The Onset of Virus Shedding and Clinical Signs in Turkeys Infected with High and Low Pathogenicity Avian Influenza Viruses

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Abstract

The possibility that avian influenza viruses may be found in raw poultry products raises concerns about the potential for human exposure via meat and eggs from flocks with unrecognized infections. This review examines the onset of virus shedding and the development of clinical signs for various avian influenza viruses in turkeys. In experimentally infected birds, some high pathogenicity avian influenza (HPAI) and low pathogenicity avian influenza (LPAI) viruses can occur in feces and respiratory secretions as early as one to two days after inoculation. HPAI viruses (HPAIVs) have also been reported in tissues, including meat, as soon as day 1 PI. LPAI viruses (LPAIVs) have never been isolated from turkey meat, although one group reported the presence of nucleic acids from an H7 LPAIV and isolated the same virus from blood. Two viruses, an H5N9 HPAIV and an H3N2 swine-origin LPAIV, have been detected in the internal contents of turkey eggs. Studies in turkeys inoculated with HPAIVs suggest that clinical signs usually develop within a few days of virus shedding; however, surveillance based solely on clinical signs might fail to detect infected flocks early after infection. During this time, avian influenza viruses may be found in turkey products unless flocks are actively monitored with laboratory tests. LPAIVs can be shed by asymptomatically infected or minimally affected flocks, but these viruses appear unlikely to cause significant human disease.

Introduction

Avian influenza viruses are a highly heterogeneous group of viruses with varying pathogenicity in different species of birds. These viruses are classified into subtypes based on two variable envelope glycoproteins, the hemagglutinin (HA) and neuraminidase (NA). Sixteen variants of the hemagglutinin (H1 to H16) and nine variants of the neuraminidase (N1 to N9) have been recognized [1]. Two pathotypes have also been defined. High pathogenicity avian influenza (HPAI) viruses cause severe and fatal infections in chickens and turkeys, while low pathogenicity (LPAI) viruses are generally much less virulent in these birds. The pathotypes are defined by the possession of certain molecular determinants in the hemagglutinin, and by their virulence in young chickens, although they also affect other species [2]. LPAIVs can contain any hemagglutinin, but HPAIVs contain either H5 or H7.

Avian influenza viruses may infect people who are in close contact with poultry. The ability of some viruses to cause severe human illness was first recognized in the late 1990s, with the emergence of a lineage of H5N1 HPAIVs in Asia. This has resulted in increased concern about the potential for avian influenza viruses to contaminate poultry products. Most confirmed human infections with the currently circulating Asian lineage H5N1 HPAIVs have been serious, and more than half have been fatal [3;4]. H7 HPAIVs have also caused human illness, although most documented cases have been limited to conjunctivitis and flu-like symptoms, and fatal cases have been rare [3;5-7]. Some other subtypes may also infect people who interact closely with poultry. Based on serological surveillance and occasional reports of clinical cases in Asia, some H9 viruses may be especially likely to infect humans [8-10]. Although the data are limited, symptomatic H9N2 infections have been clinically indistinguishable from human influenza [3;7-12]. Serological responses to H4, H5, H6, H7, H10 and H11 viruses have also been documented in healthy people who are exposed frequently to birds [9;10;13-16]. Volunteers inoculated with LPAIV subtypes including H4N8, H10N7 and H6N1 became infected, and in some cases, developed mild respiratory signs and other influenza symptoms [15].

Most human infections result from direct contact with sick or dead poultry, but other routes of transmission may be possible [7]. Although there is little evidence that ingestion is ordinarily an important route of exposure for humans, two H5N1 infections in people were linked to eating uncooked duck blood [3], and other mammals including cats [17;18], dogs [19] and red foxes (*Vulpes vulpes*) [20] have been infected when they ate contaminated poultry. Consumers might also convey the virus to mucous membranes on unwashed hands or by contact with other fomites. One recent H5N1 infection occurred in a woman who had no exposure to poultry except through the raw duck blood and chicken hearts processed in the home and sold at her husband's food stand [21]. Turkey eggs are ordinarily not sold for human consumption in the U.S.; however, they can be found in supermarkets in some countries such as the U.K. Risks to consumers can be mitigated by public education, in addition to excluding viruses from the food supply. People who follow good sanitary practices during food preparation and eat only fully cooked meat and eggs are expected to have little risk of exposure; avian influenza viruses are heat

labile and are killed by the cooking methods recommended for destroying *Salmonella* and other pathogens in poultry meat [3]. Hazards to workers who process contaminated poultry are more difficult to control. These workers could be at risk from viruses that become aerosolized when feathers contaminated with desiccated fecal material are removed during mechanical processing. Contaminated eggs could also pose an exposure hazard in hatcheries or for keepers of backyard flocks who hatch their own poults. Whether zoonotic viruses in meat, other poultry products, and fresh or desiccated feces can present a risk to consumers, poultry processors or people who handle hatching eggs depends partly on how rapidly infected flocks are identified, and how quickly viruses are shed. In this review, we examine the literature describing the onset of virus shedding in feces, respiratory secretions, meat and eggs from turkeys. We also review studies describing the onset of clinical signs in both experimentally infected turkeys and naturally infected flocks.

The onset of clinical signs in HPAIV infected turkeys

Clinical signs of HPAI are not pathognomonic: they are generally nonspecific, and their frequency and type can vary with the strain of virus [1;22-25]. Turkeys infected with HPAIVs can be found dead without prodromal signs [24-27]. Other reported clinical signs in this species include depression, unusual quietness, ruffled feathers, a pale appearance, reduced feed and/or water consumption, weight loss, oculonasal discharge, sinusitis, erythematous lesions around the head, diarrhea, hemorrhages on the shanks, and swelling of the head, wattles and/or feet [23;24;27-35]. Neurological signs are common in turkeys [24;28;30;32;36]. Avian influenza viruses that kill birds quickly may cause fewer signs than when the viral strain or dose allows the birds to survive longer [24;30]. It should be noted that some viruses classified molecularly as HPAIV are avirulent in pathogenicity tests, and may come from flocks with signs that are initially more consistent with LPAI [2;37].

The clinical picture can vary with the flock and virus; signs expected to be common in HPAIV-infected gallinaceous birds have been absent or rare in turkeys during some outbreaks [22;26;38]. Elbers et al. (2005) attempted to identify the most specific and sensitive clinical indicators for various types of poultry flocks during the 2003 H7N7 HPAI outbreak in the Netherlands. In turkey flocks, common clinical signs were increased mortality, reduced feed and water consumption, depression, respiratory signs and diarrhea. The most sensitive (100%) and specific (79%) signs in turkeys were depression, reduced growth performance, huddling, decreased vocalization, swollen sinuses, yawning, mucosal secretions from the beak, and recumbency with an extended neck. Likewise, the most prominent gross lesions can be variable [22;25;26], and may differ between turkeys and chickens infected with the same virus [30]. In some cases, lesions may be absent or minimal [24]. Turkeys should be tested for HPAIV whenever there is high mortality or other indications consistent with this disease, even if the necropsy results do not specifically suggest HPAI [26].

