in the cleavage site of the H protein. All AI viruses that have these sequences at the critical site are considered notifiable, and the viruses are denoted as HPNAI (highly pathogenic notifiable avian influenza) and LPNAI.

The known evolution of H5N1 viruses in Asia began in 1996 in southern China with the identification of an HPAI virus (Goose/GD/96) that was fatal to geese in Guangdong province. In 1997, the Hong Kong Special Administrative Region experienced the first major outbreak of H5N1; it was associated with seven human deaths, alerting the international community for the first time of the potential threat caused by this new strain of this virus as a true zoonosis. Though exact dates and figures remain unknown, retrospective analysis reveals that in mid-2003 the H5N1 virus strain of AI expanded its territorial enclave elsewhere in China. Within months – late 2003 to early 2004 – numerous countries in Asia reported its occurrence, and by late 2007 the disease was reported in over 60 countries. Though many or most countries have been able to limit its spread or occurrence in poultry, the virus has become endemic in several key countries – a worrisome reality because of three main factors: (1) high poultry density and duck-rearing practices, (2) wildlife habitats for possible maintenance in long-term or continuous virus circulation within the wild bird population and (3) unregulated trade.
In mid-2005, following a major outbreak in wild birds in Lake Qinghai located in western China, H5N1 outbreaks were detected in the Russian Federation, Kazakhstan and Mongolia. The disease was then reported in Romania, Turkey and Croatia in October 2005, confirming the westward spread of the virus and the potential threat to other countries and continents still free of the disease. The epidemic eventually reached Africa, where it was first reported in Nigeria in February 2006.

The emergence of the H5N1 strain in Asia and its subsequent spread to other continents is a result of, among other factors, years of rapid development of unregulated poultry production to meet the increased demand for animal protein. Highly concentrated domestic poultry production in densely human-populated regions and a rapid evolution of animal and farming production systems have provided the ideal conditions for the emergence of new virulent strains of avian influenza. Although there are numerous commercial-level poultry operations in countries affected by the recent H5N1 HPAI strain, the majority of such operations are still “backyard” activities where surveillance and biosecurity are minimal and farmers have little knowledge of the potential linkages between their husbandry activities and disease spread or associated human health risks. The exchange between commercial poultry species and backyard farms is often very active, allowing for the transmission of this virus between these two not-so-separate sectors. Furthermore, farmers who depend on their fowl for subsistence are reluctant to destroy their flocks or to inform the authorities about sick birds, preferring to attempt to sell them or eat them, thus contributing to the subsequent spread of the disease.

The transboundary trade in poultry and poultry products, both legal and informal (traditional), is likely to have contributed to the spread of HPAI viruses. Long land borders exist between many of the infected and at-risk countries, and the smuggling of poultry and poultry products across many of these borders is acknowledged, as is the practice of intercommunity gamecock fights.

2.1 CLINICAL SIGNS
The clinical signs of AI infection are variable and influenced greatly by the virulence of the viruses involved, the species infected, age, concurrent viral or bacterial disease and the environment. The virulence exhibited in chickens can vary during an outbreak.

Infection with non-pathogenic viruses
- There are no clinical signs in infected birds, so evidence of exposure is determined by serological conversion.
- Some of these viruses have the potential to become virulent through genetic mutation.

Infection with low or mild virulent viruses
- Clinical signs in chickens and turkeys range from inapparent to mild or severe respiratory disease and can be confused with infectious laryngotracheitis and other respiratory tract infections.
- Mortality ranges from 3 percent in caged hens (layers) to 15 percent in meat chickens (broilers).
• Egg production in layers can sometimes drop to 45 percent of the expected egg yield of a large flock, returning to normal levels of production in 2–4 weeks.
• Mutation to virulence has been demonstrated in outbreaks.

**Infection with highly pathogenic viruses**
• In peracute cases involving sudden death, as in the 2004–2005 outbreak in Viet Nam, clinical signs may not be seen and mortalities may occur within hours after onset of depression (i.e. despondence, inactivity, lethargy, etc.). Overall mortality rates for peracute/acute cases nearing 100 percent have been reported.
• In acute cases, mortalities occur as early as 24 hours after the first signs of the disease, and frequently within 48 hours. In other cases, more diverse visible signs are seen and mortalities can be delayed for as long as a week.
• Clinical signs in chickens and turkeys include severe respiratory distress with excessively watery eyes and sinusitis; cyanosis of the combs, wattle and shanks; oedema of the head and eyelids; ruffled feathers; diarrhoea and nervous signs.
• Eggs laid after the onset of illness frequently have no shells.
• Some severely affected hens may recover, but they rarely come back into lay.

The disease in turkeys is similar to that in chickens, but it is often complicated by secondary bacterial infections such as those resulting from fowl cholera (*Pasteurella multocida*), turkey coryza (*Haemophilus gallinarum*), or colibacillosis (*Escherichia coli*).
2.2 GROSS PATHOLOGY

In many cases, poultry dying from the peracute form of the disease lack visible gross pathological lesions. With acute infections in chickens, there is severe lung congestion, haemorrhage and oedema in dead chickens, while other organs and tissues appear normal. More varied visible lesions are seen in chickens surviving 3–5 days, including congestion and/or cyanosis of the comb and wattles and swollen heads. The changes in the combs and wattles progress to depressed areas of dark red to blue areas of ischaemic necrosis. Internally, the characteristics of acute infections with viruses causing HPAI are haemorrhagic, necrotic, congestive and transudative changes. The oviducts and intestines often have severe haemorrhagic changes.

As the disease progresses, the pancreas, liver, spleen, kidney and lungs can display yellowish necrotic foci. Haemorrhages (petechial and ecchymotic) cover the abdominal fat, serosal surfaces and peritoneum. The peritoneal cavity is frequently filled with yolk from ruptured ova, associated with severe inflammation of the airsacs and peritoneum in birds that survive 7–10 days. Haemorrhages may be present in the proventriculus, particularly at the junction with the ventriculus (gizzard).

In cases resulting from mild pathogenic avian influenza viruses, lesions may be seen in the sinuses, characterized by catarrhal, serofibrinous, mucopurulent or caseous inflammation. The tracheal mucosa may be oedematous with exudates varying from serous to caseous. The airsacs may be thickened and have fibrinous to caseous exudates. Catarrhal to fibrinous peritonitis and egg yolk peritonitis may be seen. Catarrhal to fibrinous enteritis

**Figure 3: Oedematous wattles dissected**  
**Figure 4: Haemorrhage in the mesentery of the small intestine**

**Figure 5: Large haemorrhages in the fat on the serosal surfaces of the abdominal organs**  
**Figure 6: Haemorrhage in the muscle and the fat around the heart**
may be seen in the caeca and/or intestine, particularly in turkeys. Exudates may be seen in the oviducts of laying birds (Easterday et al. 1997). Histopathological lesions seen in the gross changes described here are not definitive for HPAI, although vasculitis in the brain and other organs may be highly suggestive of the disease.

2.3 DIFFERENTIAL DIAGNOSIS

The following diseases must be considered in the differential diagnosis of virulent AI:

- Other diseases causing sudden high mortality:
  - Newcastle disease
  - infectious laryngotracheitis
  - duck plague
  - acute poisonings

- Other diseases causing swelling of the combs and wattles:
  - acute fowl cholera and other septicaemic diseases
  - bacterial cellulitis of the comb and wattles

Less severe forms of the disease may be confused with, or complicated by, many other diseases with respiratory or enteric signs. HPAI should be suspected in any disease outbreak in poultry that persists despite the application of preventive and therapeutic measures for other diseases, or when the epidemiological context is highly suggestive of the introduction of the infection.
3. The risk of introduction and dissemination of avian influenza

3.1 RISK OF INTRODUCTION BY MIGRATING BIRDS

The migration of water birds presents a serious risk of carrying AI viruses over long distances, with a complex network of different overlapping flyways providing the opportunity for widespread dissemination of viruses. However, extensive field studies have not been able to determine whether wild birds are spreading the H5N1 HPAI virus over long distances during their annual migrations. Current information suggests that infected birds may move short distances carrying H5N1, but long migratory movements with this strain of virus have not been confirmed.

