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A Review on Zoonosis and Avian Influenza (Bird Flu): A Literature Review

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Abstract

This article reviews literature on zoonosis infections, zoonosis in wildlife, influenza virus and subtypes, the ways zoonotic diseases spread, precautions against transmission to birds and other wildlife. Books, booklets, research proceedings, journals newspapers and fact sheets were used. A zoonosis is a disease or infection that is naturally transmitted between vertebrate animals and humans. Zoonosis have affected human health throughout times, and wildlife has always played a role. Zoonosis with a wildlife reservoir represent a major public health problem, affecting all continents. Hundreds of pathogens and many different transmission modes are involved, and many factors influence the epidemiology of the various zoonosis. The importance and recognition of wildlife as a reservoir of zoonosis are increasing. Cost effective prevention and control of these zoonosis necessitate an interdisciplinary and holistic approach and international cooperation. Surveillance, laboratory capability, research, training and education, and communication are key elements. Finally, conservation measures or biosecurity and hygiene, as well as prevention guidelines will be developed and perspectives proposed.

Keywords: Avian influenza, Orthomyxoviridae, Wildlife, Zoonosis

1. Introduction

These guidelines contain important information that can reduce the risk of visitors and researchers contracting an infection from animals when visiting wildlife from parks, an animal farm or show, petting zoo, wildlife exhibit and other similar settings offering visitors the opportunity of seeing and coming into contact with animals (Acha and Szyfres, 1987[1]; Causey and Edwards, 2008[7]). Visiting these venues does present a low, but possible, risk to visitors. Zoonotic diseases are contagious diseases spread between animals and humans. These diseases are caused by bacteria, viruses, parasites, and fungi that are carried by animals and insects. Examples are anthrax, dengue, Ebola hemorrhagic fever, Escherichia coli infection, and Lyme disease, malaria, Plague, Rocky Mountain spotted fever, salmonellosis, and West Nile virus infection. This guidance is intended to provide a practical approach to the control of zoonotic disease in zoos and wildlife parks. It covers both risks to human health and risks to other zoo animals (Webster et al., 1997[38]; Webster et al., 2006[37]). The guidance provides advice on meeting legal requirements for those managing zoos, as regards controlling the risk of infection to humans and animals and it both supplements and complements the guidance produced by the Health and Safety Executive (HSE) Managing Health and Safety in Zoos and the Department of Environment, Food and Rural Affairs (DEFRA) Standards of Modern Zoo Practice and general biosecurity guidance (Acha and Szyfres, 1987[1]). Other HSE guidance, such as that on open farms may also be relevant where zoos have similar exhibits. An approach to risk assessment is described, together with a suggested template, along with practical measures to control these risks. In addition, guidance is given on appropriate screening and monitoring for disease in animal populations and how this can contribute to the control of zoonotic disease.
1.1 Background

1.1.1 Zoonotic Infections

Zoonoses are infections that can be passed from domestic or wild animals to humans. Sources of zoonoses reported in Ethiopia include cattle, sheep, horses, goats, dogs, cats, poultry, birds, fish, rodents, amphibians, reptiles (including turtles and tortoises), bats and other species of native wildlife (Heyman, 2004[20]). Simply defined, zoonosis (plural of zoonosis’) are animal diseases that are transmissible to humans. About 75% of emerging human infectious diseases are thought to have come from animals, including wildlife (Heyman, 2004[20]). Overall, 21% of bird species are currently extinction-prone and 6.5% are functionally extinct, contributing negligibly to ecosystem processes (Sekercioglu et al., 2004) [20]. Governments in Ethiopia aim to address his threat by strengthening links between human and animal health systems. Although there are many animal-borne disease agents that can affect humans, zoonoses fortunately are not common in Ethiopia. However, for affected individuals this provides little comfort, particularly as some zoonoses have serious consequences. Most at risk of contracting a zoonosis are people in close contact with animals or animal products. This includes veterinarians, farmers, abattoir workers, shearsers and, of course, pet owners. Also at higher risk are children, the elderly and pregnant women, as well as those with impaired immunity. The occurrence of most diseases including zoonoses depends on many factors. The mere presence of a disease agent is rarely sufficient. Other important factors include the level of exposure, a mechanism to transfer the disease and host susceptibility (Webster et al., 1997[38]: Webster et al., 2006[37]).

‘Zoonosis’ comes from the Greek words zoon (animal) and osis (ill). The 2008 Communicable Diseases Intelligence Report defines zoonosis this way: ‘A zoonosis is an infection or infectious disease transmissible under natural conditions from vertebrate animals to humans (Hubbert et al., 1975[23]). Animal hosts play an essential role in maintaining the infection in nature, and humans are only incidental hosts.’ Zoonotic infections, transmissible between humans and animals, are closely associated with pastoralism and wildlife experts. Worldwide, zoonoses have important impacts on public health and livestock economies. Taylor et al. (2001) [33] reported 868 zoonotic infections representing 61% of all infectious organism known to be pathogenic to humans. Some zoonotic diseases such as rabies have been recognized since early history, others such as BSE are only now being recognized for the first time (Hugh-Jones et al., 1995[33]). Vertebrate animals (including humans) are the reservoirs of zoonotic infections, and the disease agents (bacterial, rickettsia, viral, parasitic and fungal) are transmitted directly or indirectly between them. Infection as a result of contact with an infected animal host represents a direct mode of transmission, whereas infection as a result of contact with a vector or vehicle is an indirect mode.

Reports of human illness associated with animal contact through farms, shows, zoos, petting zoos and wildlife exhibitors are infrequent in Australia. However, where illness does occur, the disease can be serious, especially for: Infants and young children, pregnant women, older adults and people with compromised immune systems (Causey and Edwards, 2008[7]).

1.1.2 The ways zoonotic diseases are spread

It should be noted, that the vast majority of contact between animals and humans do not result in any illness. But, animals may carry a range of micro-organisms (germs) potentially harmful to humans without showing any signs of illness. Zoonotic diseases can be spread by: direct contact through touching or handling animals or their carcasses, or animal bites and scratches and indirect contact with animal faces, blood and bodily fluids, aerosols, birth products or contact with contaminated objects, such as enclosures and rails, animal environments, screens, aquariums, food and water. Some animals present a higher risk of zoonoses because of increased shedding of harmful micro-organisms through their faces, urine. These include: Birthing and pregnant animals, new-born hooved animals, newly hatched chickens, some reptiles and amphibians (e.g. snakes, lizards, frogs) and animals that are stressed or unwell. There are several ways that zoonotic diseases can be spread, these are known as the different routes of transmission (Causey and Edwards, 2008[7]).

1.1.3 Zoonoses in Wildlife

People contact zoonoses from interaction with bats, birds, insects, opossums and rodents to name a few. Malaria is a classic example. It halted the first attempt to construct the Panama Canal. The canal is an engineering marvel and a triumph over wildlife disease (OIE, 2012[80]).

1.1.4 Zoonotic Diseases in Birds

Direct or indirect contact of domestic flocks with wild migratory waterfowl has been implicated as a cause of epizootics (FAO, 2004[16]; Webster et al., 1992[36]). Spread between farms during an outbreak is most likely caused by the movement of people and the transport of goods (Gilbert et al., 2006[18]). Some avian pathogens can be transmitted to humans. Among the
zoonotic, avian influenza means a severe contagious disease of poultry caused by influenza virus type A, subtype H5 and H7. There are three types of influenza viruses: A, B and C. Human influenza A and B viruses cause seasonal epidemics (World Health Organization Expert Committee, 1980[46]). Some avian pathogens can be transmitted to humans. Some have been described in detail, such as *Chlamydia psittaci*, avian influenza virus, Newcastle disease virus and avian mycobacteria. Avian zoonotic diseases are covered in detail by Carpenter and Gentz, (1997)[6] and McCluggag, (1996)[28]. If you suspect a notifiable disease (chlamydophilosis, Newcastle disease, Avian influenza, avian tuberculosis or *Salmonella enteritidis*), State Stock Diseases Acts place an obligation on you to immediately notify an inspector. Other pathogens that may be communicated to humans include: *Salmonella* and *Arizona* infections *Listeria monocytogenes* *Giardiasis*. (Giardiasis) *Encephalitozoon sp* in African lovebirds *Cryptococcus neoformans* (Cryptococcosis) (Webster et al., 1997)[38], Webster et al., 2006[37].

