

# Pathogenesis and pathobiology of avian influenza virus infection in birds

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## Summary

Avian influenza (AI) viruses vary in their ability to produce infection, disease and death in different bird species. Based on the pathobiological effect in chickens, AI viruses (AIV) are categorised as low pathogenic (LPAIV) or highly pathogenic (HPAIV). Typically, LPAIV cause asymptomatic infections in wild aquatic birds, but when introduced into domesticated poultry, infections may be asymptomatic or produce clinical signs and lesions reflecting pathophysiological damage to the respiratory, digestive and reproductive systems. The HPAIV have primarily been seen in gallinaceous poultry, producing high morbidity and mortality, and systemic disease with necrosis and inflammation in multiple visceral organs, nervous and cardiovascular systems, and the integument. Although HPAIV have rarely infected domestic waterfowl or wild birds, the Eurasian-African H5N1 HPAIV have evolved over the past decade with the unique capacity to infect and cause disease in domestic ducks and wild birds, producing a range of syndromes including asymptomatic respiratory and digestive tract infections; systemic disease limited to two or three critical organs, usually the brain, heart and pancreas; and severe disseminated infection and death as seen in gallinaceous poultry. Although experimental studies using intranasal inoculation have produced infection in a variety of wild bird species, the inefficiency of contact transmission in some of them, for example, passerines and Columbiformes, suggests they are unlikely to be a reservoir for the viruses, while others such as some wild Anseriformes, can be severely affected and could serve as a dissemination host over intermediate distances.

## Keywords

Avian diseases – Avian influenza virus – Highly pathogenic avian influenza – Low pathogenic avian influenza – Pathobiology – Pathogenesis – Pathotypes.

## Introduction

Avian influenza (AI) viruses infect a wide variety of domestic poultry, captive birds, and free-ranging wild bird species under natural and experimental conditions. Wild aquatic birds are the main reservoirs of AI viruses (AIV). These viruses are highly host-adapted, replicating in epithelial cells of the gastrointestinal and respiratory tracts, producing asymptomatic infections. Periodically, these AI viruses have been transmitted from wild aquatic to domestic birds producing subclinical infections, or, occasionally, respiratory disease and drops in egg production. This type of virus is typically termed low pathogenic avian influenza virus (LPAIV) and can carry any combination of the 16 haemagglutinin (HA) and

9 neuraminidase (NA) subtypes. However, after circulating in domestic poultry a few H5 and H7 LPAI viruses have mutated into highly pathogenic avian influenza viruses (HPAIV). These HPAIV cause severe systemic disease with lesions of necrosis and inflammation in the skin, viscera and brain of gallinaceous poultry (Order: Galliformes). Historically, HPAIV have not infected wild birds except for the die-off among common terns (*Sterna hirundo*) in South Africa in 1961 (7). However, since 2002, the Eurasian-African H5N1 HPAI viruses have caused infections, illness and death in a variety of captive, zoo and wild birds.

This review will cover the general pathobiological features of LPAIV and HPAIV infections in domestic poultry and wild birds, drawing information from multiple sources but

principally from specific summary (88, 92, 94) and original research papers (1, 3, 4, 6, 7, 13, 15, 20, 22, 23, 35, 36, 42, 43, 46, 47, 56, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 76, 77, 78, 85, 87, 89, 90, 91, 93, 95, 96, 97, 99, 115).

## Pathogenicity of avian influenza viruses

### Pathotypes

The pathogenicity of a virus refers to its ability to produce disease. Avian influenza virus infection of domestic poultry produces syndromes ranging from asymptomatic infection to respiratory disease and a drop in egg production, to severe, systemic disease with near 100% mortality. Influenza A viruses that infect poultry are classified into two pathotypes, LPAIV and HPAIV. The HPAIV produce 75% or greater mortality in intravenously inoculated chickens (*Gallus domesticus*), have a chicken intravenous pathogenicity index of 1.2 or greater, or are AIV (H5 or H7) having an HA cleavage site with a polybasic amino acid sequence similar to that of other HPAIV (112). All AIV that do not meet a highly pathogenic criterion are classified as low pathogenic. The use of the term HPAI for chickens does not indicate or imply that the AI virus strain is highly lethal for other bird species, but similar high lethality has been typically seen after infection of other gallinaceous poultry (e.g. turkey [*Meleagris gallopavo*], Japanese quail [*Coturnix japonica*], bobwhite quail [*Colinus virginianus*], pearl guineafowl [*Numida meleagris*], ring-necked pheasant [*Phasianus colchicus*] and chukar partridge [*Alectoris chukar*]).

### Pathobiological concepts applied to avian influenza virus infections

Although only two pathotypes of AIV are defined in the laboratory, natural infection by AI viruses results in a wide range of clinical outcomes, which are dependent on virus strain, host species, and environmental factors (92). In order to comprehend the complex biology of AI viruses in avian species, several pathobiological concepts need to be understood.

### Viral pathogenesis

Viral pathogenesis is the pathophysiological process that occurs when a virus infects a host.

### Infectivity

Infectivity is the ability of the virus to bind to and enter cells of the host, replicate and produce and release infectious virus progeny.

### Disease

Disease, the harmful pathobiological consequence of infection, includes abnormal physiological and anatomic changes as a result of virus replication within the cell, tissue and/or organ. In general, as virus replication increases, the severity of pathobiological changes also increases, and gross and microscopic lesions develop. Most HPAIV strains causing major cell damage and cell death, if sufficiently severe, can cause critical organ failure and death of the host.

There are four potential clinical outcomes following AI virus exposure: no infection, asymptomatic (subclinical) infection, mild disease, and severe disease leading to death. In each case, other than the first, exposure, or access to the virus, is critical to start the process. With some AI virus strains and some hosts, exposure to virus may not result in infection, especially if the route of exposure is inappropriate, the viral dose is below the threshold to initiate infection, immunity is present against the virus strain, or the virus strain is not adapted to the specific host species. The mean intranasal infectious dose for selected AI virus isolates (determined in domestic poultry under experimental conditions) has been shown to be both host- and virus-strain dependent, and measuring this infectious dose could be a means of assessing the infectivity and adaptability of the virus to a specific host (Table 1) (98).

### Host adaptation

Host adaptation, which is the result of progressive genetic changes in a virus, increases the efficiency of binding, replication and release of the virus from a specific host species. Viruses with low adaptation fail to replicate efficiently in the host species unless there is high dose exposure or accompanying secondary factors that increase host susceptibility. By comparison, an AI virus strain expressing a high degree of adaptation to a specific host species may require a low dose exposure to produce infection. Host adaptation can be maximal for only a single host species, although, in evolutionarily closely related species, the virus strain may show a gradual decrease in its level of adaptation and thus infectivity.

### Transmissibility

Transmissibility is the natural host-to-host spread and implies a specific host-adapted virus, exposure to the virus through contact with infected animals or fomites, and a naïve, susceptible host. Three factors affect transmission efficiency:

- virus shedding by the host into the environment, in terms of viral titre, duration and source (oral, cloacal)
- environmental stability of the virus, which is impacted by temperature, humidity and organic matter content
- the minimal infective dose of virus (81).

**Table I**

**Variability in intranasal infectivity of several low pathogenic avian influenza viruses (LPAIV) and highly pathogenic avian influenza viruses (HPAIV) for different poultry species, as determined by mean bird infectious dose (BID<sub>50</sub>)**

Modified from Swayne and Slemmons (98)

Virus strains	Subtype	Host species	BID <sub>50</sub>	References
<b>HPAIV</b>				
A/chicken/Rostock(Germany)/1934	H7N1	Chicken	1.2	(98)
A/tern/South Africa/1961	H5N3	"	3.4	"
A/chicken/Victoria/1975	H7N7	"	2.9	"
A/turkey/Ireland/1378/1983	H5N8	"	4.7	"
A/chicken/Pennsylvania/1370/1983	H5N2	"	3.0	(93)
A/Hong Kong/491/1997	H5N1	"	2.3	(86)
A/chicken/Italy/4580/1999	H7N1	"	2.0	(98)
A/chicken/Yamaguchi/7/04	H5N1	"	2.5	(104)
<b>LPAIV</b>				
A/emu/Texas/39924/1993	H5N2	Chicken	4.2	(90)
A/cinnamon teal/Bolivia/4537/01	H7N3	"	6.2	(79)
A/turkey/Virginia/15821/2002	H7N2	"	2.8	(105)
A/duck/Pennsylvania/454069-9/06	H5N1	"	7.5	(80)
A/chicken/Alabama/1975	H4N8	"	5.8	(98)
		Turkey	6.0	"
		Domestic duck	3.3	"
		Domestic goose	4.0	"
		Japanese quail	2.6	"
A/mallard/Ohio/338/1986	H4N8	Chicken	8.3	"
		Turkey	4.0	"
		Domestic duck	3.1	"
		Domestic goose	1.7	"
		Japanese quail	1.5	"
A/mallard/Ohio/184/1986	H5N1	Chicken	7.7	"
		Turkey	4.2	"
		Domestic duck	1.9	"
		Domestic goose	5.6	"
		Japanese quail	5.4	"

## Virulence and pathogenicity

Virulence and pathogenicity describe the relative capacity of a virus to cause disease and are determined by the capacity of a virus to grow, be invasive, infect susceptible cells, evade the immune system and cause cellular damage (108). These capacities are encoded in the viral genome by individual virulence genes. The specific property associated with virulence is termed virulence determinant. Pathogenicity of AI viruses is a polygenic trait and depends largely on an 'optimal' gene constellation affecting host and tissue tropism, replication efficacy and immune evasion mechanisms, amongst others.

## Tropism

Tropism is the capacity of a virus to infect or damage specific cells, tissues or organs, and is a fundamentally important contributor to viral pathogenesis and virulence (108). Tropism is determined by many viral and host factors, including how the virus spreads, the permissiveness of specific cell types for the virus (as defined by receptors, cellular differentiation, and intrinsic cellular resistance to infection), and the nature of innate and adaptive immune responses (108). Cell-intrinsic resistance to infection is conferred by the presence of molecules in the cells that block viral infection. Events that

are dictated by processes that occur outside of the cell are termed cell-extrinsic and many are related to the innate and adaptive immunity. An example is the death of infected cells that may result from cell-intrinsic induction of apoptosis, or cell-extrinsic immune factors such as cytokines.