Wild birds infected with AI viruses can generally shed virus for up to one month. However, studies conducted on several waterfowl species and H5N1 suggest that virus shedding occurs for only 3–4 days. During breeding season, during moult and at overwintering sites,
wild birds from different regions concentrate in wetlands or other habitats and transmission of viruses can occur. The outcome is that, over the course of a year, birds from different locations and from different flyways can potentially exchange viruses and other pathogens, resulting in the rapid spread of infectious agents across continents. In the course of the current epidemic, a large number of wild bird species have died from the H5N1 HPAI virus, but the role wild birds play in the spread of this disease remains undetermined because no reservoir species have been identified.

One would expect that if infection occurs in domestic poultry, and if breaks in biosecurity exist at the farm, it is likely that wild birds that visit the farm or are adjacent to it would test positive, or even succumb to H5N1 exposure. But the limited information that currently exists suggests that, in general, dead wild birds are not frequently found on farms or nearby locations, and testing conducted on “bridge species” (those that move between farms and wetlands or other natural habitats) has been negative for H5N1. Currently, this interface between the agriculture and wildlife sectors is nevertheless recognized as important, and further studies have been initiated.

Regardless, good biosecurity requires that physical barriers be erected between poultry and wild birds, used feed and manure waste, and that clean or treated water be provided to poultry.

An important component of preparedness in the context of the current avian influenza epidemic is to identify wild bird migratory patterns, timing and destination sites, and to assess the risk that close contact with domestic poultry may provide an entry point for establishment of avian influenza viruses.

3.2. RISK OF IMPORTATION

Many countries currently impose bans on the importation of poultry and poultry products from infected countries with notifiable AI. Given the potential for transboundary spread of the disease, it would be wise to take great care with all poultry products, especially those that can carry the virus. Live birds represent by far the greatest risk, but dressed carcasses of infected birds, eggs from infected hens, poultry waste and fomites contaminated with faeces can all be a source of infection. A detailed risk assessment for each poultry product has been carried out by the European Food Safety Authority (EFSA) and is available on its Web site (http://www.efsa.europa.eu).

Birds, such as fighting cocks, that are used for recreational purposes move from location to location and across borders, and they therefore represent a risk that should be closely monitored through regulation and inspection rather than bans, which would be likely to lead to clandestine movements of such birds. Likewise, the illegal movement of live birds represents a risk that will not be mitigated by imposing bans on legal importation.

3.3 RISK OF SPREAD FROM INFECTED POULTRY

To prevent further spread of H5N1, surveillance in poultry as well as in wild birds should be strengthened in countries at immediate risk. Resources should be focused on the reduction of close contacts between humans, poultry and wildlife through better management practices and improved biosecurity practices in poultry production enterprises, especially small and “open-air” facilities where poultry and waterfowl mingle with wild birds or local
resident bridge species. The influenza viruses are easily spread by fomites and generally survive well in water, especially in cold climates. Furthermore, certain species of ducks are able to carry influenza viruses without exhibiting any clinical signs of disease. Juvenile ducks have the highest rates of infection and shedding. High titres of virus occur in late summer, when birds leave their northern breeding areas, although these titres decrease as birds continue southward.

Once HPAI has been recognized in the marketing environment in a country, all persons working with poultry should greatly increase the level of hygienic practices to avoid bringing the virus into an operation (bioexclusion) and to prevent the virus exiting (biocontainment) if it has already entered a flock, village or region. The main ways in which the virus passes from one region to another area are: sale of infected birds to markets, departure of wild waterfowl that have commingled with infected backyard poultry units, the wearing of contaminated footwear or clothing by people working or selling poultry and the transfer of contaminated cages and egg crates to markets or poultry farms. Poultry keepers and communities must therefore take practical measures to avoid introducing the virus and to reduce the risk of spread when disease has been detected.

### 3.4 VIRUS SURVIVAL IN THE ENVIRONMENT

The survival of influenza viruses is prolonged by low relative humidity and low temperature in aerosols, whereas low temperature and high moisture levels prolong survival in faeces. Most studies on viral environmental persistence have been carried out in cool northern climates, with the following findings:

- The AI virus can survive in faeces for at least 35 days at 4 °C; it can survive within the poultry house environment for up to 5 weeks (Webster et al. 1978).
- The virus may remain infective in lake water for up to 4 days at 22 °C and over 30 days at 0 °C (Webster et al. 1978). H5N1 has been shown to survive in water between 14 and 26 days at 17 °C and between 3 and 5 days at 28 °C (Brown et al. 2006).
- As an enveloped virus, the influenza virus is susceptible to several disinfectants, including detergents.
- The virus is stable over a pH range of 5.5–8.
- The AI virus can be isolated from lake water where waterfowl are present (Hinshaw et al. 1979). Acidification of potentially contaminated drinking water to pH 2.5 or chlorination should minimize spread of infection.
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.
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

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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.

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.
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.
Preparing for an outbreak

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 (CO2). 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 (CO2) 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 CO2 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.
Preparing for an outbreak

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

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

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 (Na₂CO₃, 10H₂O): 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_summary.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

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


\(^4\) 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 train-
ing. 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 Trans-
boundary Animal Disease Information System (TADinfo). FAO, OIE and WHO have col-
laborated 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 dis-
eases 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 influ-
enza. 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 orophar-
ryngeal 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
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(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 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

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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.
Preparing for an outbreak

FIGURE 15
Classification flowchart

Substance for classification

- Have any pathogens been neutralised/inactivated?
- Is it known not to contain infectious substances?
- Are all micro-organisms present non-pathogenic for humans/animals?
- Is it dried blood/faecal occult blood?
- Is it an environmental sample, e.g. food and water that is not considered to pose a significant health risk?
- Is it for transplant/transfusion?

No to all

Yes to any

Does it meet the definition of a category A substance?

No

Yes

Is it a patient specimen for which there is only a minimal likelihood that pathogens are present?

Yes

UN2814 Infectious substance, affecting humans; or UN2900 Infectious substance, affecting animals (as appropriate)

UN3373 Biological substance category B

Subject to “Exempt human (or animal) specimen” provisions

Not subject to the provisions of the DGR unless meeting the criteria of another class or division

Preparing for highly pathogenic avian influenza

**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.

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**TABLE 3**

<table>
<thead>
<tr>
<th>UN code</th>
<th>Definition</th>
<th>Proper shipping name</th>
<th>Class/ Division</th>
<th>Packing group</th>
<th>Packing Instruction</th>
<th>Susceptible species</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>UN2814</td>
<td>Infectious substances meeting these criteria that cause disease in humans or in both humans and animals</td>
<td>Infectious Substance, affecting humans</td>
<td>6.2</td>
<td>N/A</td>
<td>Pi602</td>
<td>Human; Human and animal</td>
<td>Highly pathogenic avian influenza virus isolates, influenza A H1N1 virus isolates pathogenic to humans, Ebola virus, Nipah virus</td>
</tr>
<tr>
<td>UN2900</td>
<td>Infectious substances that cause disease only in animals</td>
<td>Infectious Substance, affecting animals</td>
<td>6.2</td>
<td>N/A</td>
<td>Pi602</td>
<td>Animal only</td>
<td>Foot-and-mouth disease virus isolates, ASF virus isolates, other influenza isolates of animal origin</td>
</tr>
<tr>
<td>UN3373</td>
<td>Infectious substances that do not meet the criteria for inclusion in Category A (UN2814 or UN2900)</td>
<td>Biological Substance, Category B</td>
<td>6.2</td>
<td>N/A</td>
<td>Pi650</td>
<td>Any</td>
<td>Samples for infectious disease diagnosis (e.g. swab, serum samples for avian/swine influenza diagnosis)</td>
</tr>
<tr>
<td>No code (not dangerous goods)</td>
<td>Substances that do not contain infectious substances or that are unlikely to cause disease in humans or animals</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>Any</td>
<td>Samples for non-infectious disease diagnosis</td>
</tr>
</tbody>
</table>


