別紙様式第2

別紙(2)-4

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 2009年5月7日	新医薬品等の区分 該当なし	厚生労働省処理欄
一般的名称	乾燥濃縮人アンチトロンビンⅢ		研究報告の公表状況	Information about Newly Emerging 2009 H1N1 Influenza Virus and Blood Safety http://www.fda.gov/cber/flu/h1n1bldsafety.htm	公表国 米国	
販売名（企業名）	アンスロビンP-ベーリング（CSLベーリング株式会社）					

研究報告の概要

問題点（2009 年の新興の H1N1 型インフルエンザウイルス感染と血液の安全性）

米国で 2009 年に新興の H1N1 型インフルエンザウイルス感染が発生していて、このウイルスが輸血により感染するか疑問視されている。米国や他の国において輸血による季節性インフルエンザが伝播した症例は報告がなく、現在まで輸血による H1N1 型インフルエンザウイルスの伝播の報告はない。FDA は継続して CDC と共同作業しており、またこのインフルエンザの発生と血液の安全性及び有用性に対するインパクトを監視するため、AABB のパンデミックインフルエンザ及び血液供給に関する組織間作業委員会と密接に連絡を取っている。今のところ、臨床上必要な場合、輸血のベネフィットが血液や血液製剤による H1N1 型インフルエンザウイルス伝播の理論的な危険性を含むリスクを上回ることを忘れないのが重要である。FDA の規制（FDA regulations at 21 CFR 640.3）において、健康でない人は献血には適していないし、血液事業者はこれらの潜在的な供血者の供血を保留しなければならない。

現在、血液事業者が実施している供血者スクリーニングにより、H1N1 型インフルエンザウイルスの症状を有する患者を同定すべきである。H1N1 型インフルエンザウイルスの人での症状は、通常のヒトインフルエンザと似ていて発熱、咳や喉の痛み、体の痛み、頭痛、寒気や疲労である。H1N1 型インフルエンザウイルスに関連した下痢や嘔吐の報告もある。メキシコや米国において重症化や死亡例が報告されている。現在実施している供血者スクリーニングは、特にヒトに H1N1 型インフルエンザが発生している地域での H1N1 型インフルエンザ伝播のリスクを減少する上で重要な手段である。さらに、良い衛生状態を維持する際に血液事業者が実施している標準的な手法や感染制御の手法は、血液事業における H1N1 型インフルエンザの起こりうる拡大を最小限にするのに役立つであろう。

2006 年 10 月の FDA ガイダンス" Biologic Product Deviation Reporting for Blood and Plasma Establishments"に従い、血液事業者は、供血者のインフルエンザ様疾患の供血後報告（a post donation report）が、既に収集された製品の適切性またはその供血者の将来の供血の適格性を評価すべきかを示していないか検討すべきである。さらに H1N1 型インフルエンザが同定された症例の国及び現地当局への通常の報告に加えて、インフルエンザの輸血による伝播に関する懸念を引き起こす症例がある血液事業者は、州及び現地健康部門と同様に適切に"Therapeutics and Blood Safety Branch of the CBER Office of Biostatistics and Epidemiology"に電話する。

新興の 2009 年の H1N1 型インフルエンザウイルスはエンベロープを有する大きなウイルスである。製造販売業者が実施したバリデーションテストでは、現在の血液製剤の製造工程により類似ウイルスが不活化・除去されることが示されている。

使用上の注意記載状況・その他参考事項等

報告企業の意見	今後の対応
本剤によるインフルエンザウイルス伝播の報告はない。 鳥インフルエンザウイルスが 60℃10 時間の液状加熱で不活化される報告があるため、本剤の製造工程でインフルエンザウイルスが不活化されると考えられる。	今後とも新しい感染症に関する情報収集に努める所存である。

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ABJ20093204

2009 H1N1 Flu Virus

Information about Newly Emerging 2009 H1N1 Influenza Virus and Blood Safety

I. Background

The ongoing outbreak of new emerging 2009 H1N1 Influenza Virus (H1N1 flu) infections in the United States has raised questions about whether this virus can be transmitted through blood transfusion. No case of transfusion transmitted seasonal influenza has ever been reported in the United States or elsewhere, and, to date, no cases of transfusion transmitted H1N1 flu have been reported. FDA is continuing to work with the Centers for Disease Control and Prevention (CDC) and is in close contact with the AABB Interorganizational Task Force on Pandemic Influenza and the Blood Supply to monitor this outbreak and its impact on blood safety and availability.

At this time, it is important to remember that, when clinically indicated, the benefits of a transfusion far outweigh the risks, including any theoretical risk of H1N1 flu transmission through blood or blood products.

II. Blood Safety Provisions

Donor Deferral

Under FDA regulations, individuals who are not in good health are not suitable to donate blood and blood establishments must defer these potential donors. (See FDA regulations at 21 CFR 640.3.) Blood donor screening procedures currently in place at blood establishments should identify persons with symptoms of H1N1 flu infection. The symptoms of H1N1 flu in people are similar to the symptoms of regular human influenza and include fever, cough, sore throat, body aches, headache, chills and fatigue. Some people have reported diarrhea and vomiting associated with H1N1 flu. Severe illness and deaths have been reported among infected individuals in Mexico and in the U.S.

The donor screening procedures in place today are important measures in reducing the theoretical risk of transfusion transmitted H1N1 flu, particularly in areas where human cases are occurring. In addition, the continued standard practice of blood establishments in maintaining good hygiene and infection control practices will help to minimize possible spread of H1N1 flu in blood establishments. Staff member hand washing between contacts with different donors is especially important.

Additional information on illness with H1N1 flu and general control strategies can be obtained at the Centers for Disease Control and Prevention (CDC) website at http://www.cdc.gov/swineflu/index.htm.

Potential Component Quarantine and Retrieval

Consistent with FDA's October 2006 Guidance on Biologic Product Deviation Reporting for Blood and Plasma Establishments (see http://www.fda.gov/cber/gdlns/devbld.htm) Medical Directors of blood establishments should consider whether a post donation report of a flu-like illness in a donor indicates that the previously collected products are unsuitable and that the donor's suitability for future donations should be assessed (e.g. deferral until well.) In addition to routine reporting of identified cases of H1N1 flu to state and local health departments, medical directors with any case

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raising concerns regarding potential transfusion transmission of influenza, may contact us at the Therapeutics and Blood Safety Branch of the CBER Office of Biostatistics and Epidemiology at 301-827-3974, as well as the CDC via state and local health departments, as appropriate.

Safety of Plasma Derivatives

The newly emerging 2009 H1N1 Influenza Virus is a large lipid-enveloped virus. Validation studies performed by the product manufacturers have shown that viruses with similar characteristics to this agent are effectively inactivated and/or removed by the manufacturing processes in place for these products.