### Avian influenza viral pathogenesis

The HA is the major determinant of virulence, but maximum expression of virulence requires an optimal combination of internal genes (9). To initiate the infection process in birds, the HA must first bind to  $\alpha$ 2,3-galactose linkage cell receptors to initiate receptor-mediated endocytosis. This is a poorly understood phenomenon but it impacts both host specificity and cell or tissue tropism (92). Changes in receptor-binding will change the host range of influenza viruses (57). In addition, fusion of the viral envelope with the endosomal membrane requires a cleaved HA. For LPAIV, trypsin-like proteases, which are restricted to epithelial cells, cleave the precursor HA0 into functional HA1 and HA2 proteins, resulting in infection and lesions in epithelial-cell-containing organs, primarily respiratory and digestive tracts. However, with HPAIV, ubiquitous furin proteases cleave HA0 to functional HA1 and HA2 proteins, resulting in infection and lesions in many cell types in numerous visceral organs, the nervous system and the cardiovascular system (82). Pathogenesis studies of AIV in chickens and ducks indicate that the NA, NS, PA, PB1, PB2, and NP proteins are also important determinants of virulence (17, 30, 31, 52, 73, 109).

### Mechanisms of cellular pathobiology

The mechanism for damage by HPAIV in avian cells is primarily cell death through necrosis or apoptosis (28, 70). A high level of virus replication, usually evident as visualisation of abundant AIV nucleoprotein in the nuclei and cytoplasm of infected cells and high AIV titres in tissue samples, has been associated with necrosis (92). Neurons of the brain, kidney tubular cells, pancreatic acinar epithelium, cardiac myocytes, adrenal cortical cells, and pulmonary epithelial cells are the most frequently reported sites of necrosis in infected chickens (85). Cell death associated with apoptosis has been confirmed in various cell culture systems and has involved cytokine expression, typically including interferon- $\beta$  and transforming growth factor- $\beta$  (27, 75, 100).

For LPAIV, the nasal cavity is the predominant initial site of virus replication, with spread to other parts of the respiratory tract and the intestinal tract (92). However, secondary bacterial, fungal or viral infections are usually necessary to produce sufficiently severe respiratory damage

to result in illness or death. Rarely, LPAIV have spread systemically, causing infection and damage in epithelial-containing tissues of visceral organs such as the kidney, pancreas, and oviduct (5).

With HPAIV, infection in chickens is initiated in the nasal epithelium within 16 h of direct intranasal exposure and, within 24 h, the nasal epithelium is necrotic, with accompanying submucosal inflammation and virus in capillary endothelial cells (92). Inflammatory cells, principally macrophages and heterophils, play important roles in the initial replication and dissemination of HPAIV, as does virus replication within endothelial cells and spread through the vascular or lymphatic systems. Such viraemia allows dissemination of HPAIV, and initiates replication in a variety of parenchymal cell types within the brain, skin and visceral organs. After intranasal exposure, replication of most HPAIV may be seen within 24 h in visceral organs. Within 48 h, the virus titres may be maximal and the lesions severe. However, some HPAIV require a longer period of time to produce illness and death. These HPAIV produce viraemia with lack of or minimal vascular endothelial cell replication but tend to extensively replicate in parenchymal cells of visceral organs. With some HPAIV, increased vascular permeability is responsible for oedema, haemorrhage and multiple organ failure with associated damaged vascular endothelial cells and accompanying microthrombosis. In some cases, the vascular damage can progress to consumptive coagulopathy with accompanying thrombocytopenia, thrombi and emboli in vessels, and altered blood clotting times. If the HPAIV infected chicken survives the peracute phase (days one and two after exposure), the virus may disseminate and replicate in multiple critical organs, causing single or multi-organ failure and death with involvement of the brain and autonomic nervous system, myocardium, endocrine tissue (e.g. adrenal gland) and/or pancreas. Avian influenza viruses can cause damage by three different pathophysiological mechanisms:

- direct virus replication in cells, tissues, and organs
- indirect effects from production of cellular mediators such as cytokines
- ischaemia from vascular thrombosis (92).

### Species variation

The ability of individual AI viruses to cause disease and the host response to these viruses vary greatly with individual bird species and virus strain. Four pre-1975 H7 HPAIV were highly lethal for chickens, but failed to induce disease, or produced only mild clinical signs, in domestic ducks (2). In addition, some differences have been observed in lethality and induced lesions between different gallinaceous bird species in experimental studies utilising

various LPAIV and HPAIV (49, 70, 111). The differences in type and severity of disease did not always result from the ability of the virus to infect or not infect a particular gallinaceous bird species, because some birds became infected but did not show disease.

## Pathobiology of low pathogenic avian influenza

### Gallinaceous species

Under both natural and experimental conditions, the majority of AIV are of low pathogenicity. Most LPAIV produce subclinical infections in experimental studies, but under natural conditions with accompanying secondary pathogens, mild to moderate disease syndromes are common. Typically, LPAIV have limited local replication in the respiratory and alimentary tracts. However, some LPAIV such as certain Asian H9N2 lineages, adapted to efficient replication in poultry, may cause more prominent signs and also significant mortality (5, 51).

### Clinical signs

Low mortality (<5%) accompanied by high morbidity (>50%) is typical. However, mortality rates can increase after co-infection with a secondary pathogen or in juvenile birds. Specific signs will differ with the virus strain, species and age of the host, and typically include pathophysiological changes in the respiratory, digestive, urinary, and reproductive systems (92). Many LPAIV infections present clinically as mild-to-severe respiratory disease with signs of coughing, sneezing, rales, rattles, and excessive ocular discharge. Hens may express increased broodiness and transient decrease in egg production. In general, birds may have decreased activity, mild weight loss and may huddle into groups. Individual birds may be lethargic and have ruffled feathers and decreased feed and water consumption, leading to mild weight loss and, occasionally, diarrhoea.

### Gross lesions

The expression of gross pathological changes is dependent upon the host species, virus strain, time to death, and the presence of secondary pathogens (92). Most frequently, rhinitis and sinusitis are observed, and if accompanied by secondary bacterial infections, swollen infraorbital sinuses and nasal discharge may be present, especially in turkeys. The tracheal mucosa may be reddened from congestion, with oedema and occasionally haemorrhages and luminal exudates. Occasionally, tracheal exudates form plugs that occlude airways resulting in peracute asphyxiation. When secondary bacterial pathogens are present, fibrinopurulent bronchopneumonia, air sacculitis and coelomitis

(‘peritonitis’) may be present. In hens, the ovaries may regress and mature ova rupture producing free yolk in the coelomic cavity or ‘egg yolk peritonitis’. The oviduct may be swollen with luminal exudates. The last few eggs produced may lack pigment and be thin-shelled and misshapen. Rarely, laying hens may have swollen kidneys with accompanying renal failure and visceral urate deposition (‘visceral gout’). Mild enteritis may be present, particularly in turkeys. In rare cases, pancreases of turkeys have been pale, mottled and contained random haemorrhages.

### Histological lesions and immunohistochemical features

Table II provides a summary of the histological lesions that have been seen in different studies. Most frequently, LPAIV produced heterophilic-to-lymphocytic rhinitis, sinusitis, tracheitis and bronchitis with common demonstration of AIV antigen in epithelial cells of the upper respiratory tract. Pneumonia has been reported and in the most severe cases was accompanied by diffuse air capillary oedema. On rare occasions, nephrosis and nephritis were present in hens and were augmented by the high calcium layer diet. In experimental studies and natural cases in turkeys during the 1999 Italian H7N1 LPAI outbreak, necrosis in the pancreas was reported (13). With other LPAIV infections, pancreatitis has been much less common in chickens than in turkeys. In birds that die from LPAIV infections, the cloacal bursa, thymus, spleen, and other areas with lymphocyte accumulations have shown depletion of lymphocytes, but without evidence of virus infection in lymphocytes.

### Non-gallinaceous bird species

In wild aquatic birds, little is known of natural LPAIV infections other than their general asymptomatic nature. In experimental studies, LPAIV preferentially infected intestinal epithelial cells and were excreted in the faeces. In one study, infected mallard hens had a transient decrease in egg production (48). In domestic ducks and geese, LPAIV infections varied from asymptomatic infections to the production of respiratory disease evident as conjunctivitis, sinusitis, and lower respiratory tract lesions. Co-infections with bacteria have been common and usually associated with production of more severe clinical disease, especially with lesions of air sacculitis, pneumonia and sinusitis. In ratites, LPAIV primarily produced respiratory disease, but in some cases digestive tract disease was also seen, evident as green diarrhoea or ‘green urine’. In emus (*Dromaius novaehollandiae*) and rheas (*Rhea americana*), the most frequent lesions included ocular and/or nasal discharge; sinusitis; tracheitis with haemorrhage, bronchitis and air sacculitis; interstitial pneumonia; and when accompanied by secondary bacterial infection, fibrinous perihepatitis and pericarditis.

**Table II**  
**Histological lesions associated with avian influenza in gallinaceous species**

Modified from Swayne and Pantin-Jackwood (94)

Organ or tissue	Lesion	References	
		HPAI	LPAI
Nasal cavity	Epithelial cell necrosis, rhinitis, sinusitis	(34)	(13, 34, 56, 87)
Trachea	Tracheitis	(56, 76, 93)	(13, 56, 78, 87)
Lung	Oedema, congestion, haemorrhage, interstitial pneumonia, bronchitis	(13, 56, 76, 87, 93)	(34, 56, 78, 87)
Heart	Myocyte necrosis, myocarditis	(13, 16, 34, 43, 56, 67, 85, 87)	NL
Brain	Oedema of neuropil, neuronal necrosis, gliosis, perivascular cuffing, meningoencephalitis	(1, 42, 56, 67)	NL
Enteric tract	Haemorrhage, lymphoid depletion, epithelial necrosis, enteritis	(13, 34, 56, 67, 87)	NL
Pancreas	Vacuolation, acinar epithelial cell necrosis, pancreatitis	(1, 13, 34, 56, 67, 87)	(13)
Spleen	Lymphoid depletion, necrosis, splenitis, hyperplasia of macrophage-phagocytic cells	(16, 34, 56, 67, 85, 87)	NL
Bursa, thymus, caecal tonsils	Lymphoid depletion, phagocytic hyperplasia, necrosis, haemorrhage	(67, 87)	(92) <sup>(a)</sup>
Adrenal glands	Necrosis, adenitis	(34)	NL
Integument	Subcutaneous oedema, epidermal and feather follicle necrosis	(67)	NL
Bone marrow	Cellular depletion, necrosis	(56,67)	NL
Skeletal muscle	Myofibre degeneration and necrosis	(1, 34, 56,67, 87)	NL
Kidney	Tubule necrosis, interstitial nephritis	(56, 87)	(77, 78, 88) <sup>(a)</sup>
Liver	Hepatocellular necrosis	(67)	NL
Reproductive organs	Necrosis, salpingitis and oophoritis	(15)	NL

a) Experimentally in intravenous route of inoculation, rare in natural cases  
NL: no lesion

Infection of a 3-month-old red-lored Amazon parrot (*Amazona autumnalis autumnalis*) with H5N2 LPAIV of the lineage causing infections in poultry since 1994 was associated with severe lethargy (26). With veterinary support care, the parrot recovered.

