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(Highly pathogenic) Avian Influenza as a zoonotic agent

Donata Kalthoff, Anja Globig, Martin Beer*

Institute of Diagnostic Virology, Friedrich-Loeffler-Institut, Südufer 10, 17493 Greifswald-
Insel Riems

*corresponding author:
Dr. Martin Beer
Friedrich-Loeffler-Institut Insel Riems
Südufer 10
17493 Greifswald-Insel Riems
Tel. 0049-38351-7-200
Fax 0049-38351-7-275
e-mail: martin.beer@fli.bund.de

Summary

Zoonotic agents challenging the world every year afresh are influenza A viruses. In the past, human pandemics caused by influenza A viruses had been occurring periodically. Wild aquatic birds are carriers of the full variety of influenza virus A subtypes, and thus, most probably constitute the natural reservoir of all influenza A viruses. Whereas avian influenza viruses in their natural avian reservoir are generally of low pathogenicity (LPAIV), some have gained virulence by mutation after transmission and adaptation to susceptible gallinaceous poultry. Those so-called highly pathogenic avian influenza viruses (HPAIV) then cause mass die-offs in susceptible birds and lead to tremendous economical losses when poultry is affected. Besides a number of avian influenza virus subtypes that have sporadically infected mammals, the HPAIV H5N1 Asia shows strong zoonotic characteristics and it was transmitted from birds to different mammalian species including humans. Theoretically, pandemic viruses might derive directly from avian influenza viruses or arise after genetic reassortment between viruses of avian and mammalian origin. So far, HPAIV H5N1 already meets two conditions for a pandemic virus: as a new subtype it has been hitherto unseen in the human population and it has infected at least 387 people, and caused severe illness and high lethality in 245 humans to date (Sep 08). The acquisition of efficient human-to-human transmission would complete the emergence of a new pandemic virus. Therefore, fighting H5N1 at its source is the prerequisite to reduce pandemic risks posed by this virus. Other influenza viruses regarded as pandemic candidates derive from subtypes H2, H7, and H9 all of which have infected humans in the past. Here, we will give a comprehensive overview on avian influenza viruses in concern to their zoonotic potential.

Introduction

The incidence of classical human influenza A infection in the human population varies from year to year and causes annually 500.000 deaths on average and subsequently substantial socio-economical losses. In the past, three influenza A pandemic viruses have been responsible for some of the most devastating diseases of mankind. Of those historical human pandemic viruses - H1N1 causing the Spanish flu in 1918, H2N2, the causative agent for the Asian flu in 1957, and H3N2 in 1968 (Hong Kong Flu) – only H1N1 may have been directly derived from an avian source whereas the other two represent reassortants between human and avian strains (see below). Generally influenza pandemics are defined as global outbreaks due to the appearance of “new” viruses in an immunological naïve population. Three conditions need to be met for a new pandemic to start: (i) the emergence (or re-emergence) of an influenza virus HA subtype, unseen in the human population for at least one generation, (ii) the infection and efficient replication in humans and (iii) the easy and sustainable spread among humans.

Avian influenza viruses (AIV), perpetuated in their natural hosts, with their high genetic divergence are suspected to be precursor viruses or even to display pandemic potential by themselves. Cases of diseased humans due to avian influenza virus infections have been documented (table 1). Nevertheless, fatal infections were caused only by HPAIV. However, in none of these cases has a sustained or efficient human-to-human transmission been observed. However, ‘bird flu’ has become an alarming term in public press.

History

Although highly pathogenic avian influenza (HPAI), previously known as ‘fowl plague’, was already recognized as infectious disease in poultry in Italy in 1878 (Perroncito 1878), it was not before 1955 that the agent was characterised as influenza A virus (Schäfer 1955). To date, all outbreaks of the highly pathogenic form have been caused by influenza A viruses of the

subtypes H5 and H7. Before 2003, outbreaks of HPAI viruses in poultry had been rare events and since 1959 only 24 primary outbreaks of subtypes H5 and H7 were reported world-wide (Werner and Harder, 2006) with the majority occurring in Europe and the Americas. However, none of the outbreaks has ever reached the size of the ongoing HPAI H5N1 Asia epizootic spreading to numerous countries in East- and Middle-Asia, Europe and Africa since 2004 and causing devastating losses to the poultry population. Besides, different HPAIV H5 and H7 outbreaks were additionally reported in poultry from various countries all around the world since 2004. So far, predominantly the HPAIV H5N1 Asia but also HPAIV of subtype H7 have been responsible for the majority of human infections with avian influenza viruses (table 1).

