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HPAI PRO-POOR HPAI RISK REDUCTION

HPAI H5N1 Transmission Risks: Pathways from Poultry to Humans

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Mekong Team Working Paper No. 10

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Preface

Since its emergence, H5N1 HPAI has attracted considerable public and media attention because the viruses involved have been shown to be capable of producing fatal disease in humans. While there is fear that the virus may mutate into a strain capable of sustained human-to-human transmission, the greatest impact to date has been on the highly diverse poultry industries in affected countries. In response to this, HPAI control measures have so far focused on implementing prevention and eradication measures in poultry populations, with more than 175 million birds culled in Southeast Asia alone.

Until now, significantly less emphasis has been placed on assessing the efficacy of risk reduction measures, including and their effects on the livelihoods of smallholder farmers and their families. In order to improve local and global capacity for evidence-based decision making on the control of HPAI (and other diseases with epidemic potential), which inevitably has major social and economic impacts, the UK Department for International Development (DFID) has agreed to fund a collaborative, multi-disciplinary HPAI research project for Southeast Asia and Africa.

The specific purpose of the project is to aid decision makers in developing evidence-based, pro-poor HPAI control measures at national and international levels. These control measures should not only be cost-effective and efficient in reducing disease risk, but also protect and enhance livelihoods, particularly those of smallholder producers in developing countries, who are and will remain the majority of livestock producers in these countries for some time to come.

With the above in mind, this document presents and discusses the potential pathways of HPAI transmission from poultry to humans.

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Disclaimer

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Keywords

Transmission Pathways, Disease Risk, HPAI, H5N1, Avian Influenza, Poultry, Humans.

More information

Please refer to the project website at www.hpai-research.net

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Executive Summary

Study Rationale

This working paper was commissioned by the Food and Agriculture Organization (FAO) with the purpose of critically reviewing published, grey literature, and accessible primary reports on HPAI, specifically focusing on highly pathogenic avian influenza (HPAI) subtype H5N1 (HPAI/H5N1) in humans. Therefore, the purpose of the following working paper is to review the epidemiology of HPAI/H5N1 in poultry and humans and to evaluate what is known about transmission patterns of HPAI/H5N1 from poultry-to-humans. Although this report focuses on HPAI/H5N1, studies which have evaluated poultry-to-human transmission for other HPAI strains (e.g., H7 outbreaks in the Netherlands, Italy and British Columbia) are included.

Background and Issues

Highly pathogenic avian influenza, subtype H5N1 (HPAI/H5N1) first crossed the species barrier in 1997 when an outbreak of 18 human cases resulting in six deaths was identified in Hong Kong [1, 2]. In 2003, HPAI/H5N1 crossed the species barrier a second time resulting in two cases and one death, again in Hong Kong [3]. Since 2003, H5N1 has been confirmed in domestic poultry and/or wild birds in 61 countries throughout Asia, Africa and Europe—largely in Viet Nam, Thailand and Egypt [4]—and in approximately 400 humans in 15 countries—largely in Indonesia and Viet Nam [5].

Preference has been given to peer-reviewed and published literature of HPAI/H5N1 transmission to and within human populations, although have included some guidelines from the World Health Organization (WHO), the Food and Agriculture Organization of the United Nations (FAO) and the World Organization for Animal Health (OIE).

Several epidemiologic studies have evaluated the risk of transmission of HPAI from poultry-to-humans including case-control studies and seroprevalence studies of social contacts, health care workers of confirmed H5N1 cases as well as poultry workers who were exposed to infected poultry. These studies have identified several risk factors that may be associated with infection including close direct contact with poultry and transmission via the environment. However, there are several important data gaps limiting our understanding of the epidemiology of H5N1 in humans. Research to date has demonstrated that despite frequent and widespread contact with poultry, transmission from poultry to humans is rare.

Introduction

Globalisation has brought an unwelcome problem – increased risk of transboundary diseases. HPAI clearly illustrates that through extending livestock supply chains, local conditions of animal production have repercussions on global human health risks.

For a vast majority of rural households in developing countries, poultry act as an important source of protein and are part of the social fabric, a situation which will not change in the near future. Therefore, global policies toward HPAI and its control necessarily implicate the rural poor majority and these people need to be recognized as part of the solution to reducing human health risk, not the problem.

It has been seen time and time again that prescriptive eradication measures fail to achieve their direct objective and that by driving the problem 'under ground', disease risk actually increases. Because of their diversity and weak institutional linkages in most of the affected countries, national policies cannot be designed and implemented effectively without close attention to local incentives. Despite international pressure to act quickly on control measures, one size will not fit all or even a significant percentage of local conditions.

To ensure effective, affordable and socially fair HPAI control programmes, national and international policy making needs to be based on stringent analysis of risks, consequences and risk management options.

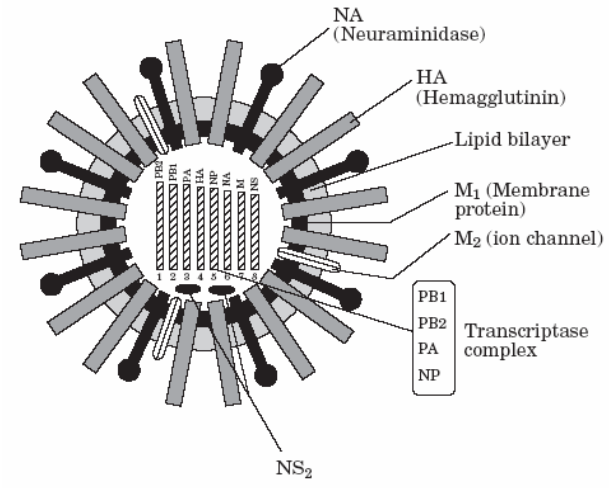
Background

The following sections will briefly review the biology of influenza "A" viruses, specifically looking into course of infections, clinical manifectations in humans, and finally, detection methodologies.

Biology of Influenza "A" Viruses

There are three types of influenza viruses – A, B and C – within the Influenzavirus genus and Orthomyxovirdae family. Only type A is capable of causing severe infections and pandemics in human populations [6], although type B can cause severe morbidity and mortality particularly in children. The central core of influenza A viruses contain eight single-stranded RNA gene segments surrounded by the surface glycoproteins hemagglutinin (HA) and neuraminidase (NA) (Figure 1) [7-9]. Influenza A viruses are classified into subtypes based on the antigenicity of HA and NA glycoproteins. There are 16 HA and nine NA subtypes. Only three HA (H1, H2, H3) and two NA subtypes (N1, N2) are widely present in humans [10].

Figure 1. Illustration of the structure of the influenza "A" virus.



Source: [9].

Influenza A viruses can infect several animal species including birds, pigs, horses, seals, cattle, and whales (Table 1). The natural host of all HA and NA subtypes are aquatic birds mainly ducks, gulls and water birds [6, 10, 11].

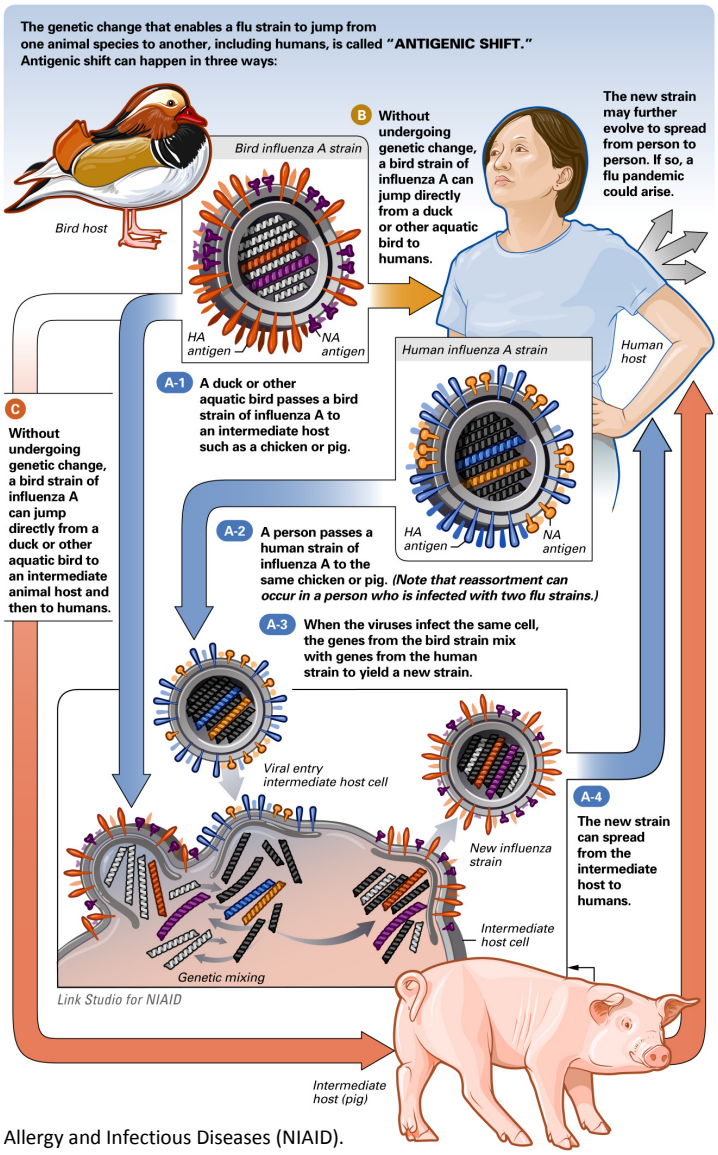
Table 1. Reservoir for HA and NA subtypes.