Avian influenza A viruses have been isolated from more than 100 different species of wild birds. Most of these viruses have been LPAI viruses. The majority of the wild birds from which these viruses have been recovered represent gulls, terns and shorebirds or waterfowl such as ducks, geese and swans. These wild birds are often viewed as reservoirs (hosts) for avian influenza A viruses. Avian influenza refers to infection of birds with avian influenza Type A viruses. These viruses occur naturally among wild aquatic birds worldwide and can infect domestic poultry and other bird and animal species. Wild aquatic birds can be infected with avian influenza A viruses in their intestines and respiratory tract, but usually do not get sick. However, avian influenza A viruses are very contagious among birds and some of these viruses can sicken and even kill certain domesticated bird species including chickens, ducks, and turkeys (FAO, 2004[46]; Causey and Edwards, 2008[7]; OIE,2012[48]).

Infected birds can shed avian influenza A viruses in their saliva, nasal secretions, and feces. Susceptible birds become infected when they have contact with the virus as it is shed by infected birds. They can also become infected by coming in contact with surfaces that are contaminated with virus from infected birds. Wild aquatic birds are the natural hosts for all known influenza type A viruses - particularly certain wild ducks, geese, swans, gulls, shorebirds and terns. Influenza type A viruses can infect people, birds, pigs, horses, dogs, marine mammals, and other animals. Influenza type A viruses are divided into subtypes on the basis of two proteins on the surface of the virus: hemagglutinin (HA) and neuraminidase (NA). For example, an “H7N2 virus” designates an influenza A virus subtype that has an HA 7 protein and an NA 2 protein. Similarly an “H5N1” virus has an HA 5 protein and an NA 1 protein. There are 17 known HA subtypes and 10 known NA subtypes (World Health Organization Expert Committee, 1980[51]; and CDC 2008[8]). Many different combinations of HA and NA proteins are possible. All known subtypes of influenza A viruses can infect birds, except subtype H17N10 which has only been found in bats. Only two influenza A virus subtypes (i.e., H1N1, and H3N2) are currently in general circulation among people. Some subtypes are found in other infected animal species. For example, H7N7 and H3N8 virus infections can cause illness in horses, and H3N8 virus infection can also cause illness in dogs (World Health Organization Expert Committee, 1980[51]; and CDC 2008[8]).

Viruses includes Orthomyxoviridae, influenza A, influenza B, influenza C and influenza A virus subtypes H1N1, H1N2, H2N2, H2N3, H3N1, H3N2, H3N8, H5N1, H5N2, H5N3, H5N6, H5N8, H5N9, H6N1, H7N1, H7N2, H7N3, H7N4, H7N7, H7N9, H9N2, H10N7 (Hoffmann et al., 2007[24]; CDC 2008[8]). Avian influenza A viruses are classified into two categories (low pathogenic and highly pathogenic) that refer to their ability to cause severe disease, based upon molecular characteristics of the virus and mortality in birds under experimental conditions. There are genetic and antigenic differences between the influenza A virus subtypes that typically infect only birds and those that can infect birds and people. Three prominent subtypes of avian influenza A viruses that are known to infect both birds and people (World Health Organization Expert Committee, 1980[49], OIE,2012[46] and CDC, 2008[8]).

Nine potential subtypes of H5 viruses are known (H5N1, H5N2, H5N3, H5N4, H5N5, H5N6, H5N7, H5N8, and H5N9). Most H5 viruses identified worldwide in wild birds and poultry are LPAI viruses. Sporadic H5 virus infection of humans, such as with highly pathogenic avian influenza A (H5N1) viruses currently circulating among poultry in Asia and the Middle East have been reported in 15 countries, often resulting in severe pneumonia with approximately 60% mortality worldwide (World Health Organization Expert Committee, 1980[49], and CDC 2008[8]).

Nine potential subtypes of H7 viruses are known (H7N1, H7N2, H7N3, H7N4, H7N5, H7N6, H7N7, H7N8, and H7N9). Most H7 viruses identified worldwide in wild birds and poultry are LPAI viruses. H7 virus infection in humans is uncommon, but has been documented in persons who have direct contact
with infected birds, especially during outbreaks of H7 virus among poultry. Illness in humans may include conjunctivitis and/or upper respiratory tract symptoms. In humans, LPAI (H7N2, H7N3, and H7N7) virus infections have caused mild to moderate illness. HPAI (H7N3, H7N7) virus infections have caused mild to severe and fatal illness (World Health Organization Expert Committee, 1980[49]; and CDC, 2008[48]).

Nine potential subtypes of H9 are known (H9N1, H9N2, H9N3, H9N4, H9N5, H9N6, H9N7, H9N8, and H9N9); all H9 viruses identified worldwide in wild birds and poultry are LPAI viruses. H9N2 virus has been detected in bird populations in Asia, Europe, the Middle East and Africa. Rare, sporadic H9N2 virus infections of humans have been reported to cause generally mild upper respiratory tract illness (World Health Organization Expert Committee, 1980[49]).

Avian means poultry including birds, chicken, ducks and geese. Diagnosis means detection of sick or dead or suspected poultry to be infected with avian influenza virus by using laboratory methodologies together with history inquiries from related persons and clinical signs observation. Laboratory Biosafety means a laboratory established with an appropriate system of construction, operation and examination as well as a safety operational procedures. Factors i.e. equipment’s, knowledge and technology shall be in place to prevent personnel, laboratory and environments from biohazards such as disease organisms, blood tissues, genetic materials and toxin which will enable to safe work with these hazardous substances (World Health Organization Expert Committee, 1980[49]).

It is caused by influenza virus type A which is a RNA virus of the family Orthomyxoviridae, genus influenza virus A. Influenza A viruses have different antigens on the envelopes which are classified into subtypes on the basis of their haemagglutinin (H) and neuraminidase (N) antigens. Avian influenza A viruses are routinely detected in wild birds. Around the world and in North America, avian influenza A outbreaks occur in poultry from time to time. Outbreaks of some avian influenza A viruses in poultry have been associated with illness and death in humans in Asia, Africa, Europe, the Pacific, and the Near East. While very rare, some avian influenza A viruses have also caused illness in humans in North America (World Health Organization Expert Committee, 1980[49]; & CDC, 2008[48]).

Highly pathogenic avian influenza H5N1 ("HPAI H5N1") first made news in 2004 and seemed to dominate headlines for several years. The alarmism belies the fact that the impact to human health has been slight. Though human outbreaks have been occurring since 1997 (WHO, 2005[47]), only 500 human cases, including 294 deaths, have been reported to the World Health Organization from 2003 through July 2010 (WHO, 2010[51]). Though there have been several confirmed cases of human-to-human transmission resulting from close, prolonged contact between family members or from an infected individual to a health care worker, nearly all other human cases – which have occurred primarily in healthy adults and children - are attributed to direct handling of infected poultry, consumption of undercooked poultry products, or contact with virus-contaminated surfaces or materials used in handling poultry (Writing Committee, 2006[51]). To date, only seven human cases of H5N1 HPAI infection appear to be related to contact with wild birds, and these resulted from the plucking of feathers from dead swans in Azerbaijan. It is not clear that all seven cases resulted from contact with the dead birds, or if one or more cases resulted from contact with those who handled the dead birds (Tsiodras et al., 2008[37]; WHO 2006[44]).