## Pathobiology of highly pathogenic avian influenza

### Gallinaceous species

Highly pathogenic avian influenza viruses produce high mortality rates in chickens and usually other gallinaceous birds, but the times to death are typically shorter in chickens and vary with individual HPAIV strains.

### Clinical signs

Clinical signs vary with the duration of the infection, the organs or organ systems affected and the degree of tissue damage. With peracute disease, birds may be found dead without prior clinical signs or with only a few birds expressing lethargy, recumbency and a comatose state. On a flock basis, the birds may show decreased activity,

produce less noise, and have decreased feed and water consumption before increased mortality is detected. In hens, egg production can drop precipitously, with cessation of egg production within six days. Faeces may be loose and contain mucus, bile or urates. Respiratory signs are less frequent than with LPAIV infections. Peracute disease is more frequent in chickens and turkeys than in other galliforme birds, but the clinical signs and the duration of morbidity may be similar.

If the clinical course is acute to subacute (3 to 10 days), birds may develop nervous signs including tremors of head and neck, torticollis, opisthotonus, nystagmus, unusual positions of head and appendages, flapping movements of the wings, paresis, paralysis, excitation, convulsions, rolling or circling movements, incoordination, loss of balance, and recumbency with pedaling movements. The occurrence of neurological signs will vary depending on the species of bird and the HPAIV strain. However, neurological signs are not pathognomonic for HPAI and can also result from velogenic Newcastle disease, other infectious diseases, or non-infectious causes.

### Gross lesions

The frequency of gross lesions varies with species of bird and virus strain, and all lesions are not present consistently

in all birds. Generally, HPAIV infections affect multiple visceral organs, the cardiovascular and nervous systems and the integument, producing necrosis, oedema and haemorrhage (Fig. 1). In peracute disease, no gross lesions may be seen. In acute disease, birds may have ruffled feathers, and swelling (oedema) of the comb, wattles, periorbital and intermandibular areas, upper neck, leg shanks and feet with accompanying subcutaneous haemorrhages, especially of the non-feathered skin (Figs 1a-1d). Some virus strains produce oedema and hyperaemia of the conjunctiva, eyelids, and trachea. The wattles, combs and snoods may contain necrotic foci and petechial-to-ecchymotic haemorrhages, or may be cyanotic. Ischaemic necrosis from vascular infarction is responsible for the cyanosis.

Internally, haemorrhages may be present on serosal or mucosal surfaces, and be accompanied by necrotic foci within multiple visceral organs (Figs 1e-1j). Haemorrhages may be especially common in the coronary fat and on the epicardium (Fig. 1f), in the serosal fat pad and in the mucosa of the proventriculus (Fig. 1g) and ventriculus, and in the pectoral muscles (Fig. 1h). Less frequently, haemorrhages will be present in the caecal tonsils (Fig. 1j) and Meckel's diverticulum, and on the inner surface of the sternum. Necrosis and haemorrhage in the pancreas may be seen as red to light orange to brown mottling (Fig. 1m). Hens may have ruptured ova with free yolk in the coelomic cavity. Unique to the recent Eurasian-African H5N1 HPAIV lineage and classic fowl plague viruses is the production of necrosis and haemorrhage in Peyer's patches of the small intestine (Fig. 1i), severe oedema and haemorrhage in the lungs (Fig. 1e), and occasionally, oedema of the brain. With many HPAIV, white foci of necrosis may be present in the heart, and occasionally, the liver and kidneys. Urate deposits may accompany the necrosis in kidney. Occasionally, the lungs may be firm from oedema and interstitial pneumonia and have congestion and haemorrhages. In young birds, the primary lymphoid organs (cloacal bursa and thymus) may be atrophic, with or without haemorrhage. The spleen may be enlarged with pale necrotic foci or be normal in size.

### Microscopic lesions

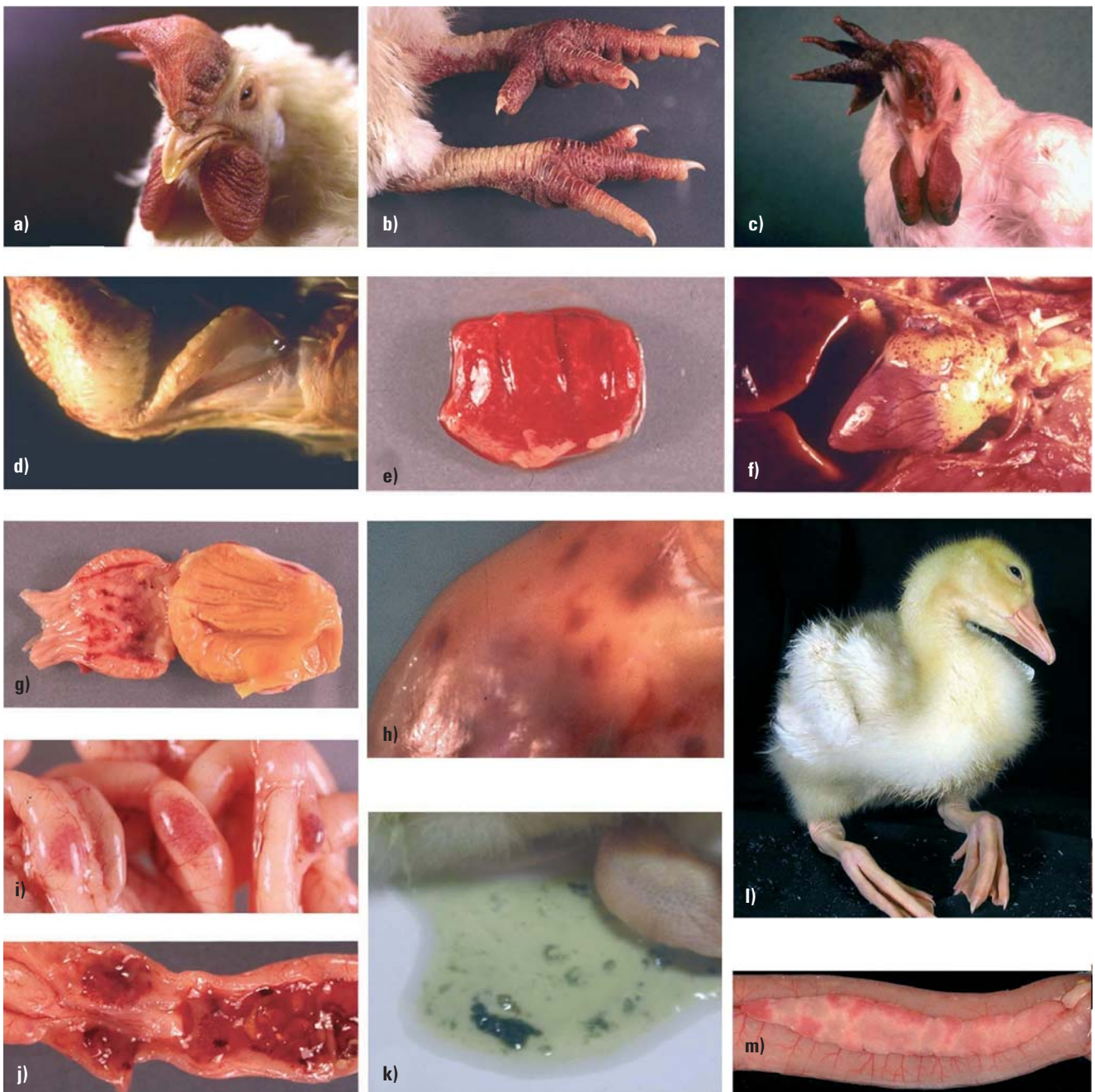
Microscopic lesions are more frequent than gross lesions in most HPAI cases. Histopathological lesions in birds from experimental studies vary with virus strain and passage history; inoculum dose and route of inoculation; and species, strain and breed of bird host. Most frequently, histological changes consist of necrosis and/or inflammation in multiple organs, most often and most severely within the skin (including feather follicles), brain, heart, pancreas, lungs, adrenal glands, and primary and secondary lymphoid organs (Table II and Fig. 2). In peracute disease, microscopic lesions are lacking in most organs, but occasionally, mild or multifocal necrotic and

inflammatory lesions are seen, with virus present principally in vascular endothelial cells and cardiac myocytes (Fig. 2). In acute disease, visceral organs may have multiple foci of necrosis, and associated inflammation, haemorrhage, and oedema. However, necrosis is less prominent and inflammation more prominent in birds that survive longest. Avian influenza virus antigen is associated with areas of necrosis and inflammation, but is not present in apoptotic lymphocytes. Common lesions are described in Table II. Lesions are similar in other gallinaceous species, but since these species survive longer than chickens or turkeys, inflammation is more common and prominent than necrosis in parenchymal organs.

### Tissue and cellular sites of viral replication and damage

Highly pathogenic avian influenza viruses grow to high titres in, and are shed from, the respiratory and intestinal tracts and spread systemically, with viral antigen demonstrated in both vascular endothelial cells and parenchymal cells of multiple organs (Fig. 2). The dominant lesion in acute disease is necrosis, which can result from vascular damage such as thrombosis and embolism, leading to infarction, especially in the skin. However, necrosis and subsequent inflammation can also result from direct viral replication and damage to the parenchymal cells, most commonly in visceral organs, nervous system and skin (28). In addition, vascular damage causes oedema, congestion and haemorrhage, visible as reddening and swelling in the heads, legs and feet (58). Pathophysiologically, viraemia and vascular damage precede the parenchymal lesions. The extent of virus spread and resulting diversity of lesions is dependent upon host survival time, which is partially related to host resistance (variable according to age, species, strain, etc.) and virus virulence (29). Highly pathogenic avian influenza virus strains may be epitheliotropic, endotheliotropic, neurotropic or pantropic depending upon the avian host. For example, A/turkey/Ontario/7732/1966 (H5N9) HPAIV was neurotropic without extensive vascular endothelium replication in turkeys (58). At a minimum, HPAIV are neuropathogenic by three different mechanisms:

- direct viral spread from the epithelium in the posterior nasal chamber through olfactory nerves to olfactory bulbs and anterior brain
- dissemination through the cardiovascular system resulting in random multifocal necrosis and microgliosis in brain parenchyma
- infection of ependymal cells resulting in associated ventriculitis and periventricular necrosis and inflammation (42).