Host	**HA Subtypes**	**NA Subtypes**
Human	H1, H2, H3, H5, H7,	N1, N2, N3, N7
Pig	H1, H3, H4, H9	N1, N2
Waterfowl	**All 16 subtypes**	**All 9 subtypes**
Horse	H3, H7	N7, N8
Seal	H4, H7	N7
Cattle	H3	N2
Whale	H3, H13	N2, N9
Cat, Tiger	H5	N1

The variability of influenza A viruses depends on the evolution of the virus through point mutations (antigenic drift) and genetic reassortment (antigenic shift) [10, 12]. Minor changes in the surface glycoproteins occur from point mutations due to the absence of proofreading mechanisms of RNA molecules as the virus replicates in the host. These point mutations occur often resulting in annual variations in the human influenza strains circulating the globe. It is these changes that require the production of new human seasonal influenza vaccines each year [13].

Humans are naturally protected from avian influenza viruses because we lack certain receptor binding sites (α 2-3 receptors) in our respiratory tracks that are required for infection to occur. Humans possess α 2-6 receptors, which are binding sites for human influenza viruses (e.g., H1N1, H3N2) but typically not susceptible to avian influenza viruses. Pigs are susceptible to both human and avian influenza viruses because they possess receptors for both avian and human influenza viruses (α 2-3 receptors and α 2-6 receptors, respectively), and therefore can serve as an 'intermediate host' (i.e., mixing vessel) (Figure 2). Antigenic shift results from the reassortment of two distinct influenza A viruses (e.g., avian and human influenza viruses) within a single host (e.g., pigs) and represents a major change in viral composition. This can result in the formation of novel viruses [10, 14, 15].

Figure 2. Illustration of antigenic shift of influenza "A" viruses.



Source: National Institute of Allergy and Infectious Diseases (NIAID).

Course of Infection of HPAI/H5N1 in Birds

Influenza A viruses occurring in birds are collectively termed avian influenza viruses. Strains of avian influenza virus are categorized as having high (HPAI) or low pathogencity (LPAI) based on the severity of disease and mortality caused in chickens [16]. LPAI strains are capable of mutating into HPAI as occurred in the Italian H7N1 outbreak in 1999-2000 [17-19]. HPAI strains replicate rapidly in the gastrointestinal tract of birds and can spread and replicate in multiple organs often resulting in rapid death [17, 19]. Chickens (order Galliformes) are more susceptible to influenza A viruses infection than ducks, geese and swans (order Anseriformes) and therefore are more likely to be diseased and die from infection [16].

HPAI/H5N1 has been further categorized into phylogenetic clades. Genetic analysis of the H5 NA genes circulating since 2003 indicate that Clade 1 strains have been circulating in Thailand, Viet Nam and Cambodia whereas Clade 2 (and several subclades 2.1-2.3) have been circulating in Indonesia (subclade 2.1), Europe, the Middle East and Africa (subclade 2.2) and China, Japan and South Korea (subclade 2.3) [20].

Symptoms of HPAI/H5N1 infection in birds range from asymptomatic, mild disease (anorexia, depression, weight loss) to severe neurological symptoms (e.g., tremors, shaking, lack of coordination, spinning, seizures) and sudden death [21]. Severe disease is usually caused by systemic virus replication affecting organs and tissues [22-25].

Experimental studies have demonstrated that chickens are almost always susceptible to HPAI/H5N1 infection with 80-100% mortality occurring within 1-5 days post inoculation (dpi) [26-29]. Experimental evidence has shown that the pathogenicity and mortality of HPAI/H5N1 in ducks has changed since 2002 and varies depending on the infecting strain [21, 23, 25, 30]. Mortality can occur faster in chickens (within 1-5 days) [27, 28] than ducks (6-7 days) [23, 27, 31]. Morbidity and mortality of HPAI/H5N1 infection in ducks also varies by age [21]. During an outbreak of commercial domestic ducks in South Korea in 2003-2004, morbidity and mortality was higher in younger ducks as compared to older animals [32].

Clinical signs are almost always present in chickens infected with HPAI/H5N1 with onset typically from 2-5 dpi until death [27, 33-35]. Tracheal viral shedding and cloacal/faecal viral shedding have been experimentally shown to begin on or before day 2 (1-3) dpi [27, 36-38]. Although the susceptibility of chickens to HPAI/H5N1 almost always leads to clinical symptoms and death, the susceptibility of wild birds and domestic ducks depends on several factors including the circulating strain [23, 25] and the age of the ducks [21]. This indicates that the pathogenicity of HPAI/H5N1 in ducks is somewhat inconsistent [21] and may be a factor in the observed differences in geographic distribution of poultry outbreaks.

In experimental studies of ducks, the onset of clinical symptoms occur 2-10 dpi [31, 39] and oropharyngeal and cloacal shedding can occur from 2-7 or up to 11-17 dpi [23, 34]. The average infectious period of ducks is estimated to be 4.3 days (95% CI 3.8-4.8) [40]. Virus titres in ducks have been found to be highest 2-3 dpi and reduce to undetectable levels by 13-20 dpi [23, 40]. Typically virus shedding is higher in symptomatic ducks. In experimental and in field settings, H5N1 virus has been detected in cloacal, tracheal and blood samples of asymptomatic ducks [41].

In wild ducks and waterfowl, H5N1 has been found to replicate in the gastrointestinal tract and infected birds can shed the virus for up to 30 days [1, 25]. Data from the Netherlands and Asia found that the virus is shed in higher doses in the pharynx than in faeces of wild ducks and mallards at 3 and 5 dpi [25, 42, 43].

The stability of HPAI/H5N1 in poultry faeces and in water is not well understood. Experimental evidence suggests that H5N1 loses infectivity in chicken faecal manure within 24 hours at 25°C and within 15 minutes at 40°C [44], indicating that the infectiousness of contaminated faecal manure may be shorter in warmer climates. However, another study suggests that H5N1 is viable in faeces for 2 days at 37°C [34] highlighting that further experimental study is necessary to understand the persistence of H5N1 in the environment under various environmental conditions. Experimental evidence has suggested that influenza A viruses are detectible in water and wet faeces for up to 6 days at 37°C [45] and H5N1 can survive in carcasses for several days at room temperature and longer in cooler (+4°C) temperatures [5, 46].

Data on the persistence of HPAI/H5N1 virus in tissues is limited. An experimental study of ducks challenged with HPAI/H5N1 demonstrated that the virus is detectable in breast and thigh tissue at 3-7 dpi, in the liver and intestine at 3-4 dpi and in the lung at 3-6 dpi. An experimental study of chickens challenged with HPAI/H5N1 found virus detectible in the trachea, lung, bone, breast and thigh tissue at 1-5 dpi [38]. These results suggest that systemic infection occurs at a faster rate in chickens than ducks and provides insight on why HPAI is more virulent in chickens.

Since wild ducks, domestic ducks and geese infected with HPAI/H5N1 can be asymptomatic; they may act as silent vectors for transmission and represent a major challenge in controlling the spread of HPAI [23, 30, 43].

Clinical Manifestations of HPAI in Humans

The pathogenicity of HPAI/H5N1 and HPAI/H7N7 in humans ranges from undetected asymptomatic or sub-clinical to severe disease resulting in death. Although the apparent case fatality rate (CFR) of HPAI/H5N1 is high (>60%), this may be an overestimate of the true CFR since relatively few seroprevalence studies have been carried out to determine the number of subclinical or asymptomatic cases in countries affected by H5N1 outbreaks in humans, domestic or wild poultry populations.

The incubation period of H5N1 in humans is believed to less than 7 days (range: 2-9 days) [47-49]. The first symptoms of H5N1 disease—typical of seasonal influenza (fever, dyspnoea, cough, sore throat) and pneumonia but sometimes including gastrointestinal symptoms (abdominal pain, diarrhoea, or vomiting)—usually appear within 1-2 days after infection, although they can take up to 8 days to appear. Among severely affected patients, severe respiratory distress syndrome can occur as well as bilateral pneumonia and multi-organ failure [49-51].

