Detecting Human-to-Human Transmission of Avian Influenza A (H5N1)

Yang Yang,* M. Elizabeth Halloran,*† Jonathan D. Sugimoto,*† and Ira M. Longini, Jr.*†

Highly pathogenic avian influenza A (HPAI) subtype H5N1 has caused family case clusters, mostly in Southeast Asia, that could be due to human-to-human transmission. Should this virus, or another zoonotic influenza virus, gain the ability of sustained human-to-human transmission, an influenza pandemic could result. We used statistical methods to test whether observed clusters of HPAI (H5N1) illnesses in families in northern Sumatra, Indonesia, and eastern Turkey were due to human-to-human transmission. Given that human-to-human transmission occurs, we estimate the infection secondary attack rates (SARs) and the local basic reproductive number, R_0. We find statistical evidence of human-to-human transmission ($p = 0.009$) in Sumatra but not in Turkey ($p = 0.114$). For Sumatra, the estimated household SAR was 29% (95% confidence interval [CI] 15%–51%). The estimated lower limit on the local R_0 was 1.14 (95% CI 0.61–2.14). Effective HPAI (H5N1) surveillance, containment response, and field evaluation are essential to monitor and contain potential pandemic strains.

Highly pathogenic avian influenza A (HPAI) subtype H5N1 is repeatedly crossing the species barrier to humans. Since December 2003, a total of 291 cases of HPAI (H5N1) have been reported in humans, resulting in 172 deaths (i.e., 59% case-fatality ratio) in 12 countries, mostly in Southeast Asia (*1*). Among these cases, 31 family clusters have been documented, ranging in size from 2 to 8 family members. How many of these clusters are due to a common avian source and how many are due to human-to-human transmission are important facts to determine. Should one of these HPAI (H5N1) strains gain the capacity for sustained human-to-human transmission, the resulting outbreak, if not contained, would spread worldwide through the global transportation network more rapidly than adequate supplies of vaccine matched to the new variant could be manufactured and distributed (*2,3*). We analyzed data from 2 of the largest of the familial clusters to ascertain if human-to-human transmission took place, and if so, how transmissible the strain was.

*Fred Hutchinson Cancer Research Center, Seattle, Washington, USA; and †University of Washington, Seattle, Washington, USA

Methods

May 2006 Human Avian Influenza Family Cluster, Indonesia

During late April and early May 2006, a cluster of 8 cases of HPAI (H5N1) was detected and investigated by the Indonesian public health surveillance system in northern Sumatra (*4–6*). All case-patients were members of the same extended family. Seven of them resided within 3 adjacent houses in the village of Kubu Sembilang. The remaining patient resided with his immediate family in the village of Kabanjahe (≈10 km away).

The index patient was a 37-year-old woman, thought to have been exposed to dead poultry and chicken fecal material before onset of illness. She also reportedly maintained a market stall that sold live chickens. Although her illness was not confirmed to have been caused by avian influenza (H5N1), her death on May 5, 2006, is suspected to be the result of HPAI (H5N1) infection because of her reported symptoms, illness progression, and prior contact with diseased or dead poultry.

Twenty members of her extended family are suspected to have been in contact with her, many during a family gathering on April 29, 2006 (*7*). At that time, she was manifesting symptoms (i.e., she had a heavy cough, was severely ill, and was prostrate). That night, 9 of these members slept in the same small room as she did (indicated by a black triangle in online Appendix Figure 1, available from www.cdc.

gov/EID/content/13/9/1348-appG1.htm). Of these 9 family members, 2 of her sons (15 and 17 years of age) and her 25-year-old brother, who lived in Kabanjhe, became ill in the next 3 weeks. The sons died. The brother was the only person from this family cluster to recover.

Of the remaining 11 family members, 4 became ill and died. The 29-year-old sister of the index patient, who lived in an adjacent house, became ill after she provided direct personal care to her ill sister (*7*). The 18-month-old daughter of this sister also became ill after she was in the presence of the index patient with her mother. The 10-year-old nephew of the index patient, who lived in the other house adjacent to hers, became ill after he attended the family gathering and frequently visited his aunt's house. The nephew's father became ill after he personally cared for his son. The possibility that HPAI (H5N1) was transmitted from the nephew to his father is also supported by genetic sequencing data (*4*). Though symptoms did not develop in the mother of the nephew, she was directly exposed to her husband during his illness. All case-patients, except for the index patient, were confirmed as influenza (H5N1) positive by PCR. The nephew's mother was confirmed as influenza (H5N1) negative. As an intervention, 54 surviving relatives and close contacts were identified and placed under voluntary quarantine (*7*). All of these persons, except for pregnant women and infants, received oseltamivir prophylactically.