N/A = Not applicable

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**TABLE 4**

<table>
<thead>
<tr>
<th>UN code</th>
<th>Item</th>
<th>Proper shipping name</th>
<th>Class/ Division</th>
<th>Packing group</th>
</tr>
</thead>
<tbody>
<tr>
<td>UN1845</td>
<td>Dry ice</td>
<td>Carbon dioxide, solid (dry ice)</td>
<td>9</td>
<td>III</td>
</tr>
<tr>
<td>UN3158</td>
<td>Liquid nitrogen</td>
<td>Gas, refrigerated liquid (liquid nitrogen)</td>
<td>2.2</td>
<td>N/A</td>
</tr>
<tr>
<td>No code (not dangerous goods)</td>
<td>Ice pack</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>
**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
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).
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

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\(^6\) 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.
Areas that have not been affected by an HPAI outbreak or that have undergone culling, disinfection and even vaccination should improve their biosecurity. Improved biosecurity, at whatever level, is cost-effective in comparison with the losses from disease, depopulation and further anguish, be it at the village level or on a commercial farm.

The most difficult environment in which to improve biosecurity and disease prevention is likely to be at village level, where poultry and other animals are often allowed to move about freely and there are no costs to animal care (feeding), but where losses due to disease or scavenging animals (e.g. dogs, cats, wildlife) are high. Under these circumstances, rural development agencies can be beneficial in promoting the advantages of keeping animals in a fenced enclosure, where environmental stresses are minimized, theft is less likely, animals are safer from scavenging animals, and the loss of valued animals through being run over by motorcycles, cars or lorries is eliminated.

5.1 RESTRICTED ACCESS KEEPS DISEASE OUT
Restricting access to a property or farm through the use of fences and enclosures creates a barrier between clean areas where poultry are kept and the outside environment. Access to places where poultry are kept should be restricted to people known by the owner, people who do not have poultry of their own and people who do not participate in events where birds congregate, such as cockfights. Particular attention needs to be paid to workers on poultry farms who keep backyard poultry at home. Best practices stipulate that no workers should have poultry of their own because this is a high-risk avenue for disease introduction.
Wild birds (resident fowl or migratory birds) should have no contact with the flock. This is achieved through the use of screens or overlying nets. Visitors wishing to see poultry should wash their hands, change their shoes and use footwear provided by the owner (e.g. rubber boots that are kept for such visitors). Visitors who have birds of their own should not be allowed near the birds.

Ducks kept in ponds or paddy fields shared with other ducks belonging to a different owner represent a high risk, unless all the duck owners agree on measures that can be taken collectively. For instance, poles with netting can be erected to separate one owner’s flock from another’s, and owners can take turns scaring away wild birds from landing or feeding within production flocks.

5.2 CLEAN AREAS MEAN HEALTHY CHICKENS, GEESE AND DUCKS
The area where the flock lives should be kept clean of garbage (e.g. food waste, plastic bottles, glass bottles, tins or drums). When the owner or care person needs to attend to chickens or other poultry (e.g. collecting eggs, feeding or watering chores, changing the bedding, repairing fencing material), a change of clothing and boots should be required. These clothes and boots can be cleaned and disinfected upon exiting the enclosure, and
thus be ready for the next use. Dirty clothes should be washed with detergent and hung out to dry in the sun; boots should be washed with chlorinated water or soapy water. Washing one’s hands with soap before entering the caged-in area is also necessary. Tools (e.g. feeding scoops, shovels, brooms) and feeding pans used in the caged areas should be cleaned daily. All manure should be removed and disposed of properly (e.g. in a compost pile). Keeping a wide pail with chlorinated or soapy water at the entrance for use before entering or exiting the enclosure is a good reminder to follow biosecurity measures.

Keeping the cages clean prevents pathogens from accumulating and causing health problems. Clean cages keep the birds and eggs clean as well, and this cleanliness translates into better market prices. Sick or dead chickens must be removed quickly, and community animal health workers or the local veterinarian must be informed of such illness or death.

5.3 BUY HEALTHY, KEEP HEALTHY
Transporting birds to the farm can represent a considerable risk. The owner should be aware not only of the “good” price obtained, but also of the fact that vehicles (e.g. trucks, motorcycles, bicycles), cages, equipment and feed may be contaminated when returning to or entering the farmer’s property. Newly purchased equipment should be thoroughly washed with soapy water or otherwise disinfected before use. Newly purchased birds should be housed in a separate enclosure for at least two weeks before being allowed to mix with birds already on the farm. Owners are advised that it is important to keep species separate and not mix ducks with chickens, chickens with pigs, or ducks with pigs. It is also good practice not to mix animals of different ages.

5.4 CLEAN EQUIPMENT KEEPS DISEASE OUT
Poultry equipment, such as cages, egg crates, shovels or rakes, should not be shared with other farms or holdings. Wooden pallets, wooden handles, or egg crates can be porous, and even though they can be treated with disinfectant, it is difficult to ensure that they are completely disinfected. Metal cages can be cleaned and disinfected; if these are borrowed because of necessity, they must be cleaned and disinfected by the owner of the birds before they are reused.

5.5 REPORT EARLY SIGNS OF A PROBLEM THAT COULD BE DEVASTATING
Many bird diseases look similar. Early detection and prompt reporting will probably help stop the spread of disease. Owners must know where to report abnormalities on the farm, and they must do this when abnormalities begin, not when they end. Signs to be reported include: sudden death; depression and decreased appetite; diarrhoea; breathing difficulties such as coughing, sneezing and gasping; nervous twitching; dropped wings; paralysis; and swelling of the head with darkened combs, wattles or legs.

Owners must be assured that the early reporting of a problem will benefit them, their families and their village in the long run. In this regard, the government – in conjunction with the poultry industry – should be prepared to react and provide proper compensation (see section 4.2.2.3 above). Failure to provide an incentive for compensation for disease reporting will undoubtedly lead to disease spread.
5.6 A PERIOD OF REST
One prevention measure that can be instituted but requires planning and several enclosures is the practice of “all-in/all-out”. This method is used in many countries and envisages a complete growth cycle of chickens (or other species) from the moment of introduction – as with day-old chicks – all the way to marketing age. At no time are other birds introduced into the enclosure. Once the entire flock is sent to market, the floor is scraped clean of faecal and feather debris, bedding and feed are removed and cages and other equipment are cleaned and prepared for the introduction of young healthy birds. It would be wise to keep the enclosures free of birds and other animals for a rest period of about seven days before bringing in the next batch of poultry.

With duck operations, the all-in/all-out operation may be more difficult, unless there is planning in the growth cycle between birds using the same pond and double netting is used between age groups. Double netting (2–3 metres apart) implies additional cost, but it decreases the opportunity for pathogens to contact susceptible ducklings. Although ducks share the same water ponds in which avian influenza viruses may survive, this measure still decreases the likelihood of disease transmission.

5.7 VACCINATION AGAINST AVIAN INFLUENZA OR OTHER DISEASES
Vaccination, in general, increases the resistance of poultry to disease but does not eliminate the possibility that infection may occur in a flock. Prevention of disease and infection can be accomplished only if other aspects of prevention and improved biosecurity are in place.

When poultry are to be vaccinated, it would be wise for the owner to ensure that the vaccination team changes clothes and cleans and disinfects all boots, gloves and equipment before entering poultry enclosures. Should the vaccination team resist such instructions, the owner should report the non-observance of biosecurity measures to the appropriate veterinary authorities.