HPAI/H7N7 in humans following an outbreak in commercial poultry farms in the Netherlands resulted in 89 infected subjects who suffered mostly from mild illness including conjunctivitis (87.6% n=78), influenza like illness (2.2% n=2), both conjunctivitis and influenza like illness (5.6% n=5), or other symptoms (4.5% n=4). However one subject (1.1%) died of acute respiratory distress syndrome and pneumonia [52].

H5N1 Detection Methods

HPAI/H5N1 infection can be detected through virologic and/or serologic testing methods. Serological tests (e.g., haemagglutination inhibition [HI] test, microneutralisation test, agar gel diffusion [AGID] test, enzyme-linked immunosorbent assay [ELISA]) detect antibodies indicating that an individual or bird has been infected in the past but cannot determine when infection occurred and are therefore indirect markers for infection [53-55]. Virological testing (e.g., rapid antigen detection tests, polymerase chain reaction [PCR] for nucleic acid detection, virus isolation after inoculation into cell cultures or embryonated eggs) assesses the presence of influenza A viruses and allows subsequent identification of specific viral subtypes [56].

Typically, suspect specimens are first tested to determine the presence of influenza A viruses or influenza A antibodies. If positive for influenza A virus or M gene detection, specimens undergo further testing to determine the subtype of the infecting strain (e.g., H5N1, H9N2, H3N2, etc). There are various tests that can be used to identify the presence of H5N1 virus. However, some methods are not appropriate for all settings because they may require highly trained staff to carry out the tests and/or require bio-safety level 3 laboratories (BSL-3) because they involve handling live HPAI viruses (e.g., virus isolation, microneutralisation tests) [57].

From all suspected H5N1 human cases, guidelines from WHO recommend collecting samples from the upper respiratory tract (e.g., nasopharyngeal and/or throat swabs) and blood samples (for serology and/or nucleic acid detection). If the patient is hospitalized and intubated, samples from the lower respiratory tract (e.g. tracheal aspirates, broncho-alveolar lavage) should be collected [58]. For suspected H5N1 in poultry populations, guidelines from OIE recommend collecting oropharyngeal samples and cloacal samples (or fresh feces) from live birds, and organ tissue (e.g., trachea, lungs, air sacs, intestine, spleen, kidney, brain, liver and heart) from dead birds [59].

Throat or nasopharyngeal swabs from suspect humans and oropharyngeal or cloacal samples from suspect birds should ideally be taken as soon as possible for the detection of H5N1 virus [58, 59]. Because antibodies require a few days to a week or longer to develop in birds [55] and sometimes more than 14 days to develop in humans [53, 54], the timing of serum sample collection for anti-H5N1 antibody detection should be considered.

Human sera tested using an H5N1 virus specific microneutralization assays are considered positive for anti-H5N1 neutralizing antibodies when titers are ≥1:80 [53]. Human sera that test positive for anti-H5N1 antibodies are then tested using Western Blot techniques or HI tests using horse red blood cells. Sensitivity and specificity is highest when a combination of microneutralization and Western Blot testing techniques are used (sensitivity 80-88%, specificity 96-100% depending on the age of the patient) [54]. The WHO requires a positive test result for both microneutralization and confirmation with Western Blot or HI to be considered positive for anti-H5 antibodies [53, 54, 60]

Epidemiology and Transmission of HPAI/H5N1 in Birds

In the following sections we will review the history of HPAI epidemics in birds, with a closer look at the geographic expanse of this disease and the number of hosts that can harbor H5N1 viruses. Towards the end, we will also examine some of the salient features of animal-to-animal transmission dynamics and their defining attributes.

History of HPAI Epidemics in Birds

All strains of influenza "A" viruses naturally infect a large variety of wild birds, including wild ducks and waterfowl, but do not usually cause disease [10]. However, there have been several instances of major outbreaks of HPAI in poultry over the last two and a half decades (Table 2) [10, 61]. HPAI/H5N1 was first detected in Hong Kong in 1997, but since 2003, HPAI/H5N1 has been confirmed in birds in 61 countries in Asia Africa and Europe (Figure 3) [62].

Table 2. Major outbreaks of HPAI (H5, H7) in poultry.

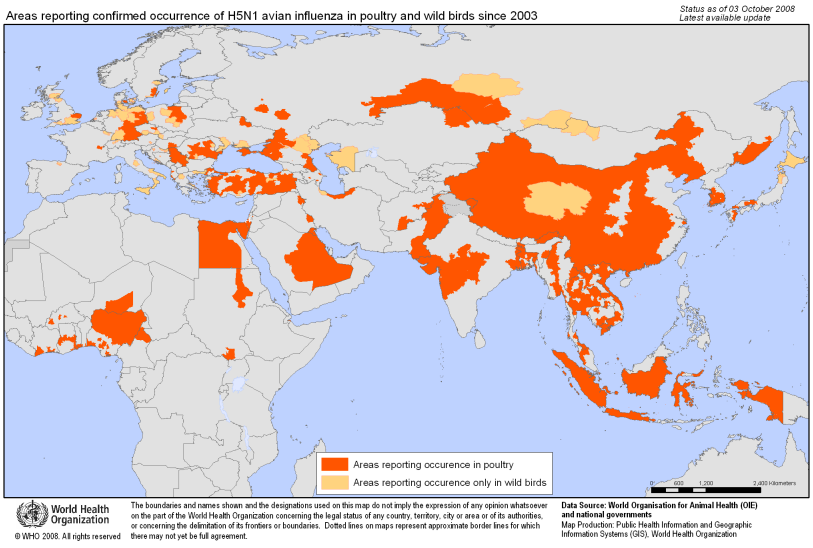
Year	Location	Subtype	Approximate number of poultry culled or dead
1983	PA, USA	H5N2	17 million (culled)
1994-2003	Mexico	H5N2	1 billion
1995-2003	Pakistan	H7N3	3.2 million (dead)
1997	Hong Kong	H5N1	1.5 million (culled in 3 days)
1999-2000	Italy	H7N1	16 million (culled)
2003	The Netherlands	H7N7	30 million (killed)
2004	British Columbia, Canada	H7N3	>19 million (culled)
2003-present	Asia, Europe, Africa	H5N1	220+ million (culled or dead)

Source: [10, 61, 63].

Expanding Geographic and Host Range of H5N1

Since 2003, the geographic and host range of HPAI/H5N1 has expanded. Figure 3 illustrates the countries which have reported H5N1 outbreaks in wild and domestic bird populations since 2003.

Figure 3. Countries reporting confirmed H5N1 in domestic and wild birds from 2003 to 2008.



Approximately 6,500 H5N1 poultry outbreaks have been reported thus far, resulting in hundreds of millions of poultry culled [62, 64]. Most outbreaks have been reported in Asia (>60% of the outbreaks reported), and to a lesser extent in Africa, the Middle East and Europe [62]. No outbreaks of H5N1 in domestic or wild birds have been reported in Australia, the Pacific Islands or the Americas.

The numbers of reported outbreaks according to OIE and Food and Agriculture Organization (FAO), vary significantly from each other because of reporting requirements making it difficult to fully understand the extent of outbreaks in wild and domestic bird populations [62, 64]. Differences in rates of detection of HPAI/H5N1 between countries may depend on the active and passive HPAI surveillance systems established and whether the focus of the surveillance system in place, if any, is on the commercial or backyard sector of poultry production. It has been suggested that it is more likely that HPAI will be detected in commercial farms as opposed to backyard flocks [65].

HPAI/H5N1 was first detected in a goose in Guangdong Province in China in 1996 and spread to poultry in Hong Kong in 1997. In humans, H5N1 was first detected in late 2003 in a family from Hong Kong that had recently travelled to Fujian Province in China. Within the first six months of 2004, H5N1 was reported among poultry in Korea, Thailand, Viet Nam, Cambodia, Laos, Japan, and Indonesia. Between July 2004 and July 2005, H5N1 was repeatedly detected in poultry in Thailand, Hong Kong, Indonesia, Viet Nam and Cambodia [3]. During this same time period, H5N1 expanded its host range to dogs, palm civets, ferrets, mice, and small and large cats [66]. Natural infection of HPAI/H5N1 was identified in tigers in a Thailand zoo that were likely infected after being fed contaminated poultry [3, 67].

Since 2003, widespread outbreaks in domestic ducks in China may have lead to the endemic situation in ducks in many countries throughout South East Asia [23, 30]. Additionally, human cases were often identified before outbreaks in poultry within many countries in Asia. This delayed detection may have also contributed to the endemic or recurrent situation in these countries [68].

HPAI/H5N1 was first detected in Europe in July 2005 in Russia and in the Middle East in early 2006. Within eight months (July 2005 to February 2006), H5N1 spread to domestic or wild poultry in 22 countries/territories including Kazakhstan, Turkey, Mongolia, Romania, Ukraine, the United Kingdom, Iraq, Italy, Slovenia, Kuwait, Bulgaria Croatia, Egypt, France, Germany, Austria, Hungary, Bosnia-Herzegovina, Slovakia, Azerbaijan, Georgia, and the West Bank/Gaza Strip [3].