Some information about the onset and sequence of clinical signs can be derived from experimentally infected birds, although detailed descriptions are generally unavailable for

turkey flocks during outbreaks. In turkeys inoculated by a natural (e.g., respiratory) route, the first sign may be sudden death [24;25], or the birds may become noticeably ill before dying [24;29;30]. The onset and expression of clinical signs can vary with the isolate (Table 1) and the dose of virus [29;39]. With some viruses, the period of illness is brief. Turkeys inoculated with A/fowl/Germany/34 (H7N1) appeared normal on day 1 PI and died suddenly on day 2 [24]. In the same study, turkeys infected with A/FPV/Dutch/1927 (H7N7) died a day or two later, and some birds had nonspecific signs of illness including depression, reluctance to move and ruffled feathers on the day before they died. Turkeys inoculated with A/fowl/Victoria/75 (H7N7) survived the longest, and developed paralysis as well as generalized signs of illness before dying, with a mean death time (MDT) of 5.2 days. Similarly, Westbury et al. (1979) reported that clinical signs developed relatively slowly (5-10 days PI) in turkeys inoculated with a closely related Australian H7N7 virus. A/turkey/Ontario/7732/66 (H5N9) killed a few turkeys suddenly within the first 24-48 hours [29]. Other birds developed depression and inappetence beginning 20 hours after inoculation, followed by diarrhea, ruffled feathers and progressive somnolence, and died 1-2 days later. In other trials with this virus, the first signs of illness occurred 2-6 days PI, with a more rapid onset when the dose was higher. Three studies examined turkeys infected with Asian lineage H5N1 viruses. One group reported that neurological signs first occurred at 1.5 days PI and increased fecal fluid and urates at 1-1.5 days, with death soon afterward (MDT of 2.5 days) [30]. Two other studies also reported that H5N1-infected turkeys usually died within the first few days [31;39]. Kilany et al. (2011) stated that the birds became reluctant to move and developed sinusitis, oculonasal discharge, edema of the face and hemorrhages on the shanks, but did not describe the timing of these signs. The clinical signs were not described in the other report [39].

Changes in production parameters as early indicators of an HPAI outbreak

Parameters often measured in commercial poultry production, such as the mortality rate, feed consumption, water consumption and egg production, can be used as indicators for disease outbreaks. The mortality rate is expected to be high in HPAIV-infected flocks, and is a useful indicator for this disease [1;26]. Based on studies in experimentally infected birds, the MDT would be expected to vary with the isolate and the dose of virus each bird receives. Reported MDTs for HPAIVs (Table 1) inoculated by intranasal, intrachoanal, oral or conjunctival routes range from 2.0 to 9.5 days [24;27;29-31;33;39]. The MDT for three different Asian lineage H5N1 viruses was relatively short (2.0 to 4.6 days) but dose dependent, with a cumulative mortality of 100% for all three viruses [30;31;39]. Similarly, the MDT for turkeys inoculated with A/turkey/Ontario/7732/66 (H5N9) was 3.8 to 9.5 days, with a cumulative mortality of 100% [29]. However, both the MDT (2.2-4.0 days) and mortality rate (40% to 100%) were dose dependent in turkeys inoculated with A/ostrich/Italy/984/00 (H7N1) [39]. Cumulative mortality rates reported for other HPAIVs in experimentally infected turkeys have varied from 20% to 100% [27;28;32;33;40].

Cumulative mortality has also been reported to reach 100% in some turkey flocks during field outbreaks [36;41;42]. During the 1999-2000 H7N1 HPAI outbreak in Italy, all birds in affected flocks died within 48-72 hours after the initial clinical signs [41]. An analysis

of the 2003 H7N7 HPAI outbreak in the Netherlands found that the mortality rate increased exponentially, and reached 3% within 2–3 days after the first day of elevated mortality [43]. Mortality rose more quickly in flocks containing older turkeys than in those with birds less than 11 weeks of age. These authors also noted a similar age-related difference for Asian lineage H5N1 viruses in turkeys in Israel. They recommended notification thresholds in the Netherlands of >0.5% mortality/day (in each individual barn) for two consecutive days, in flocks containing turkeys less than 11 weeks of age, and >1% mortality/day for two consecutive days in flocks with birds older than 16 weeks.

Decreased feed and water consumption or egg production might precede an increase in the mortality rate, but there is very little published information on their timing of such changes in turkeys. During one outbreak in the Netherlands, these signs preceded mortality by a day [43].

Table 1: Onset of morbidity and mortality in turkeys 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	30%	Sickness; MTO 6.0d	20%	6.5	Alexander et al., 1986
A/chicken/Scotland/59 H5N1	IM	2	50%	Sickness; MTO 5.2d	50%	6.0	Alexander et al., 1986
A/chicken/Hong Kong/220/97 H5N1	IN	3	100%	D 1 - 1.5: increased fecal fluid and urates D 1.5: neurological signs	100%	2.5	Perkins & Swayne, 2001
A/turkey/Turkey/1/05 H5N1	IN/IC	3	100%	D 2: first death D 7: last death	100%	2.0-4.6; dose-dependent	Aldous et al., 2010
A/chicken/Egypt/086QNLQP/2008 H5N1	IN	4	100%	D 2: first death D 4: last death	100%	2.9	Kilany et al., 2010
A/chicken/PA/1370/83 H5N2	IN	2	100%	Sickness; MTO 3.4d	100%	4.8	Alexander et al., 1986
A/chicken/PA/1370/83 H5N2	IM	2	100%	Sickness; MTO 3.7d	100%	4.9	Alexander et al., 1986
A/chicken/PA/1370/83 H5N2	DrC	2	100%	Sickness; MTO 6.6d[c]	100%	7.7[c]	Alexander et al., 1986
A/tern/South Africa/61 H5N3	IN	2	100%	Sickness; MTO 5.5d	100%	6.2	Alexander et al., 1986
A/tern/South Africa/61 H5N3	IM	2	100%	Sickness; MTO 4.0d	100%	5.1	Alexander et al., 1986
A/tern/South Africa/61 H5N3	DrC	2	20%	Sickness; MTO 7.0d[c]	20%	8.0[c]	Alexander et al., 1986

A/turkey/Ireland/1378/83 H5N8	IN	2	100%	Sickness; MTO 4.8d	100%	5.5	Alexander et al., 1986
A/turkey/Ireland/1378/83 H5N8	IM	2	100%	Sickness; MTO 2.2d	100%	2.8	Alexander et al., 1986
A/turkey/Ireland/1378/83 H5N8	DrC	2	70%	Sickness; MTO 5.8d[c]	70%	6.6[c]	Alexander et al., 1986
A/duck/Ireland/113/84 H5N8	IN	2	100%	Sickness; MTO 2.4d	100%	3.8	Alexander et al., 1986
A/duck/Ireland/113/84 H5N8	IM	2	100%	Sickness; MTO 2.0d	100%	2.4	Alexander et al., 1986
A/duck/Ireland/113/84 H5N8	DrC	2	100%	Sickness; MTO 5.1d[c]	100%	6.7[c]	Alexander et al., 1986
A/turkey/Ontario/7732/66 H5N9	IN; high dose	1 d	100%	D 2 : clin signs & first death Sickness; MTO 2.3d	100%	3.8	Narayan et al., 1969a
A/turkey/Ontario/7732/66 H5N9	IN; low dose	1 d	100%	D 6: clin signs D 9: first death Sickness; MTO 6.8d	100%	9.5	Narayan et al., 1969a
A/turkey/Ontario/7732/66 H5N9	IN	2	100%	Sickness; MTO 5.9d	100%	6.3	Alexander et al., 1986
A/turkey/Ontario/7732/66 H5N9	IN	24	100%	20 h: anorexia, listlessness, ruffled feathers D 2: first death D 3: last death	100%		Narayan et al., 1969a
A/turkey/Ontario/7732/66 H5N9	IN	26	100%		100%		Slemons & Easterday, 1972
A/turkey/Ontario/7732/66 H5N9	IN; high dose	32	100%	D 2: clin signs D 4: first death Sickness; MTO 2.5	100%	4.5	Narayan et al., 1969a
A/turkey/Ontario/7732/66 H5N9	IN; low dose	32	100%	D 6: clin signs D 9: first death Sickness; MTO 6.25d	100%	9.5	Narayan et al., 1969a
A/turkey/Ontario/7732/66 H5N9	IM	2	100%	Sickness; MTO 3.3d	100%	4.0	Alexander et al., 1986
A/turkey/Ontario/7732/66 H5N9	IM	30		24 h: sickness	Died (1 bird)		Narayan et al., 1969a
A/turkey/Ontario/7732/66 H5N9	DrC	2	90%	Sickness; MTO 10.3d[c]	90%	10.6[c]	Alexander et al., 1986
A/turkey/Ontario/7732/66 H5N9	DrC	30	100%	D 5[c]: sickness			Narayan et al., 1969a