5.8 COMPARTMENTALIZATION
In the OIE’s *Terrestrial Animal Health Code*, the term compartmentalization refers to one or more establishments under a common biosecurity management system containing an animal subpopulation with a distinct health status with respect to a specific disease or specific diseases for which required surveillance, control and biosecurity measures have been applied for the purpose of international trade (more information is available in the chapter on “zoning and compartmentalisation”: Chapter 4.3 in the 2008 edition).

In countries where the disease may be present in some areas or confined to some production systems, this concept can be applied to poultry operations that adopt strict biosecurity measures to prevent the introduction of the disease all along the production process.

In poultry operations that are tightly controlled by producers, a strict method of operation must be ensured to prevent disease from entering the operation. Besides taking the measures mentioned in the above sections of this manual, operators need to constantly monitor areas of risk and practise all-in/all-out measures. For example, the origin of fertilized eggs, certified biosecured and reliable hatcheries and their incubators, certified feed
sources and transport companies must be registered, dated and documented. The poultry operation must register a complete account of its activities and sources, which include:

- a census of production (stages and location);
- protocols for training of operators;
- instructions to operators within the farm (clothing, cleaning, vaccination, feeding, reporting, etc.);
- protocols for cleaning and disinfection;
- purchases and location of suppliers;
- vermin and insect control measures;
- egg crate circulation, management and acquisition;
- employee profiles and responsibilities;
- transport control on and off the premises;
- employee and employee-family awareness; and
- registries open to frequent regulatory inspection.

One area of concern for regulatory authorities is the reality that many highly developed production poultry operations have their own diagnostic laboratories, which may carry out diagnostic assays without reporting to the authorities. It is indispensable that such commercially associated laboratories and their managers be made aware of the importance of reporting disease occurrence and its consequences to international trade for the country and their enterprise. Commercial operators should be linked with prevention, contingency and emergency plans for national and regional success and health.
Annexes
Annex 1: Selected references for further information


**Additional reading:**


Annex 2: Sample of tender document for inactivated avian influenza vaccines

To be used for the purchase of inactivated avian influenza vaccine by governments or donor organizations for control of disease in infected countries.

INTRODUCTION
These are specifications for the purchase of inactivated avian influenza vaccine for use in poultry, including chickens and ducks. Vaccination is used to provide protection in the face of possible exposure or to reduce the viral load in an infected environment. Vaccinated birds are generally not fully protected from infection, but they have increased resistance to infection, suffer less clinical disease and shed substantially less virus.

There are several AI vaccine types and formulations. Conventional vaccine, for which this specification is written, is prepared from the allantoic fluid of infected eggs, which is inactivated and emulsified with adjuvant. Attenuated live influenza virus vaccines are not recommended, because of the risk that the vaccine virus could either mutate or reassort with other influenza viruses to become pathogenic. However, recombinant vaccines have been produced, including fowlpox virus, with the influenza haemagglutinin gene inserted and haemagglutinin produced in a baculovirus expression system. This specification does not cover the requirements for recombinant vaccines.

The virus type used for vaccine production must be of the same haemagglutinin type as the outbreak virus. For maximum potency, it is preferable for the vaccine virus to be closely related to the outbreak strain. If post-vaccination monitoring depends on serology to determine whether antibody-positive birds have been infected or vaccinated (the DIVA test), the neuraminidase type should be different from that of the outbreak strain.

TENDER SPECIFICATION

General requirements
1. Vaccine manufacture must be undertaken in accordance with OIE Guidelines – Chapters 1.1.7 and 2.7.12 of the Manual of diagnostic tests and vaccines for terrestrial animals, 5th edition, 2004. It must be produced under Good Manufacturing Practice (GMP) and under acceptable third-party-audited quality assurance.
2. In assessing the acceptability of a vaccine, [FAO or other purchaser] may require documentation to be furnished to validate GMP and quality assurance practices and the production details for a specific vaccine batch. [The purchaser] may also seek to undertake an audit of the manufacturing plant(s).
3. The vaccine must be registered, or otherwise acceptable for use, by the government of [insert country].

**Specific requirements**

1. The requirement is for [insert number] of doses of vaccine for use in [insert species].
2. The vaccine must contain haemagglutinin antigen of H [insert type – for current SE Asia epidemic, H5] type. Evidence (challenge or VN test) should be provided that the vaccine protects against the virus strains currently circulating in [insert country/region].
3. Evidence should be provided that the vaccine produced by the same means (i.e. not an individual batch requirement) in the same manufacturing plant significantly reduces virus transmission from vaccinated birds when subsequently infected.
4. The vaccine virus must be derived from an LPAI virus strain.
5. The virus should be grown in specific antibody-negative or specific pathogen-free eggs.
6. The virus is to be inactivated with formalin or beta-propiolactone.
7. The vaccine should be emulsified with a mineral oil adjuvant or with an alternative adjuvant with similar immuno-stimulating efficacy.
8. The vaccine must have undergone appropriate sterility, safety and potency tests in accordance with international standards.
9. The vaccine must have a minimum of one microgram per dose of haemagglutinin protein. Vaccine of a higher haemagglutinin concentration will be considered favourably. Alternatively, the potency of the batch may be demonstrated by live bird challenge with virulent virus or by a minimum HI antibody response of 1:32 in vaccinated birds.
10. Packaging of the vaccine should be in containers of [insert number of doses].
11. Labelling in [insert language/s] must indicate manufacturer, type of vaccine, batch identification, volume of contents, storage recommendations and expiry date. Package insert in [insert language/s] to include instructions for vaccinating poultry, recommended species to which the vaccine applies and vaccination regime and dose.
12. Vaccine to have a minimum of six months period prior to expiry, on delivery.
13. Vaccine must be delivered to cold storage in [insert place or country]. Verification will be required of continuity of appropriate storage of the vaccine from production to delivery.
Annex 3: OIE/FAO reference laboratories and experts for avian influenza

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Tel: +1 204 789 2013 Fax: +1 204 789 2038
E-mail: jpasick@inspection.gc.ca

Dr Paul W. Selleck
CSIRO, Australian Animal Health Laboratory (AAHL)
5 Portarlington Road, Private Bag 24, Geelong 3220, Victoria
AUSTRALIA
Tel: +61 3 5227 5000 Fax: +61 3 5227 5555
E-mail: paul.selleck@csiro.au

Dr David Swayne
Southeast Poultry Research Laboratory
USDA/Agricultural Research Service
934 College Station Road
Athens, Georgia 30605
UNITED STATES OF AMERICA
Tel: +1 706 546 3433 Fax: +1 706 546 3161
E-mail: david.swayne@ars.usda.gov

For more information, visit the OFFLU Web site (www.offlu.net).
Annex 4: Information for shipping international diagnostic specimens

OIE/FAO AND NATIONAL REFERENCE LABORATORY FOR AVIAN INFLUENZA AND NEWCASTLE DISEASE
ISTITUTO ZOO PROFILATTICO SPERIMENTALE DELLE VENEZIE (IZSVE)
(as of September 2008)

Types of specimen
Specimens submitted may be viruses isolated in the submitting country or clinical specimens, such as tissues or swabs, collected from diseased birds.

Packaging requirements
All materials should be placed in leakproof containers. Packaging should be composed of: (1) a primary receptacle, (2) a secondary packaging, and (3) a rigid outer packaging. Packaging of diagnostic samples should be labelled “UN3373” and comply with IATA PI650 standards. Packaging of virus isolates should be labelled “UN2814” for highly pathogenic avian influenza (HPAI) and “UN2900” for NDV and low pathogenic avian influenza (LPAI) and comply with IATA PI602 standards. Please contact your shipping agent to confirm the provision of boxes complying with these requirements.

