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REVIEW ARTICLE

The onset of virus shedding and clinical signs in chickens infected with high-pathogenicity and low-pathogenicity avian influenza viruses

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Some avian influenza viruses may be transmissible to mammals by ingestion. Cats and dogs have been infected by H5N1 avian influenza viruses when they ate raw poultry, and two human H5N1 infections were linked to the ingestion of uncooked duck blood. The possibility of zoonotic influenza from exposure to raw poultry products raises concerns about flocks with unrecognized infections. The present review examines the onset of virus shedding and the development of clinical signs for a variety of avian influenza viruses in chickens. In experimentally infected birds, some high-pathogenicity avian influenza (HPAI) and low-pathogenicity avian influenza (LPAI) viruses can occur in faeces and respiratory secretions as early as 1 to 2 days after inoculation. Some HPAI viruses have also been found in meat 1 day after inoculation and in eggs after 3 days. There is no evidence that LPAI viruses can be found in meat, and the risk of their occurrence in eggs is poorly understood. Studies in experimentally infected birds suggest that clinical signs usually develop within a few days of virus shedding; however, some models and outbreak descriptions suggest that clinical signs may not become evident for a week or more in some H5 or H7 HPAI-infected flocks. During this time, avian influenza viruses might be found in poultry products. LPAI viruses can be shed in asymptomatically infected or minimally affected flocks, but these viruses are unlikely to cause significant human disease.

Introduction

Avian influenza viruses are a highly heterogeneous group of viruses with varying pathogenicity in different species. These viruses are classified into subtypes based on two surface antigens, the haemagglutinin (H) and neuraminidase (N) proteins. Sixteen haemagglutinin antigens (H1 to H16) and nine neuraminidase antigens (N1 to N9) have been recognized (World Organisation for Animal Health, 2004; Centers for Disease Control and Prevention [CDC], 2007; Swayne, 2007). Avian influenza viruses are also divided into two pathotypes: high-pathogenicity avian influenza (HPAI) viruses, which cause severe and fatal infections in chickens, and the low-pathogenicity (LPAI) viruses, which are generally much less virulent in these birds. LPAI viruses can contain any haemagglutinin, but to date all HPAI viruses have contained either H5 or H7.

During the past two decades, avian influenza has become a zoonotic issue, prompting increased concern about the presence of viruses in poultry products. Severe and frequently fatal infections have been reported in humans infected with some Asian HPAI H5N1 viruses (CDC, 1997, 2007; Claas *et al*., 1998; World Health Organisation [WHO], 2006), and conjunctivitis, flu-like symptoms, and rare fatal cases have been reported after infection with some H7 viruses (Hinshaw *et al*., 1984; Fouchier *et al*., 2004; WHO, 2006; CDC, 2007). LPAI H9 infections have also been reported in humans; these infections generally appear to be asymptomatic or clinically indistinguishable from human influenza (Chen *et al*., 2004; Butt *et al*., 2005; WHO, 2006; CDC, 2007; Cong *et al*., 2007). Most human infections seem to result from direct contact with sick or dead poultry, but other routes of transmission may be possible (WHO, 2006). The presence of avian viruses in raw poultry products is a concern; in some circumstances, large numbers of consumers who otherwise have no contact with poultry might be exposed in the meat or eggs from a single infected flock. Although there is little evidence that ingestion is an important route of exposure for humans, two human infections with Asian HPAI H5N1 viruses were linked to the ingestion of uncooked duck blood (CDC, 2007), and other mammals including cats (Kuiken *et al*., 2004; Rimmelzwaan *et al*., 2006) and dogs (Songserm *et al*., 2006) have been infected when they ate raw poultry products. People who follow good sanitary practices during food preparation and eat only cooked eggs or meat are expected to be at low risk of exposure; avian influenza viruses are heat labile and are

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readily killed by cooking methods that destroy other pathogens found in poultry (CDC, 2007). However, consumers exposed to contaminated poultry products might convey the virus to mucous membranes on unwashed hands or by contact with other fomites. In addition, viruses in raw eggs might be a concern; although the consumption of uncooked eggs is not recommended, some consumers may disregard or be unaware of this advice when preparing foods such as mayonnaise. Workers who process poultry might be at risk from viruses that become aerosolized when feathers contaminated with desiccated faecal material are removed during mechanical processing. Whether zoonotic viruses in eggs, meat and faeces can present a risk to consumers and poultry processors depends on how rapidly infected flocks are identified, as well as how quickly viruses are shed. In the present review, we examine the literature describing the onset of virus shedding in the faeces and respiratory secretions of unvaccinated chickens, and the presence of virus in products such as meat and eggs. We also review studies describing the onset of clinical signs in these chickens infected both experimentally and naturally.

Onset of clinical signs in HPAI-infected chickens

In chickens, HPAI viruses usually cause a multisystemic disease associated with high morbidity and mortality (Hooper & Selleck, 1998; Swayne & Pantin-Jackwood, 2006; Swayne, 2007). It is difficult to describe a set of clinical signs that are consistent indicators of an HPAI outbreak; the clinical signs are generally non-specific, and their frequency and type varies with the virus (Elbers *et al.*, 2004b, 2005; Swayne, 2007). Avian influenza viruses that kill birds quickly tend to cause fewer clinical signs than when the viral strain or the dose allows the birds to survive longer (Alexander *et al.*, 1978; Bean *et al.*, 1985; Perkins & Swayne, 2001). Chickens infected with HPAI viruses are sometimes found dead with few or no preceding signs (Alexander *et al.*, 1978, 1986; Bean *et al.*, 1985; Forman *et al.*, 1986; Elbers *et al.*, 2004a; Nakatani *et al.*, 2005; Swayne, 2007; Tsukamoto *et al.*, 2007). In published outbreaks, the initial clinical signs have varied with the virus. During the H7N1 HPAI outbreak in Italy in 1999 to 2000, the initial signs were tremors and incoordination, followed by depression and anorexia (Mutinelli *et al.*, 2003). Some broiler breeder flocks also had cyanosis of the comb and wattles, or petechial haemorrhages on the hock. All of the birds died within 48 to 72 h after the first clinical signs. During the H7N7 HPAI epidemic in The Netherlands, mild respiratory signs, severe diarrhoea, severe depression, and dramatic egg-production problems were seen in one flock by the third day of illness (Elbers *et al.*, 2004a). In another flock, haemorrhage and inflammation of the trachea was reported by the second day of increased mortality, and severe respiratory signs, diarrhoea, and depression were reported by the fifth day. Elbers *et al.* (2005) attempted to identify the most specific and sensitive clinical indicators for commercial layers, broiler breeders, broilers, and backyard flocks in this outbreak In commercial layers or broiler breeders, the most sensitive indicators included increased mortality, depression, coughing, reduction in normal vocalization, or pale eggs; however, the specificity of these signs was low. Cyanosis of the head, torticollis, coughing, yawning, rales, paralysis, incoordination, excessive lacrimation, huddling, and diarrhoea or greenish faeces were less sensitive but much more specific signs in these birds. This study did not identify specific indicator signs in broiler flocks; although increased mortality and respiratory problems were seen in the few infected broiler flocks, these signs were also reported in uninfected flocks.

In experimentally infected chickens inoculated by a natural (e.g. respiratory) route, early signs have included depression, ruffled feathers, decreased feed consumption, diarrhoea, increased faecal fluid and urates, haematochezia, dyspnea, blanching of the combs, and swelling and cyanosis of the head, legs, and feet (Narayan *et al.*, 1969; Alexander *et al.*, 1978; Westbury *et al.*, 1979; Beard *et al.*, 1984; Forman *et al.*, 1986; Brown *et al.*, 1992; Perkins & Swayne, 2001; Jones & Swayne, 2004; Tsukamoto *et al.*, 2007). With the exception of depression, which is consistently present, the pattern of clinical signs varies with the virus and the study. (Narayan *et al.*, 1969; Alexander *et al.*, 1978; Westbury *et al.*, 1979; Beard *et al.*, 1984; Forman *et al.*, 1986; Brown *et al.*, 1992; Perkins & Swayne, 2001; Jones & Swayne, 2004; Tsukamoto *et al.*, 2007). In intranasally inoculated birds, the onset of clinical signs or time to death varies with the isolate (Table 1) and dose. Chickens inoculated intranasally with recent Asian H5N1 isolates often die within the first few days (Shortridge *et al.*, 1998; Suarez *et al.*, 1998; Cauthen *et al.*, 2000; Perkins & Swayne, 2001; Tumpey *et al.*, 2002; Lee *et al.*, 2005a; Nguyen *et al.*, 2005; Tian *et al.*, 2005; Swayne *et al.*, 2006; Swayne & Pantin-Jackwood, 2006; Bublot *et al.*, 2007; Swayne, 2007; Tsukamoto *et al.*, 2007). Clinical signs have rarely been specified in these birds; in one study, 4-week-old to 6-week-old chickens were reported to be depressed and have ruffled feathers on day 1 post-inoculation (p.i.), and to be dead by day 2 (Tsukamoto *et al.*, 2007). Chickens inoculated intranasally with an H5N1 virus isolated in 1959 (A/chicken/Scotland/59) became ill, on average, at 3.8 days p.i. and died at 4.6 days p.i., displaying few clinical signs before death (Alexander *et al.*, 1986). An H5N2 virus isolated during the 1983 to 1984 Pennsylvania outbreak caused depression on day 2 p.i. in one of 25 intranasally inoculated hens (Beard *et al.*, 1984). More severe depression and/or diarrhoea were seen in most of these birds on day 3 p.i., and decreased egg laying occurred on day 4 p.i., concurrently with the first deaths. Beginning the following day, no eggs were laid. A/turkey/Ontario/7732/66 (H5N9) caused listlessness, inappetence, ruffled feathers, and blanched combs on day 2 p.i. in intranasally inoculated, 10-month-old roosters (Narayan *et al.*, 1969). These birds died between days 4 and 5 p.i. Six-week-old birds inoculated with the same virus became ill beginning day 3 or day 4 p.i. and died on day 5 or 6 (Narayan *et al.*, 1969). Ruffled feathers and reluctance to move, as well as the first deaths, were reported at 24 h in 2-week-old chickens inoculated intranasally with A/fowl/Germany/34 (H7N1), and all of the birds died by day 2 (Alexander *et al.*, 1978). In the same study, chickens inoculated intranasally with A/FPV/Dutch/27 (H7N7) became ill beginning day 2 p.i., with a mean time of onset of 3.1 days, and died within 6 days; while birds inoculated with A/fowl/Victoria/75 (H7N7) became sick, on average, at 4.3 days p.i. and died within 15 days. Westbury *et al.* (1979) reported similar timing for A/fowl/Victoria/75 (H7N7): 14-week-old birds inoculated

Table 1. *Onset of morbidity and mortality in chickens infected with HPAI viruses*

Virus	Route[a]	Age[b]	Morbidity rate (%)	Onset of specified symptoms/first and last deaths	Mortality rate (%)	MDT (days)	Reference
A/chicken/Scotland/59 (H5N1)	IN	2	50	Sickness; MTO = 3.8 days	50	4.6	Alexander *et al.* (1986)
A/chicken/Scotland/59 (H5N1)	IM	2	100	Sickness; MTO = 2.7 days	100	3.2	Alexander *et al.* (1986)
A/goose/Guangdong/ 1/96 (H5N1)	IM	5	100	Day 5: last death	100		Tian *et al.* (2005)
A/goose/Guangdong/ 1/96 (H5N1)	IM	6	100	Day 5: last death	100		Tian *et al.* (2005)
A/goose/Guangdong /1/96 (H5N1)	IM	46	100	Day 5: last death	100		Tian *et al.* (2005)
A/Hong Kong/156/97 (H5N1)	IN	3	100		100	3	Shortridge *et al.* (1998)
A/Hong Kong/156/97 (H5N1)	IN	3 to 4			100; 90	2; 5	Suarez *et al.* (1998)
A/Hong Kong/156/97 (H5N1)	IN/IT	37 to 41	100		100	3	Suarez *et al.* (1998)
A/Hong Kong/156/97 (H5N1)	IV	3 to 4	100; 100		100; 100	2; 1.5	Suarez *et al.* (1998)
A/Hong Kong/156/97 (H5N1)	IV	4	100		100	2.0	Cauthen *et al.* (2000)
A/chicken/Hong Kong/220/97 (H5N1)	IN	3	100		100	2	Shortridge *et al.* (1998)
A/chicken/Hong Kong/220/97 (H5N1)	IN	3 to 4	100; 100		100; 100	2; 2	Suarez *et al.* (1998)
A/chicken/Hong Kong/ 220/97 (H5N1)	IN	4	100	Day 1 to 1.5: increased faecal fluid and urates; day 1: first death; day 2: last death	100	1.5	Perkins & Swayne (2001)
A/chicken/Hong Kong/ 220/97 (H5N1)	IN/IT	37 to 41	100		100	2	Suarez *et al.* (1998)
A/chicken/Hong Kong/ 220/97 (H5N1)	IV	3 to 4	100; 100		100; 100	1; 1	Suarez *et al.* (1998)
A/chicken/Hong Kong/ 258/97 (H5N1)	IN	3	100	Day 2: first death; day 3: last death	100		Shortridge *et al.* (1998)
A/chicken/Hong Kong/ 728/97 (H5N1)	IN	3	100		100	2	Shortridge *et al.* (1998)
A/chicken/Hong Kong/ 786/97 (H5N1)	IN	3	100		100	2	Shortridge *et al.* (1998)
A/chicken/Hong Kong/ 915/97 (H5N1)	IN	3	100	Day 2: first death; day 3: last death	100		Shortridge *et al.* (1998)
A/chicken/Hong Kong/ y385/97 (H5N1)	IN	3	100		100	2	Shortridge *et al.* (1998)
A/chicken/Hong Kong/ y388/97 (H5N1)	IN	3	100		100	2	Shortridge *et al.* (1998)
A/chicken/Hong Kong/ 1203/97 (H5N1)	IN	3	100		100	2	Shortridge *et al.* (1998)
A/chicken/Hong Kong/ w31/97 (H5N1)	IN	3	100		100	2	Shortridge *et al.* (1998)
A/chicken/Hong Kong/ w307/97 (H5N1)	IN	3	100		100	2	Shortridge *et al.* (1998)
A/chicken/Hong Kong/ w308/97 (H5N1)	IN	3	100		100	2	Shortridge *et al.* (1998)
A/chicken/Hong Kong/ p21/97 (H5N1)	IN	3	100	Day 2: first death; day 3: last death	100		Shortridge *et al.* (1998)
A/chicken/Hong Kong/ w608/97 (H5N1)	IN	3	100		100	2	Shortridge *et al.* (1998)
A/chicken/Hong Kong/ w609/97 (H5N1)	IN	3	100		100	2	Shortridge *et al.* (1998)
A/chicken/Hong Kong/ w162/97 (H5N1)	IN	3	100		100	2	Shortridge *et al.* (1998)
A/silky chicken/Hong Kong/p17/97 (H5N1)	IN	3	100	Day 2: first death; day 3: last death	100		Shortridge *et al.* (1998)
A/goose/Hong Kong/ w355/97 (H5N1)	IN	3	100		100	2	Shortridge *et al.* (1998)
A/goose/Hong Kong/ w374/97 (H5N1)	IN	3	100		100	2	Shortridge *et al.* (1998)