Emergence and spread of HPAIV H5N1 Asia – a new dimension

HPAI H5N1 Asia arose probably before 1997 in Southern China and subsequently caused numerous outbreaks in poultry farms as well as in live bird markets in Hong Kong. Additionally, human infections with HPAIV H5N1 occurred and six out of eighteen clinically affected people died – marking for the first time ever reported casualties after direct infection with a HPAIV from an avian source (table 1). From late 2003 until 2004 HPAIV H5N1 Asia spread across South-East Asia, including the Republic of Korea, Thailand, Indonesia, Viet Nam, Japan, Hong Kong, Cambodia, Lao People's Democratic Republic, People's Republic of China and Malaysia. HPAIV H5N1 was detected from poultry and wild birds, and for the first time from fatally diseased tigers and leopards. Furthermore, human infections in this period were reported from Thailand and Viet Nam. In April 2005 HPAIV H5N1 Asia caused a mass die-off in wild birds at Qinghai Lake in North Western China. Subsequently, Kazakhstan and Russia confirmed outbreaks at poultry farms with dead wild birds in the vicinity of outbreaks in July 2005. By October 2005 poultry affected outbreaks were reported from Turkey and Romania. In addition, Croatia found wild birds positive for HPAIV H5N1.

Subsequently, in early 2006 twenty European countries (Ukraine, Bulgaria, Greece, Italy, Slovenia, Germany, France, Austria, Bosnia-Herzegovina, Slovakia, Hungary, Serbia-Montenegro, Switzerland, Poland, Albania, Denmark, Sweden, Czech Republic, United Kingdom, Spain), twelve Middle-Eastern countries (Kuwait, Israel, West Bank/Gaza Strip, Iran, Iraq, Egypt, Afghanistan, Jordan, Pakistan, India, Azerbaijan, Georgia), and seven African countries (Nigeria, Niger, Cameroon, Burkina Faso, Sudan, Côte d'Ivoire, Djibouti) detected HPAIV H5N1 Asia from samples of dead poultry or dead wild birds. Meanwhile HPAI H5N1 became endemic in most South-East Asian countries with worst situation in Indonesia. Until the end of 2006, human infection caused by H5N1 virus was confirmed in 263 cases of which 158 were fatal. In 2007 and 2008 countries from East-Asia, Europe, Middle East and Africa accounted for outbreaks in poultry and wild birds. Egypt declared H5N1 to be endemic in 2008. Very recently Germany reported the infection of poultry indicating the continuing biohazard threat (Immediate notification report, Ref OIE: 7416, Report Date: 10/10/2008, Country: Germany).

In total (data until 10th September 2008) 387 human infection with 245 fatalities were reportedly caused by HPAIV H5N1, the highest number of human fatal infections occurring in Indonesia (112), Viet Nam (52), Egypt (22), China (20) and Thailand (17) (WHO timeline of major events, Cumulative Number of Confirmed Human Cases of Avian Influenza A/(H5N1) reported to WHO).

Infectious Agent

Avian Influenza Virus – Taxonomy and virus characteristics

Avian influenza viruses (AIV) are members of the family *Orthomyxoviridae* and belong to the genus *Influenza A*. On the basis of the immunogenic glycoproteins haemagglutinin (HA) and neuraminidase (NA), influenza A viruses currently cluster into sixteen HA (H1 - H16) and nine NA (N1 - N9) subtypes (Webster et al., 1992; Fouchier et al., 2005).

133 Influenza A viruses consist of eight segmented, single-stranded RNA-genomes of negative
134 polarity. The spherically or longitudinally shaped virus particles possess a host cell-derived
135 lipid envelope. The transmembrane proteins HA and NA as well as the integral M2 protein,
136 which functions as an ion channel (Lamb and Krug, 2006) are embedded in the virus
137 envelope. Along the inside, the envelope is coated by the matrix protein (M1), surrounding
138 the eight ribonucleoprotein (RNP) complexes. Each RNP complex contains one single RNA
139 segment, encapsidated by nucleoprotein (NP) molecules and the three polymerase proteins
140 PB1, PB2 and PA (Noda et al., 2006).