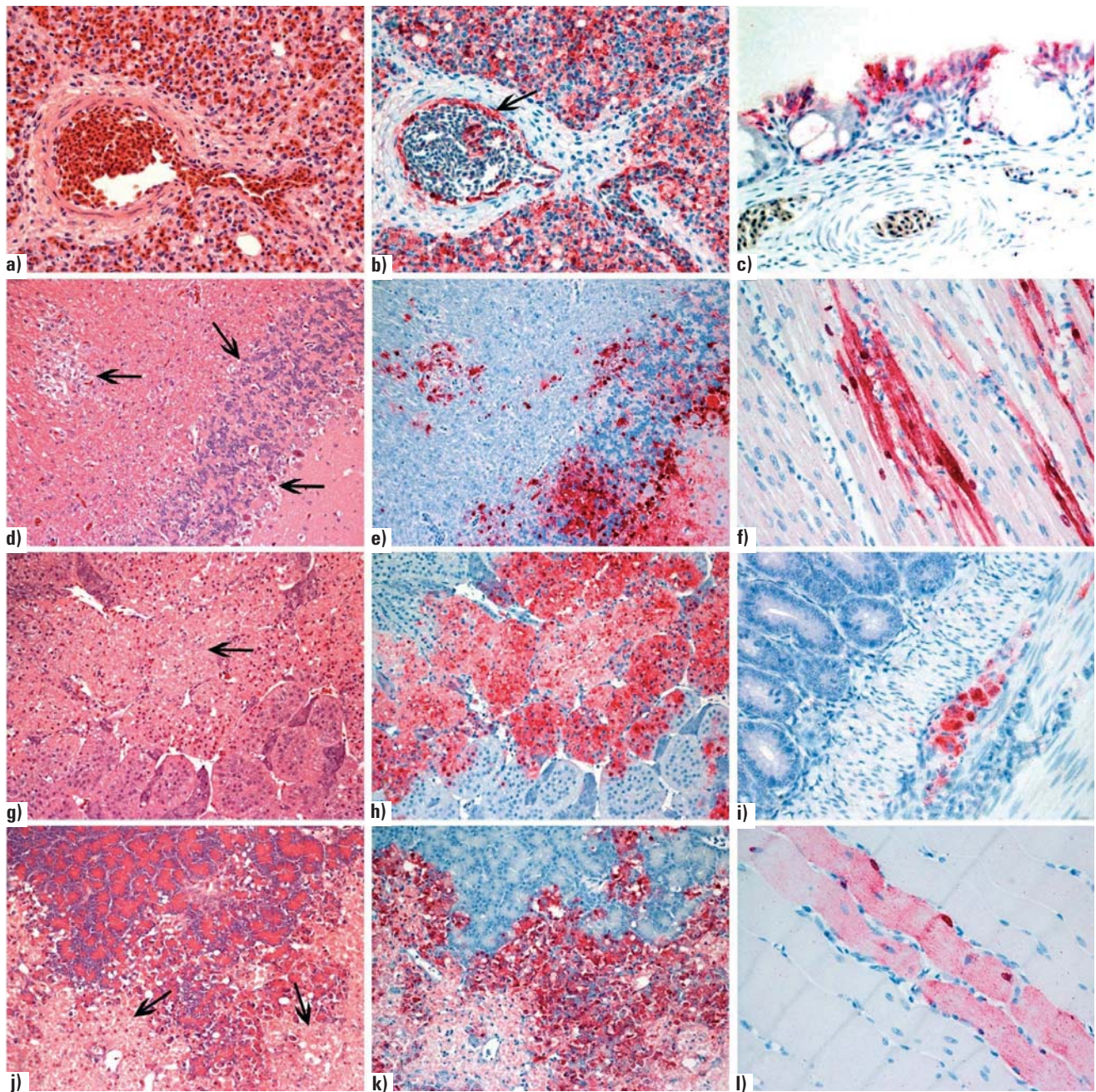


- a) Oedema of comb, wattles, and periorbital tissues, A/chicken/Puebla/1994 H5N2 HPAI virus (94)  
 b) Severe subcutaneous haemorrhage and oedema of feet and leg shanks, A/Hong Kong/156/97 (H5N1) (85)  
 c) Severe oedema, necrosis and haemorrhage of comb and wattles, highly pathogenic embryo derivative, A/chicken/NJ/12508/86 (H5N2) (92)  
 d) Thickened dermis from oedema of distal leg, A/chicken/Queretaro/14589-660/94 (H5N2)  
 e) Severe pulmonary oedema and haemorrhage in the lung, A/Hong Kong/156/97 (H5N1) (85)  
 f) Petechial haemorrhages in epicardial fat, A/chicken/NJ/12508/86 (H5N2) (91)  
 g) Submucosal haemorrhage surrounding ducts of glands in proventriculus, A/chicken/Hong Kong/156/97 (H5N1) (85)  
 h) Multifocal haemorrhage in the fascial plane of the gastrocnemius muscle (pars intermedia), A/chicken/Hong Kong/220/1997 (H5N1) (94)  
 i) Haemorrhage in lymphoid tissue of Peyer's patches and Meckel's diverticulum of the jejunum, A/Hong Kong/220/97 (H5N1)  
 j) Haemorrhage in caecal tonsils and rectum, A/Hong Kong/483/97  
 k) Bile-stained loose droppings from a 2-week-old Pekin duck, A/Egret/HK/757.2/02 (H5N1) (94)  
 l) Two-week-old Embden goose with torticollis, A/chicken/Hong Kong/220/1997 (H5N1) (68)  
 m) Mottling of the pancreas in a 2-week-old Embden goose, A/chicken/Hong Kong/220/1997 (H5N1) (68)

**Fig. 1**

**Gross lesions in chickens, ducks and geese following experimental infection with highly pathogenic avian influenza viruses**





a) Severe congestion with interstitial oedema in lung of a chicken, A/Ck/Indonesia/7/03 (H5N1). Hematoxylin and eosin stain. 400× magnification  
 b) Avian influenza viral antigen (in red) in vascular endothelium (arrow) and phagocytic leukocytes within the pulmonary parenchyma and smaller caliber interstitial vessels in the same tissue section. Immunohistochemical stain. 400× magnification  
 c) Avian influenza viral antigen (in red) in nasal epithelial cells of a 2-week-old duck, A/crow/Thailand/04 (H5N1). Immunohistochemical stain. 400× magnification  
 d) Multifocal neuronal necrosis and vacuolation (arrows) of neuropil in the cerebellum of a 2-week-old duck 2, A/egret/HK/757.2/02 (H5N1). Hematoxylin and eosin stain. 200× magnification  
 e) Avian influenza viral antigen (in red) in neurons and glial cells in the same tissue section. Immunohistochemical stain. 200× magnification  
 f) Intracellular and intracytoplasmic avian influenza viral antigen staining (in red) in cardiac myocytes in the heart of a 2-week-old duck, A/Dk/VN/218/05 (H5N1). Immunohistochemical stain. 400× magnification  
 g) Focal vacuolar degeneration to necrosis of adrenal cortical and medullary chromaffin cells (arrows) in a 2-week-old duck, A/Dk/VN/218/05 (H5N1). Hematoxylin and eosin stain. 200× magnification  
 h) Diffuse staining for avian influenza viral antigen (in red) on the same tissue. Immunohistochemical stain. 200× magnification  
 i) Avian influenza viral antigen (in red) in parasympathetic ganglia in the intestinal wall of a 2-week-old Pekin duck, A/Dk/VN/218/05 (H5N1). Immunohistochemical stain. 400× magnification  
 j) Severe vacuolation and necrosis of the pancreatic acinar epithelium of a 5-week-old duck, A/Dk/VN/218/05 (H5N1). Hematoxylin and eosin stain. 200× magnification  
 k) Diffuse staining for avian influenza virus (in red) on the same tissue. Immunohistochemical stain. 200× magnification  
 l) Avian influenza viral antigen (in red) in the myocardial cells of skeletal muscle of a 2-week-old duck, A/Dk/VN/218/05 (H5N1). Immunohistochemical stain. 400× magnification

**Fig. 2**  
**Histological lesions in chickens, ducks and geese following experimental infection with highly pathogenic avian influenza viruses**

## Non-gallinaceous bird species

### Wild birds

There were few reports of HPAIV infections and lesions in wild birds prior to 2002, except during 1961 when H5N3 HPAIV caused high mortality in common tern (*Sterna hirundo*) in South Africa (7). Since 2002, the Eurasian-African H5N1 HPAIV lineage has caused infections, illness and death in a variety of wild aquatic and terrestrial birds.

### Domestic ducks and geese

Prior to 2002 HPAIV infrequently produced disease or mortality in domestic ducks. In Italy during 1999 and 2000, natural infections with H7N1 HPAIV caused deaths in Muscovy ducks (*Cairina moschata*). The brain was the primary target, with lesions and viral antigen (14). Experimentally, A/fowl/Germany/34 (H7N1) (FPV Rostock) HPAIV produced death in domestic ducks (2). However, with most HPAIV, experimental infection of domestic Pekin ducks has resulted in either no or only limited virus replication and few clinical signs (2, 3, 68). Since 2002, the pathobiology of HPAI infections in domestic ducks has changed, with Eurasian-African lineage H5N1 HPAIV replicating systemically and causing disease and death in domestic ducks under natural and experimental conditions (24, 32, 40, 50, 59, 63, 64, 83, 84). Experimental inoculation of ringed teals (*Callonetta leucophrys*) with an H7N7 HPAIV by the intranasal and intratracheal route induced conjunctivitis (107). In geese (*Anser anser domesticus*), neurological disease and lesions were experimentally induced after inoculation with an H5N1 HPAIV from 1997 (Fig. 11) (68).

### Ratites

Ostriches (*Struthio camelus*) with natural HPAIV infections have had reduced activity and decreased appetite, and exhibited open mouth breathing, ruffled feathers, sneezing, and nervous signs, including tremors of the head and neck, torticollis, incoordination, and paralysis of the wings. Individual birds had oedema of head and neck, severe haemorrhagic enteritis, enlarged and firm pancreas from necrosis, mild to severe air sacculitis, hepatitis, peritonitis, and enlargement of kidneys and spleen.