Table 1 (*Continued*)

Virus	Route[a]	Age[b]	Morbidity rate (%)	Onset of specified symptoms/first and last deaths	Mortality rate (%)	MDT (days)	Reference
A/duck/Hong Kong/ p46/97 (H5N1)	IN	3	100	Day 2: first death; day 3: last death	100		Shortridge *et al.* (1998)
A/duck/Hong Kong/ y283/97 (H5N1)	IN	3	100	Day 2: first death; day 3: last death	100		Shortridge *et al.* (1998)
A/environment/Hong Kong/437-6/99 (H5N1)	IN	4	100		100	5.5	Cauthen *et al.* (2000)
A/environment/Hong Kong/437-4/99 (H5N1)	IV	4	100	Day 5: last death	100	3.4	Cauthen *et al.* (2000)
A/environment/Hong Kong/437-6/99 (H5N1)	IV	4	100	Day 5: last death	100	3.0	Cauthen *et al.* (2000)
A/environment/Hong Kong/437-8/99 (H5N1)	IV	4	100	Day 5: last death	100	3.3	Cauthen *et al.* (2000)
A/environment/Hong Kong/437-10/99 (H5N1)	IV	4	100	Day 5: last death	100	4.1	Cauthen *et al.* (2000)
A/duck/Anyang/ AVL-1/01 (H5N1)	IN	4	100		100	2.9	Tumpey *et al.* (2002)
A/duck/Anyang/ AVL-1/01 (H5N1)	IV	4	100		100	3	Tumpey *et al.* (2002)
A/goose/Vietnam/ 113/01 (H5N1)	IN	4	100		100	2.6	Nguyen *et al.* (2005)
A/goose/Vietnam/ 113/01 (H5N1)	IV	4	100	Day 1: first death; day 2: last death	100	1.1	Nguyen *et al.* (2005)
A/goose/Vietnam/ 324/01 (H5N1)	IN	4	100		100	2.4	Nguyen *et al.* (2005)
A/goose/Vietnam/ 324/01 (H5N1)	IV	4	100	Day 1: first death	100	1.0	Nguyen *et al.* (2005)
A/chicken/SouthKorea/ ES/03 (H5N1)	IN/IO; $10^{3.5}$ EID_{50}	3	80		80	2.8	Bublot *et al.* (2007)
A/chicken/SouthKorea/ ES/03 (H5N1)	IN/IO; $10^{5 \text{ to } 8}$ EID_{50}	3	100		100	2.0 to 2.4	Bublot *et al.* (2007)
A/chicken/Korea/ ES/03 (H5N1)	IN	4	100		100	1.9 to 2.0	Lee *et al.* (2005a)
A/chicken/Korea/ ES/03 (H5N1)	IO	4		Day 2: deaths	90		Swayne & Beck (2005)
A/chicken/Korea/ ES/03 (H5N1)	IV	4	100		100	1.0	Lee *et al.* (2005a)
A/chicken/Indonesia/ 7/03 (H5N1)	IN	3 to 6	100		100	2.1	Swayne *et al.* (2007)
A/chicken/Indonesia/ 7/03 (H5N1)	IN	6	100		100	2.2	Swayne *et al.* (2006)
A/duck/China/ E319-2/03 (H5N1)	IN	4	100	Day 3: first death; day 4: last death	100	3.4	Lee *et al.* (2007)
A/duck/China/ E319-2/03 (H5N1)	IV	4	100	Day 1: first death; day 3: last death	100	1.6 to 2.4	Lee *et al.* (2007)
A/duck/Yokohama/ aq10/03 (H5N1)	IN	6	100	Day 2: first deaths	100	4.6	Mase *et al.* (2005)
A/duck/Yokohama/ aq10/03 (H5N1)	IV	6	100		100	<3	Mase *et al.* (2005)
A/Vietnam/1203/04 (H5N1)	IN	3 to 6	100		100	1.5	Swayne *et al.* (2007)
A/chicken/Yamaguchi/ 7/04 (H5N1)	IN	4 to 6	100	Day 1: ruffled feathers, depression; day 2: all deaths	100	2.0	Tsukamoto *et al.* (2007)
A/chicken/Yamaguchi/ 7/04 (H5N1)	DrC	4 to 6	0 to 100, depending on the number of inoculated and contact birds	Day 3[c]: depression; day 3[c]: first death; day 4[c]: last death	0 to 100, depending on the number of inoculated and contact birds	3.4[c]	Tsukamoto *et al.* (2007)
A/chicken/Yamaguchi/ 7/04 (H5N1)	IrC	4 to 6	0 to 100, depending on the number of inoculated and contact birds	Day 3[c]: depression; day 4[c]: first death; day 8[c]: last death	0 to 100, depending on the number of inoculated and contact birds	5.3[c]	Tsukamoto *et al.* (2007)
A/chicken/Vietnam/ 0008/04 (H5N1)	IN	3	100	Day 2: last deaths	100		Bublot *et al.* (2007)

Table 1 (*Continued*)

Virus	Route[a]	Age[b]	Morbidity rate (%)	Onset of specified symptoms/first and last deaths	Mortality rate (%)	MDT (days)	Reference
A/chicken/Tianjing/ 65/04 (H5N1)	IM	6	100	Day 2: last deaths	100		Tian *et al.* (2005)
A/duck/Shanghai/ 16/04 (H5N1)	IM	6	100	Day2: last deaths	100		Tian *et al.* (2005)
A/crow/Thailand/ 1C/04 (H5N1)	IN	3 to 6	100		100	1.8	Swayne *et al.* (2007)
A/whooper swan/ Mongolia/05 (H5N1)	IN	3 to 6	100		100	2.3	Swayne *et al.* (2007)
A/chicken/Pennsylvania/ 1370/83 (H5N2)	IN	2	90	Sickness; MTO =4.0 days	90	5.2	Alexander *et al.* (1986)
A/chicken/Pennsylvania/ 1370/83 (H5N2)	IN	5	100	Day 5: first death; day 9: last death	100		Bean et al. (1985)
A/chicken/Pennsylvania/ 1370/83 (H5N2)	IN/IT	6	100	Day 3: first death; day 10 last death	100	6.8	Van der Goot *et al.* (2003)
A/chicken/Pennsylvania/ 1370/83 (H5N2)	IN; 10^4 EID_{50}	>26	100	Day 4: first death; day 7: last death	100		Bean *et al.* (1985)
A/chicken/Pennsylvania/ 1370/83 (H5N2)	IN; 10^5 EID_{50}	>26	100	Day4: last death	100		Bean *et al.* (1985)
A/chicken/Pennsylvania/ 1370/83 (H5N2)	IT	4		Day 3: first deaths	60	4.8	Mo *et al.* (1997)
A/chicken/Pennsylvania/ 1370/83 (H5N2)	Air sac	15		Day 3: depression, ruffled feathers; day 4: cyanotic combs and wattles, oedema of the wattles			Brown *et al.* (1992)
A/chicken/Pennsylvania/ 1370/83 (H5N2)	Air sac	>52		Day 2: first deaths, depression, ruffled feathers, dyspnea; day 3: oedema of the eyelids			Brown *et al.* (1992)
A/chicken/Pennsylvania/ 1370/83 (H5N2)	IM	2	100	Sickness; MTO =2.7 days	100	3.7	Alexander *et al.* (1986)
A/chicken/Pennsylvania/ 1370/83 (H5N2)	DrC	2	100	Sickness; MTO =8.0 days[c]	100	9.2[c]	Alexander *et al.* (1986)
A/chicken/Pennsylvania/ 1370/83 (H5N2)	DrC	6		Day 7[c]: first death; day 14[c]: last death	90	11.4[c]	Van der Goot *et al.* (2003)
A/chicken/Pennsylvania/ SERPL-PA/83 (H5N2)	IN	1 to 3 day	100		100	2.2 to 2.8	Beard *et al.* (1984)
A/chicken/Pennsylvania/ SERPL-PA/83 (H5N2)	IN	1 to 2	100		100	4.6 to 5.9	Beard *et al.* (1984)
A/chicken/Pennsylvania/ SERPL-PA/83 (H5N2)	IN	3 to 4			83 to 100	7.1 to 8.6	Beard *et al.* (1984)
A/chicken/Pennsylvania/ SERPL-PA/83 (H5N2)	IN	5			50 to 67	8.0 to 8.5	Beard *et al.* (1984)
A/chicken/Pennsylvania/ SERPL-PA/83 (H5N2)	IN/IC	Adult		Day 2: depression; day 3: severe depression, diarrhoea; day 4: decreased egg laying, last eggs laid, soft shell eggs, first death; day 20: last death	92	6.5	Beard *et al.* (1984)
A/chicken/Pennsylvania/ SERPL-PA/83 (H5N2)	IN/IC	Adult		Day 3: last eggs laid	92	6.1	Beard *et al.* (1984)
A/chicken/Queretaro/ l14588-19/95 (H5N2)	IN	4	100		100	4.9	Swayne *et al.* (1997)
A/chicken/Queretaro/ l14588-19/95 (H5N2)	IN	4	100		100	2.8	Swayne *et al.* (1997)
A/chicken/Queretaro/ 14588-19/95 (H5N2)	IN/IT/ IO	3 to 4	100		100		Horimoto *et al.* (1995)
A/chicken/Queretaro/ 14588-19/95 (H5N2)	IV	3 to 4	100		100		Horimoto *et al.* (1995)
A/tern/South Africa/61 (H5N3)	IN	2	100	Sickness: MTO =4.3 days	100	5.3	Alexander *et al.* (1986)
A/tern/South Africa/61 (H5N3)	IM	2	100	Sickness: MTO =1.6 days	100	1.9	Alexander *et al.* (1986)
A/turkey/Ireland// 1378/83 (H5N8)	IN	2	90	Sickness: MTO =3.6 days	90	4.3	Alexander *et al.* (1986)

Table 1 (*Continued*)

Virus	Route[a]	Age[b]	Morbidity rate (%)	Onset of specified symptoms/first and last deaths	Mortality rate (%)	MDT (days)	Reference
A/turkey/Ireland// 1378/83 (H5N8)	IM	2	100	Sickness: MTO = 2.4 days	100	2.8	Alexander *et al.* (1986)
A/duck/Ireland// 113/84 (H5N8)	IN	2	100	Sickness: MTO = 2.2 days	100	2.7	Alexander *et al.* (1986)
A/duck/Ireland// 113/84 (H5N8)	IM	2	100	Sickness: MTO = 2.0 days	100	2.0	Alexander *et al.* (1986)
A/duck/Ireland// 113/84 (H5N8)	DrC	2	100	Sickness: MTO = 7.5 days[c]	100	7.8[c]	Alexander *et al.* (1986)
A/turkeylOntario/ 7732/66 (H5N9)	IN	2	50	Sickness: MTO = 3.8 days	20	5.0	Alexander *et al.* (1986)
A/turkey/Ontario/ 7732/66 (H5N9)	IN; 100 EID_{50}	6	50	Day 3: sickness; MTO = 3.5 days; range 3 to 4 days; day 5: first death; day 6: last death	50	5.5	Narayan *et al.* (1969)
A/turkey/Ontario/ 7732/66 (H5N9)	IN; 10^3 EID_{50}	6	50	Day 4: sickness; MTO = 4 days; day 6: all deaths	50	6.0	Narayan *et al.* (1969)
A/turkey/Ontario/ 7732/66 (H5N9)	IN; 10^5 EID_{50}	6	100	Day 3: sickness; MTO = 3.5 days; range 3 to 4 days; day 5: first death; day 6: last death	100	5.8	Narayan *et al.* (1969)
A/turkey/Ontario/ 7732/66 (H5N9)	IN; 10^8 EID_{50}	6	100	Day 3: sickness; MTO = 3.5 days; range 3 to 4 day; day 5: first death; day 6: last death	100	5.5	Narayan *et al.* (1969)
A/turkey/Ontario/ 7732/66 (H5N9)	IN	43	100	Day 2: listlessness, anorexia, ruffled feathers, comb blanched; day 3: cyanotic combs, severe depression, swelling of the head; day 4: first death; day 5: last death	100		Narayan *et al.* (1969)
A/turkeylOntario/ 7732/66 (H5N9)	IT	4		Day 5: first deaths	20	6.7	Mo *et al.* (1997)
A/turkeylOntario/ 7732/66 (H5N9)	IM	2	90	Sickness: MTO = 4.3 days	90	4.9	Alexander *et al.* (1986)
A/turkey/Ontario/ 7732/66 (H5N9)	IM; 10 EID_{50}	6	75	Day 5: sickness; MTO = 5.7 days; range 5 to 6 days; day 6: first death; day 7: last death	50	6.5	Narayan *et al.* (1969)
A/turkey/Ontario/ 7732/66 (H5N9)	IM; 10^2 EID_{50}	6	100	Day 5: sickness; MTO = 5.5 days; range 5 to 6 days; day 6: first death; day 10: last death	100	8.0	Narayan *et al.* (1969)
A/turkey/Ontario/ 7732/66 (H5N9)	IM; 10^3 EID_{50}	6	100	Day 3: sickness; MTO = 3.0 days; day 6: first death; day 7: last death	100	6.5	Narayan *et al.* (1969)
A/turkey/Ontario/ 7732/66 (H5N9)	IM; 10^4 EID_{50}	6	100	Day 3: sickness; MTO = 3.0 days; day 6: first death; day 7: last death	100	6.5	Narayan *et al.* (1969)
A/turkey/Ontario/ 7732/66 (H5N9)	IM; 10^5 EID_{50}	6	100	Day 2: sickness; MTO = 2.5 days; range 2 to 3 days; day 5: first death; day 6: last death	100	5.5	Narayan *et al.* (1969)
A/turkey/Ontario/ 7732/66 (H5N9)	IM; 10^6 EID_{50}	6	100	Day 2: sickness; MTO = 2.0 days; day 5: first death; day 6: last death	100	5.5	Narayan *et al.* (1969)
A/turkeylOntario/ 7732/66 (H5N9)	DrC	2	20	Sickness: MTO = 8.0 days[c]	20	8.5[c]	Alexander *et al.* (1986)