December 2005 Human Avian Influenza Family Cluster, Eastern Turkey

From December 18, 2005, (*8*) to January 15, 2006 (*9*), a cluster of 8 confirmed influenza (H5N1) cases was detected in Dogubayazit District in eastern Turkey (online Appendix Figure 2, available from www.cdc.gov/EID/content/13/9/1348-appG2.htm) (*10–13*). These case-patients were among 21 members of 3 households located within 1.5 km of each other (*14*). All confirmed case-patients were hospitalized after onset of symptoms (*9*). Four of the confirmed case-patients died; the other 4 recovered (*9*). Ten of the remaining 14 household residents were hospitalized with avian influenza-like symptoms but were never confirmed to be infected with influenza (H5N1) (*9*). All but one of the hospitalized residents were children (6–15 years of age) (*9*).

Before onset of symptoms, 4 children from 1 household, 3 of whom had confirmed cases (including the index patient), were reported to have had close contact with the dead bodies of sick chickens (*15*). The 2 confirmed case-patients in the second household reportedly slaughtered a duck together on January 1, 2006, at the beginning of a die-off in the household's flock (*14*). Two of the remaining confirmed case-patients lived in the third household and had no history of contact with sick or dying poultry. The remaining confirmed case occurred in a fourth residence located near the first household (*10*), but because we lacked information on the number of household members and the case-patient's exposure history, we excluded it from these analyses. Most, if not all, of the 21 residents attended a dinner hosted by the family of the index patient on December 24, 2006, while he was symptomatic (*8*).

Statistical Methods

We used a previously developed statistical transmission model (*16,17*) to test whether human-to-human transmission occurred, and if it did, to estimate transmission parameters. In the model, persons mix with one another in households and between households. In addition, we include a common source of infection due to zoonotic exposure. Mathematical and statistical details are given in the online Technical Appendix (available from www.cdc.gov/EID/content/13/7/1348-Techapp.pdf).

Model of Probability of Transmission

We define p_1 as the probability that an infectious household member infects another household member in 1 day. If the distribution of the infectious period is known, we can obtain the household secondary attack rate (SAR_1) from p_1, defined as the probability that an infectious household member infects another household member over his or her infectious period. Similarly, we define the daily transmission probability (p_2) and the community SAR (SAR_2) for between household spread. Finally, we define the daily probability (b) that any person is infected from a zoonotic source. The contact structure used for parameter estimation is shown in the Figure. We assume that the distributions of the incubation and infectious periods are predetermined by the investigator.

We establish the likelihood function for each person and then for the whole population for statistical inference. The likelihood function for a person is equivalent to the probability of observing the realized data on that person throughout the outbreak. The likelihood function for a person labeled i is built with the following steps: 1) Obtain the probability that person i is infected by an infectious source labeled j on day t, given person i is not infected up to day $t-1$. If source j is a person, this probability is p_1, for the same household, or p_2 for exposure in the community, multiplied by the probability of person j being infectious on day t. The probability of person j being infectious on day t is derived from the symptom-onset day of person j and the distribution of the infectious period. If source j is zoonotic, the infection probability is b. The probability of escaping infection is simply 1 minus the corresponding probability of infection. 2) Take the product of the probabilities obtained in step 1 over all humans and zoonotic sources j to obtain the probability of person i escaping infection by any