Return to 2009 H1N1 Flu Virus Main Page

Updated: April 30, 2009

別紙様式第
番号 5

医薬品
医薬部外品　　研究報告　調査報告書
化粧品

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	厚生労働省処理欄
一般的名称	①ポリエチレングリコール処理抗破傷風人免疫グロブリン ②乾燥抗破傷風人免疫グロブリン			2009年4月22日	該当なし	
販売名 (企業名)	①テタノブリン－IH (ベネシス) ②テタノブリン (ベネシス)	研究報告の公表状況	CDC／MMWR 2009; 58 (DISPATCH) : 1-3		公表国 アメリカ	
研究報告の概要	米カリフォルニア南部におけるブタインフルエンザA (H1N1) ウイルス感染症例2例および感染源特定などのため現在実施中の調査に関する報告である。 2009年4月17日，米CDCは，カリフォルニア南部の隣接する地区に居住する小児2例の熱性呼吸器疾患はブタインフルエンザA (H1N1) ウイルス感染が原因であると特定した。2例からのウイルスはアマンダジンとリマンダジンに抵抗性があり，米国およびその他の国でのブタインフルエンザ又はヒトインフルエンザウイルスにおいてこれまでに報告されていない固有の遺伝子断片の組み合わせが含まれていた。両症例ともブタに接触していなかった。感染源は不明である。感染源を同定するために，他にブタインフルエンザウイルスで感染している人がいないか調査を現在進めている。 この報告は，この2症例と現在進行中の調査を簡潔に述べる。 ヒトにおけるインフルエンザAのA (H1N1) ウイルスとかなり相異する。かなりの人口が感染し，季節性インフルエンザワクチンH1N1株で予防できないかもしれない。 臨床医は，発熱性の呼吸疾患にかかっている以下に該当する患者の鑑別診断として，季節的なインフルエンザウイルス感染と同様に動物インフルエンザについても考慮すべきである。1) サンディエゴ郡およびインペリアル郡に居住する，2) これらの郡に旅行するかまたは患者がブタインフルエンザに感染していることを推測する臨床医は，呼吸器検体を採取し，州の公衆衛生研究所での検査を容易にするために国又は地方の衛生当局に連絡すべきである。 2症例ともブタに接触していないことは，この新しいインフルエンザウイルスのヒトーヒト感染が起こった可能性を大きくしている。 A (H1N1) ウイルスとは，この新しいサブタイプではないが，ブタ・インフルエンザA (H1N1) の新しい株は，ヒト・インフルエンザ				使用上の注意記載状況・ その他参考事項等 代表としてテタノブリン－IHの記載を示す。 2. 重要な基本的注意 (1) 本剤の原材料となる血液については，HBs抗原，抗HCV抗体，抗HIV-1抗体，抗HIV-2抗体陰性で，かつALT (GPT) 値でスクリーニングを実施している。更に，プールした試験血漿については，HIV-1，HBV及びHCVについて核酸増幅検査 (NAT) を実施し，適合した血漿を本剤の製造に使用しているが，当該NATの検出限界以下のウイルスが混入している可能性が常に存在する。本剤は，以上の検査に適合した高力価の破傷風抗毒素を含有する血漿を原料として，Cohnの低温エタノール分画で得た画分からポリエチレングリコール4000処理，DEAEセファデックス処理等により抗破傷風人免疫グロブリンを濃縮・精製した製剤であり，ウイルス不活化・除去を目的として，製造工程において60℃，10時間の液状加熱処理及びろ過膜処理 (ナノフィルトレーション) を施しているが，投与に際しては，次の点に十分注意すること。
米カルフォルニア南部の小児2例の熱性呼吸器疾患はブタインフルエンザA (H1N1) ウイルスによるものであり，当該ウイルスにはブタ及びヒトインフルエンザウイルスでこれまで報告されていない固有の遺伝子断片の組み合わせが含まれていたとするCDCからの報告である。 インフルエンザA (H1N1) はオルソミクソウイルス科に属し，ピリオンは球形で，直径80～120nmの脂質エンベロープを有するRNAウイルスである。万一，インフルエンザA (H1N1) が原料血漿に混入したとしてもBVDをモデルウイルスとしたウイルスバリデーション試験成績から，本剤の製造工程にて十分に不活化・除去されると考えている。		本報告は本剤の安全性に影響を与えないと考えるので，特段の措置はとらない。			

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MMWR

Dispatch

April 21, 2009 / 58 (Dispatch);1-3

Swine Influenza A (H1N1) Infection in Two Children --- Southern California, March--April 2009

On April 17, 2009, CDC determined that two cases of febrile respiratory illness occurring in children who resided in adjacent counties in southern California were caused by infection with a swine influenza A (H1N1) virus. The viruses from the two cases are closely related genetically, resistant to amantadine and rimantadine, and contain a unique combination of gene segments that previously has not been reported among swine or human influenza viruses in the United States or elsewhere. Neither child had contact with pigs; the source of the infection is unknown. Investigations to identify the source of infection and to determine whether additional persons have been ill from infection with similar swine influenza viruses are ongoing. This report briefly describes the two cases and the investigations currently under way. Although this is not a new subtype of influenza A in humans, concern exists that this new strain of swine influenza A (H1N1) is substantially different from human influenza A (H1N1) viruses, that a large proportion of the population might be susceptible to infection, and that the seasonal influenza vaccine H1N1 strain might not provide protection. The lack of known exposure to pigs in the two cases increases the possibility that human-to-human transmission of this new influenza virus has occurred. Clinicians should consider animal as well as seasonal influenza virus infections in their differential diagnosis of patients who have febrile respiratory illness and who 1) live in San Diego and Imperial counties or 2) traveled to these counties or were in contact with ill persons from these counties in the 7 days preceding their illness onset, or 3) had recent exposure to pigs. Clinicians who suspect swine influenza virus infections in a patient should obtain a respiratory specimen and contact their state or local health department to facilitate testing at a state public health laboratory.

Case Reports

Patient A. On April 13, 2009, CDC was notified of a case of respiratory illness in a boy aged 10 years who lives in San Diego County, California. The patient had onset of fever, cough, and vomiting on March 30, 2009. He was taken to an outpatient clinic, and a nasopharyngeal swab was collected for testing as part of a clinical study. The boy received symptomatic treatment, and all his symptoms resolved uneventfully within approximately 1 week. The child had not received influenza vaccine during this influenza season. Initial testing at the clinic using an investigational diagnostic device identified an influenza A virus, but the test was negative for human influenza subtypes H1N1, H3N2, and H5N1. The San Diego County Health Department was notified, and per protocol, the specimen was sent for further confirmatory testing to reference laboratories, where the sample was verified to be an unsubtypable influenza A strain. On April 14, 2009, CDC received clinical specimens and determined that the virus was swine influenza A (H1N1). The boy and his family reported that the child had had no exposure to pigs. Investigation of potential animal exposures among the boy's contacts is continuing. The patient's mother had respiratory symptoms without fever in the first few days of April 2009, and a brother aged 8 years had a respiratory illness 2 weeks before illness onset in the patient and had a second illness with cough, fever, and rhinorrhea on April 11, 2009. However, no respiratory specimens were collected from either the mother or brother during their acute illnesses. Public health officials are conducting case and contact investigations to determine whether illness has occurred among other relatives and contacts in California, and during the family's travel to Texas on April 3, 2009.

Patient B. CDC received an influenza specimen on April 17, 2009, that had been forwarded as an unsubtypable influenza A virus from the Naval Health Research Center in San Diego, California. CDC identified this specimen as a swine influenza A (H1N1) virus on April 17, 2009, and notified the California Department of Public Health. The source of the specimen, patient B, is a girl aged 9 years who resides in Imperial County, California, adjacent to San Diego County. On March 28, 2009, she had onset of cough and fever (104.3°F [40.2°C]). She was taken to an outpatient facility that was participating in an influenza surveillance project, treated with amoxicillin/clavulanate

potassium and an antihistamine, and has since recovered uneventfully. The child had not received influenza vaccir during this influenza season. The patient and her parents reported no exposure to pigs, although the girl did attend an agricultural fair where pigs were exhibited approximately 4 weeks before illness onset. She reported that she di not see pigs at the fair and went only to the amusement section of the fair. The Imperial County Public Health Department and the California Department of Public Health are now conducting an investigation to determine possible sources of infection and to identify any additional human cases. The patient's brother aged 13 years had influenza-like symptoms on April 1, 2009, and a male cousin aged 13 years living in the home had influenza-like symptoms on March 25, 2009, 3 days before onset of the patient's symptoms. The brother and cousin were not tested for influenza at the time of their illnesses.