Table 1 (*Continued*)

Virus	Route[a]	Age[b]	Morbidity rate (%)	Onset of specified symptoms/first and last deaths	Mortality rate (%)	MDT (days)	Reference
A/fowl/Germany/ 34 (H7N1)	IN	2	100	24 h: ruffled feathers, reluctance to move; Sickness: MTO = 1.6 days; day 1: first death; day 2: last death	100	1.9	Alexander *et al.* (1978)
A/fowl/Germany/ 34 (H7N1)	DrC	2	70	Day 3[c]: sickness; MTO = 3.7 days; day 3[c]: first death; day 4[c]: last death	70	3.8[c]	Alexander *et al.* (1978)
A/chicken/Chile/ 184240-1/02 (H7N3)	IN	4		Day 1: depression; day 2: first death; day 3: last death	100	2.3	Jones & Swayne (2004)
A/FPV/Dutch/ 27 (H7N7)	IN	2	100	Day 2: sickness; MTO = 3.1 days; day 6: last death	100	4.3	Alexander *et al.* (1978)
A/FPV/Dutch/ 27 (H7N7)	IM	Adult	100	15 h: malaise	100	1.5	Moses *et al.* (1948)
A/FPV/Dutch/ 27 (H7N7)	DrC	2	100	Day 4[c]: sickness; MTO = 7.4 days[c]; day 15[c]: last death	100	8.9[c]	Alexander *et al.* (1978)
A/fowl/Victoria/ 75 (H7N7)	IN	2	100	Sickness: MTO = 4.3 days; day15: last death	100	7.2	Alexander *et al.* (1978)
A/fowl/Victoria/ 75 (H7N7)	IN	14		Day 5: swelling and cyanosis of the comb, wattle, legs and feet; day 6: first death; day 13: last death	38	9.0	Westbury *et al.* (1979)
A/fowl/Victoria/ 75 (H7N7)	IN/ IO	18	100	Day 7: all deaths	100	7.0	Westbury *et al.* (1981)
A/fowl/Victoria/ 75 (H7N7)	DrC	2	80	Sickness: MTO = 11.2 days[c]	80	12.0[c]	Alexander *et al.* (1978)
A/fowl/Victoria/ 75 (H7N7)	DrC	14		Day 13[c]: one death	17	13[c]	Westbury *et al.* (1979)
A/fowl/Victoria/ 75 (H7N7)	DrC	18			44	16.4	Westbury *et al.* (1981)
A/chicken/Victoria/ 76 (H7N7)	IN	6	100	Day 3 to 5: sickness; day 7: last death	100		Forman *et al.* (1986)
A/chicken/Victoria/ A185/85 (H7N7)	IT	4		Day 2: first deaths	67	4.1	Mo *et al.* (1997)
A/chicken/Victoria/ 85 (H7N7)	IN/IC	6	100	Day 3: all chickens dead or moribund by this date	100		Hooper *et al.* (1995)
A/chicken/Victoria/ 85 (H7N7)	IN	6	100	Days 2 to 5: sickness; day 6: last death	100		Forman *et al.* (1986)
A/chicken/Victoria/ 85 (H7N7)	IN/IC	13	50	Sickness: days 5 to 9			Hooper *et al.* (1995)
A/chicken/Victoria/ 85 (H7N7)	IN	14	100	Day 3: paralysis; day 3: sudden death; day 9: last death	100	6.4	Forman *et al.* (1986)
A/chicken/Victoria/ 85 (H7N7)	IN/IC	26	50	Sickness: days 5 to 9			Hooper *et al.* (1995)
A/chicken/Victoria/ 85 (H7N7)	Air sac	6	100	Day3: last death	100		Forman *et al.* (1986)
A/chicken/Victoria/ 85 (H7N7)	IV	6	100	Day3: last death	100		Forman *et al.* (1986)
A/chicken/Victoria/ 85 (H7N7)	DrC	14	100	Day 4[c]: sickness; day 5[c]: paralysis; day 3[c]: sudden death; day 12[c]: last death	100	6.9[c]	Forman *et al.* (1986)
A/hicken/Netherlands/ 621557/03 (H7N7)	IN/IT	6	100	Day 3:first death; day 6: last death	100	4.6	Van der Goot *et al.* (2005)
A/chicken/Netherlands/ 621557/03 (H7N7)	DrC	6	100	Day 6[c]: first death; day 7[c]: last death	70	6.9[c]	Van der Goot *et al.* (2005)

MTO = mean time of onset; IN = intranasal; IM = intramuscular; IC = intraconjunctival or intraocular; IO = intraoral/feeding; IT = intratracheal; IV = intravenous; DrC = direct contact; IrC = indirect contact; EID_{50} = 50 egg infectious dose. [a]Route of inoculation; dose if variable doses were administered. [b]Age of chickens in weeks. [c]Measured from the day of inoculation for birds in contact.

with this virus first became ill on day 5 p.i., with swelling and cyanosis of the comb, wattle, legs and feet, and died between 6 and 13 days p.i. However, Forman *et al.* (1986) reported that this virus caused illness in 3 to 5 days, and death within 7 days, in 6-week-old chickens. In the same study, an Australian HPAI virus from another outbreak, A/chicken/Victoria/85 (H7N7), similarly caused illness in 2 to 5 days and death within 6 days. Marked cyanosis and oedema of the head was often seen in sick birds in this study, but paralysis was the initial sign in one chicken. Birds that developed clinical signs were usually sick for only 1 day before death.

Birds inoculated by intravenous or intramuscular injection (Table 1) usually become ill faster and die sooner than after intranasal inoculation (Narayan *et al.*, 1969; Alexander *et al.*, 1986; Lee *et al.*, 2005a; Swayne & Pantin-Jackwood, 2006). It should be noted that some viruses classified molecularly as HPAI are avirulent for chickens (Londt *et al.*, 2007). These viruses can initially be found in asymptomatically infected flocks or in flocks with clinical signs consistent with LPAI infection. (Lee *et al.*, 2005b; Pelzel *et al.*, 2006) Typical HPAI signs may be seen with time as these viruses evolve to become more pathogenic.

Increased mortality, decreased feed consumption, and decreased egg laying as indicators of an HPAI outbreak

Four parameters often measured in commercial chicken production—mortality rate, feed consumption, water consumption, and egg production—are frequently affected in HPAI-infected flocks (Mutinelli *et al.*, 2003; Elbers *et al.*, 2004a, 2005; Swayne, 2007). Of these parameters, the time of onset has been consistently described only for increased mortality. Sudden death is a prominent sign in some experimentally infected birds (Alexander *et al.*, 1978, 1986; Bean *et al.*, 1985) or infected flocks (Elbers *et al.*, 2004a; Nakatani *et al.*, 2005). Once the mortality rate has begun to rise, it often increases dramatically. During the 1999 to 2000 H7N1 outbreak in Italy, all of the birds died within 48 to 72 h of the initial signs (Mutinelli *et al.*, 2003). In the 2005 H5N1 outbreak in Thailand, the cumulative mortality was 2% within 1 day of the appearance of clinical signs, and 100% within 6 days (Tiensin *et al.*, 2007). The average daily mortality in this outbreak increased from 1% to 36%. Because the time of virus entry into a flock is usually unknown, it is rarely possible to calculate the time between infection and the initial rise in the mortality rate. However, this parameter can be estimated for different viruses using the mean death time (MDT), an estimate of the average time to death in experimentally infected birds. The MDT can vary with the isolate, dose of virus, and route of inoculation (Narayan *et al.*, 1969; Lee *et al.*, 2005a; Swayne & Pantin-Jackwood, 2006). Reported MDTs for HPAI viruses (Table 1) inoculated by intranasal, intraoral, or intraconjunctival routes range from 1.5 to 9.0 days (Narayan *et al.*, 1969; Alexander *et al.*, 1978, 1986; Westbury *et al.*, 1979, 1981; Beard *et al.*, 1984; Bean *et al.*, 1985; Hooper *et al.*, 1995; Horimoto *et al.*, 1995; Swayne *et al.*, 1997, 2006; Van der Goot *et al.*, 2003; Jones & Swayne, 2004; Lee *et al.*, 2005a; Van der Goot *et al.*, 2005). The MDT for various Asian H5N1 viruses isolated from recent outbreaks varied from 1.5 to 5.5 days, with a cumulative mortality of 100% for all viruses (Shortridge *et al.*, 1998; Suarez *et al.*, 1998; Cauthen *et al.*, 2000; Perkins & Swayne, 2001; Tumpey *et al.*, 2002; Lee *et al.*, 2005a; Mase *et al.*, 2005; Nguyen *et al.*, 2005; Swayne *et al.*, 2006; Swayne & Pantin-Jackwood, 2006; Bublot *et al.*, 2007; Lee *et al.*, 2007; Swayne, 2007; Tsukamoto *et al.*, 2007).

There is little information on the timing of decreased feed and water intake in either HPAI-infected flocks or experimentally infected birds. In 3-month-old roosters infected intranasally with A/turkey/Ontario/7732/66 (H5N9), decreased feed consumption was one of the first clinical signs; it occurred concurrently with ruffled feathers and listlessness, and preceded more severe respiratory signs and severe depression by a day (Narayan *et al.*, 1969). Decreased feed and water intake was also one of the initial signs in H7N7-infected flocks in The Netherlands in 2003 (Elbers *et al.*, 2004a). In one flock, a drop in feed and water consumption preceded a slightly increased mortality rate (1.6%) by 1 day, with all of these indicators intensifying by the third day. In another flock, reduced feed consumption was noted 2 days after increased mortality. In this flock, reduced feed consumption was reported in one house when 588 birds were dead, but feed consumption was normal until the following day in a house where 130 birds had died. In one analysis of this outbreak, decreased feed and water consumption were not among the most sensitive indicators of infection in commercial layers, broiler breeders or broilers (Elbers *et al.*, 2005).

Decreased egg production is common in HPAI-infected flocks, and decreased egg quality including pale eggs may also be seen (Bean *et al.*, 1985; Elbers *et al.*, 2004a, 2005). Limited information available in the literature suggests that, although egg production drops early, it is not the first indicator of an avian influenza infection. In hens inoculated intranasally and intraconjunctivally with an H5N2 virus from the 1983 Pennsylvania outbreak, nearly all of the birds were depressed on day 3 p.i., and some hens had green watery diarrhoea; however, egg production remained normal until day 4 p.i., when it dropped dramatically, some thin or soft-shelled eggs were laid, and the first deaths were reported (Beard *et al.*, 1984). In another study using the Pennsylvania H5N2 viruses, birds continued to lay eggs until the day of death (Bean *et al.*, 1985). There is little information on the timing of decreased egg production in naturally infected flocks; however, during the H7N7 epidemic of HPAI in The Netherlands, one flock reported a severe drop in egg production and poor shell quality by the third day of clinical signs (Elbers *et al.*, 2004a). In contrast, an H5N1-infected flock in Japan experienced no significant clinical signs or decrease in egg production before death (Nakatani *et al.*, 2005).