## Unique pathobiology of Eurasian-African lineage H5N1 highly pathogenic avian influenza virus

Over the past 12 years, individual strains of the Eurasian-African lineage of the H5N1 HPAIV have changed to express greater virulence and altered pathobiological features in certain domestic waterfowl and wild bird species (24, 40, 59, 63, 64, 83, 84, 93, 106). In experimental studies with various bird species, virus strains from the first outbreaks in Hong Kong were divided into four groups based on pathology, morbidity, mortality and the sites of virus replication (Table III) (70). Pathobiological Group One contained gallinaceous birds who had the most severe disease, i.e. systemic AIV infection, 100% morbidity, >75% mortality, severe

**Table III**

**Summary data obtained from the intranasal inoculation of multiple avian species with early Eurasian-African H5N1 lineage highly pathogenic avian influenza virus (A/chicken/Hong Kong/220/97) (70)**

Species	Pathobiological group	Morbidity <sup>(a)</sup>	Mortality <sup>(a)</sup>	Gross lesions <sup>(b)</sup>	Histological lesions <sup>(b)</sup>	Viral antigen <sup>(c)</sup>	Virus re-isolation <sup>(d)</sup>
Chickens, turkeys, quails, guineafowls pheasants, partridges and zebra finches	1	+++	+++	+++	+++	+++	+++
Domestic geese, emus, house finches and budgerigars	2	++/+++	-/++	-/+	+/++	+/+++	++
Domestic ducks, house sparrows and gulls	3	-/+	-	-	-/+	-/+	+/-
Starlings and pigeons	4	-	-	-	-	-	+/-

**a) Morbidity and mortality**

+++ = ≥ 75%  
 ++ = 50% to 74%  
 + = less than 50%  
 - = none

**b) Gross and histological lesions**

+++ = lesions common and in multiple organs  
 ++ = lesions sporadic and in few organs  
 + = lesions infrequent  
 +/- = lesions rare and mild

**c) Viral antigen**

+++ = widespread  
 ++ = multifocal  
 + = infrequent  
 +/- = rare  
 - = no viral antigen

**d) Virus re-isolation**

+++ = high viral titres ( $10^{5.0}$  mean embryo lethal dose [ELD<sub>50</sub>] per gram of tissue) obtained consistently from the brain, lung, and kidney  
 ++ = high viral titres ( $10^{5.0}$  ELD<sub>50</sub>/g tissue) obtained primarily from brain  
 +/- = low to moderate viral titres ( $10^{4.1}$  ELD<sub>50</sub>/g tissue) obtained from lung and/or kidney, negative re-isolation from brain, lung, or kidney

listlessness before death and some neurological dysfunction, but some birds died peracutely and lacked clinical signs. Pathobiologically, chickens and turkeys were most severely affected, dying within two days of inoculation, and virus replicated predominantly in vascular endothelium and phagocytic leukocytes. The other gallinaceous birds and zebra finches (*Taeniopygia guttata*) survived longer than chickens and turkeys, and had disseminated virus replication in various parenchymal cells within the brain, heart, lung, pancreas, skin and adrenal gland, along with associated necrotic and inflammatory lesions. The Eurasian-African H5N1 HPAIV have all been highly lethal in chickens, but they have expressed some variation in pathobiological features, principally a range of intravenous mean death times (MDT) from 1 to 4.1 days and intranasal MDT from 1.5 to 5.5 days (50, 59, 85, 88, 106). Group Two contained domestic geese, emus, house finches (*Carpodacus mexicanus*), and budgerigars (*Melopsittacus undulatus*) who exhibited delayed morbidity, <75% mortality, and severe lesions in two to three critical organs, predominantly the brain with associated neurological signs, and secondarily the heart and pancreas. Group Three contained domestic ducks, house sparrows (*Passer domesticus*) and laughing gulls (*Larus atricilla*) that had minimal morbidity, lacked mortality, and had low titre virus replication, usually in the respiratory tract and occasionally in the heart and gonads. Group Four contained rock pigeons (*Columba livia*) and European starlings (*Sturnus vulgaris*) that lacked morbidity and mortality, and no virus replication was evident.

## Domestic ducks

The Eurasian-African lineage of H5N1 HPAIV has evolved over the past 12 years into 10 distinct genetic lineages (clades 0 to 9) and multiple sublineages (e.g. 2.1.1, 2.1.2, etc.). Over the same period, divergent strains have developed and many have expressed distinct pathobiological features and increased virulence for various bird species. The early Hong Kong isolates (1997 to 2000) replicated only in the respiratory tract of domestic ducks and produced associated mild respiratory lesions and no morbidity or mortality (68). Similarly, intranasal inoculation of domestic ducks with H5N1 HPAIV strains isolated from ducks in China (1999 to 2002) produced respiratory and alimentary tract infection, but no illness or death (Table IV) (19). However, in 2001, an H5N1 HPAIV was isolated from frozen duck meat imported from China into South Korea (106). In intranasally inoculated domestic ducks, this virus replicated and spread systemically and was isolated and visualised in muscle, heart and brain, but did not produce clinical signs or death. Strains isolated from captive waterfowl in Hong Kong during 2002 produced high mortality in experimentally inoculated young domestic ducks, with systemic infection and high virus titres within the respiratory tract, heart and brain, while other strains from Southeast Asia produced low mortality with neurological lesions and disease (32, 50, 63, 83, 84). Domestic ducks experimentally inoculated with three H5N1 HPAIV isolates from Thailand from 2004 induced

**Table IV**

**Data from experimental studies in domestic ducks following intranasal inoculation with H5N1 highly pathogenic avian influenza virus**

Species	Virus	Sick/dead/total	MDT (days)	Virus isolation titre*		Remarks and references
				Oropharyngeal	Cloacal	
Sheldrake ducks ( <i>Tadorna</i> spp.)	DKGD/12/00	0/0/5	–	–	–	3-week-old ducks; 10 <sup>7.85-8.5</sup> EID <sub>50</sub> inoculum; titres at 3 dpi in CCID <sub>50</sub> (18)
	DKZJ/11/00	0/0/5	–	<2.0-4.3	<2.0-3.7	
	DKFJ/17/01	0/0/5	–	<2.0-2.7	–	
	DKGX/35/01	0/0/5	–	<2.0-4.0	<2.0-4.0	
	DKSH35/02 DKFJ/01/02	0/0/5 0/0/5	– –	<2.0-3.5 –	<2.0-4.3 <2.0-3.5	
Pekin ducks ( <i>Anas platyrhynchos</i> spp.)	A/chicken/Yamaguchi/7/04	0/0/3	–	5.5	NA	10 <sup>6</sup> EID <sub>50</sub> inoculum; average titres from trachea (EID <sub>50</sub> /g) (40)
	A/duck/Yokohama/03	2/0/3	–	4.7		
	HK/483/97	0/0/3	–	4.5		
	Tn/SA/6	0/0/3	–	–		
Call ducks ( <i>Anas platyrhynchos</i> )	A/chicken/Yamaguchi/7/04	4/0/9	–	NA	NA	2-week-old ducks; 10 <sup>6</sup> EID <sub>50</sub> inoculum (114)
Pekin ducks ( <i>Anas platyrhynchos</i> )	A/chicken/Suphanburi/1/04	8/5/8	6.0	2.2/3.5	3.2/3.6	4-week-old ducks; 10 <sup>6</sup> EID <sub>50</sub> inoculum; approximate titres at 4 dpi (74)
	A/quail/Angthong/71/04	8/5/8	6.0	NA		
	A/duck/Angthong/72/04	4/5/8	6.0	NA		
Cross-bred ducks ( <i>Anas platyrhynchos</i> )	A/chicken/Suphanburi/1/04	0/4/8	6.3	NA	NA	4-week-old ducks; 10 <sup>6</sup> EID <sub>50</sub> inoculum (74)
	A/quail/Angthong/71/04	0/4/8	5.3			
	A/duck/Angthong/72/04	0/6/8	4.8			

\*Titres expressed as mean embryo infective dose (EID<sub>50</sub>) per millilitre unless otherwise stated

CCID: mean cell culture infective dose

MDT: mean death time

NA: not available

50% to 75% mortality with neurological signs, but less severe disease was produced in mallard–domestic cross-bred ducks (Table IV) (74). Feather lesions have been reported in domestic ducks infected with an H5N1 HPAIV (114).

Over the past 12 years, H5N1 HPAIV in domestic ducks have changed from all being in Pathobiological Group Three to some strains in Group Two, and most recently, a few in Group One. Although some H5N1 HPAIV are Pathobiological Group One in both two-week-old ducklings and chickens, the underlying pathophysiological mechanisms are different. The viruses primarily caused severe vascular damage in chickens resulting in severe pulmonary oedema, congestion, haemorrhage and microthrombosis in capillaries (92), while in domestic ducks the virus replicated and caused damage in multiple organs including the respiratory tract, the pancreas, central nervous system, adrenal glands, and myocardium (Fig. 2) (45, 63, 94, 114). In domestic ducks, high lethality was age-dependent and most severe in young ducks, whereas in chickens, it was age-independent (63, 94). Interestingly, Vietnam isolates since 2005 have shown increased virulence in ducks compared to those prior to 2005, as evident by shorter MDT in two-week-old ducks and 100% mortality in five-week-old ducks (Table V) (94). Such enhanced virulence is the result of an expanded tissue tropism and higher virus replication titres in tissues. Field observations of increased mortality in domestic ducks infected with H5N1 HPAIV in Vietnam corroborate the experimental results of increased virulence of the newer strains for ducks (113).

## Non-poultry birds

Beginning in 2002 with isolation of new strains of H5N1 HPAIV in Hong Kong, H5N1 HPAIV have emerged with the ability to cause severe illness and death in captive water

birds of diverse genetic backgrounds. In the six years that followed, H5N1 HPAIV were isolated from individual wild birds, including gulls and shorebirds (Order: Charadriiformes); storks, herons, and egrets (Order: Ciconiiformes); pigeons and doves (Order: Columbiformes); eagles, goshawks, kestrel, falcon, buzzard and kites (Order: Falconiformes); coots, moorhen and swamphen (Order: Gruiformes); crows, magpies, sparrows, starlings, finches, mesias, munias and mynahs (Order: Passeriformes); pelicans and cormorants (Order: Pelecaniformes); flamingoes (Order: Phoenicopteriformes); grebes (Order: Podicipediformes); and owls (Order: Strigiformes) (19, 24, 25, 61). With the H5N1 HPAI outbreak in captive water birds in Hong Kong during 2002, the range of pathological changes reported resembled those previously identified in chickens infected with H5N1 virus, but with more severe lesions in the respiratory tract and more extensive neurological lesions in the captive birds than typically reported in chickens (19). After 2002, H5N1 HPAIV caused only sporadic deaths in wild birds in Southeast Asia (61) until the spring of 2005, when large numbers of bar-headed geese died in Qinghai Lake, China (19), as did various swans and other aquatic birds in Europe during the winter of 2006 (72). Neurological lesions were especially prominent.