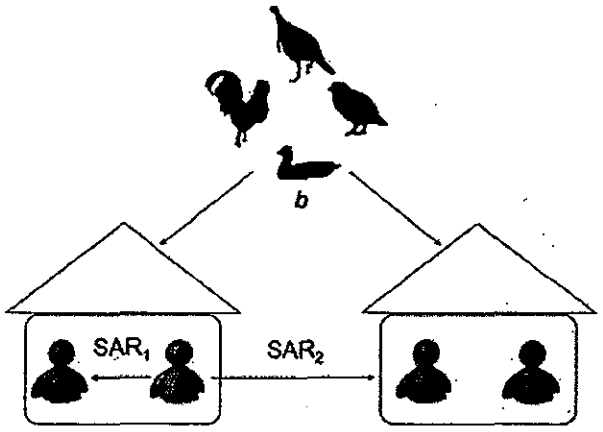

Figure. Schematic of estimation method. An infectious person (in red) infects a susceptible person (in green) in the same household with probability of household secondary attack rate (SAR_1) and infects a susceptible person in a different household with probability SAR_2. The common infectious source (i.e., avian hosts) infects a susceptible person with probability b per day. The likelihood function is constructed from symptom-onset dates and exposure information to estimate the above parameters

infectious source on day t. 3) Take the product of the probabilities obtained in step 2 over all days before and including day t to obtain the probability of person i escaping infection up to day t. 4) If person i is not infected by the end of the outbreak, the likelihood function for person i is the product of the probabilities of person i escaping infection up to the last day of observation. 5) If person i is observed to have symptom onset on day $\tilde{t}$ and the infection time is known to be t, the probability of the data regarding person i is the product of 3 pieces of information: a) the probability of person i escaping infection up to day $t-1$, b) the probability that person i is infected on day t, and c) the probability that the duration of the incubation period is $\tilde{t}-t$. Because we do not observe the infection time, the likelihood function for person i is obtained by summing the above product, a – c, over all potential values of t.

The likelihood function for the whole population is the product of all the individual likelihood functions. In the event that human-to-human transmission occurs, SAR estimates are used to estimate the local basic reproductive number (R_0), which is defined as the average number of secondary cases infected by a typical index case-patient in the beginning of the outbreak (online Technical Appendix). There is potential for sustained transmission if R_0 is >1. If human-to-human transmission is determined to be occurring, then the above parameters are estimated from the symptom dates and contact information from the population under study. Data on exposed persons who do not become ill form an important component of the inference procedure.

Statistical Test

We set up a statistical test with the null hypothesis being that no human-to-human transmission occurs, that is, $p_1 = p_2 = 0$. The alternative hypothesis is either p_1 or p_2 is not equal to 0, or both are not equal to zero. The test statistic we use is proportional to the ratio of the maximum value of the likelihood function assuming the null hypothesis is true (null likelihood) and the maximum value of the likelihood function at the estimated parameter values (full likelihood).

Specifically, we define the likelihood ratio test statistic as –2 log (the null likelihood function divided by the full likelihood function). If no human-to-human transmission occurs, the 2 likelihood functions would be roughly equal, and we expect to see a likelihood ratio close to 1, and, thus, a likelihood ratio statistic close to 0. A large value of the likelihood ratio statistic is evidence of deviation from the null hypothesis. The question is how to obtain a reference set of the likelihood ratio statistic values that we would see under the null hypothesis. Given no human-to-human transmission, all the observed case-patients must have been infected by the zoonotic source. Since the exposure to the zoonotic source is assumed constant for each person on each day, the null likelihood function will not change if we reassign the infection and symptom status of the observed case-patients to a different group of people in the population. By performing such reassignment many times, we obtained a collection of datasets that were each equally likely to have been observed had there been no human-to-human transmission. The values of the likelihood ratio statistic calculated from these datasets form the null distribution for statistical testing. This method is referred to as a permutation test. The p value is given by the proportion of the reference values that are equal to or larger than the observed likelihood ratio statistic value. More technical details are given in the online Technical Appendix.

The probability of infection by the zoonotic source may not be estimable together with SAR_1 or SAR_2 from an observed cluster. In such a situation, a statistical test of the occurrence of human-to-human transmission is still meaningful because the likelihood ratio test statistic is still estimable from the permuted datasets.