Epidemiologic and Laboratory Investigations

As of April 21, 2009, no epidemiologic link between patients A and B had been identified, and no additional cases of infection with the identified strain of swine influenza A (H1N1) had been identified. Surveillance data from Imperial and San Diego counties, and from California overall, showed declining influenza activity at the time of th two patients' illnesses. Case and contact investigations by the county and state departments of health in California and Texas are ongoing. Enhanced surveillance for possible additional cases is being implemented in the area.

Preliminary genetic characterization of the influenza viruses has identified them as swine influenza A (H1N1) viruses. The viruses are similar to each other, and the majority of their genes, including the hemagglutinin (HA) gene, are similar to those of swine influenza viruses that have circulated among U.S. pigs since approximately 1999; however, two genes coding for the neuraminidase (NA) and matrix (M) proteins are similar to corresponding genes of swine influenza viruses of the Eurasian lineage (*1*). This particular genetic combination of swine influenza virus segments has not been recognized previously among swine or human isolates in the United States, or elsewhere based on analyses of influenza genomic sequences available on GenBank.* Viruses with this combination of genes are not known to be circulating among swine in the United States; however, no formal national surveillance system exists to determine what viruses are prevalent in the U.S. swine population. Recent collaboration between the U.S. Department of Agriculture and CDC has led to development of a pilot swine influenza virus surveillance program to better understand the epidemiology and ecology of swine influenza virus infections in swine and humans.

The viruses in these two patients demonstrate antiviral resistance to amantadine and rimantadine, and testing to determine susceptibility to the neuraminidase inhibitor drugs oseltamivir and zanamivir is under way. Because these viruses carry a unique combination of genes, no information currently is available regarding the efficiency of transmission in swine or in humans. Investigations to understand transmission of this virus are ongoing.

Reported by: *M Ginsberg, MD, J Hopkins, MPH, A Marouft, MPH, G Dunne, DVM, DR Sunega, J Giessick, P McVay, MD, San Diego County Health and Human Svcs; K Lopez, MD, P Kriner, MPH, K Lopez, S Munday, MS, Imperial County Public Health Dept; K Harriman, PhD, B Sun, DVM, G Chavez, MD, D Hatch, MD, R Schechter, MD, D Vugia, MD, J Louie, MD, California Dept of Public Health. W Chung, MD, Dallas County Health and Human Svcs; N Pascoe, S Penfield, MD, J Zoretic, MD, V Fonseca, MD, Texas Dept of State Health Svcs. P Blair, PhD, D Faix, PhD, Naval Health Research Center; J Tueller, MD, Navy Medical Center, San Diego, California. T Gomez, DVM, Animal and Plant Health Inspection Svc, US Dept of Agriculture. F Averhoff, MD, F Alavrado-Ramy, MD, S Waterman, MD, J Neatherlin, MPH, Div of Global Migration and Quarantine; L Finelli, DrPH, S Jain, MD, L Brammer, MPH, J Bresee, MD, C Bridges, MD, S Doshi, MD, R Donis, PhD, R Garten, PhD, J Katz, PhD, S Klimov, PhD, D Jernigan, MD, S Lindstrom, PhD, B Shu, MD, T Uyeki, MD, X Xu, MD, N Cox, PhD, Influenza Div, National Center for Infectious and Respiratory Diseases, CDC.*

Editorial Note:

In the past, CDC has received reports of approximately one human swine influenza virus infection every 1–2 years in the United States (*2,3*). However, during December 2005--January 2009, 12 cases of human infection with swine influenza were reported; five of these 12 cases occurred in patients who had direct exposure to pigs, six in patients reported being near pigs, and the exposure in one case was unknown (*1,4,5*). In the United States, novel influenza A virus infections in humans, including swine influenza infections, have been nationally notifiable conditions since 2007. The recent increased reporting might be, in part, a result of increased influenza testing capabilities in public health laboratories, but genetic changes in swine influenza viruses and other factors also might be a factor (*1,4,5*). Although the vast majority of human infections with animal influenza viruses do not result in human-to-human

transmission (*2,3*), each case should be fully investigated to be certain that such viruses are not spreading among humans and to limit further exposure of humans to infected animals, if infected animals are identified. Such investigations should include close collaboration between state and local public health officials with animal health officials.

The lack of known exposure to pigs in the two cases described in this report increases the possibility that human-to-human transmission of this new influenza virus has occurred. Clinicians should consider animal as well as seasonal influenza virus infections in the differential diagnosis of patients with febrile respiratory illness who live in San Diego and Imperial counties or have traveled to these areas or been in contact with ill persons from these areas in the 7 days before their illness onset. In addition, clinicians should consider animal influenza infections among persons with febrile respiratory illness who have been near pigs, such as attending fairs or other places where pigs might be displayed. Clinicians who suspect swine influenza virus infections in humans should obtain a nasopharyngeal swab from the patient, place the swab in a viral transport medium, and contact their state or local health department to facilitate transport and timely diagnosis at a state public health laboratory. CDC requests that state public health laboratories send all influenza A specimens that cannot be subtyped to the CDC, Influenza Division, Virus Surveillance and Diagnostics Branch Laboratory.

Interim guidance on infection control, treatment, and chemoprophylaxis for swine influenza is available at http://www.cdc.gov/flu/swine/recommendations.htm. Additional information about swine influenza is available at http://www.cdc.gov/flu/swine/index.htm.

References

1. Vincent AL, Ma W, Lager KM, Janke BH, Richt JA. Swine influenza viruses: a North American perspective. Adv Virus Res 2008;72:127--54.
2. Myers KP, Olsen CW, Gray GC. Cases of swine influenza in humans: a review of the literature. Clin Infect Dis 2007;44:1084--8.
3. Wells DL, Hopfensperger DJ, Arden NH, et al. Swine influenza virus infections. Transmission from ill pigs to humans at a Wisconsin agricultural fair and subsequent probable person-to-person transmission. JAMA 1991;265:478--81.
4. Vincent AL, Swenson SL, Lager KM, Gauger PC, Loiacono C, Zhang Y. Characterization of an influenza A virus isolated from pigs during an outbreak of respiratory disease in swine and people during a county fair in the United States. Vet Microbiol 2009;online publication ahead of print.
5. Newman AP, Reisdorf E, Beinemann J, et al. Human case of swine influenza A (H1N1) triple reassortant virus infection, Wisconsin. Emerg Infect Dis 2008;14:1470--2.

* Available at http://www.ncbi.nlm.nih.gov/Genbank.

Date last reviewed: 4/21/2009

別紙様式第2-1

No. 29

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	新医薬品等の区分	総合機構処理欄
				2009. 4. 15	該当なし	
一般的名称	人赤血球濃厚液		研究報告の公表状況	Yu H, Zhou YJ, Li GX, Zhang GH, Liu HL, Yan LP, Liao M, Tong GZ. Virus Res. 2009 Mar;140(1-2):85-90. Epub 2009 Jan 3.	公表国	
販売名(企業名)	赤血球濃厚液-LR「日赤」(日本赤十字社) 照射赤血球濃厚液-LR「日赤」(日本赤十字社)				中国	使用上の注意記載状況・その他参考事項等 赤血球濃厚液-LR「日赤」 照射赤血球濃厚液-LR「日赤」 血液を介するウイルス、細菌、原虫等の感染 vCJD等の伝播のリスク