Onset of clinical signs in LPAI-infected chickens

LPAI viruses usually cause much milder clinical signs than HPAI viruses, and some infections are subclinical (Bano *et al.*, 2003; Swayne & Pantin-Jackwood, 2006; Okamatsu *et al.*, 2007). Although marked clinical signs are possible (Johnson & Maxfield, 1976; Johnson *et al.*, 1977; Brugh *et al.*, 1996; Swayne *et al.*, 1997; Bano *et al.*, 2003; Nili & Asasi, 2003), most LPAI viruses tend to cause disease when chickens are co-infected with other pathogens or are subject to environmental stresses (Hooper & Selleck, 1998; Bano *et al.*, 2003; Nili & Asasi, 2003). Clinical signs reported in infected chickens

include depression/lethargy, decreased feed and water consumption, decreased egg production, decreased fertility and hatchability of eggs, misshapen eggs, and increased mortality (Johnson & Maxfield, 1976; Alexander & Stuart, 1982; Bean *et al*., 1985; Hooper & Selleck, 1998; Ziegler *et al*., 1999; Kinde *et al*., 2003; Mutinelli *et al*., 2003; Nili & Asasi *et al*., 2003; Bowes *et al*., 2004; Lu *et al*., 2004; Swayne & Pantin-Jackwood, 2006). Respiratory signs including cyanosis and facial oedema, conjunctivitis, and diarrhoea have been reported at times in some infected flocks (Johnson & Maxfield, 1976; Hooper & Selleck, 1998; Ziegler *et al*., 1999; Kinde *et al*., 2003; Mutinelli *et al*., 2003; Nili & Asasi, 2003; Lu *et al*., 2004; Swayne & Pantin-Jackwood, 2006). The pattern of signs varies with the flock. The early signs have been described in published descriptions of a few outbreaks. The initial clinical signs were depression and decreased feed consumption, followed by decreased egg laying, in broiler breeder flocks or commercial layers infected with H7N1 LPAI viruses in Italy in 1999 (Mutinelli *et al*., 2003). Mild cyanosis of the comb and wattles was also reported in some birds during this time. Huddling and depression, followed within hours by the first deaths, were seen during a severe H4N8 outbreak in layers in Alabama (Johnson & Maxfield, 1976). Decreased egg production, cyanotic combs, and some diarrhoea were also reported. Anorexia and reduced water consumption, followed shortly by depression and respiratory signs, occurred during an H9N2 outbreak on Iranian broiler farms (Nili & Asasi, 2003). The signs of LPAI infections can wax and wane in a flock (Bowes *et al*., 2004).

There are few reports in the literature describing the onset of clinical signs in experimentally infected chickens (Table 2). In hens inoculated intranasally with an H5N2 LPAI virus from the 1983 Pennsylvania outbreaks, respiratory signs were first seen on day 5 (Beard *et al*., 1984). Several chickens had blue tips to the combs, and one was unable to stand. Egg production was 50% of normal on day 6 p.i. and respiratory signs continued, but most of the sick birds eventually recovered. Deaths were reported in 20% of 2-week-old chickens inoculated intranasally with a related virus from this outbreak, as well as in 10% of chickens in contact with these birds (Alexander *et al*., 1986). One of eight 4-week-old chickens inoculated with the LPAI virus A/chicken/Chile/176822/02 (H7N3) was mildly depressed from day 4 to day 6 p.i. (Jones & Swayne, 2004). It should be noted that chickens inoculated with LPAI viruses by parenteral routes occasionally develop severe disease or die, even when the virus is non-pathogenic by other routes of inoculation (Condobery & Slemons, 1992; Hooper *et al*., 1995; Jones & Swayne, 2004).

Increased mortality, decreased feed consumption, and decreased egg laying as indicators of an LPAI outbreak

Although these parameters do not usually change as dramatically as they do in HPAI-infected flocks, increased mortality, decreased egg production, and decreased feed and water consumption are common in LPAI virus-infected flocks (Johnson & Maxfield, 1976; Alexander & Stuart, 1982; Bean *et al*., 1985; Hooper & Selleck, 1998; Ziegler *et al*., 1999; Kinde *et al*., 2003; Nili & Asasi, 2003; Mutinelli *et al*., 2003; Bowes *et al*., 2004; Lu *et al*., 2004; Swayne & Pantin-Jackwood, 2006). The baseline mortality in a chicken flock varies with the timing within the production period (Elbers *et al*., 2004a). In Pennsylvania layer flocks infected with H7N2 LPAI viruses in 1997 to 1998, the mortality rate rose to two to three times baseline, with a cumulative mortality of 4% (Ziegler *et al*., 1999). In Pennsylvania broiler breeder flocks infected with an H7N2 LPAI virus in 2001, the mortality rate increased more than 10-fold in the week after disease onset, from 0.1% to 1% in hens and from less than 0.2% up to 2% to 3% in roosters (Lu *et al*., 2004). Reported mortality rates during other LPAI outbreaks typically range from 0.25% to 25% (Bean *et al*., 1985; Kinde *et al*., 2003; Mutinelli *et al*., 2003; Bowes *et al*., 2004); however, up to 69% of the chickens died in an unusual H4N8 outbreak in Alabama in 1975 (Johnson & Maxfield, 1976). During this outbreak, chickens on affected farms died within hours of the first clinical signs. Increased mortality was also an initial sign in some other outbreaks. The first signs in an H7N3-infected broiler-breeder flock in British Columbia were a sudden drop in feed consumption and a slight increase in mortality of 0.5% in 72 h (Bowes *et al*., 2004). Chickens inoculated by intranasal inoculation or other routes that mimic natural exposure (Table 2) frequently experience no mortality (Alexander *et al*., 1978, 1986; Bean *et al*., 1985; Horimoto *et al*., 1995; Swayne *et al*., 1997; Jones & Swayne, 2004; Okamatsu *et al*., 2007), but mortality rates up to 20% have been reported in some cases (Alexander *et al*., 1986; Horimoto *et al*., 1995; Mo *et al*., 1997; Swayne *et al*., 1997). The MDT in these studies varies from 6 to 9 days (Table 2).

In some outbreaks, decreased feed or water consumption has been among the initial signs noticed by the farmer. Decreased feed consumption occurred very early in LPAI H7N1-infected broiler breeder flocks and commercial layers in Italy (Mutinelli *et al*., 2003). Similarly, the first signs in a Canadian H7N3-infected flock were a sudden drop in feed consumption and a slight increase in mortality (Bowes *et al*., 2004). Anorexia and reduced water consumption were seen initially during an H9N2 outbreak on Iranian broiler farms, and were rapidly followed by depression and respiratory signs (Nili & Asasi, 2003). Chickens challenged with filtered tracheal washings containing H9N2 LPAI virus from this outbreak had decreased feed and water consumption between 8 and 14 days post challenge, with depression and respiratory signs appearing soon afterward (Nili & Asasi, 2003). A 50% decrease in feed consumption was reported in an LPAI-infected flock in England in 1982, together with decreased egg production, increased mortality and depression, but the timing of these signs was not described (Alexander & Stuart, 1982). The major signs in the 2000 to 2002 H6N2 LPAI outbreak in California included decreased egg production and increased mortality, with reduced feed consumption noticed in a few flocks (Kinde *et al*., 2003).

Decreased egg production is frequently reported in LPAI outbreaks (Johnson & Maxfield, 1976; Alexander & Stuart, 1982; Bean *et al*., 1985; Morgan & Kelly, 1990; Hooper & Selleck, 1998; Ziegler *et al*., 1999; Kinde *et al*., 2003; Mutinelli *et al*., 2003; Bowes *et al*., 2004; Lu *et al*., 2004; Swayne & Pantin-Jackwood, 2006), but most experimental studies are conducted in young birds not of laying age, and its timing is poorly understood. In one report, hens inoculated with an LPAI H5N2 virus isolated from the 1983 Pennsylvania outbreak developed

Table 2. *Onset of morbidity and mortality in chickens infected with LPAI viruses*

Virus	Route	Age[a]	Morbidity rate (%)	Onset of specified symptoms/first and last deaths	Mortality rate (%)	MDT (days)	Reference
A/duck/Victoria/ 92 (H3N8)	IV	8	25	Day 5: sickness; day 6: death	12.5		Hooper *et al.* (1995)
A/chicken/Alabama/ 7395/75 (H4N8)	IT	4	0		0		Mo *et al.* (1997)
A/chicken/Alabama/ 7395/75 (H4N8)	IV	1 day			47	6.0	Swayne & Slemons (1992)
A/mallard/Ohio/ 338/86 (H4N8)	IV	1 day			47	4.6	Swayne & Slemons (1992)
A/mallard/Ohio/ l84/86 (H5N1)	IV	1 day			15	3.4	Swayne & Slemons (1992)
A/turkey/Italy/ ZA/80 (H5N2)	IN	2	0		0		Alexander *et al.* (1986)
A/turkey/Italy/ ZA/80 (H5N2)	IM	2	0		0		Alexander *et al.* (1986)
A/chicken/Pennsylvania/ l/83 (H5N2)	IN	2	20	Sickness: MTO = 9.0 days	20	9.0	Alexander *et al.* (1986)
A/chicken/Pennsylvania/ l/83 (H5N2)	IN	>26			0		Bean *et al.* (1985)
A/chicken/Pennsylvania/ l/83 (H5N2)	IM	2	10	Sickness: MTO = 8 days	10	8	Alexander *et al.* (1986)
A/chicken/Pennsylvania/ 3/83 (H5N2)	IV	>26		Day 6: last death	50		Bean *et al.* (1985)
A/chicken/Pennsylvania/ l/83 (H5N2)	DrC	2	10	Sickness: MTO = 17 days[b]	10	17[b]	Alexander *et al.* (1986)
A/chicken/Pennsylvania0/ 21525/83 (H5N2)	IN	Adult	2	Day 5: respiratory signs (blue tips on combs), bird unable to stand; day 6: 50% decrease in egg production			Beard *et al.* (1984)
A/chicken/Pennsylvania/ 21525/83 (H5N2)	IT	4	33		20	6.0	Mo *et al.* (1997)
A/chicken/Hidalgo/ 26654-1368/94 (H5N2)	IN/IT/IO	3 to 4			0		Horimoto *et al.* (1995)
A/chicken/Hidalgo/ 26654-1368/94 (H5N2)	IN	4	6	Day 7: one death	6	7	Swayne *et al.* (1997)
A/chicken/Hidalgo/ 26654-1368/94 (H5N2)	IV	3 to 4			0		Horimoto *et al.* (1995)
A/chicken/Hidalgo/ 26654-1368/94 (H5N2)	IV	4	31		31	3.2	Swayne *et al.* (1997)
A/chicken/Queretaro/ 26654-1373/94 (H5N2)	IN/IT/IO	3 to 4			0		Horimoto *et al.* (1995)
A/chicken/Queretaro/ 26654-1373/94 (H5N2)	IV	3 to 4			0		Horimoto *et al.* (1995)
A/chicken/Mexico/ 26654-1374/94 (H5N2)	IN	4	0		0		Swayne *et al.* (1997)
A/chicken/Mexico/ 26654-1374/94 (H5N2)	IN/IT/IO	3 to 4			0		Horimoto *et al.* (1995)
A/chicken/Mexico/ 26654-1374/94 (H5N2)	IV	3 to 4			0		Horimoto *et al.* (1995)
A/chicken/Mexico/ 26654-1374/94 (H5N2)	IV	4	31		31	3.6	Swayne *et al.* (1997)
A/chicken/Jalisco/ 14589-660/94 (H5N2)	IN	4	0		0		Swayne *et al.* (1997)
A/chicken/Jalisco/ 14589-660/94 (H5N2)	IV	4	19		19	5.3	Swayne *et al.* (1997)
A/chicken//Puebla/ 14590-658/94 (H5N2)	IN/IT/IO	3 to 4			17		Horimoto *et al.* (1995)
A/chicken/ Puebla-8624-602/94[c] (H5N2)	IV	3 to 4			50		Horimoto *et al.* (1995)
A/chicken/ Puebla-8623-607/94[c] (H5N2)	IN/IT/IO	3 to 4			0		Horimoto *et al.* (1995)
A/chicken/ Puebla-8623-607/94[c] (H5N2)	IN	4	75		19	6.7	Swayne *et al.* (1997)

Table 2 (*Continued*)

Virus	Route	Age[a]	Morbidity rate (%)	Onset of specified symptoms/first and last deaths	Mortality rate (%)	MDT (days)	Reference
A/chicken/ Puebla-8623-607/94[c] (H5N2)	IV	3 to 4			63		Horimoto *et al.* (1995)
A/chicken/ Puebla-8623-607/94[c] (H5N2)	IV	4	88		25	7.3	Swayne *et al.* (1997)
A/chicken/ Ibaraki/ 1/05 (H5N2)	IV	4	0		0		Okamatsu *et al.* (2007)
A/chicken/ Ibaraki/ 1/05 (H5N2)	IN	6	0		0		Okamatsu *et al.* (2007)
A/parrot/Ulster/ 73 (H7N1)	IN	2	0		0		Alexander *et al.* (1978)
A/chicken/PA/ 3779-2/97 (H7N2	IN	23	0		0		Lu & Castro (2004)
A/chicken/PA/ 3779-2/97 (H7N2	IO	23	0		0		Lu & Castro (2004)
A/chicken/PA/ 3779-2/97 (H7N2	IC	23	0		0		Lu & Castro (2004)
A/chicken/PA/ 3779-2/97 (H7N2	IM	23	0		0		Lu & Castro (2004)
A/chicken/Chile/ 176822/02 (H7N3)	IN	4		Day 4: mild depression	0		Jones & Swayne (2004)
A/chicken/Chile/ 176822/02 (H7N3)	IV	4			25		Jones & Swayne (2004)
A/chicken/Chile/ 176822/02 laboratory derivative (02-AI-15-#9) (H7N3)	IN	4			0		Jones & Swayne (2004)
A/chicken/Chile/176822/02 laboratory derivative (02-AI-15-#9) (H7N3)	IV	4			50		Jones & Swayne (2004)
A/duck/Victoria/ 76 (H7N7)	IN	14	0		0		Westbury *et al.* (1979)
A/duck/Victoria/ 76 (H7N7)	IN	14	0		0		Westbury *et al.* (1979)
A/chicken/Iran (H9N2) (filtered tracheal washings)	IC	3		Day 8: decreased feed and water consumption	19		Nili & Asasi (2003)

MTO = mean time of onset; IN = intranasal; IM = intramuscular; IC = intraconjunctival or intraocular; IO = intraoral/ feeding; IT = intratracheal; IV = intravenous; DrC = direct contact. [a]Age of chickens in weeks. [b]Measured from the day of inoculation for birds in contact. [c]This virus would be considered HPAI by current standards, based on the cleavability of its haemagglutinin.

respiratory signs on day 5, and egg production dropped to 50% of normal the following day (Beard *et al.*, 1984). The timing of decreased egg production is rarely noted in outbreak descriptions; however, during the 1975 outbreak of severe H4N8 avian influenza in Alabama, egg laying decreased at the time of disease onset to 1 day later (Johnson & Maxfield, 1976). In H7N1-infected chickens in Italy, egg production dropped 5% to 20% in broiler breeders and 3% to 30% in layers after the initial signs of decreased feed consumption and depression (Mutinelli *et al.*, 2003). Some misshapen eggs were also reported. During this outbreak, egg production recovered to pre-disease levels in a few commercial layer flocks, but in most cases a 2% to 3% decrease continued to be seen. An initial 2% to 4% drop in egg production occurred in Pennsylvania layers infected with H7N2 LPAI viruses in 1997 to 1998 (Ziegler *et al.*, 1999). In some Pennsylvania flocks, egg production fell as low as 20% below normal within several weeks. Egg production returned to pre-disease levels in a few flocks, but it often fell again. A 10% to 20% decrease in egg production was an early sign during the 1985 H7N7 outbreak in Australia (Morgan & Kelly, 1990). A change in diet increased egg production in two sheds, while egg production continued to fall in other two, possibly from normal production losses during aging.