Data Required

A list of the inputs that are required for estimation and statistical testing are listed in the Table. Three categories of input parameters are required for this estimation model: outbreak-wide, individual level, and analysis parameters. The duration of the outbreak, the duration of the incubation period for the pathogen, and the minimum and maximum durations of the infectious period for the pathogen are the required outbreak-wide inputs. For each person, their residential location (neighborhood and household), their de-

Table. Parameters and data used in analysis

Category	Parameter/data	Required*
Entire outbreak	Outbreak begin date	X
	Outbreak end date	X
	Latent/incubation period, d†	X
	Infectious period, d†	X
All persons	Neighborhood of residence	X
	Household of residence	X
	Sex	X
	Age, y	X
	Case status (yes or no)	X
Case-patients	Whether outbreak index case-patient (yes or no)	X
	Date of illness onset	X
	Outcome (recovered, died, or don't know/still ill)	X
	Date of outcome	X
	Dates of hospitalization	O
	Period of receiving treatment (dates)	O
Non–case-patients	Dates of hospitalization	O
	Period of prophylactic treatment (dates)	O
Inter-residence visits	Identifier for visiting person	X
	Neighborhood visited	X
	Household visited	X
	Dates of the visit	X
Analysis parameters	End of exposure to the common source of infection (date)	X
	Final day of observation (date)	X
R_0 estimation	Mean no. residents per household	X‡
	Mean no. community contacts per person/d	X‡

*X, required; O, optional; R_0, basic reproduction number.
†The user defines the distribution of this period, including the minimum and maximum length of the period.
‡Required to estimate R_0.

mographic characteristics (sex and age), and whether they were a case-patient or not are required input parameters. Case-patients require additional input of their illness-onset dates, types of outcome, outcome dates, and whether or not they are the index patient in the outbreak. Hospitalization and treatment dates (considered prophylactic for nonpatients) are optional input parameters for each person. For each person who visits another residence during the outbreak period, his or her identifiers, the neighborhood and household visited, and the start and end dates of the visit are required inputs. Analysis-related inputs include the last date of community exposure to potential common sources of infection, the last date of observation, and inputs for R_0 estimation (mean number of residents per household and mean number of out-of-residence contacts per person per day). An expanded version of the model will require the input of other exposure information such as from schools or hospitals.

Results

For the outbreak in Indonesia, online Appendix Figure 1 shows that the incubation period had a probable range of 3–7 days and the infectious period, a probable range of 5–13 days. Thus, we let the incubation period have a uniform distribution of 3–7 days (mean 5 days) and the infectious period a uniform distribution of 5–13 days (mean 9 days). For the data shown in online Appendix Figure 1, only the household SAR (SAR_1) can be estimated. We determine that human-to-human spread did occur by rejecting the null hypothesis of no human-to-human transmission ($p = 0.009$). The estimated household SAR is 0.29 (95% confidence interval [CI] 0.15–0.51). Thus, a single infected person in a household infected another household member with the probability of 0.29. The average household size for rural Indonesia is ≈5 people. Because we do not have an estimate of the community SAR, we have an estimate of the lower limit of the local R_0, i.e., 1.14 with a 95% CI of 0.61–2.14. A sensitivity analysis on the distribution of the incubation and infectious period shows that the test and estimates for SAR_1 and R_0 are insensitive to uncertainty about these distributions within plausible ranges.

For the outbreak in Turkey, all the parameters are estimable, but we do not reject the null hypothesis of no human-to-human transmission ($p = 0.114$). Our estimate of the daily probability of infection from the common source is 0.011 (95% CI 0.005–0.025).

Discussion

We have presented statistical evidence that the strain of HPAI (H5N1) that caused the family cluster of human cases in northern Sumatra was spread from human to human and that the household SAR was 29%. This household

SAR is similar to statistical estimates for interpandemic influenza A in the United States (12.7%–30.6%) (*18,19*). The mean incubation period of this strain appears to have been ≈5 days, nearly twice as long as for past pandemic strains and current interpandemic strains of influenza. The CI for the estimated lower bound for the local R_0 covers 1. Therefore, even though we determined that human-to-human transmission probably occurred, whether the virus was capable of sustained human-to-human transmission is not clear. This virus may have required very close human contact to be transmitted. Even with no intervention, the finding that $R_0 = 1.14$ indicates that the chance that a single introduction would result in any further spread is ≈12%. In addition, the reported prophylactic use of oseltamivir may have played some role in limiting further spread. We did not find statistical evidence of human-to-human spread for the outbreak in eastern Turkey. This does not mean that no low-level human-to-human spread occurred in this outbreak, only that we lack statistical evidence of such spread. The power would be too low to detect such spread for an outbreak with 7 total cases and small SARs (*17*).