研究報告の概要

○中国においてブタがヒト様H1N1インフルエンザウイルスに感染しているさらなるエビデンス

典型的ブタおよびヒト様H1N1インフルエンザウイルスは世界中のブタから数多く報告されているが、ヒト様H1N1ブタ・ウイルスの報告は少ない。2006年にヒト様H1N1ブタ・ウイルス(A/swine/Guangdong/96/06)が広東省のブタから分離されたが、これは中国で初めての報告であった。ブタにおけるヒト様H1N1インフルエンザウイルス感染の更なる証拠を得るため、中国で分離された3つのヒト様ブタH1N1ウイルス(A/swine/Guangdong/96/06, A/swine/Tianjin/01/04, A/swine/Henan/01/06)の8つの遺伝子セグメントを分析した。3ウイルスにおける8つの遺伝子セグメントは、いずれも、最近(2000年頃)および早期(1980年代)のヒトH1N1インフルエンザウイルスと直接由来し、他のウイルス2種は、1980年代に循環したヒトH1N1ウイルスに由来すると考えられることが判明した。系統発生解析では、A/Swine/Guangdong/96/06は、2000年頃のヒトH1N1インフルエンザウイルスと高い相同性を示した。

我々の分離株(A/swine/Guangdong/96/06)の血清陽性率から、中国のブタにヒト様H1N1ウイルスが存在することが確認された。これらインフルエンザウイルス(特に過去のウイルスであるA/swine/Tianjin/01/04とA/swine/Henan/01/06)の存在は、ヒト様H1N1インフルエンザウイルスがブタにおいて長期間不変でいることを示しており、ブタがヒト・パンデミックを引き起こす古いインフルエンザウイルスの保有宿主である証拠を示している。

報告企業の意見	今後の対応
中国のブタからヒト様H1N1インフルエンザウイルスが検出され、ブタがヒト・パンデミックを引き起こす古いインフルエンザウイルスの保有宿主である証拠が示されたとの報告である。	日本赤十字社では、輸血感染症対策として問診時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、問診で発熱などの体調不良者を献血不適としている。更に、平成21年5月18日付薬食血発第0518001号「新型インフルエンザの国内発生に係る血液製剤の安全性確保について」に基づき、新型インフルエンザの患者又は罹患の疑いのある患者と7日以内に濃厚な接触があった人の献血を制限するほか、献血後に新型インフルエンザと診断された場合には当該製剤の回収と医療機関への情報提供を行うこととしている。今後も引き続き情報の収集に努める。

(22)

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Further evidence for infection of pigs with human-like H1N1 influenza viruses in China

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ABSTRACT

Classical swine and avian-like H1N1 influenza viruses were reported widely in swine population worldwide, but human-like H1N1 swine viruses were reported occasionally. In 2006, a human-like H1N1 swine virus (A/swine/Guangdong/96/06) was isolated from pigs in Guangdong province, which was reported in China for the first time. To get further evidence for infection of pigs with human-like H1N1 influenza viruses, we analyzed eight gene segments of three human-like swine H1N1 viruses (A/swine/Guangdong/96/06, A/swine/Tianjin/01/04 and A/swine/Henan/01/06) isolated in China. All the eight genes of the three viruses are highly homologous to recent (about 2000) and early (1980s) human H1N1 influenza viruses, respectively. Phylogenetic analyses revealed that A/Swine/Guangdong/96/06 was directly derived from about 2000 human H1N1 influenza viruses, while A/swine/Tianjin/01/04 and A/swine/Henan/01/06 seemed to be descendants of human H1N1 viruses circulating in 1980s. Seroprevalence of our isolate (A/swine/Guangdong/96/06) confirmed the presence of human-like H1N1 virus in pigs in China. Existence of these influenza viruses, especially older viruses (A/swine/Tianjin/01/04 and A/swine/Henan/01/06), indicates that human-like H1N1 influenza viruses may remain invariant for long periods in pigs and provides the evidence that pigs serve as reservoirs of older influenza viruses for human pandemics.

1. Introduction

Swine influenza is an acute respiratory disease caused by influenza A virus within the Orthomyxoviridae family. The primary clinical manifestations of viral infection are fever and acute respiratory distress. Currently, three main subtypes of influenza viruses are circulating in the swine population throughout the world: subtypes H1N1, H3N2 and H1N2 (Brown, 2000). These include classical swine H1N1, avian-like H1N1, human-like or avian-like H3N2, reassortant H3N2 and various genotype H1N2 viruses (Brown, 2000; Qi and Lu, 2006; Webby et al., 2000). These viruses have remained largely endemic in pig populations worldwide and have been responsible for one of most prevalent respiratory diseases in pigs.

China, especially southern China, is regarded as an epicenter of pandemic influenza viruses throughout history (Shortridge and Stuart-Harris, 1982). The tracheal epithelium in pigs expresses receptors for both human and avian influenza viruses, and this provides a biological basis for the susceptibility of pigs to both avian and human influenza viruses (Ito et al., 1998; Peiris et al., 2001). Pigs can therefore function as intermediate hosts or "mixing vessels" in establishing new influenza virus lineages by supporting coinfection, replication, and reassortment among human, avian, and swine influenza viruses (Brown, 2000; Landolt et al., 2003). In the past, a number of influenza viruses have been isolated from pigs in China. These mainly include classical swine H1N1 viruses, avian-like H1N1 viruses, human-like H3N2 viruses, double-reassortant H3N2 viruses containing genes from the human and avian influenza viruses, triple-reassortant H3N2 viruses containing genes from the human, classical swine and avian viruses, avian-like H9N2 viruses, and double-reassortant H1N2 virus containing genes similar to those of human and swine viruses (Guan et al., 1996; Peiris et al., 2001; Shortridge and Webster, 1979; Xu et al., 2004; Yu et al., 2008a,b).

Human H1N1 viruses can infect pigs and pig-to-pig transmission has been demonstrated under experimental conditions (Brown, 2000). Serological surveillance studies worldwide suggest that the prevailing human H1N1 strains are readily transmitted to pigs and

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have resulted occasionally in the isolation of virus (Katsuda et al., 1995; Nerome et al., 1982; Yu et al., 2007). In 2006, a human-like H1N1 swine virus (A/swine/Guangdong/96/06) was isolated from pigs in Guangdong province, which was reported by us in China for the first time (Yu et al., 2007). To get further evidence for infection of pigs with human-like H1N1 influenza viruses, we made full use of our isolate and another two human-like H1N1 swine influenza viruses isolated and sequenced by scientists of Huazhong Agricultural University of China, and we analyzed their genetic evolution. In this study, we summarize and report, for the fist time, the coexistence of recent (about 2000) human-like and early (1980s) human-like swine H1N1 influenza viruses in pigs in China.

2. Materials and methods

2.1. Viruses

A/swine/Guangdong/96/06(H1N1) was isolated from pigs in a farm of Guangdong province of southern China, by inoculation into and subsequent passage in the allantoic cavity of 10-day-old SPF embryonated chicken eggs (Yu et al., 2007). Viral gene sequencing was carried out as follows. In brief, viral RNA was directly extracted from infected allantoic fluids using RNeasy Mini Kit (Qiagen, Chatsworth, CA) and reverse transcription (RT) were carried out under standard conditions using Uni12 (AGCAAAAGCAGG) primer. PCR was performed using specific primers for eight genes (primer sequences are available on request). PCR products were purified with the QIA quick PCR purification Kit (Qiagen, Inc.) and cloned into pMD18-T vector (TaKaRa, Dalian), then sequenced using synthetic oligonucleotides by Invitrogen Company.

In addition, A/swine/Tianjin/01/04(H1N1) and A/swine/Henan/01/06(H1N1) were isolated and sequenced by scientists of Huazhong Agricultural University of China. The nucleotide sequences were made available in GenBank under accession numbers: EU004440-EU004455.

2.2. Serum samples of pigs

From 2006 to 2007, we carried out swine influenza virus surveillance in China, a total of a total of 717 serum samples were randomly collected from apparently healthy pigs from nine provinces (Heilongjiang, Henan, Shandong, Zhejiang, Anhui, Jiangxi, Guangdong, Guangxi and Beijing).