141 Trimerized HA proteins serve as viral receptor-binding protein recognising distinct terminal
142 sialic acid species present at the cell surface. Human influenza A viruses preferably bind to α -
143 2, 6-linked sialic acids (SA), whereas avian viruses particularly bind to α -2, 3-linked SA.
144 After successful attachment the virus particles are internalized into an endosome and viral
145 RNP complexes are released into the cytoplasm after HA facilitated fusion of viral
146 envelopment and cellular endosomal membrane (Skehel and Wiley, 2000). The fusogenic
147 activity of HA is restricted to mature HA after endoproteolytical cleavage through tissue
148 specific proteases. The cleavage site of the HA protein of the majority of all avian influenza
149 viruses is composed of only one to two basic amino acids at distinct positions, for example -
150 1/-4 for H5 and -1/-3 for H7 subtypes (Wood et al., 1993). Trypsin-like enzymes
151 preferentially expressed at the surface of respiratory and gastrointestinal epithelia recognise
152 this monobasic cleavage motif. Therefore efficient replication of AIV with a monobasic HA
153 cleavage site is restricted to these tissues leading to only mild disease in poultry. Those
154 viruses are of low pathogenicity (LPAIV). Contrarily, avian influenza viruses that exhibit a
155 multibasic cleavage motif (minimal consensus sequence of -R-X-K/R-R-) are recognized by
156 subtilisin-like endoproteases that are virtually present in every tissue. Hence, those viruses are
157 capable to replicate in multiple tissues (Horimoto et al., 1994; Rott et al., 1995) leading to
158 systemic infection and almost 100% mortality in galliforme species. Those viruses with a

multibasic HA cleavage site are called highly pathogenic avian influenza viruses (HPAIV), and so far have arisen only from virus subtypes H5 and H7.

After release of the RNP complexes into the cytoplasm, they are transported into the nucleus, where viral transcription and RNA replication takes place (Whittaker et al., 1996). Replication cycles are completed by assembly of nucleocapsids harbouring replicated genomic RNA and budding at the cellular membrane, into which the viral glycoproteins have previously been inserted. The NA protein is responsible for the cleavage of budding virions from sialic acid residues, which results in infectious viral progeny (Varghese et al., 1983).

Non sterile immunity, suboptimal receptor binding or antiviral drugs cause selective pressure that favour virus mutants with corresponding selective advantages. The pool of genetic variants generated during productive influenza virus infection [the viral RNA-dependent RNA polymerase lack proof reading activity, resulting in one point mutation per 1.5×10^5 nucleotides (Buonagurio et al., 1986)], may harbour mutants with selective advantages which may become dominant under a respective pressure. If driven by immunological pressures this process is referred to as '*antigenic drift*' (Fergusson et al., 2003). Exchange of whole gene segments between two viruses, by chance occurring if a single cell is infected by different virus subtypes, may lead to profound change of antigenic determinants, and is termed '*antigenic shift*'. Providing that the combination of segments results in replication competent viral progeny, '*reassortants*' carrying genetic information from two different parental viruses occur (Webster and Hulse, 2004b). The pandemic influenza A viruses of 1957 (H2N2) and 1968 (H3N2) arose through reassortment between human and avian viruses. The most devastating influenza pandemic known to date and designated as 'Spanish flu' (H1N1, 1918) appears to be caused by a virus that was entirely derived from avian origin (Belshe, 2005).

Transmission and Epidemiology

Influenza A viruses circulate in their natural hosts, wild aquatic birds predominantly of the orders *Anseriformes* (ducks, geese, swans) and *Charadriiformes* (gulls, waders, terns). Transmission occurs primarily by faecal-oral pathways through direct contact or indirect contact with contaminated surface water (Fig. 1A), (Webster et al., 1992). The majority of avian influenza viruses, including subtypes H5 and H7, are of low pathogenicity and cause subclinical infections of the intestinal or respiratory tract.

HPAIV have been proven to emerge after transmission from the wild reservoir and adaptation of LPAIV subtypes H5 or H7 to new poultry hosts and subsequent mutation to and selection of HPAI by multiple bird passages (Rohm et al., 1995, Subbarao et al., 2006). A well characterized example is the development of HPAIV H7N1 from LPAIV precursor within months in Italy 1999 (Capua and Marangon, 2000). However, nascency of highly pathogenic forms of H5 and H7 or of other subtypes has never been observed in wild birds (Webster, 1998).