We did not consider the role of heterogeneity—such as age, sex, treatment status, or quarantine—in transmission. The parameters could be made to be functions of time-dependent covariates, as we have done with similar models (*16,19,20*). We can easily extend the model used here for covariates; however, we must have sufficient data to support such models.

Computer simulations have shown that the targeted use of influenza antiviral agents could be effective in containing a potential pandemic strain of influenza at the source (*21,22*), if initiated within 3 weeks of the initial case in the community, and if the R_0 is <1.8. This strategy, known as targeted antiviral prophylaxis, involves treating identified index patients in a mixing group and offering a single course of prophylaxis to the contacts of these index patients in predefined close contact groups, i.e., households at a minimum but also possibly neighborhood clusters, preschool groups, schools, and workplaces. In addition, the voluntary household quarantine of suspected close contacts of case-patients was recommended. Targeted antiviral prophylaxis at the household and neighborhood cluster level was carried out for the outbreak in Sumatra.

Ascertaining whether a potential pandemic strain of influenza is capable of sustained human-to-human transmission and estimating key transmission parameters are important. To estimate more than the household SAR, more detailed community data need to be collected. This would include a complete census of potentially exposed households and persons in the area where immediate transmission could occur from both potential zoonotic and human sources. Such data would enable estimation of important parameters and a more complete estimate of the R_0 rather than just the lower limit.

We have developed a software application, TRANSTAT, for implementing these analyses. This application provides a stand-alone environment for the entry, storage, and analysis of data from outbreaks of acute infectious diseases. A partial list of the input information is given in the Table. The statistical methods presented here can be applied to the data along with several standard epidemiologic tools. This information system would allow for real-time analysis and evaluation of control measures for an outbreak. We would encourage outbreak investigators to use this tool, taking care to input data on the exposed nonpatients as well as case-patients. The authors will provide a link to this software upon request.

This work was supported by the National Institute of General Medical Sciences MIDAS grant U01-GM070749 and National Institute of Allergy and Infectious Diseases grant R01-AI32042.

Dr Yang is a staff scientist in the Biostatistics and Biomathematics Program in the Division of Public Health Sciences at the Fred Hutchinson Cancer Research Center, Seattle, Washington, USA. His primary research interest is in the statistical and mathematical analysis of infectious disease data and intervention studies.

References

1. World Health Organization. Confirmed human cases of avian influenza A (H5N1). Epidemic and pandemic alert and response. 2007 Apr 11. [cited 2007 Apr 25]. Available from http://www.who.int/csr/disease/avian_influenza/country/cases_table_2007_04_11/en/index.html
2. Webby RJ, Webster RG. Are we ready for pandemic influenza? Science. 2003;302:1519–22.
3. Stohr K. Avian influenza and pandemics—research needs and opportunities. N Engl J Med. 2005;352:405–7.
4. Butler D. Family tragedy spotlights flu mutations. Nature. 2006;442:114–5.
5. World Health Organization. Avian influenza—situation in Indonesia—update 12. Jakarta, Indonesia. Report no. 12. 2006 May 19. [cited 2006 Sep 22]. Available from http://www.who.int/csr/don/2006_05_16b/en/index.html
6. Soebandrio A. Indonesia: avian flu from Indonesia's experience and perspectives. Pandemic Preparedness and Infection Control. 2006 July 13–14.
7. World Health Organization. Avian influenza—situation in Indonesia—update 16. Jakarta, Indonesia. Report no. 16. 2006 Jun 2. [cited 2006 Sep 16]. Available from http://www.who.int/csr/don/2006_05_31/en/index.html
8. Recombinomics. H5N1 bird flu pandemic phase evolution. 2006 Jun 5. [cited 2007 Mar 16]. Available from http://www.recombinomics.com/news/06050601/h5n1_phase_evolution.html
9. Recombinomics. Timeline for Kocyigit Ozcan family clusters in Dogubeyazit. January 22, 2006. [cited 2006 Mar 16]. Available from http://www.recombinomics.com/news/01220601/h5n1_kocyigit_ozcan_timeline.html

