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Brief literature review for the WHO global influenza research agenda† – highly pathogenic avian influenza H5N1 risk in humans

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Highly pathogenic avian influenza A H5N1 viruses remain a significant health threat to humans given the continued rare occurrence of human cases with a high case fatality rate. This brief literature review summarizes available evidence of risk factors for H5N1 infection in humans and updates a recent systematic review published in early 2011. Several epidemiologic studies have been published to evaluate the risk factors for H5N1 infection in humans, including contact with poultry and poultry products and non-poultry-related contact such as from H5N1-contaminated water. While most H5N1 cases are attributed to exposure to sick poultry, it is unclear how many may be due to human-to-human transmission. The collective results of published literature suggest that transmission risk of H5N1 from poultry to humans may be highest among individuals who may have been in contact with the highest potential concentrations of virus shed by poultry. This suggests that there may be a 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. However, the mode of potential transmission can be quite varied throughout different countries and by study with exposures ranging from visiting a wet market, preparing infected poultry for consumption, to swimming or bathing in ponds frequented by poultry. Several important data gaps remain in the understanding of the epidemiology of H5N1 in humans and limit our ability to interpret the results of the available H5N1 seroepidemiologic studies.

Keywords exposure, H5N1, highly pathogenic avian influenza, human–animal interface, risk factors, seroprevalence.

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HPAI/H5N1 in humans

The isolation of a highly pathogenic avian influenza (HPAI) A virus, subtype H5N1 (referred to as H5N1 in this review), from a 3-year-old boy in Hong Kong in 1997 was the first detection of this virus strain in humans and raised concerns worldwide as to the potential for a pandemic of avian origin with a lethality in the range of the 1918 pandemic.[1] All of the genes found in the H5N1 viral strain in Hong Kong originated from avian viruses.[1, 2] While H5N1 has not yet demonstrated the ability to transmit efficiently from person to person, the high case fatality associated with reported infection, ongoing spread of the virus in bird populations, and the potential for influenza viruses to mutate and adapt to other hosts mean H5N1 remains a continuing public health concern.

As of August 10, 2012, H5N1 infection had been detected in 608 individuals in 15 countries.[3] The number of human cases is not evenly distributed throughout the world, and the age/gender distribution varies by country. The largest numbers of human cases reported have been from Indonesia, Vietnam, and Egypt, each having reported more than 100 cases. No human cases have yet been reported in Western Europe or the Americas. Although the apparent case fatality rate (CFR) of H5N1 is high (approximately 59%), this may be an overestimate of the true CFR because any milder cases may never be identified under current surveillance systems in countries affected by H5N1.

To date, H5N1 remains an avian epidemic with rare and sporadic spillover into the human population and other species.[4] The predominant modes of transmission from poultry to humans remain incompletely understood, and limited information on how infected individuals were exposed has restricted our ability to evaluate risk factors for human infection and implement more refined risk reduction measures. Field investigations of cases of H5N1 in humans – usually in low- or middle-income countries – are often difficult to conduct, especially in a timely manner. Conversely, in some countries, good exposure data have been collected during outbreak investigations, but have not been analyzed or published. Thus, information on potential exposures, when given, is typically limited to such general descriptions as "*recent contact with sick or dead poultry*"[5] or the "*preparation of sick birds for consumption.*"[6] Although studies to date have identified more specific variables to collect data on such investigations (World Health Organization, *WHO Minimum Data Set Report Form: Human infection with an influenza virus with pandemic potential, available upon request*), more detailed knowledge of the types of behaviors and interactions with poultry that result in virus transmission would facilitate more effective and targeted risk reduction measures at the human–animal interface.

This report summarizes a recently published review of risk factors for human H5N1 infection[4] updated with publications since that review was finalized.

Transmission of HPAI/H5N1 from poultry to humans

Several epidemiologic studies have evaluated the risk of transmission of HPAI from poultry to humans (see updated Tables 1 and 2). These studies have identified several broad risk factors that may be associated with infection including close direct contact with poultry and indirect transmission via environmental contamination. However, despite frequent and widespread contact with poultry, transmission of HPAI/H5N1 from poultry to humans continues to be rare.