Documents to be accompanied for clearing
Import permits from the Italian Ministry of Health (provided by IZSVe in advance) and a signed pro forma invoice (the template will be provided by IZSVe) should be attached firmly to the box. Shipper’s declaration (Dangerous Goods Declaration) is required for shipment in UN2814 or UN2900 categories.

Shipping procedure
Air freight to Venice Marco Polo Airport (recommended for UN3373 packages, but not for UN2814 or UN2900 packages), Milan Malpensa or Rome Fiumicino Airport. Please inform IZSVe at least one week in advance and arrange for shipments to arrive at Italian airports from Mondays to Thursdays only. Please note that a door-to-door delivery is highly recommended, so please ask your agent for this type of service.
**Important information**
It is essential to:
- contact IZSVe before shipping; and
- provide the name and details (including telephone number) of a contact person from the shipping laboratory.

**Notification of shipment**
Before shipping, please provide the following information to the IZSVe contact person:
- date of shipment;
- airline name and flight number;
- name of destination airport;
- expected date of arrival in Italy;
- air waybill number (the air waybill should be faxed or e-mailed to the contact person as soon as possible); and
- name and contact details of the person to whom the results should be communicated.

**Shipping Address**
Istituto Zooprofilattico Sperimentale delle Venezie,
Virology Department
Viale dell’Università 10,
35020 Legnaro (PD)
Italy
Tel: +39 049 8084369
Fax: +39 049 8084360

**Contact people at IZSVe**
For diagnostic samples and virus isolates:
Marta Vettore
E-mail: mvettore@izsvnezie.it

Giovanni Cattoli
E-mail: gcattoli@izsvnezie.it

Isabella Monne
E-mail: imonne@izsvnezie.it

For reagents:
William Dundon
E-mail: wdundon@izsvnezie.it

Other contact persons:
Paola De Benedictis
E-mail: pdebenedictis@izsvnezie.it
AVIAN VIROLOGY LABORATORY, VETERINARY LABORATORIES AGENCY
WEYBRIDGE, UK
FROM OUTSIDE THE EU
(as of 15 June 2009)

Availability of diagnostic services
The laboratory offers an international diagnostic service for avian influenza and Newcastle
disease. A free service for analysing viruses isolated from animals suspected to be infected
with the above-mentioned viruses is available, and testing is carried out on behalf of the
national regulatory authority, to whom results will be copied. OIE/FAO will also be informed.
General diagnostics and virus isolation of tissue samples may be charged for.

Packaging requirements
Specimens for diagnosis of the pathogens listed above must be packaged according to
PI650 requirement for UN3373 Category B classification, unless exempt due to a minimal
likelihood that pathogens are present (e.g. samples for serology). Specimens must be pack-
aged according to PI602 requirement for UN2814 or UN2900 classification if the content
of the shipment is isolated virus. It is essential that packaging ensures that the content of
containers, which may break or leak in transit, cannot contaminate the outside layer of
the parcel. Samples should be placed in a watertight primary container, which should be
individually wrapped in absorbent material and then placed in a watertight crushproof and
leakproof IATA regulation secondary container. The primary container is to be treated lightly
with disinfectant. This may be surrounded by sealed freezer packs or dry ice, and it must be
enclosed in a strong outer packaging, which should allow the release of carbon dioxide if
dry ice is enclosed. Under no circumstances should dry ice be placed in sealed containers,
due to the risk of explosion.

Labelling
The outer packaging of the parcel must be clearly labelled with the following information:

- Our import licence number:
  • For Newcastle disease: AHZ/2232/2002/5
  • For avian influenza, other viruses, avian tissue, serum, faeces and eggs:
    AHZ/2074C/2004/3

Shipping address:
Dr Ruth Manvell
Avian Virology, VLA Weybridge
New Haw, Addlestone,
Surrey KT15 3NB,
United Kingdom
In addition, the label must include the following:

- for parcels sent by air freight, the instructions must state that the package is “CARE OF TRANSGLOBAL, Unit D1 Dolphin Industrial Estate, Windmill Road, Sunbury on Thames, Middlesex TW16 7HE” (this will ensure rapid processing at the airport);
- name and telephone number of the person responsible for sending the parcel;
- infectious substance hazard label, indicating the UN code of the contents;
- package must be marked: [UN code*] – [Proper Shipping Name*] – PACKAGE ONLY TO BE OPENED AT THE AVIAN VIROLOGY SECTION, VETERINARY LABORATORIES AGENCY, WEYBRIDGE, SURREY;
- flight number;
- air waybill number; and
- dry ice label (if necessary).

Before selecting biological material, the sender should check with the VLA-Weybridge Avian Virology Laboratory about the samples required and the conditions for dispatch. A list of contents with as much history about the isolates as possible – including species, age, area/country of isolation, any clinical history, etc. – should be enclosed in a waterproof envelope between the secondary and outer packaging, not inside the container with the samples.

**Mode of transport**

The Department for Environment, Food and Rural Affairs (DEFRA) licence allows the importation of biological material to VLA-Weybridge by air freight, normally to London Heathrow Airport. The VLA-Weybridge–nominated broker will “clear” customs and deliver the shipment. If it is not possible to deliver samples by air freight, then a courier designated by VLA-Weybridge may be used, if this has been agreed to by the Avian Virology Laboratory in advance of the shipment.

**Notification of shipment**

Before dispatch, the shipment details must be agreed upon with the VLA-Weybridge Avian Virology Laboratory, which must be given the flight number, the air waybill number, the date and time of expected arrival in the UK (by fax, telephone or e-mail) before the arrival of the materials in order to facilitate an early delivery, and a point of contact for queries and to whom test results will be provided (name, telephone number, fax number, e-mail address).

**Contact should be made via:**

Fax: +44 (0)1932 357 856
Tel: +44 (0)1932 357 736
E-mail: aiwrl@vla.defra.gsi.gov.uk

If you wish to discuss a submission and options for support from the International Reference Laboratory for Avian Influenza and Newcastle Disease, please contact: Dr. I. H. Brown (tel: +44 (0)1932 357 339; fax: +44 (0)1932 357 239; e-mail: i.h.brown@vla.defra.gsi.gov.uk).

* See page 40 Table 3 for applicable UN code and proper shipping name.
AUSTRALIAN ANIMAL HEALTH LABORATORY (AAHL)  
(As of June 2009)

Type of specimens
Specimens submitted to AAHL for disease diagnosis may be either virus isolates made in the submitting country or clinical specimens, such as tissues or swabs, collected from diseased birds.

Import permit and packing
Copies of Australian import permits are available from AAHL by contacting aahl-accessions@csiro.au. All specimens must be packed in leakproof containers in accordance with the appropriate IATA regulation and appropriately labelled. Suitable transport containers and packing instructions are also available from AAHL by contacting aahl-accessions@csiro.au. Copies of the import permit and other consignment details should be attached to the outside of the package to expedite clearance through Australian customs.

Notification of shipment
If submitting specimens, please notify the accessions clerk (accessions@csiro.au), the duty veterinarian (dutyvet@csiro.au) or Dr. Peter Daniels (+61 3 5227 5000) of the consignment details so that the specimens can be collected upon arrival in Australia. Alternatively, send the information by fax to +61 3 5227 5555. Consignment details include the consignment note/air waybill number, courier/airline and expected arrival date.

Shipping address
The Director
Australian Animal Health Laboratory
5 Portarlington Road
Geelong VIC 3220
Australia
Tel: +61 3 5227 5000
Fax: +61 3 5227 5555
http://www.csiro.au/aahl

Contact for avian influenza
You may also wish to discuss the testing required with Peter Daniels (peter.daniels@csiro.au) or Paul Selleck (paul.selleck@csiro.au) at +61 3 5227 5000 prior to submitting the specimens.
NATIONAL VETERINARY SERVICES LABORATORIES (NVSL), AMES, IOWA, USA
(as of June 2008)

Import permit
Packages containing diagnostic specimens or organisms (infectious materials) imported from foreign locations into the United States of America must be accompanied by a permit issued by the U.S. Department of Agriculture. The importation permit, with proper packaging and labelling, will expedite clearance of the package through U.S. customs. One copy of the permit should be attached to the outside of the shipping container and a second copy placed just inside the lid of the outer shipping container. The importation permit can be obtained from the laboratory (NVSL, Ames, Iowa).