10. World Health Organization. Avian influenza, situation in Turkey, update 2. Ankara, Turkey. Report no. 2. Epidemic and pandemic alert and response. 2006 Jan 9. [cited 2007 Feb 27]. Available from http://www.who.int/csr/don/2006_01_09/en/index.html
11. World Health Organization. Avian influenza, situation in Turkey, update 4: sequencing of human virus. Ankara, Turkey. Report no. 4. Epidemic and pandemic alert and response. 2006 Jan 12. [cited 2007 Feb 27]. Available from http://www.who.int/csr/don/2006_01_12/en/index.html
12. World Health Organization. Avian influenza, situation in Turkey, update 5. Ankara, Turkey. Report no. 5. Epidemic and pandemic alert and response. 2006 Jan 16. [cited 2007 Feb 27]. Available from http://www.who.int/csr/don/2006_01_16/en/index.html
13. Lauer C. Turkey's bird flu outbreak: one year later. Turkish Daily News. 2007 Jan 12. [cited 2007 Mar 20]. Available from http://www.turkishdailynews.com.tr/article.php?enewsid=63680
14. World Health Organization. Shindo N. Avian influenza outbreak response in Turkey, 2006. Epidemic and pandemic alert and response. [cited 2007 Mar 20]. Available from http://www.col.ops-oms.org/servicios/influenza/reunion/docs/trad/10_turkey_summary.pdf
15. World Health Organization. Avian influenza, situation in Turkey. Ankara, Turkey. Epidemic and pandemic alert and response. Report no. 1. 2006 Jan 5. [cited 2007 Feb 27]. Available from http://www.who.int/csr/don/2006_01_05/en/index.html
16. Yang Y, Longini IM, Halloran ME. Design and evaluation of prophylactic interventions using infectious disease incidence data from close contact groups. Applied Statistics. 2006;55:317–30.
17. Yang Y, Longini IM, Halloran ME. A resampling-based test to detect person-to-person transmission of infectious disease. Annals of Applied Statistics. 2007;1:211–28. [cited 2007 Aug 1]. Available from http://projecteuclid.org/dpubs/repository/1.0/disseminate?handle=euclid.aoas/1183143736&view=body&content-type=pdfview_1
18. Longini IM, Koopman JS, Monto AS, Fox JP. Estimating household and community transmission parameters for influenza. Am J Epidemiol. 1982;115:736–51.
19. Longini IM, Koopman JS, Haber MJ, Cotsonis GA. Statistical inference for infectious diseases: risk-specific household and community transmission parameters. Am J Epidemiol. 1988;128:845–59.
20. Rampey AH, Longini IM, Haber MJ, Monto AS. A discrete-time model for the statistical analysis of infectious disease incidence data. Biometrics. 1992;48:117–28.
21. Longini IM, Nizam A, Xu S, Ungchusak K, Hanshaoworakul W, Cummings DA, et al. Containing pandemic influenza at the source. Science. 2005;309:1083–7.
22. Ferguson NM, Cummings DAT, Cauchemez S, Fraser C, Riley S, Meeyai A, et al. Strategies for containing an emerging influenza pandemic in Southeast Asia. Nature. 2005;437:209–14.

Address for correspondence: Ira M. Longini, Jr, Program in Biostatistics and Biomathematics, Fred Hutchinson Cancer Research Center, Seattle, WA 98109-1024, USA; email: longini@scharp.org

別紙様式第２－１

医薬品　研究報告　調査報告書

識別番号・報告回数			報告日	第一報入手日 平成 19 年 10 月 12 日	新医薬品等の区分 該当なし	機構処理欄
一般的名称	ﾃｸﾈﾁｳﾑ人血清ｱﾙﾌﾞﾐﾝ (^{99m}Tc)		研究報告の公表状況	Jiang Gu, et al. H5N1 infection of the respiratory tract and beyond: a molecular pathology study The Lancet Vol. 370, 29 September 2007, P 1137 -45	公表国 中国	
販売名（企業名）	テクネアルブミンキット（富士フイルムＲＩファーマ株式会社）					