Due to the capability of LPAIV H5 and H7 to mutate into highly pathogenic forms, infections of poultry with any viruses of H5 or H7 subtype regardless of their pathogenicity are nowadays classified as “notifiable avian influenza (NAI)”, and initiate official control measures (OIE, EU directive 2005/94/EC). The appropriate test to assess the pathogenicity of a certain virus strain is the determination of the “intravenous pathogenicity index (IVPI)”, according to the OIE standard protocol (OIE, Manual of Diagnostic Tests and Vaccines for terrestrial animals, 2008). The IVPI indicates the mean clinical score of ten 6-weeks-old chickens intravenously inoculated. Viruses are classified as HPAI if the IVPI is greater than 1.2 after ten days of evaluation (were birds were scored with 0 [healthy], 1 [sick], 2 [severely sick], 3 [dead]). In general viruses comprising a multibasic cleavage site in their HA sequence are confirmed by IVPI to be highly pathogenic. Nevertheless, rare exceptions are documented: H7 isolates from Chile (Suarez et al., 2004) and Canada (Pasick et al., 2005)

exhibited an unusual HA cleavage site, while being highly virulent when examined by IVPI. Vice versa, Londt and colleagues (2007) described viruses with a multibasic sequence at the cleavage site, displaying low virulence (IVPI<1.2) when inoculated intravenously into 6-week-old chickens.

To conquer HPAI H5N1 in endemic regions, poultry can be vaccinated. Thereby, vaccinated animals are protected against disease, but virus spread and intra-/ and interspecies transmission could not completely prevented since no sterile immunity is induced by commonly used inactivated vaccines (Savill et al., 2006). Therefore, highly susceptible poultry species not only loose their ‘indicator’ function in syndrome surveillance, but further contribute to virus transmission (Fig. 1B). Furthermore, viral mutants might gain unpredictable properties achieved after multiple passages in vaccinated birds.

Disease in poultry and wild birds

Generally LPAIV replicate in wild aquatic birds representing the natural host almost without causing disease. Nevertheless, e.g. migratory behaviour can be affected (van Gils et al., 2007). Clinical signs in poultry induced by viruses of this phenotype are some reduction in weight gain in fattening poultry or a slight and temporary decline in egg production in layers (Capua and Mutinelli, 2001).

In contrast, HPAI infections in gallinaceous birds result in mortalities of up to 100% within 48 hours (Swayne and Suarez 2000). Individual birds are listless, exhibit oedema, cyanosis of the comb, wattles and legs besides diarrhoea. Sudden deaths without any symptoms may also occur. Less vulnerable poultry species such as ducks, geese, ratites, and pigeons typically exhibit nervous symptoms including ataxia, torticollis and seizures (Kwon et al., 2005; Werner et al., 2007). Some duck species even show no or limited virus replication and few clinical signs (Alexander et al., 1978; 1986; Perkins and Swayne, 2002).

Before 2002, HPAIV were rarely isolated from wild birds and if so, only in close vicinity of ongoing outbreaks in poultry. The only exception was the report of the locally restricted high mortality of terns after infection with an H5N3 HPAIV (Becker, 1961). Since 2002 and in conjunction with the ongoing outbreaks of HPAIV H5N1 Asia this situation has dramatically changed as a variety of wild aquatic and terrestrial wild birds have died after infection with this virus. The large outbreak of HPAI H5N1 at Qinghai Lake in China in May 2004, which affected wild aquatic birds (Chen et al., 2005, Liu et al., 2005), characterise the striking virulence of this subtype. Since that time, fatalities due to HPAIV H5N1 Asia in wild birds were reported repeatedly from Asia, Europe and Africa (WHO timeline of major events), and experimental infections confirmed e.g. the high susceptibility of swans, wild geese and falcons (Brown et al., 2007, Kalthoff et al., 2008, Lierz et al., 2007).

Disease in mammals excluding humans

Interspecies transmission of influenza A viruses has occurred occasionally, mainly from aquatic birds to mammalian species. Marine mammals and horses have been shown to acquire influenza A viruses from avian origin (Fig.1 A; Guo et al., 1992, Ito et al., 1999), at times leading to serious disease and death. Interestingly racing dogs were recently reported of having acquired influenza A virus H3N8 from racing horses (Crawford et al., 2005). During 2007-2008, Australia, previously free from equine influenza, experienced a large-scale outbreak with H3N8 of avian origin (Patterson-Kane et al. 2008). Pigs have been postulated as intermediate host between avian and mammalian hosts as their respiratory epithelia possesses both types of sialo-receptors, providing a putative 'mixing vessel' function for genetic reassortment between human and avian viruses (Scholtissek, 1990). Although pigs remain a possible intermediate host for the transmission of AIVs to humans, recent scientific work revealed, that they probably play a less important role than quails (Perez et al., 2003; Wan and Perez, 2006). In addition, mustelids were shown to be seriously affected by a

H10N4 subtype infection in Sweden, and the isolated virus was very closely related to a virus of the same subtype isolated from chickens and a feral duck in England (Berg et al. 1990). Recently, Gillim-Ross et al. (2008) found that mice and ferrets are susceptible to some AIV H6 isolates proving that mammals can be productively and directly infected with avian H6 without prior adaptation.