2.3. Sequence analysis

All eight-gene segments of these three H1N1 swine influenza viruses were characterized and phylogenetically together with the representative sequence data available in GenBank. Sequence data were compiled and edited by using the Lasergene sequence analysis software package (DNASTAR Inc., Madison, WI). Multiple sequence alignment was carried out by using CLUSTAL W, and the unrooted phylogenetic trees were generated by the distance-based neighbor-joining method using MEGA 3.1. Bootstrap values were calculated on 1000 replicates of the alignment.

2.4. Serology tests

All sera were pretreated with the "Trypsin-Heat-Periodate" method to abolish interference by nonspecific serum inhibitors and used for hemagglutination inhibition (HAI) tests using chicken erythrocytes (World Health Organization, 2002). Neutralization tests were carried out by mixing 100 50% tissue culture infective doses of the virus with serial dilutions of serum and incubating for 2 h followed by inoculation onto MDCK cells grown in 96-well microtiter plates. After adsorption of the virus–serum mixture for 2 h, the inoculum was removed and fresh serum-free tissue culture medium containing trypsin (2 μg/ml) was added. Complete neutralization of cytopathic effect (read under an inverted microscope) was considered evidence of neutralizing antibody (Peiris et al., 2001; World Health Organization, 2002).

3. Results

3.1. Homology analysis of nucleotide sequences

Analysis of the homology of nucleotide sequences of eight genes of our isolate (A/swine/Guangdong/96/06) and another two isolates (A/swine/Tianjin/01/04 and A/swine/Henan/01/06) was performed by comparison with sequences available in GenBank (Table 1). All eight-gene segments of A/swine/Guangdong/96/06 were similar to H1N1 influenza viruses circulating in human in 2000 or 2001, with homologies ranging from 98.8 to 99.6%. But interestingly, A/swine/Tianjin/01/04 and A/swine/Henan/01/06 were closely related to human H1N1 viruses isolated in 1980s, with homologies ranging from 98.2 to 100%.

3.2. Phylogenetic relationship of H1N1 swine influenza viruses from China

In the swine influenza virus surveillance in eight provinces (Heilongjiang, Henan, Shandong, Guangdong, Zhejiang, Anhui, Jiangxi, and Beijing) during 2005–2006, one human-like H1N1 influenza virus (A/swine/Guangdong/96/06) was isolated from pigs, which was reported in China for the first time (Yu et al., 2007). Recently, the sequences of two human-like H1N1 swine viruses (A/swine/Tianjin/01/04 and A/swine/Henan/01/06) were published in GenBank. To characterize the gene segments of the three human-like H1N1 influenza viruses from pigs more precisely, we constructed the phylogenetic trees using the nucleotide sequences of the HA, NA, PB1, PB2, PA, NP, M and NS genes available in GenBank and the information from the trees was analyzed.

Phylogenetic analysis of the HA gene reveals that all of the H1N1 swine viruses isolated in China can be separated into three lineages, including human strains, classical swine strains and avian strains (Fig. 1). Previously most of the H1N1 swine influenza viruses, isolated in China, belong to classical swine or avian lineage. Classical swine lineage mainly includes A/swine/Guangdong/711/01, A/swine/Hong Kong/273/94, A/swine/Beiing/47/91, A/swine/Hong Kong/172/93 and so on. A/swine/Hong Kong/168/93 and A/swine/Hong Kong/176/93, had emerged in China, belong to avian lineage. A/swine/Guangdong/96/06, A/swine/Tianjin/01/04 and A/swine/Henan/01/06 are incorporated into the human lineage. Our isolate (A/swine/Guangdong/96/06) was closely related to A/Dunedin/2/00, while A/swine/Tianjin/01/04 and A/swine/Henan/01/06 were derived from A/Memphis/12/86.

Phylogenetic analyses of NA, PB1, PB2, PA (Fig. 2), NP, M and NS (data not shown) genes showed a clear division of each of these genes into different lineages including classical swine lineage, human lineage and avian lineage, similar to the HA gene. A/swine/Guangdong/96/06, A/swine/Tianjin/01/04 and A/swine/Henan/01/06 belong to human lineage in the seven phylogenetic trees. Because of the lack of sequence data of swine H1N1 influenza viruses isolated in China, these genes of classical swine lineage and avian lineage of China were not analyzed.

Based on the phylogenetic trees and homology of the nucleotide sequence of gene segments of the three viruses, A/Swine/Guangdong/96/06 was directly derived from about 2000 human H1N1 influenza viruses. But A/swine/Tianjin/01/04 and A/swine/Henan/01/06 seemed to be descendants of human H1N1 viruses circulating in 1980s.

Table 1
Genetic homology of the human-like swine influenza viruses isolated in China with related sequences available in GenBank.

Viruses	Gene	Virus with the highest identity	Identity (%)	GenBank accession no.
A/swine/Guangdong/96/06	HA	A/Dunedin/2/00(H1N1)	99.6	CY011584
	NA	A/Canterbury/43/00(H1N1)	99.4	CY010094
	PB1	A/New York/233/00(H1N1)	99.2	CY002646
	PB2	A/New York/443/01(H1N1)	99.4	CY003479
	PA	A/New York/443/01(H1N1)	99.1	CY003477
	NP	A/New York/234/00(H1N1)	99.3	CY002651
	M	A/New York/443/01(H1N1)	98.8	CY003473
	NS	A/New York/443/01(H1N1)	99.0	CY003476
A/swine/Tianjin/01/04	HA	A/Suita/1/89(H1N1)	99.0	D13573
	NA	A/Yamagata/120/86(H1N1)	99.1	D31948
	PB1	A/Singapore/6/86(H1N1)	99.8	CY020483
	PB2	A/New York/2924-1/86(H1N1)	99.6	CY021740
	PA	A/Fiji/15899/83(H1N1)	100.0	AJ605762
	NP	A/New York/2924-1/86(H1N1)	99.2	CY021736
	M	A/Singapore/6/86(H1N1)	98.4	CY020478
	NS	A/Chile/1/83/(H1N1)	98.2	X15282
A/swine/Henan/01/06	HA	A/Suita/1/89(H1N1)	98.9	D13573
	NA	A/Singapore/6/86(H1N1)	99.6	CY020479
	PB1	A/Singapore/6/86(H1N1)	99.9	CY020483
	PB2	A/New York/2924-1/86(H1N1)	99.3	CY021740
	PA	A/New York/2924-1/86(H1N1)	99.5	CY021738
	NP	A/New York/2924-1/86(H1N1)	99.2	CY021736
	M	A/Singapore/6/86(H1N1)	99.8	CY020478
	NS	A/Chile/1/83/(H1N1)	98.3	X15282

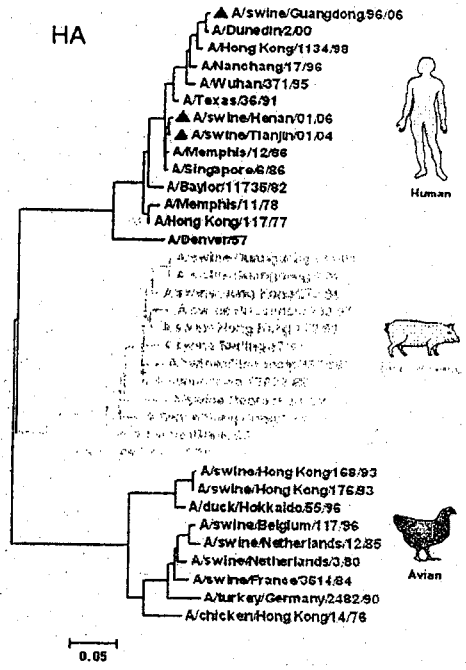

Fig. 1. Phylogenetic tree of the HA (positions 84-1061) gene of the H1N1 influenza viruses. The unrooted phylogenetic tree was generated by the distance-based neighbor-joining method using MEGA 3.1. Reliability of the tree was assessed by bootstrap analysis with 1000 replications, only bootstraps values >90% were shown. Different lineages are marked with different colors.