研究報告の概要

背景： トリインフルエンザ H5N1 のヒトへの感染は、呼吸器系の症状と高い致死率を特徴とした新興感染症である。これまでの研究ではトリインフルエンザ H5N1 のヒトへの感染は、肺とは異なる臓器もターゲットにしている事を示している。
方法： H5N1 インフルエンザウイルスに感染していた 2 人の成人(一人は男性、一人は妊娠している女性)の死後の組織と、その女性の胎児について調べた。In-situ Hybridization(血球凝集素と核蛋白のセンス、アンチセンスプローブ)と免疫組織化学染色（血球凝集素と核蛋白に対するモノクローナル抗体を用いた）が選択された組織で行なわれた。逆転写酵素(RT)PCR、リアルタイム RT-PCR、Strand-Specific RT-PCR、および塩基配列に基づく増幅(NASBA)検出法が臓器組織サンプルのウイルスの RNA を検出するために実施された。
結果： 肺の II 型上皮細胞、気管の繊毛・非繊毛上皮細胞、リンパ節の T 細胞、脳の神経細胞、胎盤のホフバウアー細胞と細胞栄養芽層でウイルスの遺伝子配列と抗原を検出した。ウイルスの遺伝子配列（ウイルス抗原は検知されなかったが）は腸管粘膜で検出された。胎児では、肺、循環する単核細胞、肝臓のマクロファージで、ウイルスの塩基配列と抗原を検出した。臓器と胎児中のウイルスの塩基配列の存在は RT-PCR, Strand-specific PCR、real-time PCR 及び NASBA でも確認された。
注釈：肺に加え、H5N1 インフルエンザウイルスは気管に感染し、脳を含む他の臓器に拡散する。又、ウイルスは胎盤を通じて母親から胎児へ感染する。

報告企業の意見	今後の対応
高病原性トリインフルエンザ（H5N1）が「胎盤を通じて母親から胎児へ感染した」ことを確認した初めての報告である。本報告では In-situ Hybrydization 法、免疫組織化学染色法、逆転写酵素 PCR 法等の手法によって、胎盤の絨毛膜中に多数の感染細胞が検出され、胎児の肺・循環する単核細胞・肝臓のマクロファージにウイルスの塩基配列の存在を確認したとあり、本報告は新たに判明した感染経路に関するもの且つ重大な感染症に関するものと判断する。	本報告はヒト血液を原料とする血漿分画製剤とは直接関連するものではなく、現時点で特に当該生物由来製品に関し、措置等を行う必要はないと判断する。血清疫学研究ではこれまでにヒトインフルエンザの経胎盤感染の証拠は示されておらず、トリインフルエンザの方が子宮や胎児に容易に到達する可能性を示唆していることから、今後も同様の情報収集が必要であると考える。

使用上の注意記載状況・その他参考事項等

特になし

MedDRA Version(10.1)

H5N1 infection of the respiratory tract and beyond: a molecular pathology study

Jiang Gu, Zhigang Xie,* Zhancheng Gao,* Jinhua Liu,* Christine Korteweg,* Juxiang Ye, Lok Ting Lau, Jie Lu, Zifen Gao, Bo Zhang, Michael A McNutt, Min Lu, Virginia M Anderson, Encong Gong, Albert Cheung Hoi Yu, W Ian Lipkin*

Summary

Background Human infection with avian influenza H5N1 is an emerging infectious disease characterised by respiratory symptoms and a high fatality rate. Previous studies have shown that the human infection with avian influenza H5N1 could also target organs apart from the lungs.

Methods We studied post-mortem tissues of two adults (one man and one pregnant woman) infected with H5N1 influenza virus, and a fetus carried by the woman. In-situ hybridisation (with sense and antisense probes to haemagglutinin and nucleoprotein) and immunohistochemistry (with monoclonal antibodies to haemagglutinin and nucleoprotein) were done on selected tissues. Reverse-transcriptase (RT) PCR, real-time RT-PCR, strand-specific RT-PCR, and nucleic acid sequence-based amplification (NASBA) detection assays were also undertaken to detect viral RNA in organ tissue samples.