A/fowl/Germany/1934 H7N1	IN	2	100%	D 2: all deaths Sickness; MTO 2.0d	100%	2.0	Alexander et al., 1978
A/fowl/Germany/1934 H7N1	DrC	2	100%	Sickness; MTO 2.6d[c]	100%	3.0[c]	Alexander et al., 1978
A/turkey/Italy/4580/1999 H7N1	IN/IO	7.1	100%	D 1: first death D 4: last death D 2: sickness	100%		Toffan et al., 2008
A/ostrich/Italy/984/00 H7N1	IN/IO	3		D 2: first death D 4: last death	40-100%; dose dependent	2.2-4.0; dose-dependent	Aldous et al., 2010
A/FPV/Dutch/1927 H7N7	IN	2	100%	D 3: nonspecific clin signs Sickness; MTO 4.0d	100%	4.5	Alexander et al., 1978
A/FPV/Dutch/1927 H7N7	DrC	2	100%	Sickness; MTO 5.0d[c]	100%	6.0[c]	Alexander et al., 1978
A/fowl/Victoria/75 N7N7	IN	2	100%	Sickness; MTO 4.1d	100%	5.2	Alexander et al., 1978
A/fowl/Victoria/75 H7N7	IN	18		D 6: first death D 10: last death	25%	8.0	Westbury et al., 1979
A/fowl/Victoria/75 H7N7	DrC	2	100%	Sickness; MTO 9.2d[c]	100%	11.0[c]	Alexander et al., 1978
A/fowl/Victoria/75 H7N7	DrC	18		D 13[c]: first death D 16[c]: last death	50%	15.0[c]	Westbury et al., 1979
A/chicken/Netherlands/621557/03 H7N7	IN/IT	12		D 5: first death D 8: last death D 18: last euthanasia of morbid bird	60-100%		Bos et al., 2008
A/chicken/Netherlands/621557/03 H7N7	DrC	12		D 8[c]: first death D 10[c]: last death D 14[c]: euthanasia of morbid bird	80-100%		Bos et al., 2008

a Route of inoculation; b Age of turkeys in weeks, unless otherwise specified; c Measured from the day of inoculation for birds in contact
MDT = mean death time; MTO = mean time of onset; IN = intranasal; IM = intramuscular; IC = conjunctival or intraocular; IO = oral/ feeding; IT = intratracheal; IV = intravenous; DrC = direct contact

The onset of clinical signs in LPAI infected turkeys

Turkeys are reported to be more susceptible to some LPAIVs than chickens , with a lower infectious dose and higher viral replication reported for some isolates [27;44-48]. In particular, turkeys may be infected more readily by some viruses from wild birds [45;46;48].

LPAIVs usually cause much milder clinical signs than HPAIVs, and some infections in turkeys are subclinical [36]. Clinical signs reported in turkeys in the laboratory or field include depression, fever, huddling, ruffled feathers, decreased feed and/or water consumption, reduced weight gain, mild to severe respiratory signs, swelling of the sinuses, conjunctivitis, diarrhea, decreased egg production, poor egg quality (e.g., soft-shelled, misshapen or unpigmented eggs) and decreased fertility and/or hatchability of the eggs [36;41;49-55]. Mortality rates vary with the outbreak. In some cases, there is little or no increase in flock mortality [50;51;55]; in others, it may be elevated [41;52-54;56-58]. Co-infections with other pathogens or environmental stressors can result in more severe illness [36;59]. There may also be differences between viruses. Although many LPAIVs do not cause any deaths in experimentally infected turkeys, and some do not cause clinical signs [24;27;28;33;44;47;58;60-65], mortality rates of 10% to 60% have been reported after respiratory inoculation of some viruses (Table 2) [27;47;63]. Age-related differences have also been reported. Lang et al. (1986) found that turkeys older than 6 weeks remained asymptomatic when they were inoculated with A/turkey/Ontario/666213/1966 (H5N9), but turkey poults less than a week of age developed depression and sinusitis, and some birds died. Similarly, older turkeys tended to recover during an H7N1 outbreak in Italy, but the mortality rate reached 40-97% in younger flocks [41]. Mortality of 40% occurred in birds up to 4 weeks of age infected with an H7N3 LPAIV in Utah [66]. In the latter outbreak, most flocks experiencing elevated mortality were co-infected with bacterial pathogens.

Outbreak descriptions provide insight into the early clinical signs seen with LPAIVs. During an H5N9 LPAI outbreak in Ontario, Canada, the earliest and most consistent signs were decreased activity, reduced feed consumption and increased broodiness of the hens [58]. The mortality rate increased to 2-13% once these signs appeared, and egg production declined concurrently. The fewest eggs were laid 8-10 days after the onset of clinical signs. Respiratory signs were reported in some flocks, but this was inconsistent. An abrupt (50%) decrease in feed and water consumption was also observed initially during an outbreak caused by an H9N2 virus (A/turkey/Wisconsin/66) [54]. This was followed by coughing and sneezing, which affected nearly all birds by the third day. Depression and huddling occurred on the third and fourth days. Other signs, with the timing not reported, included decreased egg production, diarrhea in most birds, sinusitis in approximately 2%, and severe respiratory signs or fatal convulsions in some birds. The mortality rate was 5-10%. In a flock affected by A/turkey/Wisconsin/1/68 (H5N9), clinical signs were first noted 3 days after a visit by an artificial insemination crew, the

presumed source of the infection, and included depression, coughing, mucus and urates in the droppings, a 50% drop in feed consumption, weight loss and a 30% decrease in egg production [55]. Four days later, the birds were eating very little, water intake had decreased by 50%, egg production had dropped by 80%, and coughing was widespread. In another flock affected by this outbreak, egg production decreased by 20% ten days after artificial insemination, but other signs of illness were mild, and feed consumption dropped by only 20%. In a flock of turkeys infected with A/turkey/Minnesota/67, the initial signs were mild depression of two days' duration accompanied by a sudden decrease in egg laying [50]. Egg production dropped from 59% to 16% over 4 days, with a less severe reduction in hatchability and fertility, and an increased number of unpigmented, chalky or soft-shelled eggs. During a presumed but unconfirmed H7 LPAIV infection, the first sign was a decrease in feed consumption to half of normal, followed within 2 days by respiratory signs, but no significant increase in mortality [57]. A second flock involved in this outbreak developed mild respiratory signs early, with mortality increasing on the following day from 0.1% to 11% in turkey toms and 4% in turkey hens. An unusual presentation was reported in two turkey flocks affected by an H1N1 (swine influenza) virus in North Carolina, with sudden death as the initial sign [53]. The mortality attributed to the virus was 3.3% in one flock and 4.3% in the second flock. Later, the birds exhibited depression and anorexia, and occasionally had dyspnea. No co-infections that could account for the unusual deaths, which were attributed to pulmonary damage, were detected. Other flocks affected by the same virus had decreased egg production without increased mortality.