## Captive, non-domestic ducks (Order: Anseriformes)

Relatively few studies have examined H5N1 HPAIV infection in non-domestic ducks, but the results of two such studies are summarised in Table VI. Intranasal inoculation of mallard, northern pintail, blue-winged teal, redhead, and wood ducks with H5N1 HPAIV (A/whooper swan/Mongolia/244/05 and A/duck meat/Anyang/AVL-1/01) produced disease and death only in wood ducks (10). Only wood ducks exhibited clinical signs, which included ruffled feathers, cloudy eyes, rhythmic dilation and constriction of the pupils, tremors, incoordination, severe weakness, and seizures. Individual birds shed virus

**Table V**

**Mortality, mean death times and viral replication titres from oropharyngeal and cloacal swabs of 2-week-old Pekin ducks inoculated intranasally with Asian-origin H5N1 highly pathogenic avian influenza virus**

(59, 63, 94, and unpublished data)

Virus <sup>(a)</sup>	Clade	Mortality <sup>(b)</sup>	MDT (days)	Virus isolation titres 3 days post inoculation <sup>(c)</sup>	
				Oral	Cloacal
A/duck/Vietnam/88/07	2.3.4	8/8	4	3.9	2.3
A/duck/Vietnam/10/07	1	8/8	3.2	5.5	3.0
A/duck/Vietnam/218/2005	2.3.2	8/8	2.7	6.5	3.3
A/duck/Vietnam/203/2005	2.3.4	8/8	3.4	4.8	1.5
A/Vietnam/1203/2004	1	7/8	4.2	4.9	2.0
A/goose/Vietnam/113/2001	1	0/8	–	1.8	<1.6

a) Ducks were inoculated intranasally with  $10^5$  mean embryo infective dose (EID<sub>50</sub>) of the viruses

b) Number of dead ducks/number of inoculated or exposed ducks

c) Mean log<sub>10</sub> titres expressed as EID<sub>50</sub>/ml from oropharyngeal and cloacal swabs were sampled from three individual ducks. The limit of detection was  $10^{0.9}$  EID<sub>50</sub>/ml

MDT: mean death time

from oropharynx and cloaca for four to six days and in high titres, and had severe diffuse neuronal necrosis in the brain, necrotising pancreatitis and adrenalitis, and multifocal necrosis in the heart. The mallards, northern pintail, blue-winged teal and redhead ducks lacked clinical signs but were infected, shedding low levels of virus for one to three days. Common lesions and their severity in ducks and other avian species are summarised in Table VII. In another study, six species of ducks (two species of diving ducks and four species of dabbling ducks) were infected with A/turkey/Turkey/1/2005. The infection caused clinical signs in only the diving ducks (tufted ducks

and pochards). In contrast, the remaining four species were clinically unaffected. Clinical signs, which were more severe in tufted ducks than in pochards, developed at three to four days post inoculation and consisted of laboured breathing, increased recumbency, and neurological signs (39).

### Swans (Order: Anseriformes)

Experimental studies of H5N1 HPAIV in swans are summarised in Tables VII and VIII. During the H5N1 HPAI outbreaks in Europe of 2006, swans were the most

**Table VI**  
**Data from experimental studies in wild and captive duck species following intranasal inoculation with H5N1 highly pathogenic avian influenza virus**

Species	Virus	Sick/dead/total	MDT (days)	Virus isolation titre*		Remarks and references
				Oropharyngeal	Cloacal	
Blue-winged teal ( <i>Anas discors</i> )	A/whooper swan/Mongolia/ 244/05	0/0/3	–	3.8 (2) <sup>(a)</sup>	1.0 (1)	Ducks of 10 to 16 weeks of age; 10 <sup>6</sup> EID <sub>50</sub> inoculum;
Redhead ( <i>Aythya Americana</i> )		0/0/3	–	2.8 (1-4)	1.2 (1)	titres are average maximum titres (10)
Wood duck ( <i>Aix sponsa</i> )		2/2/3	7.5	4.6 (4-6)	3.8 (2-3)	
Northern pintail ( <i>Anas acuta</i> )		0/0/3	–	1.5 (1-2)	1.0 (1)	
Blue-winged teal ( <i>Anas discors</i> )	A/duck meat/Anyang/AVL-1/01	0/0/3	–	2.0 (1-2)	–	Ducks of 10 to 16 weeks of age; 10 <sup>6</sup> EID <sub>50</sub> inoculum; titres are average maximum titres
Redhead ( <i>Aythya Americana</i> )		0/0/3	–	4.0 (4)	–	(10)
Wood duck ( <i>Aix sponsa</i> )		2/1/3	8	5.0 (7)	2.8 (4-5)	
Northern pintail ( <i>Anas acuta</i> )		0/0/3	–	1.1 (1-4)	–	
Mallard ( <i>Anas platyrhynchos</i> )		0/0/3	–	2.1 (1-2)	1.0 (1)	
Tufted duck ( <i>Aythya fuligula</i> )	A/turkey/Turkey/1/2005	4/3/4	4	2.5	–	10 <sup>4</sup> CCID <sub>50</sub> inoculum; average titres at 2 dpi in CCID <sub>50</sub> /ml
Eurasian pochard ( <i>Aythya ferina</i> )		3/1/4	4	3.0	<1.0	(39)
Mallard ( <i>Anas platyrhynchos</i> )		0/0/4	–	2.2	–	
Common teal ( <i>Anas crecca</i> )		0/0/4	–	<1.0	–	
Eurasian wigeon ( <i>Anas penelope</i> )		0/0/4	–	<1.0	–	
Gadwall ( <i>Anas strepera</i> )		0/0/4	–	–	–	

\* virus titres expressed as mean embryo infective dose per millilitre unless otherwise stated

a) Figures in parentheses indicate the duration of shedding in days

CCID: cell culture infective dose

MDT: mean death time

**Table VII**  
**Microscopic lesions and distribution of H5N1 highly pathogenic avian influenza virus antigen in tissues from experimentally infected avian species**

Location of lesion	Species of bird						Type of lesion (Viral antigen stained cell types)
	Wild ducks (10, 39)	Geese (11, 65, 68)	Swan (11, 38)	Pigeons (41, 68)	Gulls (10, 12, 69)	Crows (96)	
Nasal cavity		+	+				Epithelial necrosis, rhinitis, sinusitis (Epithelial cells, vascular endothelial cells)
Trachea		+	+				Tracheitis (Epithelial cells, vascular endothelium)
Lung	+	+	+				Oedema, bronchointerstitial pneumonia (Vascular endothelium, mononuclear cells)
Air sac	+						Epithelial necrosis (Epithelial cells)
Esophagus		+					No lesion (Epithelium, vascular smooth muscle, smooth muscle of muscularis externa, mucous glands)
Proventriculus		+	+				No lesion (Epithelium, muscularis mucosa, vascular smooth muscle, parasympathetic ganglia)
Enteric tract	+	+				+	Haemorrhage, epithelial necrosis, enteritis (Parasympathetic ganglia of the submucosa and myenteric plexi, mucosal smooth muscle, vascular endothelium)
Cecal tonsils		+	+				No lesion (Parasympathetic ganglia)
Bursa			+				Lymphoid depletion, phagocytic cell hyperplasia, necrosis (Phagocytic cells, bursa epithelium)
Thymus	+						No lesion (Thymic epithelium, phagocytic cells)
Pancreas	+	+	+		+	+	Vacuolation, pancreatic necrosis, pancreatitis (Exocrine acinar cells)
Adrenals	+	+	+		+	+	Adrenal necrosis, adrenalitis (Corticotropic and chromaffin cells)
Liver	+		+		+	+	Hepatocellular degeneration and necrosis (Hepatocyte, Kupffer's cells)
Spleen		+	+			+	Lymphoid depletion, necrosis, splenitis, hyperplasia of macrophage-phagocytic cells, hemosiderinosis (Vascular smooth muscle, endothelial cells, phagocytes)
Kidney	+	+	+				Tubule necrosis (Tubular epithelium)
Muscle	+	+			+		Myofibre degeneration (Vascular smooth muscle, myocytes)
Heart	+	+	+		+	+	Myocyte necrosis, myocarditis (Myocytes)
Brain	+	+	+	+	+	+	Oedema of neuropil, neuronal necrosis, gliosis, lymphoplasmacytic perivascular cuffing (Neurons, glial cells, ependymal cells, choroid plexus epithelium, endothelial cells, gitter cells)
Spinal cord, eye, peripheral nerves			+				No lesion (Neuron; eye retina: cells of the pigmented epithelial layer, photoreceptor cells, and cells of the outer and inner nuclear layers)
Ovary/testis	+		+				Necrosis, inflammation
Feather follicles			+				Necrosis of feather follicular epithelium (Feather follicular epithelium)

**Table VIII****Data from experimental studies in swans and geese following intranasal inoculation with H5N1 highly pathogenic avian influenza virus**