Direct routes of poultry-to-human infection of H5N1 *may* include contact with aerosolized virus, infected blood or bodily fluids via food preparation practices (e.g., slaughtering, boiling, defeathering, cutting meat, cleaning meat, removing and/or cleaning internal organs of poultry), consuming uncooked poultry products, 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)[7–10] and infection through either ingestion or conjunctival or intranasal inoculation of contaminated water and soil or via fomites on shared equipment or vehicles transporting products between farms. Live animal markets have also been shown to be a potential source of H5N1 circulation in poultry and infection source to humans.[9, 11–16] Other pathways may exist but are currently unknown.

Several epidemiologic studies have been published to evaluate the risk factors for H5N1 infection in humans, including contact with poultry and poultry products and non-poultry-related contact such as from H5N1-contaminated water (see references in Table 2). Most H5N1 cases are attributed to exposure to sick poultry, while it is unclear how many may be due to human-to-human transmission.[4, 17, 18]

Table 1. Possible risk factors for human infection with HPAI/H5N1[4, 19]

Mode of Transmission	Risk factor
Poultry-to-human transmission	Exposure to poultry at live animal/wet market
	Work in retail poultry market
	Presence of sick/dead poultry in the household
	Butchering poultry
	Preparing poultry for restaurants
	Presence of sick/dead poultry in the neighborhood
	Direct touching poultry that died unexpectedly
	Preparing/cooking (no specific practices identified) unhealthy poultry
	Feeding poultry
	>10% mortality among poultry within which poultry workers had worked within past 2 months
	Gathering poultry and placing them in cages or designated areas
Human-to-human transmission	More data needed*
Indirect transmission	Environmental contamination No water source in the household
	Swimming or bathing in ponds
	Changing bed linens
	Handling money

*No human-to-human transmission 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[4, 17, 18]).

Tables 1 and 2 summarize possible risk factors for infection identified through epidemiologic investigations of human H5N1 cases. The collective results of these studies have shown that transmission risk of H5N1 from poultry to humans may be highest among individuals who may have been contact with the highest potential concentrations of virus shed by poultry.[4, 19] This suggests that there may be a threshold of virus concentration needed for effective transmission and that circulating H5N1 strains have not yet mutated to transmit readily either from poultry to human or from human to human. However, the mode of potential transmission can be quite varied throughout different countries and by study with exposures ranging from visiting a wet market, preparing infected poultry for consumption, to swimming or bathing in ponds frequented by poultry.

Non-poultry exposure-related H5N1 exposures, defined as any contact not involving touching poultry or poultry products, for example, exposure to H5N1-contaminated

Table 2. 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[32]	Poultry workers, Hong Kong 1997	Poultry to humans	9/293 (3%) GW were seropositive 10% PW were estimated to be seropositive using MN >80 Nested case–control study conducted among 81 seropositive 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), Handling money 1·6 (1·0–2·5) Preparing poultry for restaurants 1·7 (1·1–2·7)	Limited poultry-to-human transmission among PW and GW involved in poultry culling operations
Wang *et al.*, 2006[13]	Poultry workers, Guangdong China, 2006	Poultry to humans	1/110 (0·9%) PW was seropositive using HI with turkey erythrocytes >320	Specific risk factors not identified, but subject slaughtered poultry for 5 years	Specific risk factors not identified
Oritz *et al.*, 2007[33]	Poultry workers, Kano Nigeria, 2006	Poultry to humans	0/295 PW with median 14 days exposure to H5N1 0/25 laboratory workers with exposure to H5N1 Seropositivity by MN titers if ≥ 1:80	None	No evidence of H5N1 infection for subjects with repeated exposure to infected poultry
Lu *et al.*, 2008[34]	Poultry workers, Guangdong China	Poultry to humans	2/231 (0·9%) subjects with "occupational exposure" had HI titers >1:80	Occupational exposure including raising, selling slaughtering chickens and ducks in H5N1 outbreak areas	Specific risk factors not identified
Cai *et al.*, 2009[35]	Firemen, government workers, vets for collection of dead wild birds on Ruegen Island, Germany, 2006	Poultry to humans	0/97 workers were seropositive Seropositivity by PN or MN assay if >1:20	None	No evidence of H5N1 infection for subjects with exposure to infected wild birds; use of PPE was widespread
Wang *et al.* 2009[36]	Poultry workers in China, 2007–2009	Poultry to humans	4/2191 (0·2%) using HI [no cutoff provided] had anti-H5 antibodies	None	Limited evidence
Schultsz *et al.*, 2009[37]	Poultry workers and cullers living on farms with confirmed H5N1 outbreaks in poultry in Vietnam, 04–05	Poultry to humans	0/500 (183 PW, 317 cullers) using MN and 3/500 (3 cullers) using HI >1:80 had anti-H5 antibodies	Not evaluated	Limited evidence of poultry-to-human transmission despite exposure to infected poultry
Wang *et al.*, 2009[38]	Poultry workers in LBM in Guangzhou in 2006	Poultry to humans	0/68 were seropositive using HI [no cutoff provided]	None	No evidence of H5N1 infection for subjects with repeated exposure to infected poultry