Few reports in the literature have described the onset of clinical signs in experimentally infected turkeys (Table 2), in part because many of these infections are subclinical. Depression, respiratory signs, sinusitis and mild diarrhea were found, starting on day 4 PI, in turkeys inoculated with an H7N3 LPAIV from Italy [67]. Depression and respiratory signs also began on day 4 in turkeys infected with A/turkey/Wisconsin/66 (H9N2) [68]. Other clinical signs reported in these birds included decreased feed consumption and sinusitis. Transient, mild conjunctivitis started on day 3 PI in turkeys inoculated by the conjunctival route with three different LPAI H7N2 viruses isolated from U.S. outbreaks [64]. Respiratory signs were not seen in these birds.

Table 2: Onset of morbidity and mortality in turkeys infected with LPAI viruses

Virus	Route[a]	Age[b]	Morbidity Rate	Onset of Specified Symptoms/ First and Last Deaths	Mortality Rate	MDT (days)	Reference
A/turkey/OH/313053/04 H3N2	ICh	26		D 7:decreased egg laying D 15: end egg laying	0%		Pillai et al., 2009
A/turkey/OH/313053/04 H3N2	ICh	26		D 3: decreased egg laying D 7: end egg laying			Pillai et al., 2010c

A/chicken/PA/1/83 H5N2	IN	2	20%	Sickness (mild depression); MTO 5.0d	10%	22[d]	Alexander et al., 1986
A/chicken/PA/1/83 H5N2	DrC	2	10%	Sickness (mild depression); MTO 6[c]	10%	6[d]	Alexander et al., 1986
A/chicken/PA/13609/93 H5N2	ICh	3		D 4: first death D 6: last death	33%	5	Pillai et al., 2010b
H5N2 viruses: A/turkey/MN/10734-2/95; A/turkey/CA/D0208651-C/02	ICh	3		D 3: transient mild respiratory signs	0%		Pillai et al., 2010b
A/turkey/Ontario/6213/1966 H5N9	IN	< 1		D 2: depression First week: sinusitis	up to 50%		Lang et al., 1968
A/turkey/CA/meleagrium/64 H6N5	sinus	1 d	100%	D 10: gasping, lacrimation and sinusitis			Samadieh & Bankowski, 1970
A/turkey/CA/meleagrium/64 H6N5	IT or IV	adult		D 3[f]: decreased egg laying			Samadieh & Bankowski, 1970
A/turkey/Italy/3675/1999 H7N1	IN/IO	7.1		D 3: mild respiratory signs, depression			Toffan et al., 2008
A/chicken/NJ/15086-3/1994 H7N2	IN	2			40%	7.0	Spackman et al., 2010
A/turkey/NY/445-4/1994 H7N2	IN	2			10%	12.5	Spackman et al., 2010
A/chicken/NY/3112-1/1995 H7N2	IN	2			60%	9.1	Spackman et al., 2010
A/chicken/NY/30749-3/2000 H7N2	IN	2			20%	9.0	Spackman et al., 2010
A/guinea hen/MA/148081-11/2002 H7N2	IN	2			50%	7.75	Spackman et al., 2010
A/turkey/VA/SEP-67/2002 H7N2	IN	2			33%	10.0	Spackman et al., 2010
A/chicken/MD/MinhMa/2004 H7N2	IN	2			12.5%	5.0	Spackman et al., 2010
A/chicken/MD/Minh Ma/2004 H7N2	IC	3		D 3[f]: conjunctivitis D 7: airsacculitis			Ladman et al., 2008
A/chicken/DE/Viva/2004 H7N2	IC	3		D 3[f]: conjunctivitis D 14: airsacculitis			Ladman et al., 2008
A/chicken/DE/Hobo/2004 H7N2	IC	3		D 3[f]: conjunctivitis D 7: airsacculitis			Ladman et al., 2008
A/chicken/NY/12273-11/1999 H7N3	IN	2			10%	13.0	Spackman et al., 2010
A/ruddy turnstone/DE/1538/2000 H7N9	IN	2			10%	5.0	Spackman et al., 2010

A/turkey/CA/5142/66 H9N2	sinus	1 d	100%	D 10: gasping, lacrimation, sinusitis			Samadieh & Bankowski, 1970
A/turkey/WI/66 H9N2	IN/IO/ IC	6-7		D 4: depression, respiratory signs	0%		Dundon et al., 2007
A/turkey/WI/66 H9N2	aerosols or DrC	1-19	100%		0%		Homme et al., 1970
A/turkey/MN/1012/90 H13N2	ICB	2			40%		Laudert et al., 1993a
A/water/MN/2334/90 H13N2	ICB	2			60%		Laudert et al., 1993a

a Route of inoculation; b Age of turkeys in weeks, unless otherwise specified; c Measured from the day of inoculation for birds in contact; d Based on one bird; f First day observations were recorded
MDT = mean death time; MTO = mean time of onset; IN = intranasal; ICh = intrachoanal; IM = intramuscular; IC = conjunctival or intraocular; IO = oral/ feeding; IT = intratracheal; IV = intravenous; ICB = intracerebral; DrC = direct contact

Clinical signs in turkeys infected with swine-origin H3N2 viruses

Triple reassortant H3N2 viruses from pigs have been isolated repeatedly from turkeys in the U.S. since 2003 [62;69-71]. These viruses have been associated with decreased egg production in the field, although co-infection with other pathogens may play a role [62;69-72]. In one flock, egg production stopped completely, but no other clinical signs were detected [69]. Other signs in H3N2 outbreaks included depression, anorexia, mild to severe respiratory illness, sinusitis, edema of the head and face, diarrhea, cyanosis, abnormal eggs and decreased hatchability [62;70-73]. Elevated mortality was seen in some flocks.

Changes in production parameters as indicators of an LPAI outbreak

Decreased feed and/or water consumption has been reported in a number of LPAI outbreaks and experimentally infected birds [41;50-55;57;58;68;74]. As described above, decreased feed consumption was among the earliest signs of infection in several flocks [54;55;57;58]. Reduced feed consumption preceded other clinical signs, such as respiratory signs or depression, in some flocks [54;57], but was noted concurrently with these signs in others [55]. Decreased water consumption was also observed sooner than respiratory signs or depression in one flock [54]. In another flock, a 50% decrease in water intake occurred several days after a 50% drop in feed consumption [55].

Moderately to severely decreased egg production, together with reduced egg quality, is a common syndrome in turkey breeders infected with LPAIVs [36;41;44;49-51;53;55;56;58;74-76]. This is sometimes one of the earliest signs noted by the producer

[50;55]. Decreased egg laying may be sudden and dramatic, as in one flock where production dropped from 59% to 16% over 4 days, or it may occur more gradually [50;55]. A virus may affect flocks differently. During one outbreak, egg production dropped from 70% to 25% over the course of a week in one flock, peak production was delayed in another flock that was infected just as the birds were beginning to lay, and egg production was unaffected in a flock that became ill a week before the birds began laying [54]. In another outbreak, younger flocks were less severely affected than older flocks [50]. Limited information about egg laying is available from experimental reports. In one study, decreased egg production occurred 3 days after intravenous or intratracheal inoculation with T/Calif/meleagrium/64 [49]. A virus that originated in swine, A/turkey/Ohio/313053/04 (H3N2), caused a severe drop in egg production beginning 7 days after intrachoanal inoculation, with complete cessation of egg production a week later [62]. In another study with the same virus, egg production began to decrease gradually 3 or 4 days after inoculation, and stopped completely after day 7 PI [76]. Egg production was unaffected in turkeys inoculated with a different swine origin virus, A/turkey/Illinois/04 (H3N2) [62].