1.1.5 Basics of Avian Influenza

Various avian influenza viruses are found in wild birds in virtually every country, including the United States. The subtypes are named for the 16 hemagglutinin (H) and 9 neuraminidase (N) proteins on the viral surface. The avian influenza virus of recent concern is designated as Highly Pathogenic Avian Influenza (HPAI) subtype H5N1 genotype Z, which first appeared in Asia in 2002. Other avian influenza viruses are designated “LPAI” for low pathogenicity. The degree of pathogenicity is established through testing methods developed by the World Health Organization and the International Office of Epizootics <http://www.oie.int>. The pathogenicity designation pertains only to the behavior of the virus in domestic poultry; a virus may not behave the same way in wild birds.

Many avian influenza viruses normally circulate as gastrointestinal infections in wild birds, causing little or no illness or mortality (Webster et al., 1992[46]). The H5N1 strain of HPAI has affected 152 species in 14 orders of wild birds and has caused mortality in 115 of those species (USDA, 2008[38]). Bird species in many families appear to be susceptible to infection, but because cool, wet conditions favor the persistence of the virus, and because the virus is shed in feces that contaminates their aquatic habitats, it appears that water birds, especially ducks and geese, are the most-commonly infected wild birds (Causey and Edwards, 2008[47]).

Studies have been conducted to determine if wild birds can be healthy carriers of HPAI H5N1 virus, the role of
healthy carriers in the spread of the disease, and to gather information on the routes and periods of migration of the infected wild birds. It has proved difficult to find healthy, infected birds. In 2006, none of the 39,143 wild birds of 150 species sampled in Europe were found to be infected (Pittman et al., 2007[29]). In a study that sampled 13,000+ live migratory birds in China, HPAI N5N1 was detected only six times (Chen et al., 2006[10]). Of 862 live birds tested across the Western Mongolian flyway, including 430 live birds (of 55 species) found on Erhel Lake in Mongolia where a mass mortality event killed 100 birds, none tested positive for the virus. (WCS, 2005[38]; Hoffmann et al., 2007[31]; Grund et al., 2011[109])

1.2 Basics of West Nile Virus
West Nile is an insect-borne flavivirus commonly found in Africa, western Asia and the Middle East, and, since 1999, in the Western Hemisphere. In North America, it has been detected in at least 48 species of mosquitoes and over 250 species of birds (USDA, 2008[38]). It is now found in every state except Alaska and Hawaii.

1.3 Other zoonotic pathogens
Wild birds may carry other diseases to which ornithologists and banders are susceptible and an ornithologist or a bander may easily transfer some avian pathogens from one bird to another. According to the USGS Field Manual of Wildlife Disease, “As a group, bacterial diseases pose 8 greater human health risks than viral diseases of wild birds. Of the diseases addressed in this section, chlamydiosis, or ornithosis, poses the greatest risk to humans. Avian tuberculosis can be a significant risk for humans who are immunocompromised. Salmonellosis is a common, but seldom fatal, human infection that can be acquired from infected wild birds.” However, other avian diseases rarely cause illness, much less serious illness in humans, and rarely, if ever, result in death.

According to the CDC, chlamydiosis (also known as ornithosis or psittacosis) is characterized by fever, chills, headache, myalgia, and a dry cough with pneumonia often evident on chest x-ray. Severe pneumonia requiring intensive-care support, endocarditis, hepatitis, and neurologic complications occasionally occur. Most people recover from salmonellosis in a week or less without medication though the severe dehydration that can occur can be dangerous and may require hospitalization. Human fatalities from bacterial diseases are rare due to the availability of antibiotics. There have been several severe cases among wildlife biologists (Webster et al., 1992[40]; Chen et al., 2009[9]).

The level of precaution should be commensurate with the level of risk to the individual handling the bird and to other birds. In most situations, then, hand washing and disinfecting of equipment and holding devices should be adequate. It is always helpful to recognize the signs of illness in a bird, but because birds can harbor pathogens without showing overt signs of illness, do not assume that the absence of signs indicates the absence of a pathogen (Spackman et al., 2002[32]). A researcher who becomes ill after handling wild birds should inform the physician of the possible exposure to a zoonotic pathogen.

1.4 Precautions against transmission to birds and other wildlife
To prevent transmission of any pathogen as a result of handling by researchers:
Do not re-use contaminated bags, boxes or other holding/carrying devices and other devices used to restrain birds during processing. The North American Banding Council manual states, “Launder bird bags frequently, as they must be kept clean,” and “If a diseased bird is caught, it is extremely important to put that bag aside until it has been washed and disinfected.” However, as it is not possible to determine if a bird is shedding virus, the better practice would be to carry an ample supply of bags or other holding/carrying devices so that no bag or other holding device is used more than once before laundering. Viruses can survive at cool temperatures for days, weeks, or even longer. Wash bags with hot water, detergent, and/or household bleach before reuse. When preparing specimens in the field, place waste material in a biosafety bag, seal it, and burn it, or carry it out with you and burn it later. Never re-use needles, scalpels blades, calipers, rulers, banding pliers or other equipment that touches any part of a bird unless the equipment decontaminated with a freshly prepared 10% bleach or 70% alcohol solution or alcohol wipes after use on each individual. The National Veterinary Standards Laboratory of the US Department of Agriculture, which approves pre-import treatment methods for materials of avian origin, confirmed that 70% alcohol will kill the virus. Disinfect your hands after handling each bird. Disinfectant hand wipes can be used if washing with soap and water is not possible.

For field surgeries, aseptic technique is discussed at length in Guidelines to the Use of Wild Birds in Research (Fair et al., 2010[14]).

1.5 What ornithologists and banders can do in the event of emergent avian disease or disease outbreaks?
Ornithologists and banders can and should develop relationships with their state or provincial health and
agriculture departments. For a comprehensive list of state agencies in the United States, see http://www.pandemicflu.gov/state/statecontacts.html. Should emerging infectious avian diseases arrive in your country, state, or province, or should disease outbreaks occur, you will be prepared to help persuade your state officials to continue monitoring wildlife after occurrence is confirmed, can help to share accurate scientific information about wild birds with these agencies and with the public, and can help address calls from the public or from government officials to cull wild birds. Every international and national agriculture and public health organization, including the World Health Organization and the United Nations Food and Agriculture Organization, has concluded that culling of wild birds or destruction of their habitat such as the draining of wetlands is neither practical nor feasible, from logistical, environmental, public health, and biodiversity points of view. In fact, the FAO points out that the attempt to cull or the destruction of habitat could result in the dispersion of birds and if those birds were infected, dispersion would result in spread of the virus to a wider area.

Ornithologists can also serve as experts to provide information to the general public and the media, but should be careful to avoid speculating about how or how quickly the disease might spread; if, when, and how it might arrive in the Western hemisphere or about any other matter about which information is lacking or incomplete. Speculation can lead to calls for inappropriate measures.

Ornithologists, banders, and bird observatories can greatly extend bio surveillance capacity. Contact information for organizations already involved in bio surveillance are listed below.

Avian influenza (AI) is caused by specified viruses that are members of the family Orthomyxoviridae and placed in the genus influenza virus A. There are three influenza genera A, B and C; only influenza A viruses are known to infect birds. Diagnosis is by isolation of the virus or by detection and characterization of fragments of its genome. This is because infections in birds can give rise to a wide variety of clinical signs that may vary according to the host, strain of virus, the host’s immune status, presence of any secondary exacerbating organisms and environmental conditions.

Avian influenza or “bird flu” is an infection found in birds caused by the influenza A virus. There are many different types of bird flu, some that cause disease and some that do not. In recent times, the term bird flu has often been used to describe the H5N1 avian influenza virus. In domestic poultry such as chickens or turkeys, infection with avian influenza viruses may cause two different types of illness. They are differentiated by the level of disease severity. The so-called “low pathogenic” form commonly causes only mild symptoms (ruffled feathers, a drop in egg production) and may easily go undetected. The “high pathogenic” form is more severe. It spreads very rapidly through poultry flocks, causes disease and has a death rate that can approach 100 percent, often within days.