H5N1 outbreaks in Europe have been sporadic and to date, have only occurred in animal populations. Early detection in these countries is likely due to sufficient infrastructure and ample preparation time to establish surveillance systems for the early detection of incursion of H5N1. Conversely, some countries where H5N1 has been detected have been affected by conflict or war (e.g., Afghanistan, Pakistan, West Bank/Gaza Strip). This has prevented proper HPAI surveillance due to limited financial resources, weak veterinary infrastructure and lack of access to some areas within these countries [68]. Within the Near East/North Africa region, the greatest numbers of outbreaks have occurred in Egypt, which has had outbreaks confirmed in poultry populations from almost all administrative regions in the country [69].

In sub-Saharan Africa HPAI/H5N1 was first detected in Nigeria [70]—possibly transmitted to the country through migratory birds or trade of live day-old chickens [71, 72]—in January 2006 and has sporadically spread to domestic and/or wild birds in Cameroon, Burkina Faso, Sudan, Cote d'Ivoire, Djibouti, and Benin [3]. Only two human cases of H5N1 have been identified throughout the whole of Africa, which occurred in Nigeria in early 2007 and in Djibouti in 2006. Since 2007, no further outbreaks in poultry and/or humans have been reported in Nigeria and no human cases have been reported from any of the above named countries that have reported H5N1 outbreaks in poultry populations.

Animal-to-Animal Transmission of H5N1

Animal-to-animal transmission of H5N1 can be direct via the faecal-oral route [46] or indirect through contaminated feed, clothing, and equipment (fomites) [73]. Live markets may be an important reservoir for H5N1 [74], as seen in H5N1 outbreaks in Viet Nam, Thailand and Hong Kong [75-79]. Movements of domestic poultry may also play a substantial role in viral spread. A study of the spatial distribution of HPAI outbreaks in Thailand showed a strong relationship between free-grazing ducks in rice fields and viral spread [80]. Large bodies of water such as lakes that serve as resting places for wild aquatic birds may also play a role in transmission [10] because all birds shed virus in faeces [9, 25, 81].

It is also possible that trade of commercial and domestic poultry and poultry products, often occurring across long distances is responsible for transmission between and within countries [5, 68, 82, 83]. Transmission is also likely to be occurring between wild and domestic bird populations in both directions [42].

Live bird markets (LBM) are common in Asian countries because of a cultural preference to consume freshly slaughtered meat [74, 84]. The dense concentration of live birds and a high turn-over rate of birds (i.e., hosts) in these markets provide ample conditions for virus amplification [84] and may be an important reservoir for HPAI or “hub” for circulation [85]. Additionally, LBM may be an ideal environment for transmission of avian influenza viruses from poultry-to-humans since they are frequented by large numbers of people [74].

It is unclear what role LBM has played in the circulation of HPAI/H5N1 in many Asian countries where LBM are prevalent. The close contact with live animals at such markets has been identified as a risk factor for SARS [86] and HPAI/H5N1 [87]. It has been demonstrated from investigations of past and current outbreaks and from HPAI surveillance programs in Viet Nam, Thailand, Cambodia, China and Hong Kong, that HPAI/H5N1 is circulating in the LBM [75-79, 88, 89]. It can also be assumed that HPAI/H5N1 may be circulating undetected in the markets of many other countries.

The movement of poultry through LBM has been shown to be an important factor in the circulation and spread of HPAI [77, 90]. In early 2002 in Hong Kong, an investigation into an outbreak first identified in LBM led to the discovery of the virus on rural farms that had sold chickens to the LBM [90]. Further work determined that the contact between the retail market and chicken farms via humans was a significant risk factor for infection among chicken farms [77].

Control of avian influenza viruses within LBM focuses on implementing rest days, in which poultry stalls are emptied, cleaned and restocked. These efforts, which have been implemented in Hong Kong, have shown to reduce transmission of HPAI (H9N2) and other viruses among birds in LBM [76].

Epidemiology and Transmission of HPAI/H5N1 in Humans

In the following sections we will briefly go over some of the concepts pertaining to influenza pandemics, transmission of H5N1 to human hosts, some examples of human seroprevalence studies so far done, human transmission clusters, and finally, with indirect viral transmission to humans.

History of Influenza A Pandemics in Humans

There have been several human pandemics of influenza A viruses over the last 150 years [8, 91, 92]. The pandemic of 1918-1919 (H1N1) was particularly lethal in young, otherwise healthy adults, killing an estimated 40-50 million people worldwide [6, 10, 92, 93]. Genetic analyses of specimens collected

from victims preserved in the arctic suggest that the strain was a novel avian-like virus that adapted to humans [94]. The Asian Influenza Pandemic (H2N2) in 1957 and Hong Kong Influenza Pandemic (H3N2) in 1968 were less lethal and resulted from avian-human reassortment [10, 93].

Since 1977 two influenza subtypes (H1N1 and H3N2) have been circulating in humans worldwide. The isolation of H5N1 from a 3-year-old boy in Hong Kong in 1997 was the first occurrence of a novel strain in humans and signalled the emergence of a potentially new pandemic strain of avian influenza [1]. H5N1 in Hong Kong in humans in 1997 did not emerge from reassortment; all of the genes found in this viral strain originated from an avian virus [1, 10].

Transmission of H5N1 to Humans

As of 30 December 2008, HPAI/H5N1 has infected 387 individuals in 15 countries [5]. The number of cases is not evenly distributed throughout the world. By far, the largest number of human cases reported has been from Indonesia and Viet Nam each having reported more than 100 cases (Table 3). No human cases have yet been reported in Western Europe or the Americas.

Table 3 reports the number of cases and fatalities in each country affected by H5N1 in humans, the clade or subclade that is circulating in the country and the median age and gender (% male) of the cases [49, 95]. The overall case fatality rate (CFR) is 63.1% (median 62.5% IQR: 33.3-74.6) and varies by country [95]. To date, the occurrence of cases of HPAI/H5N1 in humans is rare.

Table 3. Case fatality rate of H5N1 in humans by country as of 30 December 2008.

Country	Total		Case Fatality Rate (CFR) %	Clade or Subclade	Median age of cases (range)	% Male n/ total (%)
	Cases	Deaths				
Azerbaijan	8	5	62.5	2.2	10 & 16.5 (5-20) ‡‡	9/16 (56) ‡‡
Turkey	12	4	33.3	2.2		
Bangladesh	1	0	0	2.2	16 mo (--)	1/1 (100)
China	30	20	66.7	2.3	30 (12-41)‡	3/8 (38)‡
Djibouti	1	0	0	2.2	2 (--)	0/1 (0)
Egypt	50	22	44.0	2.2	12.5 (1-75) α	12/38 (32) α
Indonesia	137	112	81.8	2.1	18.5 (1.5-45)‡	33/54 (61)‡
Iraq	3	2	66.7	2.2	15 (3-39)	2/3 (66.7)
Lao People's Democratic Republic	2	2	100	2.3	28.5 (15-42)	0/2 (0)
Myanmar	1	0	0	NR	7 (--)	0/1 (0)
Nigeria	1	1	100	2.2	22 (--)	0/1 (0)
Pakistan	3	1	33.3	NR	25 (22-27)	3/3 (100)
Cambodia	7	7	100	1	14-22 (2-58)†	19/41 (46)†
Thailand	25	17	68.0	1		
Viet Nam	106	52	49.1	1		
Cambodia††	8	7	85.7	1	16(3-28)	3/8 (37.5) ††
Total	387	245	63.1	--	--	--

Sources: Adapted from [5, 49, 96, 97]; ***Notes:*** †Data from 2004-2005 cases only; ‡Data from 2005-2006 cases only; α Data from 2006-2007 cases only; ‡‡Data from 2006 cases only; †† Data from all cases (n=8); NR= Not released

The investigations of human H5N1 outbreaks in the field—usually in rural locations of developing countries—are difficult to conduct and have often involved collection of incomplete information about exposures. Thus data on exposure are typically limited to "recent contact with infected poultry" [98] or the preparation of sick birds for consumption [99]. The specific mode of transmission from exposure to infected poultry remains unknown and the lack of exposure information has restricted our ability to evaluate risk factors for infection. In addition, the lack of large-scale seroprevalence studies in areas where H5N1 is recurrent has limited our understanding of the extent of infection in these countries.

A small number of epidemiologic studies have been conducted throughout Asia and Africa to evaluate risk factors for human H5N1 infection. Most of these have been of a case-control design where researchers have evaluated exposure to poultry via visiting live poultry markets, through food preparation or caring or feeding poultry or contact with a confirmed human case. All of these studies, the results of which are summarized in Table 4, have included small numbers of subjects thus limiting the precision of their results.