3.3. Molecular analysis

To try to identify possible determinants of interspecies transmission of H1N1 influenza viruses from human to pigs, the deduced amino acid sequences of HA1 region were aligned. The proposed antigenic sites (Caton et al., 1982; Lubeck and Gerhard, 1981; Olsen et al., 1993), receptor-binding sites (Nobusawa et al., 1991) and potential glycosylation sites were analyzed (Fig. 3).

Antigenic sites are regions of molecules involved in antibody binding and four sites (Sa, Sb, Ca and Cb) of H1N1 influenza virus have been defined (Caton et al., 1982; Wiley et al., 1981). A/swine/Guangdong/96/06 and A/Dunedin/2/00 have the same amino acids in antigenic sites, while A/swine/Tianjin/01/04, A/swine/Henan/01/06 and A/Memphis/12/86 also have the same amino acids in antigenic sites, which indicate these three viruses may have the similar antigenicity to rece (about 2000) and early (1980s) human H1N1 influenza virus respectively.

The host range of influenza A viruses is associated with differences in specificity of HA for attachment to sialic acid-containing receptors on susceptible cells. So the receptor-binding property of the HA protein of influenza virus is an important molecular determent of host-range restrictions (Matrosovich et al., 2000; Weis et al., 1988). The amino acids at positions 91, 131–135, 150, 180, 187, 191, 192, and 221–226 (98, 134–138, 153, 183, 190, 194, 195, and 224–229 according to H3 number) are components of receptor-binding sites of the HA of H1N1 influenza viruses (Nobusawa et al., 1991). The three human-like H1N1 swine influenza viruses and the two reference human viruses (A/Dunedin/2/00 and A/Memphis/12/86) had the same amino acids at Y^{91}, G^{131}, V^{132}, A^{134}, S^{135}, W^{150}, T^{152}, H^{180}, Y^{192}, R^{221}, Q^{223}, E^{224}, G^{225}, and R^{226} (receptor-binding sites). At position 133, the three swine influenza viruses and A/Dunedin/2/00 had the same amino acids (S). At position187, A/swine/Tianjin/01/04 and A/swine/Henan/01/06 had the unique amino acid (E). The two amino acids of the three human-like swine influenza viruses at positions 191 and 222 were identical to A/Dunedin/2/00 and A/Memphis/12/86, respectively.

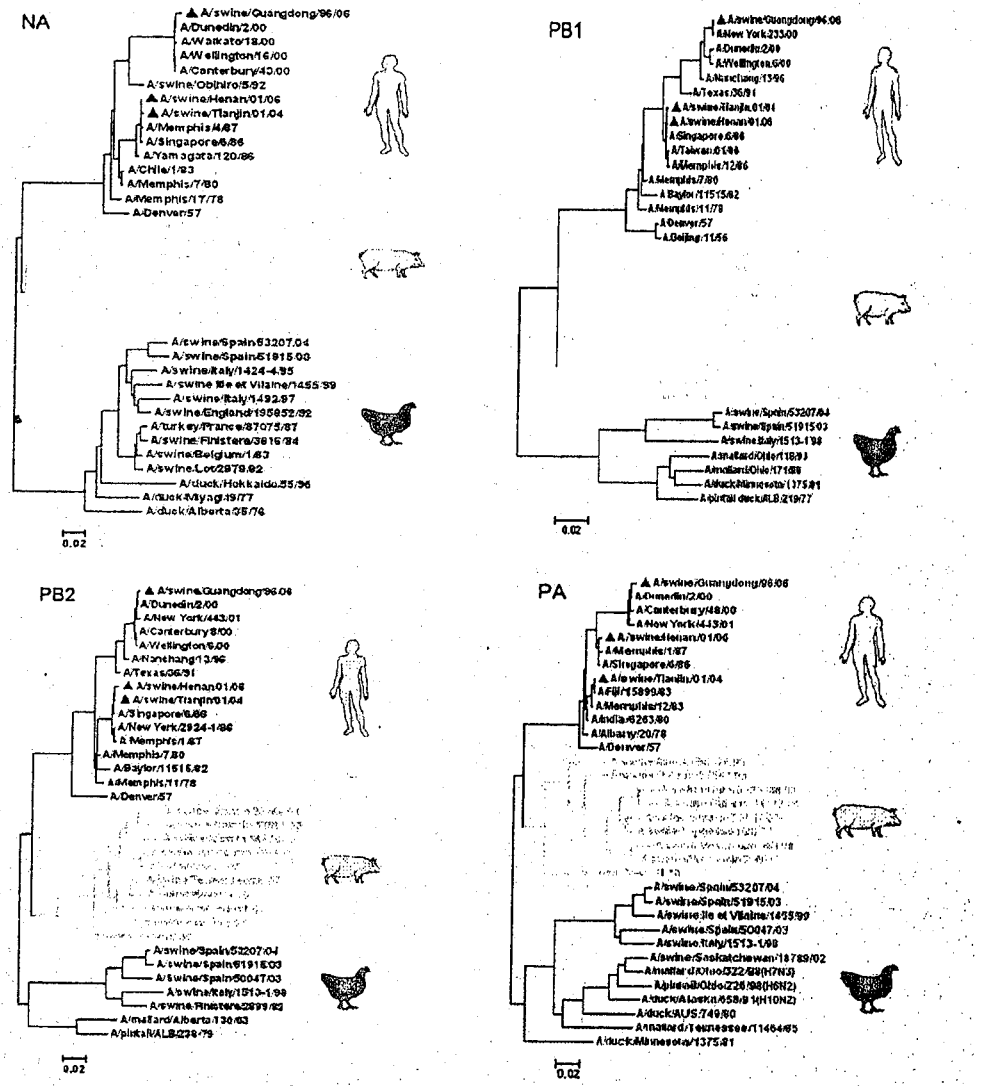

Fig. 2. Phylogenetic trees of the NA (positions 93-1415), PB1 (positions 14-2286), PB2 (positions 52-2295) and PA (positions 40-2175) genes of the H1N1 influenza viruses. The method used is as given in the legend of Fig. 1. Different lineages are marked with different colors.

Some glycosylation sites have a significant effect on receptor-binding property of the influenza virus HA protein, and glycosylation is therefore an important process in the generation of new virus (Schulze, 1997). Eight potential glycosylation sites (N-X-S/T) were conserved at positions 10, 11, 23, 54, 87, 125, 160, and 287 in the HA1 protein of the three human-like H1N1 swine influenza viruses and the two reference human viruses.

3.4. Seroprevalence of the human-like H1N1 influenza viruses in swine populations of China

The isolation and genetic characterization of human-like H1N1 influenza viruses in pigs suggested that these viruses might form a stable lineage in swine populations in China. So we conducted a serological surveillance to get some useful information about

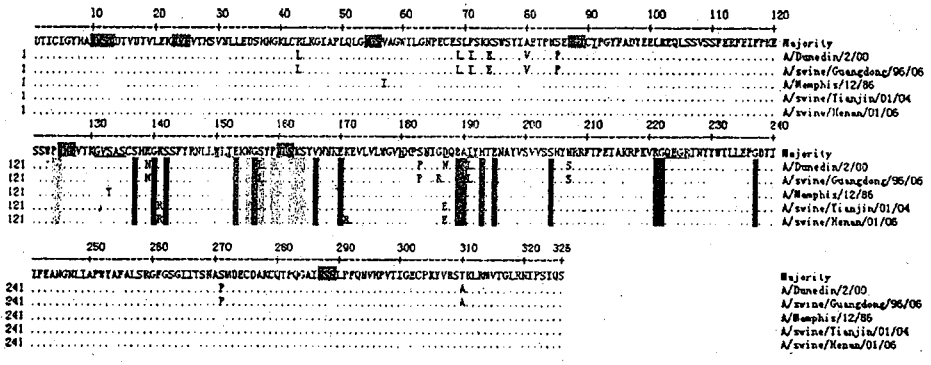

Fig. 3. Molecular analysis of HA1 amino acid sequences of the three H1N1 swine influenza viruses and reference strains. Potential glycosylation sites are marked with pink shade. Previously defined antigenic sites are indicated: site Sa (green shade), site Sb (red shade), site Ca (blue shade), site Cb (yellow shade). Underlined residues are receptor-binding sites. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Table 2
Seroprevalene of the human-like H1N1 influenza virus in swine populations of China.[a].