1.6 Influenza Viruses Types, Subtypes, and Strains

There are three types of influenza viruses: A, B, and C. Only influenza A viruses are further classified by subtype on the basis of the two main surface glycoproteins hemagglutinin (HA) and neuraminidase (NA). Influenza A subtypes and B viruses are further classified by strains. Human Influenza Viruses and Avian Influenza A Viruses Humans can be infected with influenza types A, B, and C viruses. Subtypes of influenza A that are currently circulating among people worldwide include H1N1, H1N2, and H3N2 viruses. Wild birds are the natural host for all known subtypes of influenza A viruses. Typically, wild birds do not become sick when they are infected with avian influenza A viruses. However, domestic poultry, such as turkeys and chickens, can become very sick and die from avian influenza, and some avian influenza A viruses also can cause serious disease and death in wild birds (Swayne, 2004).

Low Pathogenic versus Highly Pathogenic Avian Influenza A Viruses Avian influenza A virus strains are further classified as low pathogenic (LPAI) or highly pathogenic (HPAI) on the basis of specific molecular genetics and pathogenesis criteria that require specific testing (Spackman et al., 2002; Spackman et al., 2008).

1.6.1 Influenza Type A

Influenza type A viruses can infect people, birds, pigs, horses, seals, whales, and other animals, but wild birds are the natural hosts for these viruses. Influenza type A viruses are divided into subtypes based on two proteins on the surface of the virus. These proteins are called hemagglutinin (HA) and neuraminidase (NA). There are 15 different HA subtypes and 9 different NA subtypes. Many different combinations of HA and NA proteins are possible. Only some influenza A subtypes (i.e., H1N1, H1N2, and H3N2) are currently in general circulation among people. Other sub types are found most commonly in other animal species. For example, H7N7 and H3N8 viruses cause illness in horses. Subtypes of influenza A virus are named according to their HA and NA surface proteins. For example, an “H7N2 virus” designates influenza A subtype that has an HA 7 protein and an NA 2 protein. Similarly an
“H5N1” virus has an HA 5 protein and an NA 1 protein (Webster et al., 1997[42]; Webster et al., 2006[43]).

1.6.2 Influenza Type B
Influenza B viruses are normally found only in humans. Unlike influenza A viruses, these viruses are not classified according to subtype. Although influenza type B viruses can cause human epidemics, they have not caused pandemics.

1.6.3 Influenza Type C
Influenza type C viruses cause mild illness in humans and do not cause epidemics or pandemics. These viruses are not classified according to subtype.

1.7 Strains
Influenza B viruses and subtypes of influenza A virus are further characterized into strains. There are many different strains of influenza B viruses and of influenza A subtypes. New strains of influenza viruses appear and replace older strains. This process occurs through a type of change is called “drift” (see How Influenza Viruses Can Change: Shift and Drift). When a new strain of human influenza virus emerges, antibody protection that may have developed after infection or vaccination with an older strain may not provide protection against the new strain. Thus, the influenza vaccine is updated on a yearly basis to keep up with the changes in influenza viruses. Human Influenza Viruses versus Avian Influenza Viruses Humans can be infected with influenza types A, B, and C. However, the only subtypes of influenza A virus that normally infect people are influenza A subtypes H1N1, H1N2, and H3N2. Between 1957 and 1968, H2N2 viruses also circulated among people, but currently do not.

Only influenza A viruses infect birds. Wild birds are the natural host for all subtypes of influenza A virus. Typically wild birds do not get sick when they are infected with influenza virus. However, domestic poultry, such as turkeys and chickens, can get very sick and die from avian influenza, and some avian viruses also can cause serious disease and death in wild birds. Low Pathogenic versus Highly Pathogenic Avian Influenza Viruses H5 and H7 subtypes of avian influenza A viruses can be further classified as either highly pathogenic avian influenza (HPAI) or low pathogenic avian influenza (LPAI). This distinction is made on the basis of genetic features of the virus. HPAI is usually associated with high mortality in poultry. It is not certain how the distinction between “low pathogenic” and “highly pathogenic” is related to the risk of disease in people. HPAI viruses can kill 100% of infected chickens, whereas LPAI viruses cause less severe or no illness if they infect chickens. Because LPAI viruses can evolve in to HPAI viruses, out breaks of H5 and H7 LPAI are closely monitored by animal health officials. Most avian influenza A viruses are LPAI viruses that are usually associated with mild disease in poultry. In contrast, HPAI viruses can cause severe illness and high mortality in poultry. More recently, some HPAI viruses (e.g., H5N1) have been found to cause no illness in some poultry, such as ducks. Viruses have the potential to evolve into HPAI viruses and this has been documented in some poultry outbreaks. Avian influenza A viruses of the subtypes H5 and H7, including H5N1, H7N7, and H7N3 viruses, have been associated with HPAI, and human infection with these viruses have ranged from mild (H7N3, H7N7) to severe and fatal disease (H7N7, H5N1). Human illness due to infection with LPAI viruses has been documented, including very mild symptoms (e.g., conjunctivitis) to influenza like illness. Examples of LPAI viruses that have infected humans include H7N7, H9N2, and H7N2. In general, direct human infection with avian influenza occurs very infrequently, and has been associated with direct contact (e.g., touching) infected sick or dead infected birds (domestic poultry) (Naeem, 1999[57]; Hoffmann et al., 2007[29]; Grund et al., 2011[49]).

1.8 How Influenza Viruses Change: Drift and Shift
Influenza viruses are dynamic and are continuously evolving. Influenza viruses can change in two different ways: antigenic drift and antigenic shift. Influenza viruses are changing by antigenic drift all the time, but antigenic shift happens only occasionally. Influenza type A viruses undergo both kinds of changes; Influenza type B viruses change only by the more gradual process of antigenic drift. Antigenic drift refers to small, gradual changes that occur through point mutations in the two genes that contain the genetic material to produce the main surface proteins, hemagglutinin, and neuraminidase. These point mutations occur unpredictably and result in minor changes to these surface proteins. Antigenic drift produces new virus strains that may not be recognized by antibodies to earlier influenza strains. This process works as follows: a person infected with a particular influenza virus strain develops antibody against that strain. As newer virus strains appear, the antibodies against the older strains might not recognize the "newer" virus, and infection with a new strain can occur. This is one of the main reasons why people can become infected with influenza viruses more than one time and why global surveillance is critical in order to monitor the evolution of human influenza virus strains for selection of which strains should be included in the
Influenza A virus subtype in humans that was not currently circulating among people (see more information below under Influenza Type A and Its Subtypes). Antigenic shift can occur either through direct animal (poultry) to human transmission or through mixing of human influenza A and animal influenza A virus genes to create a new human influenza A subtype virus through a process called genetic reassortment. Antigenic shift results in a new human influenza A subtype. A global influenza pandemic (worldwide spread) may occur if three conditions are met:

A new subtype of influenza A virus is introduced into the human population. The virus causes serious illness in humans. The virus can spread easily from person to person in sustained manner. Types, Subtypes, and Strains Influenza Type A and Its Subtypes Influenza type A viruses can infect people, birds, pigs, horses, and other animals, but wild birds are the natural hosts for these viruses. Influenza type A viruses are divided into subtypes and named on the basis of two proteins on the surface of the virus: hemagglutinin (HA) and neuraminidase (NA). For example, an "H7N2 virus" designates an influenza A subtype that has an HA protein and an NA 2 protein. Similarly an "H5N1" virus has an HA 5 protein and an NA 1 protein. There are 16 known HA subtypes and 9 known NA subtypes. Many different combinations of HA and NA proteins are possible. Only some influenza A subtypes (i.e., H1N1, H1N2, and H3N2) are currently in general circulation among people. Other subtypes are found most commonly in other animal species. For example, H7N7 and H3N8 viruses cause illness in horses, and H3N8 also has recently been shown to cause illness in dogs. Only influenza A viruses infect birds, and all known subtypes of influenza A viruses can infect birds. However, there are substantial genetic differences between the influenza A subtypes that typically infect birds and those that infect both people and birds. Three prominent subtypes of the avian influenza A viruses that are known to infect both birds and people are: Influenza AH5 Nine potential subtypes of H5 are known. H5 infections, such as HPAIH5N1viruses currently circulating in Asia and Europe, have been documented among humans and sometimes cause severe illness or death. Influenza AH7 Nine potential subtypes of H7 are known. H7 infection in humans is rare but can occur among persons who have direct contact with infected birds. Symptoms may include conjunctivitis and/or upper respiratory symptoms. H7 viruses have been associated with both LPAI (e.g., H7N2, H7N7) and HPAI (e.g., H7N3, H7N7), and have caused mild to severe and fatal illness in humans (Fereidouni et al., 2008[15]; Fair et al., 2010[14]).