Table 4. Risk factors for H5N1 Infection: Summary of published case-control studies.

Study, year	Study Population	Risk Factors RR, OR, 95%CI
Mounts et al., 1999 [87]	Hong Kong 15 cases 41 matched controls	Exposure to poultry at live/wet markets was associated with a 4-fold increased risk (OR=4.5, 1.2-21.7)
Dinh et al., 2006 [100]	Viet Nam 28 cases 106 matches controls	Univariate Analysis: preparing/cooking unhealthy poultry (OR=31, 2.4-1150), having sick or dead poultry in the household (OR=7.41, 2.7-59), presence of sick/dead poultry in the neighborhood (OR=3.9, 1.0-55.7), no indoor water source in the household (OR=5.0, 1.3-77.0) Multivariate Analysis: No water in the household (OR=6.5, 1.2-34.8), sick or dead poultry in the household (OR=4.9, 1.2-20.2), prepare and cook sick or dead poultry (OR=9.0, 0.98-82.0)
Areechokchai et al., 2006 [48]	Thailand Matched case control study of 16 cases and 64 controls	Direct touching of unexpectedly dead poultry OR 29.0 (2.7—308.2)

Example 1. Hong Kong

H5N1 first crossed the animal human species barrier in 1997 in Hong Kong in a 3 year old boy and subsequently infected 17 others. A case-control study of 15 of these confirmed H5N1 cases and 41 controls matched on sex and age (±1.5 years for case subjects <18 yrs old and ±5 yrs for all other cases) found that exposure to live poultry at live/wet markets in the week before illness was associated with a 4-fold increased risk in infection with H5N1 (OR=4.5 95%CI 1.2-21.7); but did not find consumption of cooked or undercooked poultry at home or at a restaurant as risk factors for infection [87].

Example 2. Viet Nam

There have been 106 cases and 52 deaths due to H5N1 infection in Viet Nam since 2003. The majority of these cases were detected in 2004 and 2005 and incidence has declined (n=13 2006-2008), possibly due to reduced exposure resulting from control of HPAI in poultry through mass vaccination of domestic poultry populations.

A case-control study from Viet Nam (28 cases; 106 matched controls) found increased risk for human infection with H5N1 from preparing/cooking unhealthy poultry (OR=31, 95%CI 3.4-1150), having sick or dead poultry in the household (OR=7.41, 95%CI 2.7-59.0), presence of sick/dead poultry in the neighborhood (OR=3.9, 95%CI 1.0-55.7), and no indoor water source in the household (OR=5.0, 95%CI 1.3-77.0). This study did not find any other risk factors for infection including other animals in the household or neighborhood (pigs, dogs, cats, buffalo, and cows), household members working with commercial poultry, helped prepare or cook sick or dead poultry, prepared and cooked healthy poultry, or bought freshly killed poultry for household consumption [101].

Example 3. Thailand

There have been 25 cases and 17 deaths due to H5N1 infection in Thailand since 2003. All of these cases occurred in 2004-2006, none have been reported since 2007. A case-control study from Thailand evaluated risk factors for H5N1 infection in 16 confirmed patients as compared to 64 controls matched on village and age (±1 year). Cases were more likely to have touched a dead bird that died unexpectedly (i.e., death of >10% of all poultry in a household within 1 day or death >40% within 3 days) (OR=29, 95%CI 2.7-308.2); dressed poultry (no definition provided, OR=17, 95%CI 1.6-177.0); had poultry that died unexpectedly around their house (OR=5.6, 95%CI 1.5-20.7); plucked feathers from poultry (OR=14, 95%CI 1.3-152.5); stored products of sick or dead poultry in their house (OR=9.3, 95%CI 2.1-41.3); and directly touched sick poultry (OR=5.6, 95%CI 1.5-20.7). Risk factors for infection also included living ≤1 meter from sick (OR=3.8, 95%CI 1.2-11.7) or dead (OR=13, 95%CI 1.5-96.3) poultry [48].

Example 4. The Netherlands

In 2003, an outbreak of HPAI N7N1 was detected in the Netherlands affecting hundreds of poultry farms and resulting in 83 human cases. Most cases experienced only mild symptoms, (influenza-like-illness and/or conjunctivitis), but one individual died from the infection. Farm workers, mostly cullers and veterinarians involved in control procedures, became infected through handling infected poultry during outbreaks of H7N7 among 225 affected commercial poultry farms in the Netherlands [52].

Human Seroprevalence Studies

To date, a few small-scale human seroprevalence studies have been conducted in Hong Kong, China, Thailand, Nigeria, Cambodia, and Viet Nam to determine the frequency of asymptomatic or subclinical infection and evaluate risk factors for HPAI/H5N1 virus infection [41, 53, 102-110]. These studies are summarized in Annex 1 and can be categorized by the study populations evaluated in each study: occupationally exposed individuals (health care workers or poultry workers) or non-occupational settings (subjects living or working in close proximity to confirmed H5N1 case).

Occupationally exposed persons: poultry workers

The following four studies evaluated the frequency of asymptomatic or subclinical infection and poultry-to-human risk factors for H5N1 and H7N1 virus infection among poultry workers:

- *Bridges et al 2002*: The risk of H5N1 infection was evaluated among poultry workers involved in the culling of all poultry in Hong Kong following the first reported human H5N1 case in a child in Hong Kong in 1997. Among the 1525 poultry workers and 293 government workers enrolled, 83 (5.3%) poultry workers and nine (3.1%) government workers tested positive for H5N1 antibodies by both microneutralization and Western Blot techniques.

  A nested case-control study evaluated the risk factors for infection among the poultry workers (n=81) compared to unmatched controls. Risk factors associated with infection

included work in retail vs. wholesale/hatchery/farm/other poultry industry OR=2.7 (95% CI 1.5-4.9); >10% mortality among poultry with which they had worked in the previous two months OR=2.2 (95% CI 1.3-3.7); butchering poultry OR=3.1 (95% CI 1.6-5.9); feeding poultry OR=2.4 (95% CI 1.4-4.1); and preparing poultry for restaurants OR=1.7 (95% CI 1.1-2.7). The study found that subjects exposed to intense contact with poultry during the culling processes were at an increased risk for infection with H5N1. It also found that exposure through trading poultry at retail markets was associated with increased risk of H5N1 infection.

- *Ortiz et al 2007*: Upon confirmation of a H5N1 outbreak in poultry in Nigeria in 2006, the risk of H5N1 infection among poultry workers and laboratory workers in contact with H5N1 was evaluated. Two-hundred and ninety-five poultry workers who had been exposed to infected poultry occupationally and domestically participated in the study. Home exposure to poultry included owning any (54%) or sick poultry (42%) or touching live or dead poultry (81%). None of the 295 poultry workers or 25 laboratory workers tested positive for H5N1 antibodies by microneutralization and HI assay using horse red blood cells. This study found no evidence of poultry-to-human transmission among poultry and laboratory workers in contact with infected poultry.

- *Wang et al., 2006*: One hundred and ten live bird poultry market workers were tested for neutralizing antibodies of H5N1 following detection of H5N1 in a man in Guangdong Province, China. One subject, who reported slaughtering birds, tested positive using HI assay with turkey red blood cells.

- *Puzelli et al 2005*: The risk of HPAI/H7N1 and LPAI/H7N3 was evaluated among Italian poultry workers of farms affected by an outbreak of HPAI/H7N1 between 1999 and 2003. No serum samples tested positive for HPAI/H7N1 (0/672).

Occupationally exposed persons: health care workers

The following four studies evaluated the frequency of asymptomatic or subclinical infection and evaluated human-to-human transmission risk factors for H5N1 virus among health care workers:

- *Bridges et al 2000*: The risk of H5N1 among health care workers involved in the care of confirmed H5N1 patients in Hong Kong in 1997 was compared to health care workers without known exposure to confirmed cases but with similar patient responsibilities. Because diagnosis was delayed, infection control procedures were not immediately initiated. Risk factor data were collected on exposure to the case patient (provided direct care to case, physical contact, face-to-face talking, worked within two meters of patients, recalled patient coughing/sneezing, suctioned respiratory secretions from or administered breathing treatments to patients, changed bed linens or bathed patient), age, sex, occupation and exposure to poultry (shopped at live poultry market, had live or freshly cut poultry in their home in the weeks before interview).

  Among the exposed and unexposed health care workers enrolled, 4% (8/217) and 0.7% (2/309), respectively, tested positive for H5N1 antibodies using microneutralization and Western Blot techniques. Risk factors for infection included changing bed linens (no OR provided) and did not include exposure to poultry (no results provided).