Species	Virus	Sick/dead/total	MDT (days)	Virus isolation titre*		Remarks and references
				Oropharyngeal	Cloacal	
Black swan ( <i>Cygnus atratus</i> )	A/whooper swan/ Mongolia/244/05	5/5/5	2.5	6.46 (2-3) <sup>(a)</sup>	4.94 (1-2)	Birds of 5 to 6 weeks of age; 10 <sup>6</sup> EID <sub>50</sub> inoculum; titres are average maximum titres (11)
Trumpeter swan ( <i>C. buccinator</i> )		5/5/5	5.0	6.14 (4-6)	3.18 (2-5)	
Whooper swan ( <i>C. cygnus</i> )		4/4/4	4.0	6.3 (4-6)	4.25 (3-5)	
Mute swan ( <i>C. olor</i> )		5/5/5	6.5	5.58 (3-7)	4.46 (3-4)	
Mute swan ( <i>C. olor</i> )	A/Cygnus cygnus/ Germany/R65/2006	4/4/4	6.2	3.3 (1-6)	1.3 (2-6)	Birds of 1 to 4 years of age; 10 <sup>6</sup> EID <sub>50</sub> inoculum; oculo-oronasally inoculation; average titres at 3 dpi (CCID <sub>50</sub> /ml) (38)
Emden goose ( <i>Anser anser domesticus</i> )	A/chicken/HK/220/97	5/0/11	–	1.8	2.4	2-week-old birds; 10 <sup>6</sup> EID <sub>50</sub> inoculum (68)
Cackling goose ( <i>Branta hutchinsii</i> )	A/whooper swan/ Mongolia/244/05	4/3/4	6.0	5.25 (4-6)	3.05 (2-5)	12-week-old birds; 10 <sup>6</sup> EID <sub>50</sub> inoculum; titres are average maximum titres (11)
Bar-headed goose ( <i>Anser indicus</i> )	A/whooper swan/ Mongolia/244/05	5/2/5	6.5	5.10 (5-8)	2.55 (0-7)	
Canada goose ( <i>Branta canadensis</i> )	A/chicken /Vietnam/14/2005	5/5/5	~5.0	NA	NA	89-day-old birds; 1.7 × 10 <sup>5</sup> PFU inoculum; birds died or were euthanised by 5 dpi (65)
Chinese white goose ( <i>Anser cygnoides</i> )	A/Gs/HK/437-4/99	3/3/3	7-8	NA	NA	Birds of 3 to 4 weeks of age;
	A/Gs/HK/485-3/00	3/0/3	–	NA	NA	10 <sup>6.5</sup> EID <sub>50</sub> inoculum (110)

\* Virus titres are expressed as mean embryo infective dose (EID<sub>50</sub>) per millilitre unless otherwise stated

a) Figures in parentheses indicate the duration of virus shedding in days

dpi: days post inoculation

CCID: cell culture infective dose

MDT: mean death time

NA: not available

PFU: plaque forming unit

frequent birds affected and were designated as an indicator species (102, 103). Mute (*Cygnus olor*) and whooper (*Cygnus cygnus*) swans that died of natural infection had nervous signs that included somnolence, incoordination and ataxia (102). In experimental studies, multifocal pancreatic haemorrhage and necrosis, pulmonary congestion and oedema, and subpericardial haemorrhages were the most frequent gross lesions observed. Histologically, necrosis and inflammation were common in the brain, pancreas and liver, and less common in the spleen and the adrenal and submucosal lymphoid patches of the small intestine ('Peyer's patches'). Avian influenza virus antigen was present in the pancreas, adrenal gland, liver, and brain. Death was attributed to the systemic viral infection.

Experimental infection of four species of swans – whooper swan, black swan (*Cygnus atratus*), trumpeter swan (*Cygnus buccinator*), and mute swan – with an H5N1

HPAIV produced 100% mortality in the four species. Black swans were the most susceptible species with MDT of 2.5 days and clinical signs, lesions and viral distribution similar to those in gallinaceous poultry (11). In mute swans, two parallel courses of pathogenesis with predominantly endothelial or epithelial/neuronal infections were described; pre-existing avian influenza virus-specific antibodies can be an efficient modulator of the outcome of an infection with HPAIV (38). Of interest were the asymptomatic infection and shedding for 4.5 days in mute swans, which indicate their potential to transmit the virus over short migratory distances (11).

### Geese (Order: Anseriformes)

Experimental studies of H5N1 HPAIV in domestic and captive geese are reported in Tables VII and VIII. For domestic geese (*Anser anser domesticus*), neurological disease and brain and pancreatic lesions were reported following inoculation with 1997 H5N1 HPAI virus (Fig. 11

and Fig. 1m) (68). Field reports from Asia and Europe indicated that H5N1 HPAIV produced sporadic deaths in naturally infected Canada geese (*Branta canadensis*) (24, 102). Experimentally, Canada geese were susceptible to H5N1 HPAIV infection and such susceptibility was dependent on the age and immunological status of the animal (65). Juvenile birds had clinical signs including severe listlessness, inappetence, bright yellow diarrhoea, ruffled feathers, hunched posture, repetitive jerking head movements, weakness, staggering gait, distress vocalisation, wing droop, and terminal coma. The most consistently affected tissues were the brain, spinal cord, parasympathetic ganglia of the gastrointestinal tract, heart, and pancreas. Two species of geese, the bar-headed goose (*Anser indicus*) and the cackling goose (*B. hutchinsii*) presented clinical signs including severe listlessness and marked neurological signs, several of the birds died (11). Cackling geese were more susceptible than bar-headed geese. Common lesions included necrosis and heterophilic to lymphoplasmacytic inflammation along with viral antigen in the brain, pancreas, liver and adrenal glands. Survivors had transient cloudy eyes from corneal oedema.

### Gulls (Order: Charadriiformes)

Experimental studies of H5N1 HPAIV in gulls are reported in Tables VII and IX. Mortality associated with natural infections of Eurasian-African lineage H5N1 HPAIV has been reported in brown-headed gulls (*Larus brunnicephalus*), great black-headed gulls (*Larus ichthyaetus*), and black-headed gulls (*Larus ridibundus*) (24, 53). Experimentally, laughing gulls exhibited high morbidity and mortality after intranasal inoculation with two strains of H5N1 HPAI virus (10). Another study described the susceptibility of herring gulls (*Larus argentatus*) for two strains of H5N1 HPAIV, but it varied between different virus strains (12). Among the clinical signs were lethargy and weakness, and in more severe cases neurological signs consisting of seizures, head-tilt, head tremors, torticollis and imbalance. Laughing gulls had cloudy eyes and petechial haemorrhages in the ventriculus, apex of the heart, cerebrum and pancreas. Necrotising pancreatitis and cerebral neuronal necrosis were the most common lesions, with some having necrotising adrenalitis (10). Gulls that survived to 14 days had lymphoplasmacytic perivascular encephalitis and heterophilic pancreatitis. Herring gulls had similar lesions, with the addition of moderate myocardial degeneration and necrosis with mild heterophilic myocarditis (12). This study also provided preliminary experimental evidence to indicate that herring gulls can be infected by some H5N1 HPAIV by ingesting infective chicken meat and, potentially, could contribute to the geographical spread of virus.

### Pigeons (Order: Columbiformes)

Experimental studies of H5N1 HPAIV in rock doves (pigeons, *Columba livia*) are reported in Tables VII and X.

Historically, pigeons have been considered resistant to infection, illness and death following exposure to HPAIV (37). Sporadic cases of fatal infection have been identified in pigeons with the Eurasian-African H5N1 HPAIV. Intranasal inoculation of pigeons with A/chicken/Hong Kong/220/1997 H5N1 HPAIV failed to produce infection, and inoculation with two Chinese H5N1 HPAIV produced rare infection without clinical signs (55, 68). However, recent studies in pigeons inoculated intranasally using high doses ( $10^6$  and  $10^8$  EID<sub>50</sub>/bird) of three H5N1 HPAIV isolated from Thailand (2004) and Indonesia (2003) produced infrequent illness and death (41, 88). Although most pigeons did not become ill, some of the asymptomatic birds were infected, and virus was shed from the oropharynx and cloaca. Neurological signs were observed in some pigeons, with the most severe lesions and highest virus titres in the brain. Lesions included severe neuronal necrosis in the brain, moderate necrosis of autonomic ganglia with accompanying lymphocytic ganglioneuritis, severe degeneration and necrosis in cardiac myocytes, mild necrosis of the adrenal cortical cells and rare foci of pancreatic acinar necrosis. Viral antigen was common in neurons and ependyma of the brain, autonomic neurons, and cardiac myocytes, and AIV antigen was sporadic in skeletal muscle fibres, adrenal cortical cells, granulocytes, capillary endothelium, pancreatic acinar cells and smooth muscle of large arteries. Contact exposed pigeons did not become infected, suggesting that transmission is inefficient and pigeons are unlikely to be a reservoir. In other studies, young and adult pigeons did not become infected after experimental exposure to five different isolates of H5N1 HPAIV (54), and pigeons inoculated with four different H5N1 HPAIV did not show clinical disease. Virus recovery varied between the virus strains, with one strain unable to be reisolated (8).

### Perching or song birds (Order: Passeriformes)

Experimental studies of H5N1 HPAIV in perching or song birds (Order: Passeriformes) are reported in Tables VII and X. Natural infections with deaths have been associated with H5N1 HPAIV for corvids (Family: Corvidae) including crows, magpies and rooks (44, 101). American crows (*Corvus brachyrhynchos*) intranasally inoculated with an H5N1 HPAIV from Thailand developed listlessness, hunched posture, ruffled feathers and loss of appetite (88). The most severely affected birds had pancreatitis with necrosis, non-suppurative encephalitis, splenic and hepatic haemochromatosis, myocarditis with necrosis, lymphocytic adrenalitis, and myenteric ganglioneuritis. Viral antigen was demonstrated in neurons, pancreatic acinar cells, and granulocytes and precursors in bone marrow. These lesions were similar to those reported for H5N1 HPAIV natural infections in large-billed crows (*Corvus macrorhynchos*) and the Korean magpie (*Pica pica sericea*) (44, 101).