Influenza AH9 Nine potential subtypes of H9 are known; Influenza A H9 has rarely been reported to infect humans. However, this subtype has been documented only in a low pathogenic form.

Influenza Type B Influenza B viruses are usually found only in humans. Unlike influenza A viruses, these viruses are not classified according to subtype. Influenza B viruses can cause morbidity and mortality among humans, but in general are associated with less severe epidemics than influenza A viruses. Although influenza type B viruses can cause human epidemics, they have not caused pandemics.

Influenza Type C Influenza type C viruses cause mild illness in humans and do not cause epidemics or pandemics. These viruses are not classified according to subtype. Strains Influenza B viruses and subtypes of influenza A virus are further characterized into strains. There are many different strains of influenza B viruses and of influenza A subtypes. New strains of influenza viruses appear and replace older strains. This process occurs through antigenic drift. When a new strain of human influenza virus emerges, antibody protection that may have developed after infection or vaccination with an older strain may not provide protection against the new strain. Therefore, the influenza vaccine is updated on a yearly basis to keep up with the changes in influenza viruses.

Notifiable avian influenza (NAI) is caused by infection with viruses of the family Orthomyxoviridae placed in the genus influenza virus A. Influenza A viruses are the only orthomyxoviruses known to naturally affect birds. Many species of birds have been shown to be susceptible to infection with influenza A viruses; aquatic birds form a major reservoir of these viruses, and the overwhelming majority of isolates have been of low pathogenicity (low virulence) for chickens and turkeys. Influenza A viruses have antigenically related nucleocapsid and matrix proteins, but are classified into subtypes on the basis of their haemagglutinin (H) and neuraminidase (N) antigens (World Health Organization Expert Committee, 1980[40]). At present, 16 H subtypes (H1–H16) and 9 N subtypes (N1–N9) are recognized (Swayne & Halvorson, 2008[43]).
viruses that produce acute clinical disease in chickens, turkeys and other birds of economic importance have been associated only with the H5 and H7 subtypes. Most viruses of the H5 and H7 subtype isolated from birds have been of low virulence for poultry. As there is the risk of a H5 or H7 virus of low virulence becoming virulent by mutation, all H5 and H7 viruses have been designated as NAI viruses.

Depending on the species, age and type of bird, specific characteristics of the viral strain involved, and on environmental factors, the highly pathogenic disease, in fully susceptible birds, may vary from one of sudden death with little or no overt clinical signs to a more characteristic disease with variable clinical presentations including respiratory signs, such as ocular and nasal discharges, coughing, snicking and dyspnoea, swelling of the sinuses and/or head, apathy, reduced vocalisation, marked reduction in feed and water intake, cyanosis of the unfeathered skin, wattles and comb, incoordination and nervous signs and diarrhoea. In laying birds, additional clinical features include a marked drop in egg production, usually accompanied by an increase in numbers of poor quality eggs. Typically, high morbidity is accompanied by high and rapidly escalating unexplained mortality.

However, none of these signs can be considered pathognomonic. In certain host species such as Pekin ducks some HPAI viruses do not necessarily provoke significant clinical disease. In addition, low pathogenicity avian influenza (LPAI) viruses, which normally cause only a mild or no clinical disease, may in certain circumstances produce a spectrum of clinical signs, the severity of which may approach that of highly pathogenic avian influenza (HPAI), particularly if exacerbating infections and/or adverse environmental conditions are present. Confirmatory diagnosis of the disease, therefore, depends on the isolation or detection of the causal virus. Testing sera from suspect birds using antibody detection methods may supplement diagnosis, but these methods are not suitable for a detailed identification. Diagnosis for official control purposes is established on the basis of agreed official criteria for pathogenicity according to in-vivo tests or to molecular determinants (i.e. the presence of a cleavage site of the haemagglutinin precursor protein HA0 consistent with HPNAI virus) and haemagglutinin subtyping. These definitions evolve as scientific knowledge of the disease increases. NAI are subject to official control. The viruses that cause NAI have the potential to spread from the laboratory if adequate levels of biosecurity and biosafety are not in place. Consequently, a risk assessment should be carried out to determine the level of biosecurity needed for laboratory diagnosis and chicken inoculation; characterization of the HPAI virus should be conducted at biocontainment level 3 and LPNAI at biocontainment level 2 (at least). The facility should meet the requirements for the appropriate Containment Group as determined by the risk assessment and as outlined in Chapter 1.1.2 Biosafety and biosecurity in the veterinary microbiology laboratory and animal facilities. Countries lacking access to such a specialized national or regional laboratory should send specimens to an OIE Reference Laboratory.

1.9 Identification of the Agent
Suspensions in antibiotic solution of oropharyngeal and cloacal swabs (or faeces) taken from live birds, or of faeces and pooled samples of organs from dead birds, are inoculated into the allantoic cavity of 9 - 11-day-old embryonated chicken eggs. The eggs are incubated at 37°C (range 35–39°C) for 2–7 days. The allantoic fluid of any eggs containing dead or dying embryos during the incubation and all eggs at the end of the incubation period are tested for the presence of haemagglutinating activity. The presence of influenza A virus can be confirmed by an immunodiffusion test between concentrated virus and an antiserum to the nucleocapsid and/or matrix antigens, both of which are common to all influenza A viruses. Isolation in embryos has recently been replaced, under certain circumstances, by detection of one or more segments of the influenza A genome using real-time reverse-transcription polymerase chain reaction (rRT-PCR) or other validated molecular techniques (Das et al., 2006[12]; Webster et al., 1997[42]; Webster et al., 2006[41]).

For subtyping the virus, a reference laboratory should conduct haemagglutination and neuraminidase inhibition tests against a battery of polyclonal or monospecific antisera to each of the 16 haemagglutinin (H1–16) and 9 neuraminidase (N1–9) subtypes of influenza A virus, or identify the genome of specific H and N subtypes using RNA detection technologies with subtype specific primers and probes (e.g. rRT-PCR) or sequencing and phylogenetic analysis. As the term highly pathogenic avian influenza and the historical term ‘fowl plague’ refer to infection with virulent strains of influenza A virus, it is necessary to assess the virulence of an isolate for domestic poultry. Any highly pathogenic avian influenza isolate is classified as notifiable avian influenza (NAI) virus. Although all naturally occurring virulent strains isolated to date have been either of the H5 or H7 subtype, most H5 or H7 isolates have been of low virulence. Due to the risk of a low virulent H5 or H7 becoming virulent by mutation in poultry hosts, all H5 and H7 viruses have also been classified as NAI viruses. The methods used
for the determination of strain virulence for birds have evolved over recent years with a greater understanding of the molecular basis of pathogenicity, but still primarily involve the intravenous inoculation of a minimum of eight susceptible 4 to 8-week-old chickens with infectious virus; strains are considered to be highly pathogenic if they cause more than 75% mortality within 10 days or inoculation of 10 susceptible 4-to 8-week-old chickens resulting in an intravenous pathogenicity index (IVPI) of greater than 1.2. Characterization of suspected virulent strains of the virus should be conducted in a virus-secure biocontainment laboratory. All virulent AI isolates are designated as highly pathogenic notifiable avian influenza (HPNAI) viruses. Regardless of their virulence for chickens, H5 or H7 viruses with a HA0 cleavage site amino acid sequence similar to any of those that have been observed in virulent viruses are considered HPNAI viruses. H5 and H7 isolates that are not pathogenic for chickens and do not have an HA0 cleavage site amino acid sequence similar to any of those that have been observed in HPNAI viruses are designated as low pathogenicity notifiable avian influenza (LPNAI) viruses and non-H5 or non-H7 AI isolates that are not highly pathogenic for chickens are designated as low pathogenicity avian influenza (LPAI) viruses.