Approving the exceptional virulence of HPAIV H5N1 Asia, this subtype infected feline species (tigers, leopards and domestic cats) as well as dogs after consumption of infected poultry (Fig. 1B; Keawcharoen et al., 2004; Songserm et al., 2006a, 2006b; Giese et al., 2008, Vahlenkamp et al., 2008). Clinical signs in cats consisted of high fever and respiratory distress followed by death. Infected domestic cats in endemically infected areas pose a special threat as they might be a link between wild birds, poultry and humans. Fatal infection of other mammalian carnivores (stone marten, oston`s palm civet) were recently reported for HPAI H5N1 (Klopfleisch et al. 2007, Robertson et al., 2006). Surprisingly, the susceptibility of pigs to HPAI H5N1 viruses was shown to be low (Lipatov et al., 2008), and experimental infection of calves resulted in subclinical seroconversion only (Kalthoff et al., 2008).

Disease in humans

Besides the fact, that the human pandemic viruses of 1918, 1957 and 1968 arose either by reassortment between viruses present in the human population and AIV, or by direct introduction into humans from birds, five avian influenza subtypes are known to have induced sporadic human disease after direct transmission from an avian source: HPAIV H5N1, LPAIV and HPAIV H7N3, LPAIV and HPAIV H7N7, LPAIV H9N2, and LPAIV H10N7 (table 1). Prior to 1996, there had been only three cases of AIV directly infecting humans (table1), and the reduced replication efficacy in human and non-human primates of such strains has been explained by the existing interclass-barrier (Hinshaw et al., 1983, Murphy et al., 1982, Beare and Webster 1991). Recently, human infections with AIV subtypes H5 and

H7 have been reported more frequently. The clinical spectrum of human infection ranged from asymptomatic infection to severe pneumonia and multi-organ dysfunction resulting in death [restricted to H5N1 Asia (245 cases) and to H7N7 (1 case, 2003), table 1]. HPAIV H5N1 Asia is exceptionally virulent and pathogenic in humans with an estimated case fatality proportion of close to 60%. However, the prerequisite for infection is very close contact with infected birds, e.g. the intensive inhalation or ingestion of infectious droplets or self-inoculation of the conjunctival or upper respiratory tract mucosa.

The majority of humans infected with low or highly pathogenic H7 viruses developed conjunctivitis (table 1). Although this ocular tropism dominated, some people showed additional flu-like symptoms. The single case of a fatal infection with HPAIV H7N7 occurred after pneumonia and respiratory failure (Kemink et al., 2004).

Person-to-person transmission of HPAIV H7N7 (Du Ry van Beest Holle et al., 2003) and of HPAIV H5N1 (Ungchusak et al., 2005; Wang et al., 2008) are reported as very rare events, although family clusters of human infection with HPAIV H5N1 infection were reported (Ungchusak et al., 2005; Olsen et al., 2005). Whether this cluster was related to genetic susceptibility of affected humans or reflected close contact through intensive care by family members is still point of discussion (Pitzer et al., 2007, Nicoll 2008). Viral genetic markers - besides the HA cleavage site sequence - identified as determinants of viral pathogenicity were reviewed very recently by deWit and Fouchier, 2008.

LPAIV H9N2 and LPAIV H10N7 have caused infections in humans that were associated only with mild clinical symptoms Lin et al., 2000, Butt et al., 2005).

Conclusions

Avian influenza viruses are primarily affecting wild birds and poultry. However, defined subtypes, and especially HPAIV, have the potential to directly infect other species including humans. The high natural variability of avian influenza viruses in combination with a broad

capacity of adaptation are the main risk factors, also interfering with immunological and therapeutic protection. In addition, an enhanced zoonotic potential can result on one hand from the transfer of genetic elements of avian viruses to human adapted viruses, or on the other hand from the generation of avian virus variants that replicate and spread efficiently in the human population. Nevertheless, both scenarios are not restricted to HPAIV, but are also possible features of LPAIV. The pandemics of the last century (H1N1, H2N2, H3N2) were all related to AIVs. Therefore, virus candidates of the next human pandemic could be besides HPAIV H5N1, in particular AIVs of the H2-, H7- or H9-subtype. In conclusion, active surveillance and collection of circulating AIV subtypes from different species is necessary to get hands on precursors of potential pandemic viruses which enables the development of tailored vaccines and further examination of viral genetic markers predictive of pandemic potential.