Onset of virus shedding in respiratory secretions, faeces, and poultry products for HPAI and LPAI viruses

Influenza viruses shed from the body in secretions and excretions, particularly faeces, may be found on the surface of eggs. Shed viruses can also contaminate meat and other tissues during processing. In addition, some isolates may localize in the skeletal muscle (meat) and/or the internal contents of eggs from infected birds. The risk that avian influenza viruses will contaminate tissues may vary with the isolate and its pathotype. HPAI and LPAI viruses differ in the structure of their haemagglutinin, the protein that must be cleaved for the virus to enter cells. This structural difference has important ramifications for the distribution of virus in poultry

products. The haemagglutinin of an LPAI virus is cleaved by trypsin-like enzymes found in epithelial cells and respiratory secretions, or by certain bacterial proteases (Swayne, 2007). As a result, LPAI viruses that enter the body by a natural route (e.g. inhalation) are thought to remain localized in the respiratory and gastrointestinal tracts (Hooper & Selleck, 1998; Swayne & Beck, 2005; Zepeda & Salman, 2007). In contrast, the haemagglutinin of an HPAI virus is cleaved by the furin family of enzymes, which are found throughout the body, and HPAI infections are systemic (Swayne, 2007). This difference in tissue distribution explains, at least in part, the increased severity of clinical signs with HPAI viruses. It also suggests that HPAI viruses are more likely to be found in tissues such as skeletal muscle. The onset of virus shedding has been extensively studied for both HPAI and LPAI viruses in experimentally infected birds.

HPAI and LPAI viruses in faeces and respiratory secretions

HPAI viruses are typically found in both the faeces and respiratory secretions of chickens (Moses *et al*., 1948; Alexander *et al*., 1978; Westbury *et al*., 1979, 1981; Beard *et al*., 1984; Horimoto *et al*., 1995; Shortridge *et al*., 1998; Tumpey *et al*., 2002; Van der Goot *et al*., 2003; Jones & Swayne, 2004; Lee *et al*., 2005a; Swayne & Beck, 2005; Tian *et al*., 2005, Van der Goot *et al*., 2005, Swayne *et al*., 2006; Bublot *et al*., 2007). Many HPAI viruses appear to be shed within a day or two (Table 3) in experimentally inoculated chickens (Westbury *et al*., 1979; Forman *et al*., 1986; Shortridge *et al*., 1998; Jones & Swayne, 2004; Lee *et al*., 2005a; Swayne & Beck, 2005; Van der Goot *et al*., 2005; Swayne *et al*., 2006; Bublot *et al*., 2007). Shedding of viruses is rarely examined before the second day p.i.; however, some studies found that intranasally inoculated chickens could shed A/chicken/Pennsylvania/1370/83 (H5N2) (Van der Goot *et al*., 2003; Swayne & Beck, 2005) and A/chicken/Netherlands/621557/03 (H7N7) (Van der Goot *et al*., 2005) by day 1 p.i. in both respiratory secretions and faeces.

LPAI viruses have also been detected in the faeces and respiratory secretions of some chickens inoculated by intranasal, intraoral or intratracheal routes (Table 4). Some viruses have been found in the faeces as early as day 2 p.i. (Horimoto *et al*., 1995; Lu & Castro, 2004; Swayne & Beck, 2005) and in respiratory secretions as early as day 1 (Van der Goot *et al*., 2003; Swayne & Beck, 2005). When both faecal and respiratory shedding were examined, most studies report that viruses are excreted at least occasionally by both routes (Westbury *et al*., 1979; Slemons & Swayne, 1990; Shalaby *et al*., 1994; Swayne *et al*., 1997; Lu *et al*., 2003; Van der Goot, *et al*., 2003; Lu & Castro, 2004; Okamatsu *et al*., 2007). Some studies have found that certain LPAI viruses are shed mainly in respiratory secretions (Horimoto *et al*., 1995; Van der Goot *et al*., 2003; Swayne & Beck, 2005). Swayne and Beck (2005) reported that A/turkey/Virginia/158512/02 (H7N2) replicated poorly in the gastrointestinal tract, and virus was detected in cloacal samples from only one chicken on day 5, but A/chicken/New York/21586-8/99 (H7N2) replicated well in the gastrointestinal tract, and virus was found consistently beginning day 2 p.i. Both viruses were found in tracheal swabs starting on day 1 p.i. Van der Goot *et al.* (2003) reported that an H5N2 LPAI virus isolated early in the 1983 to 1984 Pennsylvania outbreak was shed consistently in respiratory secretions and rarely detected in cloacal swabs, while an HPAI virus isolated later in the outbreak was shed readily from both the trachea and the cloaca. Similarly, Horimoto *et al*. (1995) reported that some less pathogenic Mexican LPAI H5N2 viruses were found only in tracheal swabs, but a more pathogenic LPAI isolate, as well as an HPAI virus from this outbreak, occurred in both tracheal and faecal swabs. Swayne *et al*. (1997) reported that both H5N2 viruses tested, including one detected only in respiratory secretions in the previous study, were shed consistently by both routes in either intranasally or intravenously inoculated chickens (Swayne *et al*., 1997). In this study, greater quantities of virus were found in oropharyngeal than cloacal swabs during the clinical stage, but viruses were more consistently detected in the faeces than respiratory secretions after recovery. Interestingly, intravenously inoculated A/mallard/Ohio/184/86 (H5N1), a virus isolated directly from waterfowl, was found consistently in cloacal swabs but rarely in tracheal swabs (Slemons & Swayne, 1990). Whether chickens in naturally infected flocks shed LPAI or HPAI viruses as rapidly as experimentally infected birds is unknown, as the day the virus enters a flock can rarely be determined.

HPAI and LPAI viruses in meat and other tissues

HPAI viruses cause systemic infections; these viruses and their antigens have been isolated from numerous tissues including the skeletal muscle (meat), blood, bone marrow, upper and lower respiratory tract, kidney, spleen, liver, thymus, pancreas, bursa, adrenal gland, gastrointestinal tract, ovary, testis, comb, wattles, feather follicles, and brain of experimentally infected chickens (Moses *et al*., 1948; Bean *et al*., 1985; Forman *et al*., 1986; Brown *et al*., 1992; Hooper *et al*., 1995; Mo *et al*., 1997; Suarez *et al*., 1998; Perkins & Swayne, 2001; Tumpey *et al*., 2002; Jones & Swayne, 2004; Lee *et al*., 2005a, 2007; Swayne *et al*., 2007). HPAI viruses reported to occur in the skeletal muscle (meat) of experimentally infected chickens include A/duck/Anyang/AVL-1/01 (H5N1) (Tumpey *et al*., 2002), A/chicken/Korea/ES/03 (H5N1) (Swayne & Beck, 2005) and A/chicken/Pennsylvania/1370/83 (H5N2) (Brown *et al*., 1992; Mo *et al*., 1997; Swayne & Beck, 2005). Antigens of A/turkey/Ontario/7732/66 (H5N2), A/chicken/Victoria/85 (H7N7) and A/chicken/Victoria/92 (H7N3) have also been reported in skeletal muscle, although virus isolation has not been confirmed (Hooper *et al*., 1995; Mo *et al*., 1997). There is limited information on when HPAI viruses first appear in meat. In intranasally inoculated chickens, A/chicken/Pennsylvania/1370/83 (H5N2) was detected in bone marrow, breast, and thigh meat beginning day 1 p.i. (Swayne & Beck, 2005). Another study found antigens from this virus in the ocular muscles, as well as in numerous other tissues, beginning on day 1 p.i. in chickens inoculated into the air sac (Brown *et al*., 1992). Studies that did not specifically address the presence of virus in meat reported that A/chicken/Hong Kong/220/97 (H5N1), A/chicken/Korea/ES/03 (H5N1), A/duck/CHN/E319-2/03 (H5N1), A/chicken/Pennsylvania/1370/83 (H5N2), and A/chicken/Chile/184240-1/02 (H7N3) can be detected in numerous tissues on either the first or the second day p.i. (Perkins & Swayne, 2001; Jones & Swayne, 2004; Lee *et al*.,

Table 3. *First occurrence of HPAI viruses in faeces, respiratory secretions, and tissues of infected chickens*

Virus	Route	Age[a]	Faecal/ cloacal shedding	Tracheal shedding	Virus/viral antigen in tissues	Reference
A/goose/Guangdong/ 1/96 (H5N1)	IM	5	Day 3[b]	Day 3[b]		Tian *et al.* (2005)
A/goose/Guangdong/ 1/96 (H5N1)	IM	6	Day 3[b]	Day 3[b]		Tian *et al.* (2005)
A/goose/Guangdong/ 1/96 (H5N1)	IM	46	Day 3[b]	Day 3[b]		Tian *et al.* (2005)
A/chicken/Hong Kong/ 220/97 (H5N1)	IN	4			Day 1: upper respiratory tract, lung, intestinal tract, spleen, liver, thymus, feather follicle, bone marrow, brain, heart, kidney, adrenal gland, bursa, pancreas; +: ovary, testis	Perkins & Swayne (2001)
A/duck/Anyang/ AVL-1/01 (H5N1)	IN	4	+	Day 1 to 3	Day 2 to 3[b]: skeletal muscle (breast meat, thigh meat), brain, lung, kidney	Tumpey *et al.* (2002)
A/chicken/SouthKorea/ ES/03 (H5N1)	IN	3	Day 2[b]	Day 2[b]		Bublot *et al.* (2007)
A/chicken/Korea/ ES/03 (H5N1)	IN	3			+: skeletal muscle (breast meat)	Swayne & Beck (2005)
A/chicken/Korea/ ES/03 (H5N1)	IN	4	Day 2[b]	Day 2[b]	Day 2[b]: heart, brain, lung	Lee *et al.* (2005a)
A/chicken/Korea/ ES/03 (H5N1)	IO	4	+	+		Swayne & Beck, 2005)
A/chicken/Indonesia/ 7/03 (H5N1)	IN	6	Day 2[b]	Day 2[b]		Swayne *et al.* (2006)
A/duck/China/ E319-2/03 (H5N1)	IN	4			48 h[b]: trachea, lung, kidney, liver, heart, bursa, brain, spleen, pancreas	Lee *et al.* (2007)
A/chicken/Vietnam/ 0008/04 (H5N1)	IN	3	Day 2[b]			Bublot *et al.* (2007)
A/chicken/Tianjing/ 65/04 (H5N1)	IM	6	Day 3[b]	Day 3[b]		Tian *et al.* (2005)
A/duck/Shanghai/ 16/04 (H5N1)	IM	6	Day 3 [b]	Day 3 [b]		Tian *et al.* (2005)
A/chicken/Pennsylvania/ 1370/83 (H5N2)	IN	3 to 4	Day 1	Day 1	Day 1: lung, bone marrow, muscle (breast meat, thigh meat); day 3: air sac	Swayne & Beck (2005)
A/chicken/Pennsylvania/ 1370/83 (H5N2)	IN	5	Day 2			Bean *et al.* (1985)
A/chicken/Pennsylvania/ 1370/83 (H5N2)	IN/IT	6	Day 1	Day 1		Van der Goot *et al.* (2003)
A/chicken/Pennsylvania/ 1370/83 (H5N2)	IN	>26	Day 2		Day 2[b]: brain, kidney, lung, intestine, blood; last day of life: eggs	Bean *et al.* (1985)
A/chicken/Pennsylvania/ SERPL-PA/83 (H5N2)	IN/IC	Adult	+	+	Day 3: eggs	Beard *et al.* (1984)
A/chickenlPennsylvania/ 1370/83 (H5N2)	IT	4			+: skeletal muscle, brain, heart, spleen, pancreas, kidney, lung, trachea; day 4[b]: pooled tracheal/cloacal swabs	Mo *et al.* (1997)
A/chicken/Pennsylvania/ 1370/83 (H5N2)	Air sac	15			Day 1: spleen, thymus, bursa, heart, brain, lung, trachea, testis; day 2: comb, wattles, ocular muscles; +: liver, kidney, pancreas	Brown *et al.* (1992)
A/chicken/Pennsylvania/ 1370/83 (H5N2)	Air sac	>52			Day 1: comb, wattles, heart, thymus, brain, ocular muscles; +: spleen lung, liver, kidney, pancreas, trachea, ovary, bursa	Brown *et al.* (1992)
A/chicken/Pennsylvania/ 1370/83 (H5N2)	DrC	6	Day 3[c]	Day 3[c]		Van der Goot *et al.* (2003)
A/chicken/Queretaro/ 14588-19/95 (H5N2)	IO/IN/IT	3 to 4	+	+	+: brain, lung, spleen, liver, pancreas, kidney, colon, blood	Horimoto *et al.* (1995)