Province or city	Number of sera collected	HAI positive rate (%)	NT positive rate[b] (%)
Henan	68	17.6	11.8
Shandong	123	5.7	0
Heilongjiang	54	3.7	0
Zhejiang	92	7.6	6.5
Anhui	30	0	0
Jiangxi	44	4.5	2.3
Beijing	38	7.9	5.3
Guangxi	110	9.1	6.4
Guangdong	158	20.8	13.9

[a] HAI and neutralization positives were taken as titers of 1/80 or more.
[b] NT, neutralization test.

seroprevalence of the human-like H1N1 influenza viruses in swine populations of China. A collection of 717 pig serum samples from nine provinces in China was analyzed in HAI and neutralization tests for the presence of antibody to human-like H1N1 swine influenza virus (A/swine/Guangdong/96/06) (Table 2). Serological surveillance results indicated that the human-like H1N1 swine influenza virus might sporadically infect pigs in China. In the HAI test antibody to A/swine/Guangdong/96/06 was detected with prevalence ranging from 0 to 20.8%, while in the neutralizing test antibody to the H1N1 virus was relatively low with prevalence ranging from 0 to 13.9%.

4. Discussion

Influenza virus infection is an important cause of respiratory disease among pigs throughout the swine producing regions of the world (Karasin et al., 2000). Swine influenza was first observed in 1918 at the time of the human pandemic and the virus was isolated and identified in 1930 by shope (Brown, 2000; Shope, 1931). This virus was the prototype strain of a group of viruses now known as classical swine influenza viruses. Virologic and serological surveillance has shown that classical swine H1N1 is prevalent throughout the major pig population of the world (Brown, 2000; Chambers et al., 1991; Guan et al., 1996; Hinshaw et al., 1978). Since 1979, classical swine influenza viruses have been replaced by avian-like H1N1 viruses that are antigenically distinguishable from classical swine H1N1 viruses in Europe. Human H1N1 viruses can infect pigs and pig-to-pig transmission has been demonstrated under experimental conditions. Serological surveillance studies worldwide suggest that the prevailing human H1N1 strains are readily transmitted to pigs (Brown, 2000), but there are a few reports about isolation of the human-like swine H1N1 viruses. In China, classical swine H1N1 viruses were the predominant influenza virus infecting pigs and circulated in pigs in China in northern, central (Henan and Jiangxi), and southern (Guizhou and Guangdong) provinces (Guo et al., 1992). Since 1993, avian-like swine influenza viruses had been isolated from pigs and circulated with classical H1N1 viruses (Guan et al., 1996). In 2006, human-like swine H1N1 influenza viruses were reported by us for the first time. In this study, we summarized and reported coexistence of recent (about 2000) and early (1980s) human-like swine H1N1 influenza viruses, which provides further evidence for infection of pigs with human-like H1N1 influenza viruses in China.

Serological surveillance had indicated that classical swine H1 and human-like H3 subtype influenza infections widely existed in the pig populations in China, and avian H4, H5 and H9 influenza viruses had been transmitted to pig populations in southeastern China (Li et al., 2004; Ninomiya et al., 2002). No type of swine influenza vaccine has been used in pigs in China, and therefore the serological surveillance of human-like H1N1 swine influenza viruses conducted in this study could reflected the real situation of swine influenza infection. In this study, a total of 717 pig serum samples from nine provinces in China were detected in HAI and neutralization tests for the presence of antibody to human-like H1N1 swine influenza virus (A/swine/Guangdong/96/06). In the HAI test antibody to A/swine/Guangdong/96/06 was detected with prevalence ranging from 0 to 20.8%, while in the neutralizing test antibody to the H1N1 virus as relatively low with prevalence ranging from 0 to 13.9%. All these indicated that the human-like H1N1 swine influenza virus might sporadically infect pigs in China.

Influenza virus genomes are well known to undergo antigenic drift or antigenic shift that enable escape from preexisting immunity and cause new outbreaks of influenza in animals and even humans (Chi et al., 2005; Potter, 2001; Subbarai and Joseph, 2007), so influenza viruses exhibit the greatest genetic diversity and change every year. In this study, we analyzed eight gene segments of three human-like swine H1N1 viruses (A/swine/Guangdong/96/06, A/swine/Tianjin/01/04 and A/swine/Henan/01/06) isolated in China. Why were all the eight genes of the three viruses closely related to recent (about 2000) or early (1980s) human H1N1 influenza viruses? A possible explanation may be that these influenza viruses were introduced into

pigs at the time they circulated in humans and have persisted in pigs without antigenic drift. In China, Pigs have a short lifespan (approximately 6 months) and are not inoculated any type of swine influenza vaccine. Once the influenza viruses were introduced into pigs, these viruses might appear to have been under less immune selection pressure and all genes evolved more slowly than in humans and poultry. We describe here genetic relatedness of these swine isolates with recent (about 2000) or early (1980s) human H1N1 influenza viruses and provide evidence of long term conservation of human H1N1 influenza viruses in pigs.

Of the four pandemic strains of human influenza A virus occurred in the 20th century, the 1977 pandemic strain was very similar in all eight genes to a 1950 human H1N1 strain (Kilbourne, 2006). Therefore, pandemic strains of influenza A virus could arise by re-emergence of these older viruses that may have caused an epidemic many years earlier. In this study, we phylogenetically analyzed eight gene segments of three human-like H1N1 influenza viruses isolated from pigs in China. A/Swine/Guangdong/96/06 was directly derived from about 2000 human H1N1 influenza viruses. But A/swine/Tianjin/01/04 and A/swine/Henan/01/06 seemed to be descendants of human H1N1 viruses circulating in 1980s, which showed the possibility that pigs serve as reservoirs for older influenza viruses.

China, especially Southern China, is thought to be the epicenter for the human influenza pandemics throughout history (Shortridge and Stuart-Harris, 1982). The special environment and lifestyle in southern China provide more chances for wild aquatic birds, domestic poultry, pigs and humans to contact closely, and create the opportunity for interspecies transmission and generation of new reassortment influenza viruses. Although, it is virtually impossible to prevent new outbreaks of influenza in human and animals, it is now well recognized that animal influenza virus surveillance can play a key role in the early recognition of outbreak threats. So it is of great significance to carry out swine influenza virus surveillance. Existence of these influenza viruses, especially older viruses (A/swine/Tianjin/01/04 and A/swine/Henan/01/06), in pigs provides the evidence that pigs serve as reservoirs of older influenza viruses for human pandemics and emphasizes the importance of reinforcing swine influenza virus surveillance in China.