Increased mortality is an inconsistent indicator in LPAIV infected flocks. While mortality is often relatively low, reported mortality rates from outbreaks vary from essentially 0% to as high as 97% (in young birds) [41;44;50-58;66]. In one outbreak, the mortality rate in one flock increased from 0.1% to 11% in turkey toms and 4% in turkey hens, the day after the onset of mild respiratory signs [57]. However, other flocks infected with this virus developed clinical signs without elevated mortality. Similarly, sudden deaths were seen initially in two turkey flocks affected by an H1N1 (swine influenza) virus, but some flocks had clinical signs without increased mortality [53]. When deaths have been reported in experimentally infected turkeys (Table 2), the MDT varied from 5 to 22 days, but this is sometimes based on a single bird [27;47;63].

The onset of virus shedding in respiratory secretions and feces

Influenza viruses shed from the body in secretions and excretions, particularly feces, may contaminate the surface of eggs, as well as meat and other tissues during processing.

HPAI viruses in feces and respiratory secretions

HPAIVs are shed in both feces and respiratory secretions of experimentally infected turkeys, although viruses are occasionally detected at only one of these sites [24;27;32;33;39]. Some HPAIVs have been excreted within a day or two (Table 3) of inoculation [31;32;39]. Turkeys inoculated via the respiratory tract shed an Asian lineage H5N1 virus, an H7N1 virus and an H7N7 virus in respiratory secretions on day 1 PI, with shedding first reported in feces on day 2 [32;39]. Another Asian lineage H5N1 virus was also found on day 1, using combined tracheal and fecal sampling [31]. In one study, which began sampling intranasally inoculated turkeys on day 2 PI, Australian H7N7 HPAIVs were detected in tracheal secretions on that day, and in feces on the following sampling day, day 4 [33]. Alexander et al. (1986) reported that intranasally inoculated

turkeys shed some HPAIVs earlier than others. Sampling was begun on day 4 in this experiment, and performed thereafter at 3-4 day intervals. A/turkey/Ireland/1378/83 (H5N8) and A/chicken/Pennsylvania/1370/83 (H5N2) were detected in the respiratory secretions and feces on day 4 PI, but A/tern/South Africa/61 (H5N3) was first found at both sites on day 11. A/chicken/Scotland/59 (H5N1) was detected in tracheal secretions on day 7 and in feces on day 11, while A/turkey/Ontario/7732/66 (H5N9) was isolated in the feces beginning day 4, but was not found in tracheal secretions.

Table 3: First occurrence of HPAI viruses in feces, respiratory secretions and tissues

Virus	Route[a]	Age[b]	Fecal/ Cloacal Shedding[c]	Tracheal Shedding[c]	Virus /Viral Antigen in Tissues[c]	Reference
A/chicken/Scotland/59 H5N1	IN	2	18	7		Alexander et al., 1986
A/chicken/Scotland/59 H5N1	IM	2	7	7		Alexander et al., 1986
A/chicken/Hong Kong/220/97 H5N1	IN	3			D 1: resp & intest tracts, adrenal gl, spleen, liver, thymus, feather follicle epithelium, bone, brain, heart, kidney, pancreas	Perkins & Swayne, 2001
A/turkey/Turkey/1/05 H5N1	IN/IC	3	2	1		Aldous et al., 2010
A/chicken/Egypt/086Q-NLQP/2008 H5N1	IN	4			D 1: combined fecal/ tracheal swabs	Kilany et al., 2010
A/chicken/PA/1370/83 H5N2	IN or IM	2	4[d]	4[d]		Alexander et al., 1986
A/chicken/PA/1370/83 H5N2	DrC	2	7	7		Alexander et al., 1986
A/tern/South Africa/61 H5N3	IN	2	11	11		Alexander et al., 1986
A/tern/South Africa/61 H5N3	IM	2	4[d]	4[d]		Alexander et al., 1986
A/tern/South Africa/61 H5N3	DrC	2	4[d]	NF		Alexander et al., 1986
A/turkey/Ireland/1378/83 H5N8	IN or DrC	2	4[d]	4[d]		Alexander et al., 1986
A/turkey/Ireland/1378/83 H5N8	IM	2	(4)‡	(4)‡		Alexander et al., 1986
A/duck/Ireland/113/84 H5N8	IN or IM	2	(4)‡	(4)‡		Alexander et al., 1986
A/duck/Ireland/113/84 H5N8	DrC	2		4[d]		Alexander et al., 1986
A/turkey/Ontario/7732/66 H5N9	IN	2	4[d]	NF		Alexander et al., 1986
A/turkey/Ontario/7732/66 H5N9	IN	24-32	+	+	16-24 h: viremia +: egg yolk; brain, heart, lung, liver, kidney, spleen, ovary, testis	Narayan et al., 1969a
A/turkey/Ontario/7732/66	IM	2	(4)‡	(4)‡		Alexander

H5N9						et al., 1986
A/turkey/Ontario/7732/66 H5N9	DrC	2	14	14		Alexander et al., 1986
A/turkey/Italy/4580/1999 H7N1	IN/IO	7.1			D 1:breast & thigh muscles, lungs	Toffan et al., 2008
A/ostrich/Italy/984/00 H7N1	IN/IC	3	2	1		Aldous et al., 2010
A/FPV/Dutch/1927 H7N7	DrC	2	4[d]	4[d]		Alexander et al., 1978
A/fowl/Victoria/75 H7N7	IN or DrC	2	4[d]	4[d]		Alexander et al., 1978
A/fowl/Victoria/75 H7N7	IN or DrC	18	4	2[d]		Westbury et al., 1979
A/chicken/Netherlands/621557/03 H7N7	IN/IT	12	2	1		Bos et al., 2008
A/chicken/Netherlands/621557/03 H7N7	DrC	12	5	3		Bos et al., 2008

a Route of inoculation; b Age of turkeys in weeks, unless otherwise specified; c Measured in days after inoculation (including birds inoculated by contact with inoculated birds); d Virus was isolated on the first day virus shedding was examined
+ virus found, day(s) not specified
‡ Bird died before first sampling day
NF = virus was not found on any days examined; IN = intranasal; IM = intramuscular; IC = conjunctival or intraocular; IO = oral/ feeding; IT = intratracheal; IV = intravenous; DrC = direct contact

LPAI viruses in feces and respiratory secretions

Some LPAIVs have also been detected in respiratory secretions [44;60;61;77] and feces [44] as early as day 1 PI (Table 4). Homme et al. (1970) recovered an LPAIV at 6 hours in inoculated birds, and within 24 hours from turkeys in contact with these birds. Many studies began sampling birds on day 2 PI, and detected numerous viruses in respiratory secretions and feces on that day [33;46;47;60;62;63;78;79]. However, the shedding of some viruses was reported to begin on day 4 [33;46], between days 4 and 7 [27], or between days 7 and 11 [27]. When both fecal and respiratory shedding were examined, most studies reported that LPAIVs are excreted at least occasionally by both routes in turkeys inoculated via the respiratory tract [27;33;44;46;47;52;60;62;63;65;67;77;78;80]. Some isolates from waterfowl and shorebirds were detected in respiratory secretions but not feces [46;77], or in feces but not respiratory secretions [47]. Similarly to HPAI viruses, shedding sometimes began slightly earlier at one site, typically the respiratory tract in birds inoculated by that route [27;33;47;60;63;67;77].

Whether turkeys 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.