- *Apisarnthanarak et al 2005*: Occupational exposure to H5N1 of 49 health care workers with a confirmed H5N1 patient in a university hospital setting in Thailand was evaluated in a seroprevalence study. Health care workers were classified as exposed (n=25) and non-

exposed (n=24) to the patient and did not differ by demographic characteristics or exposure to poultry (contact with ill poultry, shopping at live poultry market, had live or freshly cut poultry in their home in the two weeks before interview or history of living on a poultry farm). The use of personal protective equipment (PPE, surgical mask, gown and gloves) was not initiated until 48 hours after the case was admitted to the hospital. No health care workers tested positive for H5N1 antibodies using microneutralization and Western Blot techniques and thus there was no evidence of person-to-person transmission of H5N1 in this study.

- *Schultsz et al 2005*: Occupational exposure to H5N1 was evaluated among health care workers exposed to confirmed H5N1 patients in a Ho Chi Minh City hospital, Viet Nam. None of the 60 health care workers involved in the care of H5N1 patients tested positive for H5N1 antibodies using ELISA or microneutralization and Western Blot techniques despite 25.4% having reported contact with the patients secretions, approximately half (29/59) reporting to have spent >12 hours with the patient and limited use of control measures or personal protective equipment (e.g., gloves). No evidence of human-to-human or poultry-to-human transmission of H5N1 occurred among health care workers.

- *Thanh Lim et al 2005*: Occupational exposure to H5N1 of health care workers exposed to four confirmed and one probable H5N1 patients in a Hanoi hospital was evaluated in a seroprevalence study. None of the 83 health care workers who provided a single blood sample and completed a questionnaire to obtain information on demographic characteristics, medical history, use of protective equipment while in contact with the case, exposure to the cases, or exposure to poultry tested positive for H5N1 antibodies using microneutralization and Western Blot techniques.

  The use of PPE was high among subjects with 94.8% reporting that they always wore a mask while examining or caring for H5N1 patients, while 31.6% reported that they always wore eye protection, 61.5% reported that they always wore gloves while in contact with H5N1 patients.

Non-occupational exposure: household and social contacts

The following five studies have evaluated the frequency of asymptomatic or subclinical infection and evaluated poultry-to-human risk factors for HPAI/H5N1 infection among subjects living or working in close proximity to confirmed H5N1 cases in human and domestic poultry populations:

- *Katz et al 1999*: The frequency of asymptomatic or sub-clinical H5N1 infection was evaluated among household or social contacts of 17 confirmed human H5N1 cases in Hong Kong. Six of the 51 household contacts and none of the 26 social contacts (26 social contacts who participated in a 4 day tour with one case plus 23 co-workers) tested positive for H5N1 antibodies using microneutralization and Western Blot techniques. Although not statistically significant, the authors suggest that exposure to poultry in their homes was a likely risk factor for infection.

- *Vong et al 2006*: The frequency of asymptomatic or sub-clinical H5N1 infection was evaluated among residents living within a 1km radius where a man was confirmed with H5N1 infection in Cambodia. Three-hundred and fifty one subjects were recruited in the study; however none tested positive for H5N1 antibodies using microneutralization and Western Blot techniques despite frequent contact with poultry and 96 of 262 (36.6%) households with probable H5N1 infection in chickens.

- *Hinjoy et al, 2008*: A seroprevalence study in rural Thailand [109] was conducted to evaluate asymptomatic infection among poultry farmers in rural areas where H5N1 outbreaks had been confirmed. No farmers in rural Thailand (n=322) tested positive for anti-H5 antibodies by microneutralization and Western Blot techniques.

- *Lu et al*., 2008: A seroprevalence study was conducted in Guangdong Province, China among individuals living within 3 km of H5N1 outbreaks in poultry populations. Out of 1,214 subjects enrolled in the study, 14 (1.15%) of the subjects had HI titers >1:80 using HI and microneutralization tests. Among those, 2/231 (0.9%) were classified as having occupational exposure to poultry (individuals responsible for raising, selling and slaughtering poultry in outbreak areas) while, 1.2% (12/983) were classified as "general citizens" who lived in areas where the outbreak occurred, but did not report handling live poultry. Further risk factors for infection were not evaluated.

- *Vong et al 2009*: The frequency of asymptomatic or sub-clinical H5N1 infection was evaluated among residents living within a 1km radius of two human H5N1 cases in two rural villages in Cambodia. Among the 674 subjects recruited, seven (1.0%) tested positive for H5N1 antibodies by microneutralization and Western Blot. All seven cases were ≤18 years old and six of the seven were male (85.7%). Risk factors for infection—including handling poultry, practices involved in the preparation of food, contact with confirmed cases, hand hygiene after contact with poultry and general health—were evaluated in a retrospective matched case-control study of the seven subjects and 24 matched controls (for sex, age [±3 yrs], village of residence and households with H5N1).

  Risk factors associated with testing positive for H5N1 antibodies included swimming or bathing in ponds OR=11.3 (95% CI 1.25-102.18) and gathering poultry and placing them in cages or designated areas OR=5.8 (95% CI 0.98-34.12). These results taken in conjunction with recent evidence of H5N1 virus in the surrounding areas where poultry died from H5N1 infection [111] indicate that swimming or bathing in ponds located around the household where poultry typically have access may be a risk factor for infection. It is worth noting that one case had only spent five days in the village during the study period (approximately three months) and had reported preparing poultry for consumption and cleaning poultry feces in his house yard during that 5-day period.

- *Weekly Epidemiologic Record, 2006*: Following an outbreak of HPAI/H5N1 in wild birds in Azerbaijan in 2006, active surveillance of residents in settlements where these nine cases resided was initiated. A total of 52 residents were sampled (20 residents with suspect H5N1 infection + 32 contacts) and clinical specimens were tested for the presence of influenza A/H5 using RT-PCR, HI test, and virus isolation at the NAMRU-3 field laboratory and the National Institute for Medical Research in the UK for confirmation. Nine patients tested positive, all of whom were from related or neighboring families. These nine individuals likely became infected with H5N1 while defeathering wild swans [112].

Seroprevalence studies of human infection with HPAI other than H5N1

Described below are three seroprevalence studies conducted in humans following poultry outbreaks of HPAI/H7N1 in Italy in 1999-2000, the Netherlands in 2003 and HPAI/H7N3 poultry outbreaks in British Columbia, Canada in 2004:

- *Capua et al., 2002* [115]: Following outbreaks of LPAI and HPAI H7N1 in 1999-2000 affecting hundreds of farms in Veneto and Lombardia regions of Northern Italy, a seroprevalence survey was conducted among individuals with close contact to poultry involved in the outbreaks (e.g., farmers, technicians, veterinarians, and abattoir employees). None of the

serum samples from 765 employees tested positive for anti-H7 antibodies using microneutralization and single radial haemolysis tests.

- *Du Ry Van Beest Holle, et al 2003 [116]*: Following an outbreak of HPAI/N7N7 in hundreds of farms in the Netherlands in 2003, human-to-human transmission was evaluated in a retrospective cohort study of household contacts of infected poultry workers. Among the 56 household contacts of 25 H7N7 confirmed poultry workers included in the study, 58.9% (n=33) tested positive for antibodies against H7. The serologically positive household contacts were from 15 households. Risk factors associated with testing positive for H7 antibodies included having ≥2 toilets in the home RR=3.8 (1.1-13.5), having a pet bird inside the home RR 1.9 (1.4-2.5), using a cloth handkerchief RR 1.7 (1.1-2.5), having burning sensation in eyes RR 1.8 (1.4-2.3), smoking 1.8 (1.4-2.3), use of oseltamivir RR 1.8 (1.4-2.3) and having conjunctivitis RR 1.8 (1.4-2.3), suggesting that transmission may have occurred by person-to-person or by contaminated items (fomites).

- *Tweed et al., 2004* [117]: A seroprevalence study was conducted in British Columbia, Canada following an HPAI/H7N3 outbreak among commercial poultry farms in 2004. More than 2,000 individuals were involved in the culling procedures. Seventy-seven individuals reported symptoms, however only 2 of were confirmed to be infected with HPAI/H7N3. A case-control study to evaluate risk factors for infection was not initiated.

Clusters of H5N1 in humans

Clusters of epidemiologically linked H5N1 cases have occurred among blood relatives in several countries, including Indonesia, China, Turkey, Azerbaijan, Viet Nam and Thailand, suggesting that human-to-human transmission between family members may have occurred [112, 118-123]. An early investigation in Viet Nam, suggested that between January 2004 and July 2005, 15 suspected family clusters occurred among the first 109 cases, of which nine clusters had at least two laboratory confirmed H5N1 cases [118].

A family cluster in mainland China occurred in a father and son, the former likely infected through close, unprotected contact via care at a hospital of his son during his illness [122]. Similarly in Thailand, a mother and aunt of an infected patient likely became infected through unprotected hospital care of their daughter/niece [120]. In Turkey, several members of the same family became infected with H5N1; however transmission was probably poultry-to-human rather than human-to-human since they all shared the same living space with poultry [119].