Table 2. (*Continued*)

Study, year	Study Population & Year of Outbreak	Transmission	Seroprevalence Results (% seropositive)	Risk Factors RR, OR, 95%CI	Comments
Robert *et al.* 2010[39]	Poultry and farm workers, Indonesia, 2007	Poultry to humans	0/495	None	No evidence of H5N1 infection for subjects with repeated exposure to infected poultry
Huo *et al.* 2012[40]	Poultry workers, Jiangsu China, 2010	Poultry to humans	306 were seropositive using horse red blood cell HI $\geq$1:160	Raising poultry OR 2·39 (1·00–5·69)	Increasing poultry numbers; however, 40% of subjects were $\geq$60 years old
Occupationally Exposed Persons: Health Care Workers					
Bridges *et al.*, 2000[41]	Healthcare workers, Hong Kong 1997	Human to human; poultry to human	10/526 (2%) (8/21 exposed; 2/309 non-exposed HCW) using MN >1:80, confirmed by WB	Bathing patients or changing the bed linen of cases (no OR provided); controlled for poultry exposure	Limited human-to-human transmission
Apisarnthanarak *et al.*, 2005[42]	Healthcare workers, Thailand 2004	Human to human; poultry to human	0/25 among HCW in direct contact with H5N1 patient; seropositivity tested using MN >1:80, confirmed by WB	None	No serologic evidence of H5N1 among HCW with direct contact with human H5N1 patient
Thanh Liem *et al.*, 2005[43]	Healthcare workers, Vietnam 2004	Human to human; poultry to human	0/83 among HCW, 95% of which had direct contact with confirmed H5N1 patients Seropositivity tested using MN >1:40 in 2 independent assays	None	No serologic evidence of H5N1 among HCW with direct contact with human H5N1 patient
Schultsz *et al.*, 2005[44]	Healthcare workers, Vietnam 2004	Human to human; poultry to human	0/60 HCW in contact with confirmed H5N1 patients Seropositivity tested using MN >1:80 and ELISA >1:80	None	No serologic evidence of H5N1 among HCW with direct contact with human H5N1 patient
Non-occupational Exposure: Household and Social Contacts					
Katz *et al.*, 1999[45]	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 Seropositivity tested using MN or ELISA >1:80, confirmed by WB	Nonsignificant; however, 21% of seropositive had contact to poultry vs. 5% of seropositive with no poultry contact, $P = 0{\cdot}13$	Human-to-human transmission was limited
Vong *et al.*, 2006[46]	Rural Cambodian villagers living in the same villages as two confirmed H5N1 human cases in 2005	Poultry to human	0/351 villagers tested positive for H5N1 antibodies $\geq$1:80 using MN and WB	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

Table 2. (*Continued*)