**Table IX**  
**Data from experimental studies in gulls following intranasal inoculation with H5N1 highly pathogenic avian influenza virus**

Species	Virus	Sick/dead /total	MDT (days)	Virus isolation titre*		Remarks and reference
				Oropharyngeal	Cloacal	
Laughing gull ( <i>Larus atricilla</i> )	A/chicken/HK/220/97	0/0/8	–	<0.97	<0.97	Birds of 2 to 3 weeks of age; 10 <sup>6</sup> EID <sub>50</sub> inoculum (69)
	A/whooper swan/Mongolia/244/05	3/2/3	7.5	4.2 (7-8) <sup>a)</sup>	2.6 (4-7)	12-week-old birds; 10 <sup>6</sup> EID <sub>50</sub> inoculum; titres are average maximum titres (10, 12)
	A/duck meat/Anyang/AVL-1/01	3/2/3	7.5	5.0 (6-10)	2.0 (3-6)	
Herring gull ( <i>Larus argentatus</i> )	A/whooper swan/Mongolia/244/05	3/2/3	5.0	3.89 (4.3)	2.07 (3.0)	
	A/duck meat/Anyang/AVL-1/01	3/0/3	–	3.89 (5.0)	1.96 (2.3)	

\*Virus titres are expressed as mean embryo infective dose (EID<sub>50</sub>) per millilitre

a) Figures in parentheses indicate the duration of shedding in days

MDT: mean death time

**Table X**  
**Data from experimental studies in sparrows, starlings, pigeons and crows following intranasal inoculation with H5N1 highly pathogenic avian influenza virus**

Species	Virus	Sick/dead/total	MDT (days)	Virus isolation titre (EID <sub>50</sub> /ml)		Remarks and references
				Oropharyngeal	Cloacal	
American crow ( <i>Corvus brachyrhynchos</i> )	A/crow/ Thailand/1C/04	2/2/2	8-9	<4 (1-8) <sup>a)</sup>	<2 (2-8)	10 <sup>6</sup> EID <sub>50</sub> inoculum (88)
Zebra finch ( <i>Taeniopygia guttata</i> )	A/chicken/Hong Kong/220/97	7/7/9	3-5	NA	NA	Adult birds; 10 <sup>6</sup> EID <sub>50</sub> inoculum (71)
House finch ( <i>Carpodacus mexicanus</i> )		7/4/11	6-13	NA	NA	
House sparrow ( <i>Passer domesticus</i> )		3/0/7	–	NA	NA	
Starling ( <i>Sturnus vulgaris</i> )		0/0/4	–	NA	NA	
Budgerigar ( <i>Melopsittacus undulatus</i> )		6/6/10	5-9	NA	NA	
House sparrow ( <i>Passer domesticus</i> )	A/duck/Thailand/144/05	3/3/3	4.2	2.4	1.7	Adult birds; 10 <sup>6</sup> EID <sub>50</sub> inoculum; titres at 2 dpi (8)
	A/quail/Thailand/551/05	3/3/3	6.3	1.0	<1	
	A/CM/HK/645/06	2/2/3	<6.3	2.6	0.8	
	A/JW/HK/1038/06	3/3/3	<6.3	2.1	<1	
Starling ( <i>Sturnus vulgaris</i> )	A/duck/Thailand/144/05	0/0/3	–	3.8	0.8	
	A/CM/HK/645/06	0/0/3	–	3.3	0.8	
	A/JW/HK/1038/06	0/0/3	–	2.5	1	
Pigeon ( <i>Columba livia</i> )	A/chicken/HK/220/97	0/0/10	–	NA	NA	4-week-old birds; 10 <sup>6</sup> EID <sub>50</sub> inoculum (68)
Carneau pigeon ( <i>Columba spp.</i> )	A/duck/Thailand/144/05	0/0/3	–	<1	<1	6-week-old birds; 10 <sup>6</sup> EID <sub>50</sub> inoculum; titres at 3dpi (8)
	A/quail/Thailand/551/05	0/0/3	–	<1	0.5	
	A/CM/HK/645/06	0/0/3	–	1.9	<1	
	A/JW/HK/1038/06	0/0/3	–	0.5	<1	
Pigeon ( <i>Columbia livia f. domestica</i> )	A/chicken/Indonesia/2003	5/5/14	11.4	NA	NA	4-month-old birds; 10 <sup>8</sup> EID <sub>50</sub> inoculum (41)
Pigeon ( <i>Columba livia</i> )	A/chicken/HK/220/97	0/0/6	–	NA	NA	10 <sup>6</sup> EID <sub>50</sub> inoculum (88)
	A/crow/Thailand/1C/2004	1/1/6	6			
	A/pigeon/Thailand/1B/04	0/0/6	–			
Pigeon ( <i>Columba livia</i> )	A/Ck/Huabei/H5N1/04	0/0/13	–	0	0	Adult birds; 5 × 10 <sup>4</sup> EID <sub>50</sub> inoculum (54)

a) Figures in parentheses indicate the duration of shedding in days

dpi: days post inoculation

EID<sub>50</sub>: mean embryo infective dose

NA: not available

Natural infections and deaths in small passerine birds such as tree (*Passer montanus*) and house sparrows (*Passer domesticus*) have been reported. In a quarantine station, 10% mortality was seen in mesias (*Leiothrix* sp.) with isolation or identification of H5N1 HPAIV, while samples from mynahs and black-throated laughing thrush (*Garrulax chinensis*) in the same facility were negative for HPAIV (21). In experimental intranasal inoculation studies with 1997 Hong Kong H5N1 HPAIV, zebra finches developed anorexia, listlessness, and >75% mortality within five days of inoculation (71). Histologically, necrosis with associated viral antigen was observed in multiple organs, especially the nasal cavity, brain, pancreas, spleen, adrenal glands, and ovary. Experimentally, house finches exhibited 64% morbidity, 36% mortality, anorexia, listlessness, and neurological signs. Typically, birds were moribund or dead within two days of the onset of clinical signs, with severe necrotic lesions in the brain and pancreas. By contrast, house sparrows had mild transient listlessness, no mortality, lacked gross lesions, and AIV antigen and histological lesions were observed only in the heart and testicle of a few birds. European starlings lacked clinical disease, mortality and gross and histological lesions.

By contrast, intranasal inoculation of house sparrows with four different H5N1 HPAIV isolated in Hong Kong in 2005 and 2006 caused death in 66% to 100% of the infected birds and high virus loads were detected in the brain and lung of deceased sparrows. In contrast, inoculation of European starlings with three of these viruses caused no deaths. However, virus was re-isolated from oral and cloacal swabs, indicating that starlings were infected by the viruses tested (8). In both species, the H5N1 HPAIV were not transmitted to contact birds.

### Parrots (Order: Psittaciformes)

Natural cases have not been reported but experimental infection with H5N1 HPAIV caused high mortality in budgerigars (*Melopsittacus undulatus*) with multifocal necrosis in the brain and associated viral antigen in necrotic neurons (33, 71). In a private quarantine station in the United Kingdom, H5N1 HPAIV infections were confirmed in passerines imported from Asia, but H5N1 HPAIV infection was not identified in any of the psittacines in the same facility (21).

## Pathogénie et pathobiologie de l'infection des oiseaux par le virus de l'influenza aviaire

M.J. Pantin-Jackwood & D.E. Swayne

### Résumé

La capacité des virus de l'influenza aviaire de provoquer l'infection, la maladie ou la mort varie en fonction des souches virales et des espèces aviaires affectées. Les virus de l'influenza aviaire se répartissent en deux groupes, en fonction des caractéristiques pathobiologiques observées chez les poulets : virus de l'influenza aviaire faiblement pathogènes (IAFP) et virus de l'influenza aviaire hautement pathogènes (IAHP). Généralement, les virus de l'IAFP provoquent une infection asymptomatique chez l'avifaune aquatique, mais une fois introduits dans les populations de volailles domestiques, ils causent des infections soit asymptomatiques, soit accompagnées de signes cliniques et de lésions témoignant d'une atteinte pathophysiologique des systèmes respiratoire, digestif et reproducteur. Les virus de l'IAHP ont d'abord été observés dans les populations de gallinacées, où l'infection entraîne une forte morbidité et mortalité et une maladie systémique caractérisée par la nécrose et l'inflammation de nombreux organes viscéraux, du système nerveux, du système cardiovasculaire ainsi que du tégument. Bien que les virus de l'IAHP affectent exceptionnellement les oiseaux aquatiques domestiques ou les oiseaux sauvages, l'évolution très particulière qu'a connue la souche eurasiennne/africaine H5N1 du virus de l'IAHP depuis une dizaine d'années en fait désormais un virus infectieux et pathogène pour les canards domestiques et les oiseaux sauvages, avec divers signes cliniques dont une atteinte asymptomatique du système respiratoire et du digestif, une maladie systémique affectant deux ou trois organes vitaux, généralement le cerveau, le cœur et le

pancréas, et, chez les gallinacées, une grave infection généralisée, suivie de la mort. L'infection expérimentale par inoculation intra-nasale du virus a été réalisée avec succès chez diverses espèces d'oiseaux sauvages; néanmoins, le caractère marginal de la transmission par contact chez certaines de ces espèces, par exemple les passereaux et les Colombiformes, indique que ces espèces ne jouent probablement pas un rôle de réservoir, contrairement à certains membres sauvages de l'ordre des Anseriformes qui sont sensibles à des formes graves de l'infection et pourraient jouer un rôle dans la dissémination du virus sur des distances moyennes.

#### **Mots-clés**

Influenza aviaire faiblement pathogène – Influenza aviaire hautement pathogène – Maladie aviaire – Pathobiologie – Pathogénie – Pathotype – Virus de l'influenza aviaire.



## **Patogénesis y patobiología de la infección por virus de la influenza aviar en las aves**

M.J. Pantin-Jackwood & D.E. Swayne

#### **Resumen**

La capacidad de los virus de la influenza aviar (IA) para causar infección, enfermedad y muerte varía en función de la especie avícola de que se trate. Atendiendo a las características patobiológicas observadas en pollos, los autores distinguen entre virus de la IA levemente patógena (virus IALP) y virus de la IA altamente patógena (virus IAAP). Por regla general, los virus IALP causan infecciones asintomáticas en las aves acuáticas salvajes, pero si son introducidos en bandadas domésticas puede ocurrir que provoquen infecciones asintomáticas o bien que induzcan lesiones y signos clínicos reveladores de trastornos patofisiológicos en los sistemas respiratorio, digestivo y reproductor. El virus IAAP, observado en un principio en aves gallináceas, provoca índices elevados de morbilidad y mortalidad, así como una enfermedad sistémica con necrosis e inflamación de varios órganos viscerales, los sistemas nervioso y cardiovascular y el integumento. Aunque el virus IAAP rara vez ha infectado a aves acuáticas domésticas o aves salvajes, en el último decenio los virus de la cepa H5N1 euroasiático-africana han adquirido la singular capacidad de infectar a los patos domésticos y las aves salvajes y de causar enfermedades que provocan una serie de síndromes, en particular infecciones asintomáticas del sistema respiratorio y el tracto digestivo, una enfermedad sistémica limitada a dos o tres órganos vitales, en general cerebro, corazón y páncreas, y una grave infección generalizada que resulta letal, observada en gallináceas de corral. Aunque a veces, en condiciones experimentales, se ha logrado provocar la infección de diversas especies de aves salvajes por inoculación intranasal, la ineficiencia de la transmisión por contacto en ciertos casos, por ejemplo en aves passeriformes y columbiformes, lleva a pensar que muy probablemente éstas no sean un reservorio del virus, mientras que otras aves, como las anseriformes salvajes, pueden resultar gravemente afectadas y podrían constituir anfitriones que propagaran el virus a media distancia.

#### **Palabras clave**

Enfermedades aviares – Influenza aviar altamente patógena – Influenza aviar levemente patógena – Patobiología – Patogénesis – Patotipos – Virus de la influenza aviar.



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