In Indonesia, there have been 11 clusters of H5N1 among blood relatives with each cluster involving 2-7 blood relatives [121, 123]. Among the first three clusters, which occurred in 2005, limited human-to-human transmission may have occurred in two of the three clusters. Exposure to the virus via a contaminated environment, through contact with contaminated poultry manure or with infected poultry could not be ruled out [121]. In a detailed analysis of all human H5N1 cases in Indonesia, the authors examined direct and indirect exposure to poultry and could not rule out a common source of infection in the clusters since family members usually have similar opportunities for exposure to the virus. While there may have been limited human-to-human transmission in some clusters, the authors suggest that genetic variation between families could result in the occurrence of clusters because of a predisposition to infection [123]. Cluster investigations have suggested that some individuals may be genetically more susceptible to infection. Interpretations of the family clusters are often difficult because not all of the suspected patients may have been tested for H5N1.

Indirect-transmission of H5N1 to humans

It is possible for HPAI/H5N1 to be transmitted to humans indirectly via contact with fomites or through the environment [107, 111, 124-126]. Since birds are known to shed high concentrations of virus into water sources, transmission from poultry-to-humans through contaminated water is possible [126]. The epidemiologic investigation of two H5N1 cases in a single family in Viet Nam suggested that exposure to possibly contaminated canal water via swimming or washing may have played a role in infection. However, the role of water in transmission could not be confirmed nor extrapolated since no further follow-up studies were conducted [124]. More recently, results from environmental sampling within a village with confirmed H5N1 in domestic poultry flocks and one human case as well as results from a human seroprevalence study from the same villages in Cambodia identified contaminated water as a potential risk factor for H5N1 infection [107, 111].

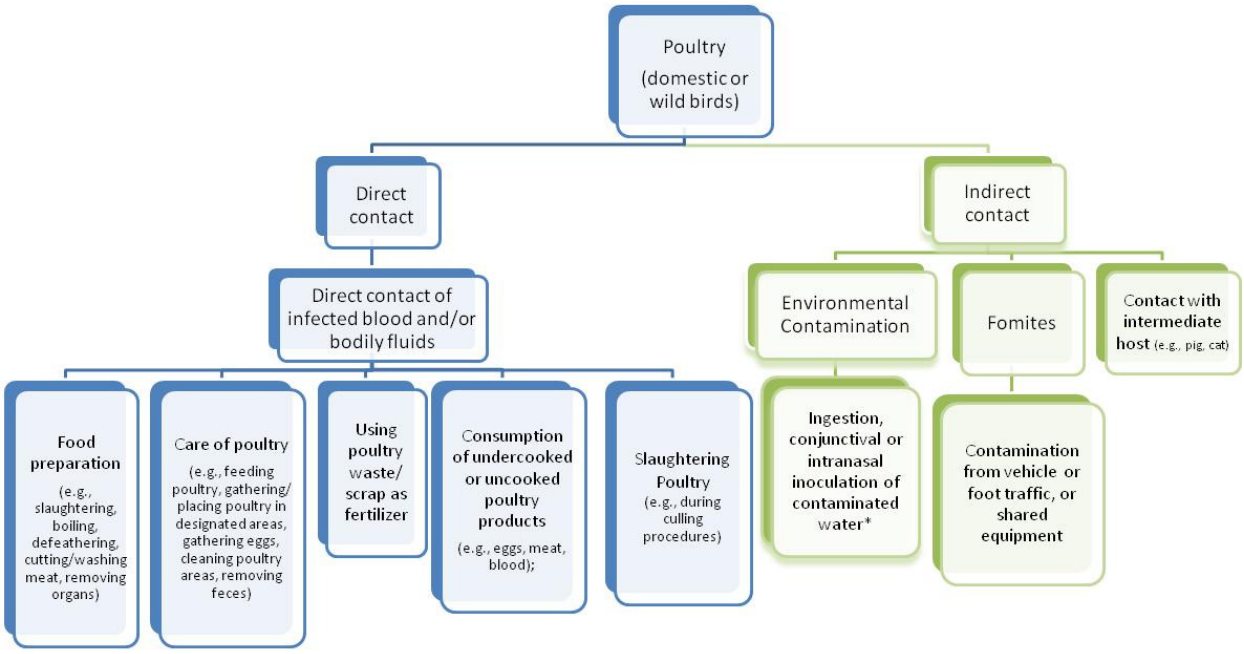
Conclusions and Discussion

Several epidemiologic studies have evaluated the risk of transmission of HPAI from poultry-to-humans. These studies have identified several risk factors that may be associated with infection including close direct contact with poultry and indirect transmission via the environment. However, despite frequent and widespread contact with poultry, transmission from poultry to humans is rare.

An illustration of possible pathways of poultry-to-human infection of HPAI, particularly subtype H5N1, is shown in Figure 4. Direct routes may include contact with infected blood or bodily fluids via food preparation practices [127] (e.g., slaughtering, boiling, defeathering, cutting meat, cleaning meat, removing and/or cleaning internal organs of poultry); consuming uncooked poultry products (e.g., raw duck blood) [102, 124, 128] or through the care of poultry (either commercially or domestically). Little is understood about H5N1 transmission via indirect routes, though recent studies have suggested an association between exposure to a contaminated environment (e.g., water; cleaning poultry cages or their designated areas; using poultry feces for fertilizer) and infection either through ingestion, conjuctival or intranasal inoculation of contaminated water, soil [111, 124] or via fomites on shared equipment or vehicles transporting products between farms [125]. Other pathways may exist but are currently unknown.

HPAI is transmissible from poultry-to-humans directly via contact with contaminated environments, through close contact with infected poultry or possibly through other animal species (e.g., pig, cat, dog, tiger) that serve as a mixing vessel [12, 52, 67, 129, 130]. Intimate contact with infected poultry (e.g., slaughtering, removing internal organs, licking wounds of fighting cocks) is believed to be required for transmission of H5N1 from poultry to humans [5, 101]. However, the extent of these behaviors is currently unknown and there is reluctance of individuals to disclose information on possible exposure from illegal activities. For example, an outbreak investigation in Azerbaijan in early 2006 found that the likely source of H5N1 in nine (eight confirmed, one probable) human cases was infected wild swans, with transmission probably occurring as a result of the illegal activity of de-feathering these birds [112].

Figure 4. Known and suggested pathways to infection from poultry to humans.



via swimming/bathing in water frequently used by domestic and/or wild poultry.

Table 5 summarizes possible risk factors for infection identified through epidemiologic investigations of human HPAI/H5N1 cases. The collective results of these studies have shown that transmission of HPAI/H5N1 from poultry-to-humans is currently limited to individuals who may have been contact with the highest potential concentrations of virus shed by poultry. This suggests that there may be threshold of virus concentration needed for effective transmission and that circulating H5N1 strains have not yet mutated to transmit readily from either poultry-to-human or from human-to-human. The mode of transmission can be quite varied throughout different countries ranging from exposure to poultry during a visit to a wet market to preparing infected poultry to swimming or bathing in ponds, which are frequented by poultry.

Table 5. Possible risk factors for human infection with HPAI/H5N1 from seroprevalence studies.

Mode of Transmission	Risk factor	Citation
Poultry-to-human Transmission	Exposure to poultry at live/wet market	Mounts et al., 1999 Wang et al., 2006
	Work in retail poultry market	Bridges et al 2002
	Presence of sick/dead poultry in the household	Dinh et al., 2006
	Butchering poultry	Bridges et al 2002
	Preparing poultry for restaurants	Bridges et al 2002
	Presence of sick/dead poultry in the neighborhood	Dinh et al., 2006
	Direct touching poultry that died unexpectedly	Areechokchai et al., 2006
	Preparing/cooking (no specific practices identified) unhealthy poultry	Dinh et al., 2006
	Feeding poultry	Bridges et al 2002
	>10% mortality among poultry within which poultry workers had worked within past 2 months	Bridges et al 2002
	Gathering poultry and placing them in cages or designated areas	Vong et al., 2009
Human-to-human transmission	None†	
Indirect transmission	No water source in the household	Dinh et al., 2006
	Swimming or bathing in ponds	Vong et al., 2009
	Changing bed linens	Bridges et al 2000
	Handling money	Bridges et al 2002

†No human-to-human risk factors for infection were identified from seroprevalence studies; however possible human-to-human transmission may have occurred in several clusters in other countries (see Section 3.4)

It is likely that direct and indirect human-poultry contact patterns differ between countries. It has been shown that there is substantial variation in the frequency of different poultry contact practices amongst populations in rural Cambodia living in close proximity to poultry [131]. Such differences demonstrate that the potential risk of transmission of H5N1 from poultry-to-humans is not uniform across age and gender and therefore may not be uniform within or across countries [131]. The demographic differences in human cases of H5N1 to date between countries may be because contact patterns with poultry differ between countries. However, it is also suggestive that the variation in H5N1 incidence by age may not be due to exposure alone and that there may be differences by age in intrinsic immunologic susceptibility to infection, pre-existing immunity against human influenza A virus and/or clinical presentation of disease.