Study, year	Study Population & Year of Outbreak	Transmission	Seroprevalence Results (% seropositive)	Risk Factors RR, OR, 95%CI	Comments
Lu *et al.*, 2008[34]	Poultry workers, Guangdong China	Poultry to humans	12/983 (1·2%) "general citizens" had HI or MN titers ≥1:20	Subjects were general citizens without direct contact with poultry	Specific risk factors not identified
Hinjoy *et al.* 2008[47]	Rural poultry farmers in Thailand, 2004	Poultry to human	0/322 farmers tested positive for H5N1 antibodies; using MN >1:80, confirmed by WB or ELISA	None	No evidence of H5N1 infection among subjects living in villages with conformed H5N1 in domestic poultry flocks
Vong *et al.*, 2009[24]	Rural Cambodian villagers living in the same villages as confirmed H5N1 human case, 2006	Poultry to human	7/674 (1%) seropositive for H5N1 antibodies using MN ≥1:80 6/7 (85·7%) male All ≤18 years old Matched case–control study conducted with 7 seropositive 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
Dejpichal *et al.*, 2009[48]	Residents in 4 Thai villages with human cases in 2005	Poultry to human	0/901 tested positive for anti-H5 antibodies using MN confirmed by Immunofluorescence >1:40	None	No evidence of H5N1 infection among subjects living in villages with conformed H5N1 in domestic poultry flocks
Santhia *et al.*, 2009[49]	Residents in 38 villages and 3 LBM in Bali, 2005	Poultry to human	0/841 tested positive for anti-H5N1 antibodies using MN >1:80	None	Despite H5N1 exposure from poultry outbreaks, no evidence of poultry-to-human transmission
Cavailler *et al.*, 2010[8]	Rural Cambodian villagers living in the same villages as c onfirmed H5N1 human case, 2007	Poultry to human	18/700 (2·8%) seropositive for H5N1 antibodies using MN ≥1:80	Swam/bathed in pond OR 2·52 (95% CI, 0·98–6·51) No other risk factors identified	Poultry-to-human transmission was low; possible transmission from the environment to humans via contaminated water
Kurskaia *et al.* 2009[50]	Residents of West Siberia	Poultry to human	0/265 using HI and MN	None	No evidence

PPE, personal protective equipment including masks, gloves, eye protection; PW, poultry workers; GW, government workers; HCW, healthcare workers; MN, microneutralization (MN) assay; HI, hemagglutination–inhibition assay; WB, Western blot assay; PN, plaque neutralization.

environments, may also lead to H5N1 infection.[7–9, 20–22] Exposure to H5N1 virus in contaminated feces in garden fertilizer has been reported as a source of human infection.[23] Because birds are known to shed high concentrations of virus into water sources, transmission from poultry to humans through contaminated water is also possible.[21] The epidemiologic investigation of two H5N1 cases in a single family in Vietnam suggested that exposure to possibly contaminated canal water via swimming or washing may have resulted in infection. However, the role of water in transmission could not be confirmed.[20] More recently, results from environmental sampling within Cambodian villages with confirmed H5N1 in domestic poultry flocks and one human case as well as results from a human seroprevalence study from the same village identified contaminated water as a potential risk factor for H5N1 infection.[7, 8, 24]

Conclusions

Direct and indirect human–poultry contact patterns differ between countries[25–29], which demonstrates 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. 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 only be due to exposure and that there may be differences by age in intrinsic immunologic susceptibility to infection, preexisting immunity against human influenza A virus, and/or clinical presentation of disease.

Several important data gaps remain in the understanding of the epidemiology of H5N1 in humans and limit our ability to interpret the results of the available H5N1 seroepidemiologic studies:

1. First, there remains considerable scope for underreporting of human cases (both mild and severe) 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.
2. Second, the number of asymptomatic H5N1 infections identified via seroprevalence studies may be overestimated because of differences and inconsistencies in assays used to test for antibodies used by various laboratories.[30]
3. Third, 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, the clusters are difficult to interpret because all suspected family members may not have been tested for H5N1 and family members may have had a common non-human source of exposure.
4. Fourth, 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.

To fully evaluate the occurrence of human-to-human transmission, standardized case investigations with detailed exposure history need to be collected from *all* suspected cases and their contacts.[31] Direct and indirect exposure to poultry by species should also be standardized across epidemiologic studies to facilitate pooled or meta-analyses.

Collaboration between human and animal health sectors is essential to understand the risk of transmission between domestic poultry and humans. Current understanding of exposure remains too general to explain the current pattern or to predict future cases of H5N1 infection in human populations; however, the results of the available studies indicate that indirect exposure to poultry through the environment may play a role in transmission.[10] 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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Conflict of interest

The author has no potential conflicts to declare.

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