Findings We detected viral genomic sequences and antigens in type II epithelial cells of the lungs, ciliated and non-ciliated epithelial cells of the trachea, T cells of the lymph node, neurons of the brain, and Hofbauer cells and cytotrophoblasts of the placenta. Viral genomic sequences (but no viral antigens) were detected in the intestinal mucosa. In the fetus, we found viral sequences and antigens in the lungs, circulating mononuclear cells, and macrophages of the liver. The presence of viral sequences in the organs and the fetus was also confirmed by RT-PCR, strand-specific RT-PCR, real-time RT-PCR, and NASBA.

Interpretation In addition to the lungs, H5N1 influenza virus infects the trachea and disseminates to other organs including the brain. The virus could also be transmitted from mother to fetus across the placenta.

Lancet 2007; 370: 1137–45

See Comment page 1106

*Contributed equally to the study as first authors

Infectious Disease Centre (Prof J Gu MD, L T Lau PhD, J Lu PhD, Prof E Gong MD, Prof A C H Yu PhD), Department of Pathology, School of Basic Medical Sciences (Prof J Gu, Z Xie MD, C Korteweg MD, J Ye BS, Prof Z Gao MD, Prof B Zhang MD, Prof M A McNutt MD, M Lu MD, V M Anderson MD, Prof E Gong), People's Hospital (Prof Zh Gao MD), Neuroscience Research Institute (Prof A C H Yu, L T Lau, J Lu), Peking University, Beijing, China; College of Veterinary Medicine, China Agricultural University, Beijing, China (Prof J Liu PhD); Department of Pathology, State University of New York, Health Science Center at Brooklyn, New York, NY, USA (Prof J Gu, V M Anderson); Mailman School of Public Health, Columbia University, New York, NY, USA (Prof W I Lipkin MD)

Correspondence to:
Prof Jiang Gu, Infectious Disease Centre, Peking University, Beijing, 100083 China
jianggu@bjmu.edu.cn

Introduction

A pandemic outbreak of human infection with avian influenza H5N1 currently poses a potentially serious health threat worldwide. Since the outbreak of infection with avian influenza H5N1 virus in 2003, WHO has reported 277 laboratory confirmed cases in ten countries with a mortality rate of about 60%.[1] So far the virus has spread only from animals to human beings. However, human-to-human transmission potentiated by viral genomic mutation and reassortment of genomic subunits could be imminent. Recently, the first cases of probable human-to-human transmission have been reported.[2,3] The H5N1 influenza A virus is a negative-stranded RNA virus in which the genome consists of eight segments encoding ten viral proteins including haemagglutinin, neuraminidase, polymerase proteins, and nucleoprotein.[4]

Little is known about the specific effects in organs and cells targeted by the virus. The infection initially seemed to be restricted to the lungs, but later reports[5,6] have suggested that influenza A H5N1 could disseminate beyond the lungs. For various reasons (eg, religion), full autopsies of H5N1-infected human cases can often not be obtained. Accordingly, only a few reports[2,5,7–9] have described histopathology and virus distribution in H5N1 cases. Studies using in-situ hybridisation to detect viral genomic sequences in target cells have not been reported thus far.

We present clinicopathological data from H5N1 autopsies of two unrelated Chinese cases, as well as the histopathological changes and pattern of infection in the placenta and fetus from one of the patients, who was pregnant at the time of death. To gain further insight into the tissue tropism of influenza A H5N1 virus, we used in-situ hybridisation and immunohistochemistry to analyse viral localisation in various organs. Reverse transcription (RT) PCR, real-time RT-PCR, and nucleic acid sequence-based amplification (NASBA) H5 detection assays were also done to detect viral RNA in tissue samples, as well as strand-specific RT-PCR.

Methods

Patients

The clinical data of patient 1 have previously been published in detail.[10] A 24-year-old Chinese woman from China's Anhui province who was 4 months pregnant presented with a 6-day history of fever, cough, and dyspnoea. 2 weeks before admission, she had handled birds, several of which had died. On admission, she was lymphopenic, confused, and irritable, had bilateral infiltration on chest radiograph, and substantially reduced oxygen saturation. She was placed on a ventilator and treated with antibiotics, corticosteroids (hydrocortisone 400 mg on day 6 and day 7, and methylprednisolone 160 mg on day 8 and 240 mg on day 9), and fluids, but died 62 h after admission, 9 days after the onset of symptoms. No antiviral treatment was given.