Packaging requirements
All materials should be in leakproof containers and packaged to withstand breakage. All materials should be properly labelled.

Shipping address
Director
National Veterinary Services Laboratories
Diagnostic Virology Laboratory
1800 Dayton Avenue
Ames, Iowa, USA 50010

Notification of shipment
Please notify the Diagnostic Virology Laboratory with shipping information (date of arrival, airline/courier, waybill number, etc.) as soon as it is available. Fax information to +1 (515) 663-7348 or telephone +1 (515) 663-7551.

Contact for avian influenza
Dr. Beverly J Schmitt
Tel: +1 515/663-7532
Fax: +1 515/663-7348
E-mail: beverly.j.schmitt@usda.gov
FRIEDRICH-LOEFFLER INSTITUTE, FLI, ISLE OF RIEMS, GERMANY
(as of April 2008)

Before shipment of samples
Contact the FLI at timm.harder@fli.bund.de (Timm Harder, head AI lab, tel: ++49 38351 7152) or christian.grund@fli.bund.de (head ND lab, tel: ++49 38351 7106) and announce/discuss shipment of diagnostic materials and diagnostic cultures at least one week ahead of shipment. Designate a contact person in your institute and provide contact details. In case of e-mail communication, please be informed that the FLI firewall will not accept e-mails from Yahoo or similar providers if the e-mail carries an attachment; such e-mails will be automatically returned unread to sender.

What to ship
Virus isolates made in your country and clinical specimens – including tissues, swabs, and sera of avian or mammalian origin with regard to AI/ND-specific diagnostic measures – can be sent.

Packaging requirements
Packaging must comply with OIE and IATA regulations (see http://www.oie.int/eng/normes/mmanual/2008/pdf/1.1.01_COLLECTION.pdf for details). Leakproof containers are to be used throughout. Packages are composed of (1) a primary receptacle, (2) a secondary packaging and (3) a rigid outer packaging. “Diagnostic samples” in IATA PI 650 standard boxes are encoded UN3373 (“Biological Substance Category B”). These comprise all clinical material and uncharacterized virus cultures (diagnostic culture). “Virus isolates” in IATA PI 602 standard boxes are encoded UN2814 (“Infectious substance affecting humans”) for culture material containing HPAIV and UN2900 (“Infectious substance affecting animals only”) for NDV. Dry ice, if used, is to be placed between the secondary packaging and the rigid (not airtight) outer packaging. Declare dry ice content on outer package as UN1845. More details in Spanish, French or English can be found at http://www.who.int/csr/resources/publications/biosafety/WHO_HSE_EPR_2008_10/en/index.html.

Contact your local branch of international shipping agents to ensure compliance with packaging requirements. The shipper is responsible and held liable for accidents resulting from violation of these rules.

Direct air freight or couriers to Berlin-Tegel Airport (door-to-door delivery is highly recommended) or to Frankfurt Rhein-Main Airport (courier only); select the appropriate import permission. Arrange for shipments to arrive in Germany from Monday to Thursday only.

Documents
Permissions for import via airports Berlin-Tegel or Frankfurt Rhein-Main are provided by the FLI upon contact and must accompany the shipment in an envelope attached to the outside of the outer packaging. In addition, a signed pro forma invoice (template provided by the FLI upon contact) must be provided. For UN2814 and UN2900 shipments, a shipper’s declaration for dangerous goods has to be completed and signed by an authorized person. Check whether export certificates from your national veterinary authorities/ministries are required.
Shipping address:
Friedrich-Loeffler Institute, Institute of Diagnostic Virology
OIE and National Reference Laboratories for AI/ND
Suedufer 10, D-17493 Greifswald-Insel Riems
Germany
Tel: +49 38351 70
Fax: +49 38351 7275

Notification of shipment
While shipping, please provide your contact person at the FLI with:
- embarkation date;
- airline and flight number;
- name of the destination airport;
- date of arrival in Germany;
- airway bill number; and
- contact person to whom the results should be sent (name, phone and fax number, e-mail address).

Additional information
According to rules of the OIE, all results concerning the detection of notifiable diseases will be forwarded to OIE headquarters, Paris.
Annex 5: Examples of packing and marking for Category A and B infectious substances

Example of packing and marking for category A infectious substances
(See Packing Instruction 602 for additional requirements)

Notes: The smallest external dimension of the outer packaging must not be less than 100 mm.
The primary receptacle or the secondary packaging must be capable of withstanding, without leakage, an internal pressure producing pressure differential of not less than 95 kPa.
Example of packing and marking for category B infectious substances
(See Packing Instruction 650 for additional requirements, e.g. drop test)

Notes: At least one surface of the outer packaging must have a minimum dimension of 100 mm x 100 mm. The primary receptacle or the secondary packaging must be capable of withstanding, without leakage, an internal pressure producing pressure differential of not less than 95 95 kPa.

Annex 6: Criteria for defining infected areas and disease control zones

**Infected area**
An area classified as an *infected area* (IA) will be a defined area (e.g. village, farm) in which highly pathogenic avian influenza (HPAI) disease or a highly virulent strain of AI virus exists or is believed to exist. An IA will be subject to quarantine. A low pathogenic AI (LPAI) virus may be declared an agent for eradication if it has the potential to mutate into the more virulent HPAI.

**Dangerous contact place**
An area classified as a *dangerous contact place* (DCP) will be one that contain birds, poultry products or poultry wastes that have recently been introduced from an IA (usually up to 21 days before the premises were declared infected) and are likely to be infected or contaminated, or one that contains any of these items that may have been in substantial contact with people, vehicles and equipment associated with an IA within three days.

**Suspect place**
An area classified as a *suspect place* (SP) will be one that contains birds that have possibly been exposed to an AI virus, such that quarantine and surveillance, but not pre-emptive slaughter, are warranted. In addition, a place containing birds not known to have been exposed to an AI virus but showing clinical signs requiring differential diagnosis will be classified SP.

This is a temporary classification, and an area designated SP should be treated as infected until determined otherwise. High priority should be given to clarifying the status of the suspect birds so that the area can be reclassified either as an IA where appropriate quarantine and movement controls can be implemented, or as free from disease, in which case no further disease control measures are required.

**Restricted area**
A *restricted area* (RA) will be a relatively small, declared area compared with a *control area* (CA) around an IA. Movement out of the area will, in general, be prohibited, while movement into the area would be allowed. Multiple RAs may exist within one CA.

The RA does not need to be circular but can have an irregular perimeter, provided the boundary is initially an appropriate distance from the nearest IA, DCP or SP. This distance will vary depending on the size and nature of the potential source of virus, but it 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 control area (CA) will be a larger declared area around the RA(s). Initially, it could be as large as a province. Restrictions in the CA will reduce the risk that disease can spread from the RA(s). 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 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.

The declaration of a CA also helps to control the spread of the outbreak from within the RA. 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, the movement of possibly contaminated things and materials within the CA is allowed, but movement out of the CA without CVO approval is prohibited. This type of control area allows reasonable commercial activities to continue.

**NB: In declaring RAs and CAs, 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.**

**International considerations**
Under OIE Code definitions, an infected zone is a clearly defined territory in which a disease (listed in the Code) has been diagnosed. This area must be clearly defined and decreed by the veterinary authorities in accordance with the environment and the different ecological and geographical factors, as well as all the epidemiological factors and the type of husbandry being practised. The territory in question should have a radius from the centre or centres of the disease of at least 10 km in areas with intensive livestock raising, and 50 km in areas where extensive livestock raising is practised.