Several important data gaps currently limit our understanding of the transmission of HPAI/H5N1 from poultry to humans.

- First, there remains considerable scope for underreporting of human cases and poultry outbreaks and we currently lack sufficient exposure data from the confirmed H5N1 cases around the world to fully evaluate other potential risk factors (e.g., the environment) for infection. The seroprevalence studies that have evaluated the frequency of asymptomatic or subclinical infection and risk factors for H5N1 infection have identified few asymptomatic individuals with anti-H5N1 antibodies, indicating previous infection with H5N1. However, it is not possible to determine whether this is a true reflection of HPAI/H5N1 infection given the limited geographical scope of such studies to date.

- Second, the influence of genetic and/or immunological factors on transmission is poorly understood. Although there have been several suspected clusters of H5N1 infection (largely among blood relatives) where H5N1 may have been transmitted between humans [118-122], the clusters are difficult to interpret because all suspected family members may not have been tested for H5N1. In an analysis of 11 suspected clusters of H5N1 among blood relatives in Indonesia, the authors suggest that there may have been limited human-to-human transmission in some clusters. However genetic variation in families could result in the occurrence of clusters because of a predisposition to infection [123].

  While no health care workers exposed to H5N1 patients in Viet Nam or Thailand were infected from the care of these patients [102, 106], a father may have been infected through contact during the care of his dying son infected with H5N1 at a hospital in China [122], and a mother and aunt may have become infected from similar contact with their dying daughter/niece in a hospital in Thailand [120].

- Third, improved knowledge is needed on all potential routes of transmission of H5N1 from poultry-to-humans and the prevalence of risky practices in human populations. Studies to date have evaluated what are believed to be the main potential routes through which people can become infected with H5N1, but we currently lack sufficient data from the confirmed H5N1 cases around the world to fully evaluate other potential risk factors for infection such as the role of water and other environmental factors. Transmission could also include oral ingestion, conjunctival or intranasal inoculation from contaminated water while drinking, swimming or bathing or from feces while caring for poultry [107] and may explain why more children than adults are infected. Furthermore, asymptomatic cases may occur because of low concentrations of viruses in the environment.

In order to fully evaluate the occurrence of human-to-human transmission, a detailed exposure history needs to be collected from all suspected cases and their contacts. Direct and indirect exposure to poultry by species should also be standardized across epidemiologic studies to facilitate pooled or meta-analyses. Bird and Farrar have developed a data collection form that could be used during all future human outbreak investigations, which includes not only information on contact with poultry by species and a suspect case, but includes questions regarding the timing of the contact [132]. However this questionnaire covers only general exposure information (e.g., handling sick or dead poultry, handling feces or fertilizer from sick or dead poultry, slaughtering poultry) and does not include any potential transmission via the environment (e.g., contaminated water). In order to build a database from which more robust analysis can be conducted, detailed exposure information should be systematically collected from all suspect cases.

Collaboration between human and animal health sectors is essential to understand the risk of transmission between domestic poultry and humans. Current exposure estimates remain too general to explain the current pattern or to predict future cases of H5N1 infection in human populations [131]; however the results of the available studies indicate that indirect poultry exposure through

the environment may play a role in transmission [107]. Rapid, systematic and standardized collection of detailed information on poultry contact patterns in suspected human outbreaks of H5N1 would improve our understanding of transmission from poultry to humans. Detailed exposure information detailing direct and indirect contact should be included in all future human outbreak investigations as well as seroprevalence studies.

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Annexes

Annex 1. Results of seroprevalence studies to determine the frequency of asymptomatic or subclinical infection and evaluate risk factors for H5N1 virus infection.

Study, year	Study Population & Year of Outbreak	Transmission	Seroprevalence Results (% seropositive)	Risk Factors RR, OR, 95%CI	Comments
Occupationally Exposed Persons: Poultry Workers					
Bridges et al., 2002 [104]	Poultry workers, Hong Kong 1997	Poultry-to-humans	9/293 (3%) government workers were seropositive 81/1525 (5.3%) poultry workers were seropositive Nested case-control study conducted among 81 cases and 1231 controls	Work in retail vs. wholesale/ hatchery/farm/other poultry industry 2.7 (1.5-4.9) >10% mortality among poultry 2.2 (1.3-3.7) Jobs: -Butchering poultry 3.1 (1.6-5.9) · Feeding poultry 2.4 (1.4-4.1) · Handling money 1.6 (1.0-2.5) · Preparing poultry for restaurants 1.7 (1.1-2.7)	Limited poultry-to-human transmission among poultry and government workers involved in poultry culling operations
Wang et al., 2006 [79]	Poultry workers, Guangdong China, 2006	Poultry-to-humans	1/110 poultry workers were seropositive	Specific risk factors not identified, but subject slaughtered poultry for 5 years	Specific risk factors not identified
Oritz et al., 2007 [105]	Poultry workers, Kano Nigeria 2006	Poultry-to-humans	0/295 poultry workers with median 14 days exposure to H5N1 0/25 laboratory workers with exposure to H5N1	None	No evidence of H5N1 infection with subjects with repeated exposure to infected poultry
Lu et al., 2008 [113]	Poultry workers, Guangdong China	Poultry-to-humans	2/231 subjects with "occupational exposure" had titers >1:80	Occupational exposure including raising, selling slaughtering chickens and ducks in H5N1 outbreak areas	Specific risk factors not identified
Occupationally Exposed Persons: Health Care Workers					
Bridges et al., 2000 [103]	Health care workers, Hong Kong 1997	Human-to-human; poultry-to-human	10/526 (8/21 exposed; 2/309 non exposed HCW)	Changing the bed linen of cases (no OR provided); controlled for poultry exposure	Limited human-to-human transmission
Apisarnthanarak et al., 2005 [102]	Health care workers, Thailand 2004	Human-to-human; poultry-to-human	0/25 among health care workers in direct contact with H5N1 patient	None	No serologic evidence of H5N1 among health care workers with direct contact with human H5N1 patient
Thanh Liem et al., 2005 [106]	Health care workers, Viet Nam 2004	Human-to-human; poultry-to-human	0/83 among health care workers, 95% of which had direct contact with confirmed H5N1 patients	None	No serologic evidence of H5N1 among health care workers with direct contact with human H5N1 patient

Hinjoy et al., 2008 [108]	Health care workers, Viet Nam 2004	Human-to-human; poultry-to-human	0/60 healthcare workers in contact with confirmed H5N1 patients	None	No serologic evidence of H5N1 among health care workers with direct contact with human H5N1 patient
Non-Occupational Exposure: Household and Social Contacts					
Katz et al., 1999 [53]	Household and Social contacts of H5N1 patients, Hong Kong 1997	Human-to-human; poultry-to-human	6/51 (12%) household contacts 0/47 co-workers tested positive for H5 antibodies	None significant; however 21% of seropositive had contact to poultry vs. 5% of seropositive with no poultry contact, p=0.13	Human-to-human transmission was limited
Vong et al., 2006 [41]	Rural villagers living in the same villages as two confirmed H5N1 human cases 2005	Poultry-to-human	0/351 villagers tested positive for H5N1 antibodies	None	No evidence of H5N1 infection among subjects living in villages with conformed H5N1 in domestic poultry flocks; poultry-to-human transmission was low in this setting
Lu et al., 2008 [113]	Poultry workers, Guangdong China	Poultry-to-humans	12/983 "general citizens" had titers >1:80	Subjects were general citizens without direct contact with poultry	Specific risk factors not identified
Hinjoy et al. 2008 [109]	Rural poultry farmers in Thailand, 2004	Poultry-to-human	0/322 farmers tested positive for H5N1 antibodies	None	No evidence of H5N1 infection among subjects living in villages with conformed H5N1 in domestic poultry flocks
Vong et al., 2009 [107]	Rural villagers living in the same villages as confirmed H5N1 human case 2006	Poultry-to-human	7/674 (1%) seropositive for H5N1 antibodies ≥1:80 85.7% (6/7) male All ≤18 years old Matched case-control study conducted with 7 cases and 24 controls	Swim/bathe in ponds OR 11.3 (1.25-102.2) Water source 6.8 (0.68-66.4) Gathered poultry and placed in cages or designated areas 5.8 (0.98-34.1) Removed/cleaned feces from cages or poultry areas 5.0 (0.69-36.3)	Poultry-to-human transmission was low; possible transmission from the environment to humans via contaminated water
WER, 2006 [114]	Residents in settlements of confirmed cases Azerbaijan, 2006	Poultry-to-human	9/52 residents tested positive for H5N1 virus	No case-control was initiated, but contact with infected wild birds (defeathering) likely cause of infection	All cases were from related or neighboring families

Notes: PPE = personal protective equipment including masks, gloves, eye protection.