Table 4: First occurrence of LPAI viruses in feces, respiratory secretions and tissues

Virus	Route[a]	Age[b]	Fecal/ Cloacal Shedding[c]	Tracheal Shedding[c]	Virus/ Viral Antigens in Tissues[c]	Reference
A/ruddy turnstone/NJ/749/02 H1N9	ICh	3	4	4		Morales, et al., 2009
A/mallard/OH/30/86 H2N1	IV	2			D 3/5: kidney, bursa, pancreas, ileocecal junction	Laudert et al., 1993b
A/mallard/OH/48/86 H3N2	IV	2			D 3/5: kidney, liver, bursa, ileocecal junction	Laudert et al., 1993b
A/turkey/OH/313053/04 H3N2	ICh	26	2[d]	2[d]	D 7[d]: oviduct,- infundibulum, magnum, isthmus, uterus	Pillai et al., 2009
A/turkey/OH/313053/04 H3N2	ICh	26			D 2: egg shell surface, egg albumin D 3[d]: oviduct	Pillai et al., 2010c
A/turkey/IL/04 H3N2	ICh	26	2[d]	2[d]	D 7[d]:oviduct – infundibulum, magnum	Pillai et al., 2009
A/shorebird-environment/DE/251/2005 H3N6	IT/IC	2	NF	1		Ladman et al., 2010
A/blue-winged teal/OH/305/86 H3,4N6	IV	2			D 3/5: kidney, liver, bursa, ileocecal junction	Laudert et al., 1993b
A/turkey/MN/1248/80 H4N2	IN		3[d]	3[d]		Karunakaran et al., 1987
A/mallard/OH/83/86 H4N6	IV	2			D 3/5: kidney, liver, bursa, pancreas, jejunum, ileocecal junction	Laudert et al., 1993b
A/blue-winged teal/LA/240B/88 H4N6	ICh	3	NF	2[d]		Morales et al., 2009
A/mallard/MN/198/99 H4N6	ICh	3	2[d]	2		Morales et al., 2009
A/mallard/OH/338/86 H4N8	IV	2			D 3/5: brain, lung, kidney, liver, jejunum, bursa, ileocecal junction	Laudert et al., 1993b
A/mallard/MN/263/99 H4N9	ICh	3	NF	2[d]		Morales et al., 2009
A/mallard/OH/184/86 H5N1	IV	2			D 3/5: thymus, kidney, bursa, ileocecal junction	Laudert et al., 1993b
A/mallard/MD/1159/2006 H5N1	IT/IC	2	NF	1		Ladman et al., 2010
A/mute swan/MI/451072-2/06 H5N1	IN	3	2[d]	2[d]		Spackman et al., 2007
A/mute swan/MI/451072-2/06	ICh	3	2[d]	2[d]		Pillai et al.,

H5N1						2010b
A/mute swan/MI/451072-2/06 H5N1	DrC	3	3	1		Spackman et al., 2007
A/mute swan/MI/451072-2/06 H5N1	DrC	3	4	2[d]		Pillai et al., 2010b
A/turkey/Italy/ZA/80 H5N2	IN	2	11	7		Alexander et al., 1986
A/turkey/Italy/ZA/80 H5N2	DrC	2	18	11		Alexander et al., 1986
A/turkey/MN/1630/81 H5N2	IV	2			D 3/5: lung, kidney, liver, bursa, ileocecal junction	Laudert et al., 1993b
A/turkey/MN/1700/82 H5N2	IT			2[d]	D 2: trachea, lung	Kodihalli et al., 1994
A/chicken/PA/1/83 H5N2	IN	2	21	7		Alexander et al., 1986
A/chicken/PA/1/83 H5N2	DrC	2	7	7		Alexander et al., 1986
A/mallard/OH/345/88 H5N2	IV	2			D 3/5: brain, kidney, liver	Laudert et al., 1993b
H5N2 viruses: A/chicken/PA/13 609/93 A/emu/NY/12716/94 A/turkey/MN/10734-2/95 A/pheasant/NJ/1355/98 A/avian/NY/31588/00 A/duck/ME/151895-7A/02 A/turkey/CA/8651-C/04 A/parrot/CA/406032/04	ICh or DrC	3	2[d]	2[d]		Pillai et al., 2010b
H5N2 viruses: A/pheasant/MD/4457/98 A/mallard/MN/182742/98 A/duck/NJ/117228-7/01 A/duck/NY/185 502/02	ICh	3	2[d]	2[d]		Pillai et al., 2010b
A/pheasant/MD/4457/98 H5N2	DrC	3	4	2[d]		Pillai et al., 2010b
A/mallard/MN/182742/98 H5N2	DrC	3	2[d]	4		Pillai et al., 2010b
A/duck/NY/185 502/02 H5N2	DrC	3	2[d]	4		Pillai et al., 2010b
A/parrot/CA/6032/04 H5N2	IN	3	2[d]	2[d]		Pillai et al., 2008
A/mallard/MN/479/00 H5N3	ICh	3	4	2[d]		Pillai et al., 2010b
A/mallard/MN/479/00 H5N3	DrC	3	2[d]	2[d]		Pillai et al., 2010b
A/ruddy turnstone/NJ/2242/00 H5N3	ICh	3	2[d]	4		Pillai et al., 2010b
A/ruddy turnstone/NJ/2242/00 H5N3	DrC	3	2[d]	NF		Pillai et al., 2010b
A/chicken/TX/167280-4/02 H5N3	ICh	3	4	2[d]		Pillai et al., 2010b
A/chicken/TX/167280-4/02 H5N3	DrC	3	4	2[d]		Pillai et al., 2010b
A/mallard/MN/3 5581/00 H5N5	ICh	3	2[d]	2[d]		Pillai et al., 2010b
A/mallard/MN/3 5581/00 H5N5	DrC	3	2[d]	NF		Pillai et al., 2010b
A/ruddy turnstone/DE/2046/01	ICh	3	2[d]	2[d]		Pillai et al.,

H5N8						2010b
A/ruddy turnstone/DE/2046/01 H5N8	DrC	3	2[d]	NF		Pillai et al., 2010b
A/ruddy turnstone/DE/85/03 H5N9	ICh or DrC	3	2[d]	2[d]		Pillai et al., 2010b
A/turkey/MN/524/78 H6N1	IV	2			D 3/5: brain, lung, spleen, kidney, liver, bursa, ileocecal junction	Laudert et al., 1993b
A/turkey/MN/584/78 H6N1	IT			2[d]	D 2: trachea, lung	Kodihalli et al., 1994
A/mallard/DE/415/2005 H6N2	IT/IC	2	NF	1		Ladman et al., 2010
H6N2; strain not given	IT/ IO	3	3[d]	3[d]		Zarkov, 2008
A/northern pintail/TX/828197/02 H6N4	ICh	3	NF	2[d]		Morales et al., 2009
A/parrot/Ulster/1973 H7N1	IN	2	4[d]	4[d]		Alexander et al., 1978
A/turkey/Italy/3675/1999 H7N1	IN/IO	7.1			D 2 : lung, blood, breast meat, thigh meat	Toffan et al., 2008
H7N2 viruses: A/chicken/NJ/15086-3/1994; A/turkey/NY/445-4/1994; A/chicken/NY/3112-1/1995; A/chicken/PA/9801289/1998; A/chicken/NY/30749-3/2000; A/guinea hen/MA/148081-11/2002; A/turkey/VA/SEP-67/2002; A/chicken/MD/MinhMa/2004	IN	2	2[d]	2[d]		Spackman et al., 2010
A/turkey/VA/158512/02 H7N2	IN	4 & 8	1	1		Tumpey et al., 2004
A/pheasant/MN/934/80 H7N3	IV	2			D 3/5: lung, kidney, liver, bursa, pancreas, jejunum, ileocecal junction	Laudert et al., 1993b
H7N3 viruses: A/chicken/NY/12273-11/1999; A/pintail/MN/423/1999	IN	2	2[d]	2[d]		Spackman et al., 2010
A/turkey/Italy/8000/02 H7N3	IN	10	5	3[d]		Capua et al., 2004
A/mallard/DE/418/2005 H7N3	IT/IC	2	3	1		Ladman et al., 2010
A/duck/Victoria/76 H7N7	IN	18	4	2[d]		Westbury et al., 1979
A/duck/Victoria/76 H7N7	IN	18	4[d]	4		Westbury et al., 1979
A/mallard/OH/421/1987 H7N8	IN	2	4	2[d]		Spackman et al., 2010
A/ruddy turnstone/DE/1538/2000 H7N9	IN	2	2[d]	2[d]		Spackman et al., 2010
A/turkey/WI/66 H9N2	aerosols or DrC	1-19		1		Homme et al., 1970
H9N2 viruses: A/mallard/MN/232/98 A/ruddy turnstone/NJ/452/03	ICh	3	NF	2[d]		Morales et al., 2009