Future Prospects

The development of reverse genetics provides the tool to evaluate molecular mechanisms of efficient replication in different tissues and species. Elucidating the pathogenesis of influenza virus infection hopefully results in novel therapeutic approaches and improved prophylactic measure for humans as well as for veterinary practice.

Conflict of Interest Statement

None.

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548 **Table 1:** Documented human infections with avian influenza virus* (modified from Werner
549 and Harder, 2006)

Strain	Country/ Area	Date	Cases (deaths)	Symptoms	Source
H7N7 hp	USA	1959	1	respiratory	overseas travel
H7N7 hp	Australia	1977	1	conjunctivitis	Unknown
H7N7 lp	USA	1978-9	1	conjunctivitis	seals
H7N7 lp	UK	1995	1	conjunctivitis	pet ducks
H5N1 hp	Hong Kong SAR	1997	18 (6)	respiratory	poultry
H9N2 lp	China (Guangdong)	1998	5	unknown	unknown
H9N2 lp	Hong Kong SAR	1999	2	respiratory	poultry, unknown
H7N2 lp	Virginia, USA	2002	1	respiratory	poultry
H5N1 hp	China	2003	1	respiratory	unknown
	Hong Kong SAR		2		poultry
	Vietnam		3		poultry
H7N2 lp	New York	2003	1	respiratory	unknown
H7N7 hp	Netherlands, Belgium, Germany	2003	89 (1)	conjunctivitis (pneumonia, respiratory failure in fatal case)	poultry
H9N2 lp	Hong Kong SAR	2003	1	respiratory	poultry
H5N1 hp	Thailand	2004	17 (12)	respiratory	poultry
	Vietnam		29 (20)		
H7N3 hp	Canada	2004	2	conjunctivitis	poultry
H10N7	Egypt	2004	2	Respiratory, fever	Poultry/wild ducks
H5N1 hp	Cambodia	2005	4 (4)	respiratory	
	China		8 (5)		
	Indonesia		20 (13)		poultry
	Thailand		5 (2)		
	Vietnam		61 (19)		
H5N1 hp	Azerbaijan	2006	8 (5)	respiratory	
	Cambodia		2 (2)		
	China		13 (8)		
	Djibouti		1		
	Egypt		18 (10)		poultry
	Indonesia		55 (45)		wild birds
	Iraq		3 (2)		
	Thailand		3 (3)		
	Turkey		12 (4)		
H7N3 lp	United Kingdom	2006	1	conjunctivitis	poultry
H5N1 hp	Cambodia	2007	1 (1)	respiratory	
	China		5 (3)		
	Egypt		25 (9)		
	Indonesia		42 (37)		poultry
	Laos		2 (2)		birds
	Myanmar		1		unknown
	Nigeria		1 (1)		
	Pakistan		3 (1)		
	Vietnam		8 (5)		
H7N2 lp	United Kingdom	2007	4	conjunctivitis	poultry
H5N1 hp	Bangladesh	2008	1	respiratory	poultry

	China	3 (3)	unknown
	Egypt	7 (3)	
	Indonesia	20 (17)	
	Vietnam	5 (5)	

550 *Source: WHO, Cumulative Number of Confirmed Human Cases of Avian Influenza

551 A/(H5N1), Reported to WHO 10 September 2008, <http://www.who.int/csr/disease/>

552 avian_influenza/country/cases_table_2008_09_10/en/index.html.; Lin et al., 2000, Butt et al.,

553 2005

554 # lp low pathogenic isolate; hp highly pathogenic isolate.

555 **Figure legends**

556

557 **Figure 1:** Schematic illustration of (A) influenza A virus cross-species transmission and (B)
558 infectious routes of HPAI H5N1 in particular

Figure 1A

H1-H16
sporadic to endemic

H1-H16

H1,H3 endemic
H2,H4,
H5,H7,
H9 } sporadic

H1,H2,H3 endemic
H5,H7,
H9,(H10) } sporadic

H3,H7

H3

H13,H1,H3

H7,H4

Influenza A

Figure 1B