1.10 Serological tests: As all influenza A viruses have antigenically similar nucleocapsid and matrix antigens, agar gel immunodiffusion tests are used to detect antibodies to these antigens. Concentrated virus preparations containing either or both type of antigens are used in such tests.

Not all species of birds develop demonstrable precipitating antibodies. Haemagglutination inhibition tests have also been employed in routine diagnostic serology, but it is possible that this technique may miss some particular infections because the haemagglutinin is subtype specific. Enzyme linked immunosorbent assays have been used to detect antibodies to influenza A type-specific antigen in either species-dependent (indirect) or -independent (competitive) test formats.

1.11 Requirements for vaccines and diagnostic biological
Historically, in most countries, vaccines specifically designed to contain or prevent HPNAI were banned or discouraged by government agencies because they may interfere with stamping-out control policies. The first use of vaccination in an avian influenza eradication programme was against LPAI and LPNAI. The programme used inactivated oil-emulsion vaccines with the same haemagglutinin and neuraminidase subtypes, and infected flocks were identified by detection of virus or antibodies against the virus in non-vaccinated sentinel birds. During the 1990s the prophylactic use of inactivated oil-emulsion vaccines was employed in Mexico and Pakistan to control widespread outbreaks of NAi, and a recombinant fowl poxvirus vaccine expressing the homologous HA gene was also used in Mexico, El Salvador and Guatemala. During the 1999–2001 outbreak of LPNAI in Italy, an inactivated vaccine was used with the same haemagglutinin type as the field virus, but with a different neuraminidase. This allowed the differentiation of non-infected vaccinated birds from vaccinated birds infected with the field virus and ultimately resulted in eradication of the field virus (Capua & Alexander, 2008[5]). Prophylactic use of H5 and H7 vaccines has been practiced in parts of Italy, aimed at preventing LPNAI infections, and several countries in Asia, Africa and the Middle East as an aid in controlling HPNAI H5N1 virus infections. HPNAI viruses should not be used as the seed virus for production of vaccine. If HPNAI is used in challenge studies, the facility should meet the OIE requirements for Containment Group 4 pathogens.

1.12 Diagnostic Techniques
Identification of the agent (the prescribed test for international trade)
Samples taken from dead birds should include intestinal contents (faeces) or cloacal swabs and oropharyngeal swabs. Samples from trachea, lungs, air sacs, intestine, spleen, kidney, brain, liver and heart should also be collected and processed either separately or as a pool. Samples from live birds should include both oropharyngeal and cloacal swabs. To avoid harming them, swabbing of small delicate birds should be done with the use of especially small swabs that are usually commercially available and intended for use in human paediatrics. Where these are not available, the collection of fresh faeces may serve as an alternative.

The samples should be placed in isotonic phosphate-buffered saline (PBS), pH 7.0–7.4 with antibiotics or a solution containing protein and antibiotics. The antibiotics can be varied according to local conditions, but could be, for example, penicillin (2000 units/ml), streptomycin (2 mg/ml), gentamycin (50 μg/ml) and mycostatin (1000 units/ml) for tissues and oropharyngeal swabs, but at five-fold higher concentrations for faces and cloacal swabs. It is important to readjust the pH of the solution to pH 7.0–7.4 following the addition of the antibiotics. It is recommended that a solution for transport of the swabs should contain protein to stabilize the virus (e.g. brain–heart infusion, up to 5% cattle serum, 0.5% bovine albumen or similar commercially available transport
media). Faeces and finely minced tissues should be prepared as 10–20% suspensions in the antibiotic solution. Suspensions should be processed as soon as possible after incubation for 1–2 hours at room temperature. When immediate processing is impracticable, samples may be stored at 4°C for up to 4 days. For prolonged storage, diagnostic samples and isolates should be kept at −80°C. Repeated freezing and thawing should be avoided.

The preferred method of growing avian influenza A viruses is by the inoculation of specific pathogen free (SPF) embryonated chicken eggs, or specific antibody negative (SAN) eggs. The supernatant fluids of faeces or tissue suspensions obtained through clarification by centrifugation at 1000 g are inoculated into the allantoic sac of three to five embryonated SPF or SAN chicken eggs of 9–11 days’ incubation. The eggs are incubated at 37°C (range 35–39°C) for 2–7 days. Eggs containing dead or dying embryos as they arise, and all eggs remaining at the end of the incubation period, should first be chilled to 4°C for 4 hours or overnight, and the allantoic fluids should then be recovered and tested with a screening test (such as haemagglutination [HA] test), influenza A type-specific test (such as agar gel immunodiffusion test [AGID] or solid-phase antigen-capture enzyme-linked immunosorbent assays [ELISA]) or influenza A subtype-specific test (such as haemagglutinin inhibition [HI] and neuraminidase inhibition [NI] tests) or a molecular test to detect influenza A specific nucleic acid signatures (such as real-time reverse transcriptase polymerase chain reaction [rRT-PCR] test) as described later. Detection of HA activity, in bacteria-free amnio-allantoic fluids verified by microbiological assay, indicates a high probability of the presence of an influenza A virus or of an avian paramyxovirus. Fluids that give a negative reaction should be passage into at least one further batch of eggs.

The presence of influenza A virus can be confirmed in AGID tests by demonstrating the presence of the nucleocapsid or matrix antigens, both of which are common to all influenza A viruses. The antigens may be prepared by concentrating the virus from infective allantoic fluid or extracting the infected chorioallantoic membranes; these are tested against known positive antisera. Virus may be concentrated from infective allantoic fluid by ultracentrifugation, or by precipitation under acid conditions. The latter method consists of the addition of 1.0 M HCl to infective allantoic fluid until it is approximately pH 4.0. The mixture is placed in an ice bath for 1 hour and then clarified by centrifugation at 1000 g at 4°C. The supernatant fluid is discarded. The virus concentrates are resuspended in glycin/sarcosyl buffer: this consists of 1% sodium lauroyl sarcosinate buffered to pH 9.0 with 0.5 M glycine. These concentrates contain both nucleocapsid and matrix polypeptides.

Preparations of nucleocapsid-rich antigen can also be obtained from chorioallantoic membranes for use in the AGID test (Beard, 1970[23]). This method involves removal of the chorioallantoic membranes from infected eggs that have allantoic fluids with HA activity. The membranes are then homogenised or ground to a paste. This is subjected to three freeze–thaw cycles, followed by centrifugation at 1000 g for 10 minutes. The pellet is discarded and the supernatant is used as an antigen following treatment with 0.1% formalin.

Use of the AGID test to demonstrate nucleocapsid or matrix antigens is a satisfactory way to indicate the presence of avian influenza virus (AIV) in amnioallantoic fluid, but various experimental and commercial rapid, solid-phase antigen-capture ELISAs (AC-ELISAs) are an effective alternative (Swayne & Halvorson, 2008[34]). Most AC-ELISAs have been licensed and marketed to detect human influenza A virus in clinical specimens. Some have demonstrated effectiveness for detection of AIV, but many of these commercial tests have had low sensitivity (Woolcock & Cardona, 2005[46]). Those validated for veterinary use are preferred. Any HA activity of sterile fluids harvested from the inoculated eggs is most likely to be caused by an influenza A virus or an avian paramyxovirus, but a few strains of avian viruses, as well as nonsterile fluid containing HA of bacterial origin can cause the agglutination of RBCs. There are currently 10 recognized serotypes of avian paramyxoviruses (Miller et al., 2010[26]). Most laboratories will have antiserum specific to Newcastle disease virus (avian paramyxovirus type 1), and in view of its widespread occurrence and almost universal use as a live vaccine in poultry, it is best to evaluate its presence by haemagglutination inhibition (HI) tests (Newcastle disease).