A/ruddy turnstone/ DE/1070/02 H9N4	ICh	3	NF	2[d]		Morales et al., 2009
H13N2 viruses: A/turkey/MN/1012/90; A/water/MN/2334/90	IN/IC	2	3[d]			Laudert et al., 1993a

a Route of inoculation; b Age of turkeys in weeks, unless otherwise specified; c Measured in days after inoculation (including birds inoculated by contact with inoculated birds); d Virus was isolated on the first day virus shedding was examined
NF = virus was not found on any days examined; IN = intranasal; IM = intramuscular; IC = conjunctival or intraocular; IO = oral/ feeding; IT = intratracheal; IV = intravenous; DrC = direct contact

LPAI and HPAI viruses in meat and eggs

Some avian influenza viruses may localize within the skeletal muscle (meat) and/or the internal contents of eggs from infected birds. The risk of localization within meat or eggs is influenced by the hemagglutinin precursor protein HA0. This protein, which must be cleaved for the virus to enter cells, differs in LPAI and HPAI viruses. The HA0 of LPAIVs is cleaved by trypsin-like enzymes found in the respiratory and intestinal tracts (or by certain bacterial proteases) [1;81;82]. This characteristic is generally thought to limit LPAIVs to these locations [36;83]. In contrast, the HA0 of an HPAIV is cleaved intracellularly by proteases of the subtilisin family (e.g., furin) [82]. Such proteases are found in cells throughout the body, and HPAIV infections are systemic [1;81]. For this reason, HPAIVs are more likely to be found in tissues such as skeletal muscle.

HPAI viruses in meat and other tissues

In experimentally infected turkeys, HPAIVs or their antigens have been found in numerous tissues including blood, skeletal muscle (breast and thigh meat), respiratory and intestinal tracts, heart, liver, kidney, spleen, pancreas, adrenal gland, thymus, bone, feather follicle epithelium, brain, ovary and testis [29;30;40]. Some viruses disseminate to the tissues very soon after infection. Antigens from an Asian lineage H5N1 HPAIV were found in numerous sites on day 1 PI [30]. Narayan et al. (1969) detected A/turkey/Ontario/7732/66 (H5N9) in blood 16-24 hours after inoculation. Virus localization in meat was examined in only one study, which reported the isolation of A/turkey/Italy/4580/1999 (H7N1) from breast and thigh meat on day 1 PI [40]. Other HPAIVs are also likely to occur in turkey meat, given the widespread distribution of these viruses in tissues, and the detection of HPAIVs in meat from other species, including ducks [84-86], chickens [83;84;87-89] and quail [86]. It is noteworthy that vaccination prevented A/turkey/Italy/4580/1999 (H7N1) from localizing in turkey meat, though it did not prevent virus replication in the lungs [40]. The authors noted that viruses from the respiratory tract could contaminate meat during processing.

LPAI viruses in meat and other tissues

No studies have reported finding viable LPAIVs in the meat of turkeys. In one study, nucleic acids from the LPAIV A/turkey/Italy/3675/1999 (H7N1) were detected in muscle, viremia occurred in one of 12 turkeys, and nucleic acids were detected in the blood of 2 birds. [40] However, live virus could not be isolated from breast or thigh muscles. Although the presence of nucleic acids does not necessarily indicate that intact, live virus is present, these findings suggest that additional studies may be warranted. A risk analysis in chickens suggests that the risk of LPAIVs in meat is probably minimal [90]. The corresponding analysis has not been done for turkey meat.

HPAI viruses in eggs

Eggshells laid by influenza virus-infected flocks could be contaminated by viruses from feces. Eggs may also, in some cases, contain viruses in the albumen or yolk. HPAIVs have been found in the internal contents of eggs from quail [91] and chickens [92-95], suggesting that they may also occur in turkey eggs. One virus (A/turkey/Ontario/7732/66; H5N9) was isolated from the yolk of three eggs laid by contact-exposed turkeys [29]. Antigens from an Asian lineage H5N1 HPAIV (A/chicken/Hong Kong/220/97) were detected in numerous tissues from intranasally inoculated turkeys on day 1 PI, although the ovary and its associated tissues were not examined [30]. It is possible, therefore, that developing eggs might be contaminated soon after infection.

LPAI viruses in eggs

The swine origin, triple reassortant virus, A/turkey/Ohio/313053/04 (H3N2) was recently detected in the internal contents of eggs from intrachoanally inoculated turkeys [76]. This virus was first found in the albumin on day 2 PI in one trial and on day 3 in another trial. It also occurred on the surface of the eggshell on day 2. High titers of this virus were reported in the oviduct after intrachoanal inoculation, with low and inconsistent titers on day 3 PI and higher titers on day 5 [76]. There are no other reports of LPAIVs in the internal contents of turkey eggs, but fecal 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?

HPAI viruses

For a clinical sign to be effective in preventing the distribution of contaminated poultry products, it must consistently occur at the same time as, or precede, virus shedding. The onset of clinical signs, mortality and virus shedding in turkeys inoculated with Asian lineage H5N1 HPAIVs is summarized in Table 5. Although the data are limited, they

suggest that these viruses may be shed as soon as one day after infection, and can be found in tissues on that day, at least in experimentally infected birds [30;31;39]. They also suggest that clinical signs or dead birds would be expected to appear within a day or two of the onset of virus shedding, perhaps with slightly slower development of mortality in birds receiving lower doses [30;31;39]. Some viruses belonging to other lineages also appear to be shed quickly and kill birds rapidly [39]. In the only published report of an HPAIV in turkey meat, an H7N1 virus was found in breast and thigh muscle on day 1 PI, the same day severe clinical signs appeared [40]. Similarly, viremia began 16-24 hours after inoculation of turkeys with A/turkey/Ontario/7732/66 (H5N9), and some birds began showing clinical signs on that day [29]. Other HPAIVs might, however, be excreted for a few days before the clinical signs appear [24;33]. In one study, turkeys inoculated intranasally with an Australian H7N7 virus became ill 5-10 days later, but began shedding virus on day 2 [33]. A closely related virus was excreted by day 4 in turkeys infected by direct contact, but the mean time of onset for clinical signs was 9.2 days [24]. Turkeys infected with a Dutch H7N7 HPAIV began shedding virus on the first or second day after inoculation, and deaths occurred on days 5 to 21 [32].

Collectively, these studies suggest that some HPAIVs may be found in feces, respiratory secretions, and tissues including meat within a day or two after infection, with clinical signs appearing shortly thereafter. Other viruses might be shed in feces or respiratory secretions for several days before individual birds become ill or die. Whether they also localize in meat at that time is unknown. There is no information about the onset of HPAIV shedding in the yolk or albumin of eggs.