Table 3 (*Continued*)

Virus	Route	Age[a]	Faecal/ cloacal shedding	Tracheal shedding	Virus/viral antigen in tissues	Reference
A/turkey/Ontario/ 7732/66 (H5N9)	IN	43			48 h: blood	Narayan *et al.* (1969)
A/turkeylOntario/ 7732/66 (H5N9)	IT	4			+: skeletal muscle, brain, heart, spleen, pancreas, kidney, lung; day 4[b]: pooled tracheal/cloacal swabs	Mo *et al.* (1997)
A/chicken/Victoria/ 92 (H7N3)	IN/IC	6 to 8			+: pancreas, heart, brain, kidney	Hooper *et al.* (1995)
A/chicken/Victoria/ 92 (H7N3)	IN/IC	24			+: pancreas, heart, brain, muscle, kidney	Hooper *et al.* (1995)
A/chicken/Chile/ 184240-1/02 (H7N3)	IN	4	Day 2	Day 2	Day 2: upper respiratory tract, lung, heart, bone marrow, oesophagus, intestines, caecal tonsil, liver, pancreas, thymus, spleen, brain, kidney, adrenal, eyelid, gonad	Jones & Swayne (2004)
A/FPV/Dutch/27 (H7N7)	IN	2	Day 4[b]	Day 4[b]		Alexander *et al.* (1978)
A/FPV/Dutch/27 (H7N7)	IM	Adult	24 h	18 h	15 hrs: blood; 35 to 37 h: brain, lung, spleen, kidney, proventriculus, ileum; 35 h: egg in oviduct; 37 h: ovary, sessile ovum	Moses *et al.* (1948)
A/FPV/Dutch/27 (H7N7)	DrC	2	Day 4[bc]	Day 4[bc]		Alexander *et al.* (1978)
A/fowl/Victoria/ 75 (H7N7)	IN	2	Day 8	Day 8		Alexander *et al.* (1978)
A/fowl/Victoria/ 75 (H7N7)	IN	14	Day 2[b]	Day 2[b]		Westbury *et al.* (1979)
A/fowl/Victoria/ 75 (H7N7)	IN/ IO	18	+	+	+: heart blood	Westbury *et al.* (1981)
A/fowl/Victoria/ 75 (H7N7)	DrC	2	Day 8[c]	Day 13[c]		Alexander *et al.* (1978)
A/fowl/Victoria/ 75 (H7N7)	DrC	14	Day 2[bc]	Day 4[c]		Westbury *et al.* (1979)
A/fowl/Victoria/ 75 (H7N7)	DrC	18	+	+	+: heart blood	Westbury *et al.* (1981)
A/chicken/Victoria/ A185/85 (H7N7)	IT	4			+: skeletal muscle, brain, heart, spleen, pancreas, kidney, lung, trachea; day 4[b]: pooled tracheal/ cloacal swabs	Mo *et al.* (1997)
A/chicken/Victoria/ 85 (H7N7)	IN/IC	6			+: pancreas, heart, muscle, brain, kidney	Hooper *et al.* (1995)
A/chicken/Victoria/ 85 (H7N7)	IN/IC	13			+: pancreas, heart, muscle, brain, kidney	Hooper *et al.* (1995)
A/chicken/Victoria/ 85 (H7N7)	IN	14	Day 2		+: trachea, intestinal contents	Forman *et al.* (1986)
A/chicken/Victoria/ 85 (H7N7)	IN/IC	26			+: pancreas, heart, muscle, brain, kidney	Hooper *et al.* (1995)
A/chicken/Victoria/ 85 (H7N7)	DrC	14	Day 3[c]		+: trachea, intestinal contents	Forman *et al.* (1986)
A/chicken/Netherlands/ 621557/03 (H7N7)	IN/IT	6	Day 1	Day 1		Van der Goot *et al.* (2005)
A/chicken/Netherlands/ 621557/03 (H7N7)	DrC	6	Day 3	Day 2		Van der Goot *et al.* (2005)

+, virus found, day(s) not specified. IN = intranasal; IM = intramuscular; IC = intraconjunctival or intraocular; IO = intraoral/ feeding; IT = intratracheal; IV = intravenous; DrC = direct contact. [a]Age of chickens in weeks. [b]Virus was isolated on the first day virus shedding was examined. [c]Measured from the day of inoculation for birds in contact.

Table 4. *First occurrence of LPAI viruses in faeces, respiratory secretions, and tissues of infected chickens*

Virus	Route[a]	Age[b]	Faecal/cloacal shedding	Tracheal shedding	Virus/viral antigens in tissues	Reference
A/duck/Victoria/ 92 (H3N8)	IV	8			+: kidney	Hooper *et al.* (1995)
A/blue-winged teal/ OH/305/86 (H3,4N6)	IV	6	+		Day 5[c]: brain, thymus, spleen, pancreas, gonads, kidneys, lungs	Condobery & Slemons (1992)
A/mallard/OH/ 183/86 (H4N1)	IV	6	+		Day 4[c]: thymus, spleen, pancreas, kidney	Condobery & Slemons (1992)
A/grey teal/WA/ 1840/79 (H4N4)	IV	8			+: kidney	Hooper *et al.* (1995)
A/chicken/Alabama/ 7395/75 (H4N8)	IT	22	Day 1.5/3[c]	Day 1.5/3[c]	Day 1.5/3[c]: ovary, oviduct, kidney, lung	Shalaby *et al.* (1994)
A/chicken/Alabama/ 7395/75 (H4N8)	IV	1 day	Day 5[d]		Day 5[c]: kidney	Swayne & Slemons (1992)
A/chicken/Alabama/ 7395/75 (H4N8)	IV	22	Day 1.5/3[c]	Day 1.5/3[c]	Day 1.5/3[c]: ovary, oviduct, kidney, lung)	Shalaby *et al.* (1994)
A/mallard/Ohio/ 338/86 (H4N8)	IV	1 day	Day 5[c]		Day 5[c]: kidney	Swayne & Slemons (1992)
A/mallard/OH/338/ 86 (H4N8)	IV	6	+		Day 5[c]: thymus, spleen, pancreas, gonads, kidneys, lungs	Condobery & Slemons (1992)
A/mallard/Ohio/ l84/86 (H5N1)	IV	1 day	Day 5[c]			Swayne & Slemons (1992)
A/mallard/Ohio/ 184/86 (H5N1)	IV	6	Day 1	Day 3/rare	Day 1: kidney, spleen, pancreas, lung, heart, jejunum, ileum, bursa	Slemons & Swayne (1990)
A/chicken/Pennsylvania/ 21525/83 (H5N2)	IN/IT	6	Day 3/ uncommon	Day 1		Van der Goot *et al.* (2003)
A/chicken/Pennsylvania/ 21525/83 (H5N2)	IT	4			+: lung, trachea; day 4[c]: pooled tracheal/ cloacal swabs	Mo *et al.* (1997)
A/chicken/Pennsylvania/ 21525/83 (H5N2)	DrC	6	NF	Day 3[d]		Van der Goot *et al.* (2003)
A/chicken/Hidalgo/ 26654-1368/94 (H5N2)	IO/IN/IT	3 to 4	NF	Day 2		Horimoto *et al.* (1995)
A/chicken/Hidalgo/ 26654-1368/94 (H5N2)	IN	4	Day 3[c]	Day 3[c]		Swayne *et al.* (1997)
A/chicken/Hidalgo/ 26654-1368/94 (H5N2)	IV	4	Day 3[c]	Day 3[c]		Swayne *et al.* (1997)
A/chicken/Queretaro/ 26654-1373/94 (H5N2)	IO/IN/IT	3 to 4	NF	Day 2		Horimoto *et al.* (1995)
A/chicken/Mexico/ 26654-1374/94 (H5N2)	IO/IN/IT	3 to 4	NF	Day 2		Horimoto *et al.* (1995)
A/chicken/Mexico/ 26654-1374/94 (H5N2)	DrC	3 to 4	NF	Day 5[d]		Horimoto *et al.* (1995)
A/chicken/Mexico/ 26654-1374/94 (H5N2)	IrC	3 to 4	NF	Day 7[d]		Horimoto *et al.* (1995)
A/chicken/Puebla/ 8623-607/94[e] (H5N2)	IO/IN/IT	3 to 4	Day 2	Day 2	+: brain, lung, spleen, liver, pancreas, kidney, colon, blood	Horimoto *et al.* (1995)
A/chicken/Puebla-8623-607/ 94[e] (H5N2)	IN	4	Day 3[c]	Day 3[c]		Swayne *et al.* (1997)
A/chicken/Puebla-8623-607/ 94[e] (H5N2)	IV	4	Day 3[c]	Day 3[c]		Swayne *et al.* (1997)
A/chicken/Puebla/14590-658/ 94 (H5N2)	IO/IN/IT	3 to 4	Day 3[c]	Day 3[c]		Horimoto *et al.* (1995)
A/chicken/I Ibaraki/ 1/05 (H5N2)	IN	6	Day 5	Day 3[c]	Day 3[c]: trachea, rectum; day 5: lung, kidney; day 7: pancreas	Okamatsu *et al.* (2007)
A/chicken/I Ibaraki/ 1/05 (H5N2)	DrC	6	Day 5[d] (faeces)			Okamatsu *et al.* (2007)
A/chicken/I Ibaraki/ 1/05 (H5N2)	IrC	6			Day 5[d]: drinking water in cage	Okamatsu *et al.* (2007)
A/Eurasiancoot/WA/ 2729/79 (H6N2	IV	8			+: kidney	Hooper *et al.* (1995)
A/whistling swan/Shimane/ 35/80 (H6N3	IT	5			Day 3[c]: brain, lungs, spleen, kidney, jejunum, rectum; day 5: liver	Otsuki *et al.* (1982)
A/whistling swan/Shimane/ 35/80 (H6N3	IP	5			Day 3[c]: lungs, kidney, rectum; day 5: brain, liver, jejunum	Otsuki *et al.* (1982)

Table 4 (*Continued*)

Virus	Route[a]	Age[b]	Faecal/cloacal shedding	Tracheal shedding	Virus/viral antigens in tissues	Reference
A/parrot/Ulster/73 (H7N1)	IN	2	Day 4[c]	NF		Alexander *et al.* (1978)
A/parrot/Ulster/73 (H7N1)	DrC	2	Day 4[cd]	NF		Alexander *et al.* (1978)
A/chicken/PA/3779-2/97 (H7N2)	IO/IN/IC	5	Day 2[c]			Lu *et al.* (2003)
A/chicken/PA/3779-2/97 (H7N2)	IN; $10^{4.7}$ ELD_{50}	5	Day 4			Lu & Castro (2004)
A/chicken/PA/3779-2/97 (H7N2)	IN; $10^{5.7}$ ELD_{50}	5	Day 7			Lu & Castro (2004)
A/chicken/PA/3779-2/97 (H7N2)	IC; $10^{4.7}$ ELD_{50}	5	Day 7			Lu & Castro (2004)
A/chicken/PA/3779-2/97 (H7N2)	IC; $10^{5.7}$ ELD_{50}	5	Day 2[c]		+: pooled trachea/lung/ kidney (rare)	Lu & Castro (2004)
A/chicken/PA/3779-2/97 (H7N2)	IO; $10^{4.7}$ ELD_{50}	5	Day 7			Lu & Castro (2004)
A/chicken/PA/3779-2/97 (H7N2)	IO; $10^{5.7}$ ELD_{50}	5	Day 2[c]			Lu & Castro (2004)
A/chicken/PA/3779-2/97 (H7N2)	IO/IN/IC	10	Day 2[c]	Day 2[c]		Lu *et al.* (2003)
A/chicken/PA/3779-2/97 (H7N2)	IO/IN/IC	14	Day 2[c]	Day 2[c]		Lu *et al.* (2003)
A/chicken/PA/3779-2/97 (H7N2)	IO/IN/IC	23	Day 3[c]	Day 3[c]		Lu *et al.* (2003)
A/chicken/PA/3779-2/97 (H7N2)	IC/IN/IO	23	Day 3[c]	Day 5	+: trachea, lung, intestine, oviduct	Lu & Castro (2004)
A/chicken/PA/3779-2/97 (H7N2)	IM; $10^{4.7}$ ELD_{50}	5	Day 2			Lu & Castro (2004)
A/chicken/PA/3779-2/97 (H7N2)	IM; $10^{5.7}$ ELD_{50}	5	Day 2[c]			Lu & Castro (2004)
A/chicken/PA/3779-2/97 (H7N2)	DrC; $10^{4.7}$ ELD_{50}	5	Day 2[cd]			Lu & Castro (2004)
A/chicken/PA/3779-2/97 (H7N2)	DrC; $10^{5.7}$ ELD_{50}	5	Day 7[d]			Lu & Castro (2004)
A/chicken/New York/ 21586-8/99 (H7N2)	IN	3 to 4	Day 2	Day 1	Day 3: trachea, lung; day 5: air sac	Swayne & Beck (2005)
A/turkey/Virginia/ 158512/02 (H7N2	IN	3 to 4	Day 5/ uncommon	Day 1	Day 3: trachea, lung	Swayne & Beck (2005)
A/duck/Victoria/ 76 (H7N7)	IN	14	Day 4	Day 2		Westbury *et al.* (1979)
A/duck/Victoria/ 76 (H7N7)	DrC	14	Day 4[d]	Day 4[d]		Westbury *et al.* (1979)
A/whistling swan/Shimane/ 42/80 (H7N7)	IT	5			Day 3[c]: lungs, spleen, kidney, jejunum, rectum; day 5: liver, brain	Otsuki *et al.* (1982)
A/whistling swan/Shimane/ 42/80 (H7N7)	IP	5			Day 3[c]: lungs, spleen, kidney, jejunum, rectum, liver, brain	Otsuki *et al.* (1982)
A/black-tailed gull/Tottori/ 61/80 (H7N7)	IP	5			Day 3[c]: lungs, spleen, brain, liver, kidney, rectum; day 5: jejunum	Otsuki *et al.* (1982)
A/black-tailed gull/Tottori/ 61/80 (H7N7)	IT	5			Day 3[c]: lungs, kidney, jejunum, rectum; day 6: liver, spleen	Otsuki *et al.* (1982)
Various isolates from wild ducks, United States (H2N1, H3N2, H3N8, H3,4N6, H4N1, H4N2, H4N6, H4N8, H6N2, H11N1, H11N2. H11N3, H11N9)	IV	6	Day 1	Day 1	Day 1: thymus, spleen, pancreas, gonads, kidney, blood, lung, jejunum, ileum, bursa, air sac, liver; day 3: brain, bone marrow	Condobery & Slemons (1992)

+, virus found, day(s)not specified. NF = virus was not found on any days examined; IN = intranasal; IM = intramuscular; IC = intraconjunctival or intraocular; IO = intraoral/ feeding; IT = intratracheal; IV = intravenous; DrC = direct contact; IrC = indirect contact; ELD50 = 50% embryo lethal dose. [a]Route of inoculation; dose if variable doses were administered. [b]Age of chickens in weeks. [c]Virus was isolated on the first day virus shedding was examined. [d]Measured from the day of inoculation for birds in contact. [e]This virus would be considered HPAI by current standards, based on the cleavability of its haemagglutinin.