Alternatively, the presence of influenza virus can be confirmed by the use of RT-PCR or rRT-PCR using nucleoprotein-specific or matrix-specific conserved primers (Altmuller et al., 1991[22]; Spackman et al., 2002[32]). Also, the presence of subtype H5 or H7 influenza virus can be confirmed by using H5- or H7-specific primers (Monne et al., 2008[27]; Spackman et al., 2008[31]).

Antigenic subtyping can be accomplished by mono specific antisera prepared against purified or recombinant H and N subtype-specific proteins, used in HI and NI tests, or polyclonal antisera raised against a
battery of intact influenza viruses and used in HI and NI tests. Genotyping can be accomplished using H and N subtype specific primers in RT-PCR and rRT-PCR tests; or 4) using sequence analysis of H and N genes. Subtype identification by these techniques is beyond the scope of most diagnostic laboratories not specializing in influenza viruses (Starick et al., 2000[33]).

1.13 Assessment of pathogenicity

The term HPAI relates to the assessment of virulence in chickens and implies the involvement of virulent strains of virus. It is used to describe a disease of fully susceptible chickens with clinical signs such as ocular and nasal discharges, coughing, snicking and dyspnoea, swelling of the sinuses and/or head, listlessness, reduced vocalization, marked reduction in feed and water intake, cyanosis of the unfeathered skin, wattles and comb, incoordination, nervous signs and diarrhoea. In laying birds, additional clinical features include a marked drop in egg production usually accompanied by an increase in numbers of poor quality eggs. Typically, high morbidity is accompanied by high and rapidly escalating unexplained mortality. However, none of these signs can be considered pathognomonic and high mortality may occur in their absence. In addition, LPAI viruses that normally cause only mild or no clinical disease, may cause a much more severe disease if exacerbating infections or adverse environmental factors are present and, in certain circumstances, the spectrum of clinical signs may mimic HPAI (Spackman et al., 2008[31]).

The historical term ‘fowl plague’ has been abandoned in favor of the more accurate term HPAI. Because all naturally occurring HPAI viruses to date have been H5 and H7 subtypes and genomic studies have determined HPAI viruses arise by mutation of H5 and H7 LPAI viruses, all H5 and H7 LPAI have been recognized as potentially pathogenic. Pathogenicity shifts have been associated with changes to the proteolytic cleavage site of the haemagglutinin including: 1) substitutions of non-basic with basic amino acids (arginine or lysine); 2) insertions of multiple basic amino acids from codons duplicated from the haemagglutinin cleavage site; 3) short inserts of basic and non-basic amino acids from unknown source; 4) recombination with inserts from other gene segments that lengthen the proteolytic cleavage site; and 5) loss of the shielding glycosylation site at residue 13 in combination with multiple basic amino acids at the cleavage site. Amino acid sequencing of the cleavage sites of H5 and H7 subtype influenza isolates of low virulence for birds should identify viruses that have the capacity, following simple mutation, to become highly pathogenic for poultry (FAO, 2004[16]; Elvinger et al., 2007[13]; Swayne, 2004[38]).

The following criteria have been adopted by the OIE for classifying an AIV as HPNAI: a) One of the two following methods to determine pathogenicity in chickens is used. A HPNAI virus is: i) any influenza virus that is lethal for six, seven or eight of eight 4- to 8-week-old susceptible chickens within 10 days following intravenous inoculation with 0.2 ml of a 1/10 dilution of a bacteria-free, infective allantoic fluid or ii) any virus that has an intravenous pathogenicity index (IVPI) greater than 1.2. The following is the IVPI procedure:

- Fresh infective allantoic fluid with a HA titre >1/16 (>24 or >log2 4 when expressed as the reciprocal) is diluted 1/10 in sterile isotonic saline.
- 0.1 ml of the diluted virus is injected intravenously into each of ten 4- to 8-week-old SAN susceptible chickens; if possible, SPF chickens should be used.
- Birds are examined at 24-hour intervals for 10 days. At each observation, each bird is scored 0 if normal, 1 if sick, 2 if severely sick, 3 if dead. (The judgement of sick and severely sick birds is a subjective clinical assessment. Normally, ‘sick’ birds would show one of the following signs and ‘severely sick’ more than one of the following signs: respiratory involvement, depression, diarrhoea, cyanosis of the exposed skin or wattles, oedema of the face and/or head, nervous signs. Dead individuals must be scored as 3 at each of the remaining daily observations.)
- The IVPI is the mean score per bird per observation over the 10-day period. An index of 3.00 means that all birds died within 24 hours, and an index of 0.00 means that no bird showed any clinical sign during the 10-day observation period.
- For all H5 and H7 viruses of low pathogenicity in chickens, the amino acid sequence of the connecting peptide of the haemagglutinin must be determined. If the sequence is similar to that observed for other highly pathogenic AI isolates, the isolate being tested will be considered to be highly pathogenic (found at: http://www.offlu.net/OFFLU%20Site/Projects/Table%20HPAI%20cleavage%20site%20sequences.pdf). The OIE has the following classification system to identify viruses for which disease reporting and control measures should be taken: a) All AI isolates that meet the above criteria are designated as HPNAI. b) H5 and H7 isolates that are not virulent for chickens and do not have an HA0 cleavage site amino acid sequence similar to any of those that have been observed in HPAI viruses are designated as low pathogenicity...
notifiable avian influenza (LPNAI). c) Non-H5 or non-H7 AI isolates that are not virulent for chickens are designated as LPAI (Starick et al., 2000[33]).

1.13.1 Psittacosis (Ornithosis, Chlamydiosis): Psittacosis is caused by the bacteria *Chlamydia psittaci*. *C. psittaci* is common in wild birds and can occur in laboratory bird colonies. Infected birds are highly contagious to other birds and to humans. The organism is spread to humans by aerosolization of respiratory secretions or feces from the infected birds. Typical symptoms in the bird are diarrhea, ocular discharge, and nasal discharge. The infection in humans by *C. psittaci*, can cause fever, headache, myalgia, chills, and upper and lower respiratory disease. Serious complications can occur and include pneumonia, hepatitis, myocarditis, thrombophlebitis and encephalitis. It is responsive to antibiotic therapy but relapses can occur in untreated infections.

Prevention: Only disease-free flocks should be allowed into the research facility. Wild-caught birds or birds of unknown status should be treated prophylactically for 45 days with chlortetracycline. Animal Biosafety Level 2 practices are recommended for personnel working with naturally infected birds or experimentally infected birds. Wearing NIOSH certified dust masks should be considered in rooms housing birds of unknown health status (Fouchier et al., 2010[17]; Capua & Alexander, 2008[44]).

Newcastle Disease: Newcastle disease is caused by a paramyxovirus and can be seen in birds both wild and domestic. Transmission is mainly by aerosol but contaminated food, water and equipment can also transmit the infection within bird colonies. Pathogenic strains produce anorexia and respiratory disease in adult birds. Young birds often show neurologic signs. In humans the disease is characterized by conjunctivitis, fever, and respiratory symptoms. Prevention: The disease can be prevented by immunizing susceptible birds and obtaining birds from flocks free of infection. Good personal-hygiene practices which include hand washing after handling animals or their waste should be in place (Carpenter and Gentz, 1997[16]; Chen et al., 2005[10]).

Salmonellosis: Along with a variety of other species, Salmonella, and other enteric bacteria are capable of causing disease in humans. Salmonellae are transmitted by the fecal-oral route. Infection produces an acute enterocolitis and fever with possible secondary complications such as sepsicaemia. Prevention: Use of protective clothing, personal hygiene which include hand washing after contact with animals or their waste, and sanitation measures prevent the transmission of the disease.