Table 5: The onset of clinical signs, mortality, and virus shedding in H5N1 Asian lineage HPAI virus-infected turkeys

Clinical Signs Reported	Reluctance to move (Kilany et al., 2010), recumbency (Aly et al., 2008), facial edema (Aly et al., 2008; Kilany et al., 2010), conjunctivitis (Aly et al., 2008), sinusitis (Kilany et al., 2010), oculonasal discharge (Kilany et al., 2010), hemorrhages on the shanks (Aly et al., 2008; Kilany et al., 2010), comb/wattle cyanosis (Aly et al., 2008), diarrhea (Aly et al., 2008); neurological signs (Aly et al., 2008)
Morbidity Rate, Turkeys Inoculated IN	100% (Perkins & Swayne, 2001; Kilany et al., 2010; Aldous et al., 2010)
Mortality Rate, Turkeys Inoculated IN	100% (Perkins & Swayne, 2001; Kilany et al., 2010; Aldous et al., 2010)
MDT (IN)	2.0-4.6 (Perkins & Swayne, 2001; Kilany et al., 2010; Aldous et al., 2010); dose-dependent (Aldous et al., 2010)
Virus Shedding from Intranasally Inoculated Turkeys	
Tracheal Shedding	On day 1 PI (Kilany et al., 2010; Aldous et al., 2010)
Fecal Shedding	On or before day 2 (Kilany et al., 2010; Aldous et al., 2010)
Virus or Viral Antigens in Tissues	
On day 1 PI	Respiratory tract, intestinal tract, adrenal gland, spleen, liver, thymus, feather follicle epithelium, bone, brain, heart, kidney, pancreas (Perkins & Swayne, 2001)

LPAI viruses

Clinical indicators do not appear to be reliable for preventing the distribution of poultry products from LPAIV infected flocks. Although turkeys infected with these viruses may become symptomatic more often than chickens [41;58;63;75;96], LPAIVs are also shed by clinically normal turkeys and birds with minimal signs [28;33;36;46;48;58;60;65;77;78]. Changes in some nonspecific parameters, particularly decreased egg production and reduced feed and water consumption, seem to occur early [49;50;54;55;57;58;62], and may be helpful in recognizing some flocks and triggering an investigation. However, an analysis of LPAI outbreaks in a poultry-raising area of Italy found that active surveillance (periodic sampling of all flocks) was more likely to detect infections in unvaccinated meat turkey flocks than passive surveillance (i.e., surveillance based mainly on the detection of clinical signs) [97]. During the study period, which encompassed several outbreaks, 114 infected flocks were found by active sampling and

90 by passive surveillance. The maximum likelihood predicted values for the detection of infections was 50% for active surveillance and 40% for passive surveillance.

Virus transmission and its effect on the recognition of infected flocks

In large commercial flocks, diseases may be recognized only when enough birds are affected to influence production parameters. Commercial flocks often experience a persistent 'baseline' level of morbidity and mortality. In these flocks, the recognition of an outbreak can be triggered by a change in 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. Relatively little is known about avian influenza virus transmission in turkeys; however, some general conclusions based on experiments in chickens are likely to apply to turkeys, and a few studies have evaluated transmission parameters in turkeys.

Environmental factors influencing virus spread may include the stocking density, size of the room, temperature, humidity and airflow [27;45;98]. Avian influenza viruses are expected to spread efficiently among birds housed in groups, such as turkeys, compared to birds kept in individual cages [41;43;99-102]. Nevertheless, one model, which used transmission parameters from experiments in chickens, suggested that recognizing an HPAI outbreak by an increase in the mortality rate may take more than a week in group-housed birds [103]. In this model, infected chicken flocks were likely to be recognized 10-12 days after the introduction of a single infected bird into the flock, if disease was recognized when the mortality rate reached 0.01% to 0.5% per day, on two consecutive days. Some descriptions of outbreaks affecting chickens also suggest that the flock mortality rate may not rise significantly enough for farmers to recognize an infected flock for an average of 5 days (range 1-8 days) to a week or more in some cases [99;104].

Transmission parameters may vary between avian influenza viruses. Some viruses do not appear to spread easily between birds that are physically separated. Narayan et al. (1969a) reported that HPAIV A/turkey/Ontario/7732/1966 (H5N9) was transmitted readily between experimentally infected turkeys in contact, but virus spread was markedly reduced if the birds were separated by a distance of 1 meter. During an outbreak, this virus was not transmitted between turkey houses on an infected farm, although the only precautions were simple quarantine measures. Similar findings have been reported in chickens, with some viral strains spreading between caged birds by airborne means, but others seeming to require close contact between birds or contact with infected feces to be transmitted efficiently [105-109]. Some studies suggest that viruses which kill chickens or turkeys rapidly with few clinical signs, and thus are not excreted for long periods, are likely to spread more slowly [27;110]. In contrast, one study found that an HPAIV was infectious longer in chickens and was transmitted more readily than a closely related LPAIV from the same outbreak [98].

The transmission rate may also differ between species of poultry [111]. A few studies have quantified the transmission rate parameter β for HPAIVs in turkeys. This parameter is defined as the number of birds infected by each infectious bird per unit of time, in a fully susceptible population. In two studies that used HPAIV A/chicken/Netherlands/621557/03 (H7N7), β in turkeys (1.26 birds/ day) was estimated to be comparable to and somewhat lower [32] than the same parameter in chickens (1.72) [112] cited in [32]. In a model of the 1999-2000 H7N1 HPAI outbreak in Italy, β was estimated to be 1.43 birds/ day in turkey flocks and 1.19 birds/day in broilers, although this difference was not statistically significant [111]. In a similar model of the 2003 H7N7 HPAI outbreak in the Netherlands, β appeared to be higher in chickens (4.50 birds/ day) than turkey flocks (3.37 birds/day), but this difference also did not reach significance [113]. These few measurements show no pattern for the magnitude of β in turkeys compared to chickens, and suggest that it might differ with the specific virus and its adaptation to each species.

Overall, these studies indicate that some viruses might spread more rapidly than others within a turkey flock. Together with studies describing the onset of clinical signs and virus shedding in experimentally infected turkeys, this suggests that HPAIVs might sometimes occur in poultry products before infections are detected by mortality or other clinical parameters. Whether infected meat would reach consumers or poultry processors would depend on whether a flock is sent to slaughter before the infection is recognized. The possibility of virus distribution in the contents of eggs cannot be evaluated, as there is little or no information on the onset of virus shedding in turkey eggs.

Conclusions

Recognizing infected turkey flocks before the onset of virus shedding, based solely on clinical signs, appears to be unreliable for HPAIVs as well as LPAIVs. Some HPAI and LPAI viruses can be shed in the feces and respiratory secretions within the first 1-2 days after infection, although the excretion of other viruses may begin later. There are also reports of HPAIVs in meat and eggs from turkeys. Based mainly on the expected behavior of LPAIVs in turkeys and other birds, the risk of LPAIVs in meat appears to be low. However, the identification of nucleic acids from an H7 LPAIV in turkey meat and the isolation of the same virus from blood suggest that further studies may be advisable. One swine origin H3N2 LPAIV was detected inside turkey eggs. Active surveillance, with periodic laboratory testing and immediate testing of sick and dead birds, appears to be the most effective method of recognizing both HPAI and LPAI virus infected flocks. Studies examining the onset of decreased feed and water consumption, decreased egg laying or other clinical signs may reveal changes in these parameters that could be useful in recognizing HPAIV infected flocks before mortality begins to rise.

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