2005a, 2007). Although the isolation of HPAI viruses has not yet been reported from the meat of naturally infected chickens, Asian HPAI H5N1 viruses have been found in meat from naturally infected ducks (Tumpey *et al*., 2002; Mase *et al*., 2005).

Although some LPAI viruses inoculated intravenously may bypass natural barriers and spread to other tissues (Slemons & Swayne, 1990; Condobery & Slemons, 1992), these viruses are generally thought to remain localized in the respiratory and gastrointestinal tract when not inoculated by intravenous or intramuscular injection (Hooper & Selleck, 1998; Swayne & Beck, 2005; Zepeda & Salman, 2007). Some LPAI viruses have been isolated from a limited number of other tissues including the pancreas, kidneys, and oviduct of intranasally inoculated chickens (Lu & Castro, 2004; Okamatsu *et al*., 2007), and from the kidneys, ovary, and oviduct of intratracheally inoculated birds (Shalaby *et al*., 1994). Unusually, one study reported that three H6N3 or H7N7 viruses isolated from asymptomatically infected wild birds were recovered from many tissues including the respiratory tract, intestinal tract, spleen, kidney, liver, and brain of intratracheally inoculated chickens (Otsuki *et al*., 1982). No studies have reported the occurrence of LPAI viruses in meat. One study examining the distribution of HPAI and LPAI viruses in meat found that neither A/turkey/Virginia/158512/02 (LPAI; H7N2) nor A/chicken/New York/21586-8/99 (LPAI; H7N2) spread beyond the respiratory tract in intranasally inoculated chickens (Swayne & Beck, 2005). Similarly, A/chicken/Ibaraki/1/05 (H5N2) was not found in the muscle, brain, or spleen of intranasally inoculated chickens despite its occurrence in the kidney and pancreas (Okamatsu *et al*., 2007). To date, the risk for LPAI viruses in meat appears to be minimal (Zepeda & Salman, 2007).

HPAI and LPAI viruses in eggs

The surfaces of eggs laid by hens in flocks infected with avian influenza viruses may be contaminated by infective faeces. Eggs may also, in some cases, contain viruses in the albumen or yolk. To date, two HPAI viruses, A/chicken/Pennsylvania/83 (H5N2) (Beard *et al*., 1984; Bean *et al*., 1985) and A/FPV/Dutch/27 (H7N7) (Moses *et al*., 1948), have been isolated from the internal contents of eggs from experimentally infected hens. A/FPV/Dutch/27(H7N7) was found in the ovary and the yolk mass of a sessile ovum from an intramuscularly inoculated hen that died at 37 h, as well as in the yolk and albumen of a fully formed egg found in the oviduct of a hen that died at 35 h (Moses *et al*., 1948). H5N2 HPAI viruses have been found in eggs from intranasally inoculated hens. In one study, an H5N2 HPAI virus was recovered from 12 out of 14 eggs laid by infected hens on day 3 p.i., concurrently with the onset of significant clinical signs, but from not from eggs laid on day 2 (Beard *et al*., 1984). Eleven of these eggs contained virus on the shell surface, possibly from faecal contamination, but nine eggs contained virus in the yolk and 11 eggs had virus in the albumen. Virus was also found in all three eggs laid on day 4. Bean *et al.* (1985) isolated a closely related H5N2 virus from the albumen and the yolk of three eggs laid by intranasally inoculated hens on the day of their death. Of the 37 eggs laid throughout the experiment, only these three eggs contained virus. An H5N2 HPAI virus isolated from the same outbreak was also found in the ovaries of hens inoculated into the air sac (Brown *et al*., 1992).

Only H5N2 viruses have been reported, to date, in the yolk and albumen of eggs from naturally infected chicken flocks. Cappucci *et al.* (1985) isolated H5N2 viruses from the yolk and albumen of eggs from five HPAI-infected Pennsylvania flocks. In these flocks, 17% to 56% of the eggs collected after the onset of clinical signs contained virus internally, and 9% to 50% contained virus on the shells. It is not known when virus shedding began; however, in one flock, virus was not found in the yolk or albumen of eggs collected 1 day after the onset of clinical signs, but it was present in eggs collected 4 days later. Interestingly, some of the viruses recovered from eggs were not as virulent in the intravenous pathogenicity test as viruses from clinical specimens. It should be noted that viruses were not recovered from the eggs of two HPAI-infected flocks in this study. Beard *et al.* (1984) also tested eggs from four infected flocks identified during this outbreak, and did not recover avian influenza viruses from dead embryos, non-fertile eggs or 214 live chicks that hatched. Other HPAI viruses have not yet been recovered from eggs, but A/chicken/Hong Kong/220/97 (H5N1) antigens were detected within 1 to 2 days in the ovary of 4-week-old, intranasally inoculated chickens (Perkins & Swayne, 2001), and A/chicken/Chile/184240-1/02 (H7N3) antigens were found on day 2 p.i. in the gonads of intranasally inoculated chickens (Jones & Swayne, 2004). Asian HPAI H5N1 viruses have been found in the internal contents of quail eggs (Promkuntod *et al*., 2006).

Although reproductive lesions including salpingitis and oophoritis have been reported in some LPAI-infected flocks (Johnson & Maxfield, 1976; Johnson *et al*., 1977; Hooper & Selleck, 1998; Ziegler *et al*., 1999; Kinde *et al*., 2003; Mutinelli *et al*., 2003; Swayne & Pantin-Jackwood, 2006), as well as in intravenously inoculated hens (Shalaby *et al*., 1994), there is no definitive evidence that LPAI viruses are shed in eggs. The most direct evidence for shedding is the recovery of a non-pathogenic H5N2 virus (intravenous patho genicity index = 0) from the internal contents of two of 120 eggs laid by an asymptomatically infected, commercial layer flock in Virginia during the H5N2 (HPAI) Pennsylvania outbreak (Cappucci *et al*., 1985). H7 and H4 LPAI viruses have also been found in the oviduct of naturally infected flocks (Ziegler *et al*., 1999), and in the oviduct and/or ovary of experimentally inoculated chickens (Shalaby *et al*., 1994; Lu & Castro, 2004); however, no virus was recovered from the internal contents of 20 eggs laid by the hens in one of these studies (Shalaby *et al*., 1994). According to a personal communication by P. Dunn cited in Swayne & Beck (2004), no virus-positive eggs were found in the albumen of nearly 10 000 eggs from H7N2 (LPAI)-infected Pennsylvania flocks between 1996 and 1998. LPAI viruses were also absent from shell swabs, albumen, and yolk samples from 120 eggs collected during an LPAI H7N2 outbreak in 2001/2002 (Lu *et al*., 2004). It can be inferred from these data that LPAI viruses are either absent from the internal contents of eggs or present at very low levels. Nevertheless, the occurrence of LPAI viruses in ovarian tissues makes shedding in eggs a possibility. Furthermore, faecal contamination of the shell can occur in all infected flocks.

Can infected flocks be recognized by clinical signs or changes in production parameters before virus shedding begins?

If a rise in the mortality rate, a decrease in feed and water consumption, a reduction in egg laying, or another sign is to be used as an indicator to prevent contaminated poultry products from being distributed, it must consistently occur at the same time as or precede virus shedding. Because LPAI viruses are often shed from clinically normal chickens and birds showing minimal clinical signs (Westbury *et al*., 1979; Otsuki *et al*., 1982; Slemons & Swayne, 1990; Condobery & Slemons, 1992; Lu *et al*., 2003; Lu & Castro, 2004; Okamatsu *et al*., 2007), there is no consistent sign that would precede the shedding of these viruses. Decreased egg production, decreased feed and water consumption, increased mortality, or the occurrence of clinical signs should trigger an investigation, and may result in the recognition of an infected flock. Nevertheless, some flocks infected with LPAI viruses will be detected only by routine testing.

Few studies have examined the shedding of HPAI viruses in faeces and respiratory secretions relative to the onset of clinical signs. In those that have, virus shedding began slightly earlier than (Westbury *et al*., 1979; Forman *et al*., 1986) or around the same time as (Beard *et al*., 1984; Jones & Swayne, 2004; Lee *et al*., 2005a) the first clinical signs appeared. In two experiments, a few birds exhibited depression the day before virus shedding began (Beard *et al*., 1984; Jones & Swayne, 2004); however, in both studies it is questionable whether these signs would have been apparent in a large flock. Beard *et al.* (1984) reported that a high pathogenicity H5N2 virus was recovered beginning day 3 p.i. from hens, 1 day after depression was first seen. However, only one of the 25 birds was depressed on day 2 p.i., while the majority of the hens exhibited clinical signs beginning day 3 p.i. Similarly, Jones and Swayne (2004) reported that one of eight 4-week-old chickens inoculated with A/chicken/Chile/184240-1/02 (H7N3) exhibited mild depression on day 1 p.i., the day before virus was first found throughout the body and most clinical signs appeared. Some studies have reported that HPAI viruses may be shed a few days before clinical signs begin. Westbury *et al.* (1979) found that 14-week-old, intranasally inoculated chickens shed two Australian H7N7 viruses from both pharyngeal and cloacal swabs by day 2 p.i., but the first signs occurred on day 5. Similarly, A/chicken/Victoria/85 (H7N7) was recovered for 1 to 4 days before death in intranasally inoculated, 14-week-old chickens; most of these birds died suddenly, although some were ill for a day before death (Forman *et al*., 1986).

HPAI viruses can be found in meat very soon after experimental inoculation, and may be present before the clinical signs become obvious. In both older hens and 15-week-old male chickens inoculated with HPAI A/chicken/Pennsylvania/1370/83 (H5N2), viral antigens first appeared in the ocular muscles 1 day before the onset of clinical signs (day 2 p.i. in the hens and day 3 p.i. in the younger birds) (Brown *et al*., 1992). Breast or thigh meat was not examined in this study; however, some other tissues also contained viral antigens as early as day 1 p.i. In another study, the same virus was found in bone, breast, and thigh meat of intranasally inoculated chickens beginning day 1 p.i. (Swayne & Beck, 2005). Whether HPAI viruses are shed in eggs before the onset of clinical signs is unknown. As discussed earlier, although antigens from Asian HPAI H5N1 viruses have been detected in the ovary of some intranasally inoculated chickens within the first 2 days p.i. (Perkins & Swayne, 2001; Jones & Swayne, 2004), two studies that address virus shedding within fully formed eggs suggest that HPAI viruses are not shed until clinical signs have become apparent (Beard *et al*., 1984; Bean *et al*., 1985). The onset of clinical signs, mortality, and virus shedding in chickens infected with Asian HPAI H5N1 viruses is summarized in Table 5.

Table 5. *Onset of clinical signs, mortality, and virus shedding in Asian HPAI H5N1-infected chickens*

Clinical signs reported in H5N1-infected chickens	Sudden death (Nakatani *et al*., 2005; Swayne, 2007; Tsukamoto *et al*., 2007), depression (Perkins & Swayne, 2001; Jones & Swayne, 2004; Lee *et al*., 2007; Tsukamoto, 2007), ruffled feathers (Lee *et al*., 2007; Tsukamoto *et al*., 2007), respiratory signs (De Benedictis *et al*., 2007), cyanotic combs and wattles (De Benedictis *et al*., 2007), excessive lacrimation (Lee *et al*., 2007), swelling of the head and leg joints (Shortridge *et al*., 1998), oedema of the limbs (De Benedictis *et al*., 2007), subcutaneous haemorrhages (Shortridge *et al*., 1998; De Benedictis *et al*., 2007; Lee *et al*., 2007), increased faecal fluid and urates (Perkins & Swayne, 2001), haematochezia (Perkins & Swayne, 2001), weakness (Shortridge *et al*., 1998), neurological signs (Shortridge *et al*., 1998; De Benedictis *et al*., 2007)
Morbidity rate (%), chickens inoculated intranasally	80 to 100
Mortality rate (%), chickens inoculated intranasally	80 to 100
MDT (days) (intranasally)	1.5 to 5.5
MDT (days) (intravenously)	1 to 4.1
Virus shedding from intranasally inoculated chickens	
Tracheal shedding	On or before day 2 p.i. (Lee *et al*., 2005; Swayne *et al*., 2006; Bublot *et al*., 2007)
Faecal shedding	On or before day 2 p.i. (Lee *et al*., 2005; Swayne *et al*., 2006; Bublot *et al*., 2007)
Virus or viral antigens in tissues	
On day 1 p.i.	Upper respiratory tract, lung, spleen, liver, thymus, feather follicle, bone marrow, brain, heart, kidney, bursa, pancreas (Perkins & Swayne, 2001)
On or before day 2 p.i.	Lung, trachea, heart, liver, spleen, kidney, pancreas, bursa, brain (Lee *et al*., 2007)
On or before day 2 to 3 p.i.	Skeletal muscle (breast meat, thigh meat), lung, kidney, brain (Tumpey *et al*., 2002)
Time not specified	Skeletal muscle (breast meat) (Swayne & Beck, 2005), ovary, testis, adrenal gland (Perkins & Swayne, 2001)

Together, these studies suggest that experimentally infected chickens shed HPAI viruses in faeces and meat either at the same time as, or a few days before, the clinical signs appear. Limited information, mainly from one virus, suggests that HPAI viruses are not found in eggs until the clinical signs have become apparent in the infected bird. In a naturally infected flock, the onset of clinical signs or changes in production parameters is also affected by virus transmission within the flock, a factor addressed in the final section of this review.