Campylobacter: Campylobacter species can be found in pet and laboratory animal species. Transmission to humans is by the fecal-oral route and can produce an acute enteritis. Symptoms include diarrhea, abdominal pain, fever, nausea, and vomiting. Prevention: Use of personal protective clothing, good personal hygiene, and sanitation measures will help to prevent the transmission of the disease.

1.14 Zoonotic Diseases in Mice, Rats, Hamsters and other rodents

Lymphocytic Choriomeningitis Virus: Lymphocytic choriomeningitis virus infects wild mice world-wide and laboratory animal species including mice, hamsters and guinea pigs. Humans can be infected by inhalation and by contact with tissues or fluids from infected animals. Symptoms include fever, myalgia, headache and malaise. More severe symptoms can occur such as lymphadenopathy, meningoencephalitis and neurologic signs. Prevention: Serologic surveillance of animal colonies at risk and screening of all tumors and cell lines intended for animal passage will help to prevent LCM. Personnel should wear gloves when handling animals and practice appropriate personnel hygiene which includes hand washing.

Leptospirosis: Leptospirosis is widely distributed in domestic and wild animals. The possibility of transmission to humans from most animal species maintained in the laboratory should be considered but livestock and dogs would be the most common reservoirs. Transmission of the organism to humans can occur through skin abrasions and mucous membranes by contact with urine or tissues of animals infected with Leptospirosis. Inhalation or ingestion of organisms can also transmit the diseases. Disease can vary from asymptomatic infection to severe disease ranging from flu-like symptoms to liver and kidney failure, encephalitis, and pulmonary involvement. Prevention: Control of this infection in laboratory animal populations along with use of protective clothing and gloves by persons working with and caring for infected animals will help prevent disease (OIE,2012[50]).

Rat-Bite Fever: Rat-bite fever is caused by Streptobacillus moniliformis or Spirillum minro. These organisms are in the respiratory tracts and mouths of rodents, especially rats. Most human infections are the result of a bite wound. Symptoms include chills, fever, malaise, headache and muscle pain. A rash can develop along with painful joints, abscesses, endocarditis, pneumonia, hepatitis, pyelonephritis, and enteritis.
Prevention: Animals need to be handled properly to prevent bites.

Campylobacter: Campylobacter species can be found in pet and laboratory animal species. Transmission to humans is by the fecal-oral route and can produce an acute enteritis. Symptoms include diarrhea, abdominal pain, fever, nausea, and vomiting. Prevention: Use of personal protective clothing, good personal hygiene, and sanitation measures will help to prevent the transmission of the disease (Chua et al., 2007[11]).

Salmonellosis: Along with a variety of other species, Salmonella, and other enteric bacteria are capable of causing disease in humans. Salmonellae are transmitted by the fecal-oral route. Infection produces an acute enterocolitis and fever with possible secondary complications such as septicemia. Prevention: Use of protective clothing, personal hygiene which include hand washing after contact with animals or their waste, and sanitation measures will prevent the transmission of the disease (Webster et al., 1997[42]; Webster et al., 2006[41]).

Campylobacter: Campylobacter species can be found in pet and laboratory animal species. Transmission to humans is by the fecal-oral route and can produce an acute gastrointestinal illness. Symptoms include diarrhea, abdominal pain, fever, nausea, and vomiting. Prevention: Use of personal protective clothing, good personal hygiene, and sanitation measures will help to prevent the transmission of the disease (Webster et al., 1992[40]).

1.15 Zoonotic Diseases in Rabbits

Cryptosporidiosis: Cryptosporidium species have a worldwide distribution and can be found in many animal species including rabbits. Cryptosporidiosis is caused by a protozoan parasite which lives in the intestines of mammals. Cryptosporidiosis is transmitted by the fecal-oral route and can cause diarrhea in humans. Usually the diarrhea is self-limiting but in immune compromised individuals the disease can have a prolonged course. Prevention: Appropriate personal-hygiene practices which include washing hands after contact with animals or their waste should prevent spread of this organism.

Leptospirosis: is widely distributed in domestic and wild animals. The possibility of transmission to humans from most animal species maintained in the laboratory should be considered but livestock and dogs would be the most common reservoirs. Transmission of the organism to humans can occur through skin abrasions and mucous membranes by contact with urine or tissues of animals infected with Leptospirosis. Inhalation or ingestion of organisms can also transmit the diseases. Disease can vary from asymptomatic infection to severe disease ranging from flu-like symptoms to liver and kidney failure, encephalitis, and pulmonary involvement (Causey and Edwards, 2008[8]).

Prevention: Control of this infection in laboratory animal populations along with use of protective clothing and gloves by persons working with and caring for infected animals will help prevent disease.

Ringworm: Dermatophytes, which are fungi, cause ringworm in humans and animals. Infection in animals may be in apparent and is transmitted to humans by direct contact with infected animals or by indirect
contact with contaminated equipment or materials. Dermatophytes produce flat, circular lesions that are clear in the center and crusted and red on the periphery. Prevention: The use of protective clothing, disposable gloves, and hand washing along with good personal hygiene will help to reduce the spread of dermatophytosis in a laboratory animal facility.

1.15 Zoonotic Diseases in Fish
Cryptosporidia: Cryptosporidium species have a worldwide distribution and can be found in many animal species including fish. Cryptosporidiosis is caused by a protozoan parasite is transmitted by the fecal-oral route and can cause diarrhea in humans. Usually the diarrhea is self-limiting but in immunocompromised individuals the disease can have a prolonged course (OIE, 2012[58]).

Prevention: Appropriate personal-hygiene practices which include washing hands after contact with animals or their waste should prevent spread of this organism

Mycobacteriosis/Nocardiosis: Mycobacteriosis and nocardiosis are bacterial diseases of fish. In the fish external as well as internal lesions can be found resulting in anorexia, popeye, shin discoloration and external lesions such as ulcers, and fin rot. Transmission to humans is by bacteria entering abrasions. Persons infected with these bacteria may develop cysts or abscesses at the site of the abrasion that may ulcerate and scar (OIE, 2012[58]).

Prevention: Wear protective gloves when cleaning fish aquaria or tanks as well as when handling or gutting fish.

1.17 Zoonotic Diseases in Reptiles and Amphibians
Cryptosporidia: Cryptosporidium species have a worldwide distribution and can be found in many animal species. It is transmitted by the fecal-oral route and can cause diarrhea in humans. Usually the diarrhea is self-limiting but in immunocompromised individuals the disease can have a prolonged course. Prevention: Appropriate personal-hygiene practices which include washing hands after contact with animals or their waste should prevent spread of this organism (OIE, 2012[58]).

Salmonellosis: Along with a variety of other species, Salmonella, and other enteric bacteria are capable of causing disease in humans. Salmonellae are extremely common in reptiles and are transmitted by the fecal-oral route. Infection produces an acute enterocolitis and fever with possible secondary complications such as septicemia. Prevention: Use of protective clothing, personal hygiene which include hand washing after contact with animals or their waste, and sanitation measures prevent the transmission of the disease (OIE, 2012[58]).

2. References
12. Das A, Spackman E, Senne D, Pedersen J, Suarez DL. Development of an internal positive control for rapid diagnosis of avian influenza virus infections by real-time reverse transcription-PCR


24. Internet Health Directory (http://www.internethealthdirectory.com/Conditions_and_Diseases_Infectious_Diseases_Zoonoses.html) --- A website that links together a large number of health related websites, including a variety of sites related to zoonotic diseases.


32. Spackman E, Senne DA, Myers TJ, Bulaga LL, Garber LP, Perdue ML, Lohman K, Daum LT, Suarez DL. Development of a real-time reverse transcriptase PCR assay for type A influenza virus and the avian H5 and H7 hemagglutinin