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References

Brown, I.H., 2000. The epidemiology and evolution of influenza viruses in pigs. Vet. Microbiol. 74, 29–46.
Caton, A.J., Brownlee, G.G., Yewdell, J.W., Gerhard, W., 1982. Antigenic structure of the influenza virus A/PR/8/34 hemagglutinin (H1 subtype). Cell 31, 417–427.
Chambers, T.M., Hinshaw, V.S., Kawaoka, Y., Easterday, B.C., Webster, R.G., 1991. Influenza viral infection of swine in the United States 1988–1989. Arch. Virol. 116, 261–265.
Chi, X.S., Bolar, T.V., Zhao, P., Tam, J.S., Rappaport, R., Cheng, S.M., 2005. Molecular evolution of human influenza A/H3N2 virus in Asia and Europe from 2001 to 2003. J. Clin. Microbiol. 43, 6130–6132.
Guan, Y., Shortridge, K.F., Krauss, S., Webster, R.G., 1996. Emergence of avian H1N1 viruses in pigs in China. J. Virol. 70, 8041–8046.
Guo, Y., Webster, R.G., Zhuge, Y.H., 1992. Swine (H1N1) viruses isolated from pigs in China and studies on the origin of isolates. Chin. J. Clin. Exp. Virol. 6, 347–353.
Hinshaw, V.S., Bean, J., Webster, R.G., Easterday, B.C., 1978. The prevalence of influenza viruses in swine and the antigenic and genetic relatedness of influenza viruses from man to swine. Virology 84, 51–62.
Ito, T., Couceiro, J.N., Kelm, S., Baum, L.G., Krauss, S., Castrucci, M.R., Donatelli, I., Kida, H., Paulson, J.C., Webster, R.G., Kawaoka, Y., 1998. Molecular basis for the generation in pigs of influenza A viruses with pandemic potential. J. Virol. 72, 7367–7373.
Karasin, A.I., Schutten, M.M., Cooper, L.A., Smith, C.B., Subbarao, K., Anderson, G.A., Carman, S., Olsen, C.W., 2000. Genetic characterization of H3N2 influenza viruses isolated from pigs in North America 1977–1999: evidence for wholly human and reassortant virus genotypes. Virus Res. 68, 71–85.
Katsuda, K., Sato, S., Shirahata, T., Lindstrom, S., Nerome, R., Ishida, M., Nerome, K., Goto, H., 1995. Antigenetic and genetic characteristics of H1N1 human influenza virus isolated from pigs in Japan. J. Gen. Virol. 76, 1247–1249.
Kilbourne, E.D., 2006. Influenza pandemics of the 20th Century. Emerg. Infect. Dis. 12, 9–14.
Landolt, G.A., Karasin, A.I., Phillips, L., Olsen, C.W., 2003. Comparison of the pathogenesis of two genetically different H3N2 influenza A viruses in pigs. J. Clin. Microbiol. 141, 1936–1941.
Li, H.Y., Yu, K.Z., Xin, X.G., Yang, H.L., Li, Y.B., Qin, Y.N., Bi, Y.Z., Tong, G.Z., Chen, H.L., 2004. Serological and virologic surveillance of swine influenza in China from 2000 to 2003. Int. Congress Series 1263, 754–757.
Lubeck, M.D., Gerhard, W., 1981. Topological mapping antigenic sites on the influenza A/PR/8/34 virus hemagglutinin using monoclonal antibodies. Virology 113, 64–72.
Matrosovich, M., Tuzikov, A., Bovin, N., Gambaryan, A., Klimov, A., Castrucci, M.R., Donatelli, I., Kawaoka, Y., 2000. Early alterations of the receptor-binding properties of H1, H2, and H3 avian influenza virus hemagglutinins after their introduction into mammals. J. Virol. 74, 8502–8512.
Nerome, K., Ishida, M., Oya, A., Kanai, C., Suwicha, K., 1982. Isolation of an influenza H1N1 virus from a pig. Virology 117, 485–489.
Ninomiya, A., Takada, A., Okazaki, K., Shortridge, K.F., Kida, H., 2002. Seroepidemiological evidence of avian H4, H5, and H9 influenza A virus transmission to pigs in southeastern China. Vet. Microbiol. 88, 107–114.
Nobusawa, E., Aoyama, T., Kato, H., Suzuki, Y., Tateno, Y., Nakajima, K., 1991. Comparison of complete amino acid sequences and receptor-binding properties among 13 serotypes of hemagglutinins of influenza A viruses 182, 475–485.
Olsen, C.W., McGregor, M.W., Cooley AJ, A.J., Schantz, B., Hotze, B., Hinshaw, V.S., 1993. Antigenic and genetic analysis of a recently isolated H1N1 swine influenza virus. Am. J. Vet. Res. 54, 1630–1636.
Peiris, J.S.M., Guan, Y., Markwell, D., Ghose, P., Webster, R.G., Shortridge, K.F., 2001. Cocirculation of avian H9N2 and contemporary "human" H3N2 influenza A viruses in pigs in southeastern China: potential for genetic reassortment? J. Virol. 75, 9679–9686.
Potter, C.W., 2001. A history of influenza. J. Appl. Microbiol. 91, 572–579.
Qi, X., Lu, C.P., 2006. Genetic characterization of novel reassortant H1N2 influenza A viruses isolated from pigs in southeastern China. Arch. Virol. 151, 2289–2299.
Schulze, I.T., 1997. Effects of glycosylation on the properties and functions of influenza virus hemagglutinin. J. Infect. Dis. 1 (Suppl.), S24–S28.
Shope, R.E., 1931. Swine influenza. III. Filtration experiments and etiology. J. Exp. Med. 54, 373–385.
Shortridge, K.F., Stuart-Harris, C.H., 1982. An influenza epicentre? Lancet 11, 812–813.
Shortridge, K.F., Webster, R.G., 1979. Geographical distribution of swine (Hsw1N1) and Hong Kong (H3N2) influenza virus variants in pigs in southeast Asia. Intervirology 11, 9–15.
Subbarai, K., Joseph, T., 2007. Scientific barriers to developing vaccines against avian influenza viruses. Nature 7, 267–278.
Webby, R.J., Swenson, S.L., Krauss, S.L., Gerrish, P.J., Goyal, S.M., Webster, R.G., 2000. Evolution of swine H3N2 influenza viruses in the United States. J. Virol. 74, 8243–8251.
Weis, W., Brown, J.H., Cusack, S., Paulson, J.C., Skehel, J.J., Wiley, D.C., 1988. Structure of the influenza virus haemagglutinin complexed with its receptor, sialic acid. Nature 333, 426–431.
Wiley, D.C., Wilson, I.A., Skehel, J.J., 1981. Structural identification of the antibody-binding sites of Hong Kong influenza haemagglutinin and their involvement in antigenic variation. Nature 289, 373–378.
World Health Organization, 2002. WHO Manual on Animal Influenza Diagnosis and Surveillance. World Health Organization, Department of Communicable Diseases Surveillance and Control, Geneva, pp. 28–50.
Xu, C., Fan, W., Wei, R., Zhao, H., 2004. Isolation and identification of swine influenza recombinant A/Swine/Shandong/1/2003(H9N2) virus. Microbes Infect. 6, 919–925.
Yu, H., Hua, R.H., Wei, T.C., Zhou, Y.J., Tian, Z.J., Li, G.X., Liu, T.Q., Tong, G.Z., 2008a. Isolation and genetic characterization of avian origin H9N2 influenza viruses from pigs in China. Vet. Microbiol. 131, 82–92.
Yu, H., Hua, R.H., Zhang, Q., Liu, T.Q., Liu, H.L., Li, G.X., Tong, G.Z., 2008b. Genetic evolution of swine influenza A (H3N2) viruses in China from 1970 to 2006. J. Clin. Microbiol. 46, 1067–1075.
Yu, H., Zhang, G.H., Hua, R.H., Zhang, Q., Liu, T.Q., Liao, M., Tong, G.Z., 2007. Isolation and genetic analysis of human origin H1N1 and H3N2 influenza viruses from pigs in China. Biochem. Biophys. Res. Commun. 356, 91–96.