Virus transmission and the recognition of clinical signs in a naturally affected flock

Commercial flocks often experience a persistent 'baseline' level of morbidity and mortality. In these flocks, the recognition of an outbreak is triggered by a change in a production parameter such as the mortality rate, feed and water consumption or egg production, or by the occurrence of clinical signs in a significant percentage of the flock. When this happens depends not only on the virulence of the virus, but also on the dose each individual bird receives, and the rate of virus transmission through the flock. Host factors that may influence transmission and the development of clinical signs include the birds' breed, age and existing immunity to influenza viruses, as well as any concurrent infections (Allan *et al*., 1974; Alexander *et al.*, 1986; Van der Goot *et al*., 2003). Environmental factors that may influence transmission include the stocking density, size of the room, temperature, and airflow (Alexander *et al*., 1986; Van der Goot *et al*., 2003). For instance, avian influenza viruses are thought to propagate more slowly in caged flocks, where the virus must be transmitted between spatially separated cages, than in loose-housed flocks (flocks housed without barriers between the birds); this is supported by descriptions of some H5N1 and H7 HPAI outbreaks (Mutinelli *et al*., 2003; Sims *et al*., 2003; Nakatani *et al*., 2005; Elbers *et al*., 2007) and by models and analyses of avian influenza transmission (Savill *et al*., 2006; Tiensin *et al*., 2007). In loose-housed birds, HPAI viruses are thought to spread rapidly; however, there have been a few reports of slow transmission during the initial stage of the infection. In Japan, an H5N1 HPAI virus (A/chicken/Yamaguchi/7/04) spread slowly on one farm for more than 1 week (Tsukamoto *et al*., 2007). On this farm, eight dead chickens without typical avian influenza lesions were found at two sites near windows on 28 December. Although a few more dead chickens were found near these sites each day, no typical clinical signs were seen in the majority of the flock until the mortality rate reached 200 chickens per day on 5 January. At this point, deaths increased dramatically, with 70% of the chickens dead by 13 January. Westbury *et al.* (1981) similarly reported that, during the 1975 to 1976 H7N7 HPAI outbreak in Australia, susceptible chickens in some flocks did not become infected, although they were exposed to infected birds for up to 3 weeks. Interestingly, the possibility of situations like these was predicted in 1986, by Alexander (2003).

A partial explanation for the reported variability in transmission may lie with the individual virus. Alexander *et al.* (1978) studied the transmission of several HPAI viruses in chickens, and found that onset of clinical signs or death varied with the isolate. A/fowl/Germany/34 (H7N1) killed all inoculated birds day 2 p.i., without many prodromal signs. Naïve chickens allowed to mix freely with these birds remained healthy until day 3, when one bird was found dead and another was ill; 70% of the uninoculated birds were dead by day 4, while the remaining birds did not become sick. Chickens inoculated with A/FPV/Dutch/27 (H7N7) died more slowly and displayed more marked clinical signs: sick birds were seen beginning day 2 p.i., and all inoculated birds were dead by day 6. Contact birds became ill starting day 4, usually died within 48 h of the onset of illness, and were all dead by day 15. Chickens inoculated with A/fowl/Victoria/75 (H7N7) displayed severe clinical signs, but some of the birds remained alive until day 15 p.i. Four contacts had clinical signs before death, while four others died without prior signs on days 10, 11, and 14, and two birds remained healthy.

Some experimental studies suggest that certain viral strains require close contact with infected birds or faeces for transmission (Westbury *et al*., 1981; Forman *et al*., 1986; Shortridge *et al*., 1998), while other viruses spread readily between cages (Horimoto *et al*., 1995; Okamatsu *et al*., 2007). Two LPAI viruses, A/chicken/Ibaraki/1/05 (H5N2) (Okamatsu *et al*., 2007) and A/chicken/Mexico/26654-1374/94 (H5N2) (Horimoto *et al*., 1995), appear to spread rapidly to birds in nearby cages by both contact and airborne transmission. In contrast, a recently isolated, high-pathogenicity Asian H5N1 virus spread to chickens in the same cage as inoculated birds, as well as to chickens in the cage below, but did not infect birds in an adjacent cage (Shortridge *et al*., 1998). Similar results were reported with two viruses isolated during the 1975 to 1976 HPAI outbreak in Australia. In experimental studies, these viruses spread to uninoculated chickens in direct contact, but did not infect chickens suspended in cages one bird height above the floor (Westbury *et al*., 1981). Interestingly, A/duck/Victoria/76 (H7N7), which did not cause clinical signs, spread more rapidly and completely than the virulent isolate A/fowl/Victoria/75 (H7N7). Similarly, A/chicken/Mexico/26654-1374/94 (LPAI; H5N2), which was not lethal in chickens, spread readily by both contact and airborne transmission, while a more virulent H5N2 virus from this outbreak was not isolated from uninoculated birds sharing the same cage (Horimoto *et al*., 1995). Some studies suggest that viruses that kill birds rapidly with few clinical signs, and thus are not excreted for long periods, are less likely to spread readily (Alexander *et al*., 1978; Alexander *et al*., 1986; Alexander, 2003). In contrast, Van der Goot *et al.* (2003) found that A/chicken/Pennsylvania/1370/83 (H5N2), an HPAI virus, was infectious for a longer period than a closely related LPAI virus from the same outbreak, and transmission occurred more readily from these birds to susceptible contacts.

The number of infected and uninfected chickens influences the likelihood of transmission in some studies. A recent study examined contact and airborne transmission of A/chicken/Yamaguchi/7/04 (H5N1) when the number of inoculated and uninoculated chickens in direct contact and in nearby cages was varied (Tsukamoto *et al*., 2007). This study found that both contact and airborne transmission occurred slowly and inefficiently when only one or two chickens were infected initially, but more efficiently when additional chickens were inoculated. When one inoculated chicken was

added to four uninoculated chickens, the inoculated chicken died by day 2 p.i., but neither the chickens in the same cage nor four chickens in a nearby cage had any sign of illness. With two inoculated and two uninoculated chickens sharing a cage, and four chickens in the separated cage, the inoculated chickens and one contact chicken died by day 3 p.i., but the remaining chickens did not become infected. However, when four inoculated and four uninoculated chickens were placed in one cage, and four chickens were in a nearby cage, all four inoculated chickens died by day 2 p.i., the four contact chickens died during the following 2 days, and three chickens infected by airborne transmission died on days 4 and 8 p.i. These findings may not apply to all avian influenza viruses. In experiments with the HPAI virus A/turkey/Ontario/7732/66 (H5N9), increasing the number of infected birds and placing the birds in crowded conditions did not increase transmission (Narayan *et al.*, 1969).

Models of transmission. Although studies in small groups of birds provide useful insights into the transmission of specific isolates, it is difficult to extrapolate from these studies to naturally infected flocks. A few studies have attempted to model virus transmission in flocks. One model, developed to back-calculate the day an H7N7 HPAI virus is introduced into a flock, suggests that recognizing an HPAI outbreak by an increase in the mortality rate may take more than 1 week in loose-housed birds (Bos *et al.*, 2007). This study found that if a single infected bird, not yet excreting virus, introduces the virus into a flock of 10 000 birds, and reporting occurs when the mortality rate is at least 0.5% per day on two consecutive days, the virus was probably introduced 12 days (range 11 to 15 days) before detection. A noticeable rise ($>0.5\%$) in the number of dead chickens does not occur until day 10, and most of the susceptible chickens became infected at 9 to 12 days. Increasing the detection threshold to 0.01% on two consecutive days only decreases the interval to 10 days (range 7 to 12 days). One interesting aspect of this model is that the flock size does not have a major effect on the detection time. Although the range varies slightly, the mean time to detection is the same in a flock of 100, 4000, or 40 000 birds. The parameters of the model (1 to 2 days between infection and virus shedding; infectious period of 6.3 days) are based on experimental transmission of the H7N7 virus A/chicken/Netherlands/621557/03 within a limited number of birds. This model concurs with a summary of the 1997 to 2002 H5N1 HPAI outbreak in Hong Kong, which suggested that the flock mortality rate may not rise significantly for a week or more (Sims *et al.*, 2003). Another analysis of the 2003 H7N7 HPAI outbreak in the Netherlands concluded that the mortality rate increased exponentially, with a five-fold to 10 fold rise from baseline mortality on the first day, a five-fold to 10 fold rise on the subsequent day, and a 3% flock mortality rate within 2 to 3 days after the first day of elevated mortality (Elbers *et al.*, 2007). A regression model of 10 affected farms during the 2003 to 2004 H5N1 HPAI epidemic in Korea suggests that these farmers first recognized an increased mortality rate approximately 5 days (range 1 to 8 days) after a virus entered the flock, with the entire flock expected to die in 12.5 days (range 7 to 16 days) of virus entry (Yoon *et al.*, 2005). In contrast to the study by Bos *et al.* (2007), this analysis also suggested that the disease spread more rapidly on farms with larger numbers of chickens.

Within-flock transmission can be quantified by the basic reproduction number (R_0), an estimate of the number of secondary cases from each infected bird in a completely susceptible flock, and the transmission rate parameter (β), the average rate at which animals become infected. Models usually estimate R_0 and β from transmission experiments in small numbers of experimentally infected birds. In one model described above, Bos *et al.* (2007) estimated β to be 33 new birds infected per day. When modelling the spread of Asian HPAI H5N1 viruses, Savill *et al.* (2006) estimated R_0 to be approximately 66 for unvaccinated floor-reared birds and approximately 25 for caged birds with eight birds per cage, five cages in a column, and 250 cages in a row. Because outbreaks in the field can be influenced by the type of housing, flock management, or other factors, their R_0 and β values could differ (Van der Goot *et al.*, 2003; Tiensin *et al.*, 2007). Recently, a study estimated β to vary from a mean of 2.26 new birds infected per day (for a 1-day infectious period) to 0.66 birds per day (for a 4-day infectious period), during the 2005 H5N1 epidemic in Thailand (Tiensin *et al.*, 2007). In this study, R_0 varied from 2.26 to 2.64.

Overall, these models suggest that increased mortality may remain unrecognized for a week, and possibly longer, after some HPAI viruses enter a flock. Small-scale transmission studies in experimentally infected birds suggest that the transmission rate may vary with the virus, with some viruses spreading readily in aerosols and others transmitted mainly during close contact. Models or studies estimating the onset of other parameters such as decreased feed and water consumption, depression, or decreased egg laying have not been published. Extrapolating from studies in experimentally infected birds, these parameters might be expected to change from one to several days before the onset of increased mortality. It appears likely that virus shedding would be present in the meat or eggs of some HPAI-infected flocks before this time. Whether infected meat would reach consumers or poultry processors depends on whether a broiler flock is sent to slaughter before the infection is recognized. Whether eggs could reach consumers varies with the time before eggs reach the market and with the type of marketing (continuous or in batches). One possibility would be to hold eggs in cold storage while the flock continues to be observed for clinical signs or changes in production parameters. After the maximum potential interval between shedding of virus in the egg and observation of clinical signs has elapsed, the eggs in cold storage could be considered free of virus.

Conclusions

Although some HPAI and LPAI viruses can be shed in the faeces and respiratory secretions of unvaccinated chickens within the first 1 to 2 days after infection, different viral strains appear to vary in their transmissibility as well as in the clinical signs they cause. Outbreak descriptions and studies in experimentally infected birds suggest that some HPAI viruses are likely to be found in the meat and eggs, and could occur in these poultry products before an infected flock is recognized. In contrast, there appears to be little or no

risk of transmission of LPAI viruses in meat. The shedding of LPAI viruses in the yolk or albumen of eggs at a low incidence has not been proven or disproved, and it would be valuable to clarify whether LPAI viruses can be shed in eggs. It should be noted that the present review has examined studies on virus shedding and clinical signs only in unvaccinated birds. Vaccination would be expected to decrease virus shedding (Tian *et al.*, 2005; Swayne *et al.*, 2006; Bublot *et al.*, 2007) and transmission (Van der Goot *et al.*, 2005), and reduce the severity of clinical signs (Tian *et al.*, 2005; Swayne *et al.*, 2006; Bublot *et al.*, 2007). Depending on the challenge dose and other factors, it would be possible for vaccinated chickens to become infected and shed virus while remaining asymptomatic or minimally affected (Tian *et al.*, 2005; Swayne *et al.*, 2006; Bublot *et al.*, 2007). Currently, the most effective method for recognizing the first occurrence of avian influenza in a flock appears to be periodic laboratory testing of the flock, and immediate testing of ill and dead birds. Studies examining the first occurrence of decreased feed and water consumption, decreased egg laying, or depression may reveal changes in these parameters that could also be useful in early recognition.

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