In May 1993, the European Union published a decision laying down the criteria for classifying “third countries” with regard to avian influenza and Newcastle disease. Annex C, point 4, of this decision states:

*Around confirmed outbreaks of disease a protection zone with a minimum radius of 3 km and a surveillance zone with a minimum radius of 10 km shall be implemented. In these zones stand-still measures and controlled movements of poultry shall be in force until at least 21 days after the end of disinfection operations on the infected holding. Before lifting the measures in these zones the authorities shall carry out the necessary inquiries and sampling of the poultry holdings to confirm that disease is no longer present in the region concerned.*

Nonetheless, the practicality of declaring a zone, the intensity of the industry and the transmissibility of virus causing an outbreak might mean a decision is taken to declare larger areas than those defined by the EU.
Annex 7: Leaflet example

**BIRD FLU (HIGHLY PATHOGENIC AVIAN INFLUENZA)**

The purpose of this leaflet is to provide the public, especially poultry farmers and those responsible for meat markets, with basic information on avian influenza, commonly known as “bird flu”. This leaflet also informs them about the rights and responsibilities in case of eventual disease outbreaks.

**WHAT IS BIRD FLU?**

Bird flu (avian influenza) is a highly contagious viral disease of poultry and other birds. According to the virulence (the severity of disease manifestations), the bird flu virus is often characterized as:

- **highly pathogenic** – causing illness with high a death rate (over 75 percent); or
- **low pathogenic** – causing mild signs of disease, but in case of secondary infections causing serious problems with death rates of up to 50 percent.

Some low pathogenic types can change over time to become highly pathogenic.

A current strain of avian flu virus, known as H5N1, is circulating in many countries in Southeast Asia and has been confirmed in the Russian Federation, Romania, Croatia, Turkey and Egypt. The H5N1 can also cause disease in humans.

**WHO CAN BE INFECTED BY BIRD FLU?**

Domestic poultry – chickens and turkeys – are most often affected, while ducks and geese often develop only mild signs of the disease. Wild birds, especially wild ducks, may be naturally resistant to the infection and do not show clinical signs of the disease.

Birds that do not show signs of the disease can still carry the virus, and they therefore represent a danger for the introduction of the virus in poultry operations.

**HOW DOES THE DISEASE SPREAD?**

The main sources of infection in poultry are the following:

- live infected birds, even if they do not appear sick;
- faecal droppings and discharges from sick birds;
- dead birds; and
- contaminated objects, including equipment (e.g. egg crates, cages), shoes or clothing and contaminated ground.

It is believed that the disease can be spread over long distances by migrating birds (especially wild ducks and geese, but possibly other waterbirds or shorebirds). If infected wild birds have contact with domestic poultry during their resting times along their migrating routes, transmission of the virus could occur.

Within a country, the disease is most commonly spread in poultry through the movement of people, birds and goods in an infected area, and through marketing practices that cause infected poultry to come into contact with healthy birds. Humans play a very important role in spreading the disease, because the virus can easily be carried on dirty clothes, shoes, contaminated equipment and vehicles, and it can be spread through the transportation of sick poultry.

**HOW TO RECOGNIZE THE DISEASE IN THE FLOCK**

Bird flu spreads very quickly within the flock, so almost all units will be infected in a very short time and birds may die within days of being infected. The spread of the disease is likely to be slower in layers (chickens that are used to produce eggs) because they are often in cages and do not mix with each other.

Signs of disease are:

- depression and lack of appetite;
- drastic drop in egg production;
- swollen head and neck;
- dark and swollen wattles and comb;
- bleeding under the skin; and
- sudden death, which can reach 100 percent of the flock.

If any of your birds show these signs, you should immediately report to the nearest veterinary station or to the veterinary inspector. Failure to notify the authorities could put more animals – and even humans – at risk of becoming infected.

Though this means of spreading is rare, the disease can be introduced by importing healthy live poultry or poultry products (meat, unprocessed feathers, laying eggs, etc.). The virus can also be introduced to an unaffected area or country through illegal trade, especially trade in live birds.
HOW TO PREVENT BIRD FLU
Basic prevention measures include the following:

- Decrease the opportunity for wild birds to come into contact with domestic poultry through the use of protective nets, or by keeping the poultry in enclosed and protected buildings.
- Apply biosafety measures:
  - Fence the farms to keep unwanted animals and visitors out. Lock doors!
  - Disinfect vehicles before they enter the farm.
  - Prohibit the entrance of unauthorized people to the farm.
  - Establish disinfection areas (e.g. for foot baths) at the entrance to the farm or in each of the poultry houses.
  - Use boots and outer clothing that can be cleaned or changed between houses or farms. Use rubber gloves as well.
  - Clean and disinfect all surfaces regularly (e.g. cages, walls, poultry eating and watering areas).
  - Do not borrow equipment from other farms, as these may be contaminated.
  - Disinfect with detergents or hypochlorite solutions.
  - Replace animals from within the flock or from controlled and healthy flocks.
  - Apply the principle of “all in/all out”. This means that all animals in a poultry house are taken off the farm at the same time (for the market), then the ground and house must be cleaned and disinfected, and only then can young stock be introduced into the cleaned house. Lock doors!
  - Avoid keeping ducks, chickens and turkeys in the same yard.
  - Immediately report an outbreak to the veterinary authorities. Do not sell your animals, and do not eat sick or dead birds or feed them to other animals.

- Increase control over the movement of people, mechanical equipment, vehicles, etc. from contaminated yards, farms, and settlement areas.
- Implement other measures ordered by the veterinary inspectors.

Owners will be compensated for slaughtered birds as part of the measures for preventing the spread of the disease. Compensation will also be paid for destroyed poultry products and equipment according to their market value.

IS BIRD FLU A THREAT FOR HUMAN HEALTH?
The bird flu virus rarely infects people. If it does, the disease is usually not serious and is often characterized by a reddening of the eyes (conjunctivitis) with mild respiratory symptoms.

However, the highly pathogenic avian influenza H5N1 strain can be a serious health problem and can kill people who are infected. You should therefore avoid contact with birds that are thought to be affected.

HOW DO PEOPLE BECOME INFECTED?
Avian influenza is not a food-borne disease. The bird flu virus is killed by the heat of normal cooking, so there is no risk of contracting avian influenza from properly cooked poultry meat and eggs. Nonetheless, sick chickens should not be eaten, as a sick bird often releases toxins and has other micro-organisms that may pose a danger to you and your family.

WHICH GROUPS OF PEOPLE ARE AT RISK?
People who are at higher risk for infection include those who work with poultry (e.g. breeders, buyers, transporters, slaughterhouse workers and veterinarians) and those who are in contact with infected poultry material or are employed in disease control activities (e.g. veterinarians employed in laboratories). Hunters and ornithologists are at risk only if they have handled sick animals or animals that have died from the avian influenza virus.

HOW CAN PEOPLE BE PROTECTED?
Currently, there is no medicine that can fully protect people from bird flu. The current seasonal vaccines against human flu are effective only against known circulating human influenza. Should you or anyone in your family have a fever with flu-like symptoms, it is advised to seek medical attention immediately.

WHOM DO I CONTACT?
This area to be used for information about the local or regional veterinary authority, diagnostic laboratory, help desk, or call-free number.

NB: This leaflet was designed by the authorities of the former Yugoslav Republic of Macedonia.
Annex 8: Avian influenza vaccine producers and suppliers

This list was compiled by FAO’s Emergency Prevention System for Transboundary Animal and Plant Pests and Diseases (EMPRES). The manufacturers/suppliers and their vaccines are not necessarily endorsed by FAO, and it is the (importing) country’s responsibility to establish independent quality assurance and quality control for safety, purity, potency and efficacy.
<table>
<thead>
<tr>
<th>Vaccine type</th>
<th>Laboratory</th>
<th>Affiliation</th>
<th>Strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monovalent inactivated H5N2 vaccines</td>
<td>Avimex laboratories, Mexico</td>
<td>Avimex laboratories, Mexico</td>
<td>A/Chicken/Mexico/232/94/CPA</td>
</tr>
<tr>
<td>Monovalent inactivated H5N2 vaccines</td>
<td>Boehringer Ingelheim Vetmedica S.A. de C.V., Mexico</td>
<td>Boehringer Ingelheim Vetmedica GmbH, Ingelheim am Rhein, Germany</td>
<td>A/Chicken/Mexico/232/94/CPA</td>
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<tr>
<td>Monovalent inactivated H5N2 vaccines</td>
<td>Ceva Mexico</td>
<td>Ceva Santé Animale SA, Z.I. La Ballastière B.P.126-33501 Libourne, France</td>
<td>A/Chicken/Mexico/232/94/CPA</td>
</tr>
<tr>
<td>Monovalent inactivated H5N2 vaccines</td>
<td>Fort Dodge Animal Health</td>
<td>Fort Dodge Animal Health, Overland Park, USA</td>
<td>A/California/20902/2002</td>
</tr>
<tr>
<td>Monovalent inactivated H5N2 vaccines</td>
<td>Chengdu Jianghua Bioproducts Co. Ltd, Ziyang City, Sichuan Province, China</td>
<td>Jianghua Group</td>
<td>A/Turkey/England/N-28/73</td>
</tr>
<tr>
<td>Monovalent inactivated H5N2 vaccines</td>
<td>Guangdong Yongshun Bio- pharm Co. Ltd, Guangdong Province, China</td>
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<td>A/Turkey/England/N-28/73</td>
</tr>
<tr>
<td>Monovalent inactivated H5N2 vaccines</td>
<td>Harbin Veterinary Research Institute, Harbin, Heilongjiang Province, China</td>
<td>National Veterinary Research Institute and National Reference laboratory for Avian Influenza, Harbin, Heilongjiang Province, China</td>
<td>A/Turkey/England/N-28/73</td>
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<tr>
<td>Monovalent inactivated H5N2 vaccines</td>
<td>Lohmann Animal Health</td>
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<td>A/Turkey/Minnesota/3689-1551/81</td>
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<td>Monovalent inactivated H5N2 vaccines</td>
<td>Kyoto Biken Laboratories, Inc, Japan</td>
<td></td>
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<td>Monovalent inactivated H5N2 vaccines</td>
<td>Intervet, Boxmeer, The Netherlands</td>
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<td>A/Duck/Potsdam/1402/86</td>
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<tr>
<td>Monovalent inactivated H5N2 vaccines</td>
<td>Investigação Aplicada S.S. (IASA), Tehuacan, Puebla, Mexico</td>
<td></td>
<td>A/Chicken/Mexico/238/94/CPA</td>
</tr>
<tr>
<td>Monovalent inactivated H5N2 vaccines</td>
<td>Laprovet S.A.S, Tours Cedex 2, France</td>
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<td>A/Chicken/Mexico/232/94/CPA</td>
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<td>Liaonong Yikang Bioengineering Co. Ltd, Liaoyang City, Liaoqing Province, China</td>
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<td>Monovalent inactivated H5N2 vaccines</td>
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<td>National Animal Quarantine Institute of the Ministry of Agriculture</td>
<td>A/Turkey/England/N-28/73</td>
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### Annex 8: Avian influenza vaccine producers and suppliers

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<th>Commercial name</th>
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<td>H5N2, LP</td>
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<td><a href="http://www.avimex.com.mx">www.avimex.com.mx</a></td>
<td>Avian Influenza H5</td>
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<td>H5N2, LP</td>
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<td><a href="http://www.lineavolvac.com">www.lineavolvac.com</a></td>
<td>Volvac AI KV</td>
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<td>H5N2, LP</td>
<td>Inactivated, oil adjuvant</td>
<td><a href="http://www.ceva.com">www.ceva.com</a></td>
<td>FLU-KEM</td>
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<td>“KYOTO BIKEN” POULSAVER AI</td>
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<td>Inactivated, oil adjuvant</td>
<td><a href="http://www.iasa.com.mx">www.iasa.com.mx</a></td>
<td>Aerovac AI</td>
<td>Undiluted spraying only (fine spray)</td>
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<td>ITA-FLU</td>
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## Preparing for highly pathogenic avian influenza

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<th>Strain</th>
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<td>Merial Italia Spa</td>
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<td>A/Chicken/Italy/22A/98</td>
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<td>Monovalent inactivated H5N1 vaccines</td>
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<td>A/CK/Legok/2003</td>
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<td>Ministry of Agriculture, Republic of Indonesia</td>
<td>A/CK/Legok/2003</td>
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<td>Ministry of Food, Agriculture and Livestock, Province of Sindh, Pakistan</td>
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<td>Monovalent inactivated H5N1 vaccines</td>
<td>Biolabs (pvt) Ltd, Islamabad, Pakistan</td>
<td>Private company</td>
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<td>Gallimune Flu H5N9</td>
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<td>China Animal Husbandry Group</td>
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<td>Live Newcastle disease virus (LaSota) and H5 A/Barheaded goose/ Qinghai/3/2005</td>
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<td>H7 vaccines</td>
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**Prepared for highly pathogenic avian influenza**
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### FAO ANIMAL PRODUCTION AND HEALTH MANUALS

1. Small-scale poultry production, 2004 (E, F)
2. Good practices for the meat industry, 2006 (E, F, S, Ar)
3. Preparing for highly pathogenic avian influenza, 2006 (E, Ar, S*, F*, M*)
4. Revised version, 2009 (E)
6. Wild birds and avian influenza – an introduction to applied field research and disease sampling techniques, 2007 (E, F, R, Id, Ba, S**)
7. Compensation programs for the sanitary emergence of HPAI-H5N1 in Latin America and the Caribbean, 2008 (E*, S*)
8. The AVE systems of geographic information for the assistance in the epidemiological surveillance of the avian influenza, based on risk, 2009 (E*, S*)
9. Preparation of African swine fever contingency plans, 2009 (E)

### Availability: November 2009

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### FAO ANIMAL HEALTH MANUALS

3. Epidemiology, diagnosis and control of helminth parasites of swine, 1998
4. Epidemiology, diagnosis and control of poultry parasites, 1998
5. Recognizing peste des petits ruminant – a field manual, 1999 (E, F)
7. Manual on the preparation of rinderpest contingency plans, 1999 (E)
8. Manual on livestock disease surveillance and information systems, 1999 (E)
12. Manual on procedures for disease eradication by stamping out, 2001 (E)
13. Recognizing contagious bovine pleuropneumonia, 2001 (E, F)
14. Preparation of contagious bovine pleuropneumonia contingency plans, 2002 (E, F)
15. Preparation of Rift Valley fever contingency plans, 2002 (E, F)
17. Recognizing Rift Valley fever, 2003 (E)
Highly pathogenic avian influenza (HPAI) represents a threat to poultry industries worldwide and to people’s livelihoods, and a potential threat to human health. The international community has a vested interest in minimizing the spread of this disease. Countries may be under threat of introduction of HPAI through unregulated poultry trade and marketing practices and, on rare occasions, exposure of poultry to wild birds, especially waterfowl.

The Food and Agriculture Organization of the United Nations and the World Organisation for Animal Health prepared the first edition of this manual to help national animal health authorities and other stakeholders prepare for a possible incursion of HPAI, detect disease as soon as possible and respond as rapidly as possible to contain the disease. This second edition reflects lessons learned and provides additional details.

The manual offers practical advice on disease identification, pathology and diagnosis; detection, response and control strategies; and biosecurity measures to prevent outbreaks. It is an invaluable source of useful information for anyone involved in poultry-keeping and animal health practices.