Veterinary Microbiology 148 (2011) 413–418

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Short communication

Serological evidence of pig-to-human influenza virus transmission on Thai swine farms

Pravina Kitikoon [a,c], Donruethai Sreta [a,c], Ranida Tuanudom [a,c], Alongkorn Amonsin [a,c], Sanipa Suradhat [a,c], Kanisak Oraveerakul [a], Yong Poovorawan [b], Roongroje Thanawongnuwech [a,c,*]

[a] Faculty of Veterinary Science, Chulalongkorn University, Henri-Dunant Rd., Bangkok 10330, Thailand
[b] Faculty of Medicine, Chulalongkorn University, Henri-Dunant Rd., Bangkok 10330, Thailand
[c] Emerging and Re-emerging Infectious Disease in Animals, Research Unit (CUEIDAs), Chulalongkorn University, Henri-Dunant Rd., Bangkok 10330, Thailand

ARTICLE INFO

Article history:
Received 14 June 2010
Received in revised form 15 September 2010
Accepted 21 September 2010

Keywords:
Influenza
Interspecies transmission
Hemagglutination inhibition
Antibody

ABSTRACT

We investigated influenza interspecies transmission in two commercial swine farms in Thailand. Sera from swine-exposed workers ($n = 78$), age-matched non-swine-exposed healthy people ($n = 60$) and swine populations in both farms ($n = 85$) were studied. Hemagglutination-inhibition (HI) assay was performed on Thai swine H1 viruses (swH1N1 and swH1N2) isolated from both farms. Thai human H1N1 (huH1N1) and pandemic H1N1 2009 (pH1N1) were also used as test antigens. The hemagglutinin (HA) 1 genes of swH1N1 and swH1N2 viruses were sequenced and shown to be genetically distinct from the Thai huH1N1 and pH1N1 viruses. Evidence of pig-to-human influenza virus transmission was found in farm workers with increased odds of elevated antibody titers to both swH1N1 (OR 42.63, 95% CI, 14.65–124) and swH1N2 (OR 58, 95% CI, 13.12–256.3) viruses. No evidence of human-to-pig influenza virus transmission was detected in this study.

1. Introduction

Occasionally, swine influenza virus (SIV) transmission to humans, and vice versa, has been documented (Karasin et al., 2006; Katsuda et al., 1995). Previously documented SIV-infected human cases were nonfatal and human-to-human transmission was rare (Myers et al., 2007). In addition, a large number of cases involved swine farmers, people who lived on swine farms or were in close contact with pigs. At present, three SIV subtypes, H1N1, H1N2 and H3N2 are commonly found in swine throughout the world (Easterday and Van Reeth, 1999). The first subtype isolated was named "classical-swine" influenza virus as all eight gene segments were of swine origin. Classical swine H1N1 (cH1N1) was known as the dominant virus in North American swine populations for over 60 years (Easterday and Van Reeth, 1999). Recent data indicated that the HA1 genes of classical-swine subtypes could now be grouped into three separate clusters, swine H1-alpha (swH1α), swine H1-beta (swH1β) and swine H1-gamma (swH1γ) (Vincent et al., 2009). The HA1 gene of swH1γ is known as the progenitor of the pandemic H1N1 2009 (pH1N1) viruses (Kingsford et al., 2009). In contrast, H1N1 virus originally introduced into the European pig population is often referred to as "avian-like" virus since it contains entire avian genes that are genetically distinct from the cH1N1 viruses. Currently, European H1N1 isolates contain an HA1 segment from both human and avian lineages while the remaining gene segments are still of avian origin (Zell et al., 2008). Thai H1N1 SIV contains surface HA1 and NA1 antigens from the North American (classical swine)- and Eurasian (avian-like)- swine lineages, respectively (Sreta

---

[*] Corresponding author at: Faculty of Veterinary Science and Emerging and Re-emerging Infectious Disease in Animals, Research Unit (CUEIDAs), Chulalongkorn University, Henri-Dunant Rd., Pathumwan, Bangkok 10330, Thailand. Tel.: +66 0 2218 9615; fax: +66 0 2252 0779.
E-mail address: roongroje.t@chula.ac.th (s.$. Thanawongnuwech).

0378-1135/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetmic.2010.09.019

et al., 2009; Takemae et al., 2008). Notably, this feature is uniquely shared among the pH1N1 viruses that emerged in early 2009 (Kingsford et al., 2009).

Swine production system in Thailand is different from the systems in North America and Europe. The production size in Thailand ranges from large industrialized farms (>1000 pigs) to backyard farms (<50 pigs). Pig housing in Thailand also consists of both closed housing or evaporation cooling system and open housing where natural air flow ventilates the units. Normally, Thai swine farm owners and farm workers including their spouses and children live on the farm. Such an environment provides an excellent human-animal interface for influenza virus cross-species transmission. The aim of this study was to investigate the serological evidence of influenza virus interspecies transmission among Thai swine workers and pigs on the farm. Tests for hemagglutination-inhibition (HI) antibodies against the Thai swine H1N1 viruses (swH1N1 and swH1N2) isolated from both farms and the representative Thai human H1N1 (huH1N1) and pH1N1 were performed on both human and swine populations. To evaluate the genetic diversity of the viruses utilized as HI test antigen, the HA1 genes of swH1N1 and swH1N2 viruses were sequenced followed by phylogenetic analysis adding huH1N1, pH1N1 and other reference H1 influenza viruses from GenBank.

2. Materials and methods

2.1. Study population

2.1.1. Human population

From 2008 to early 2009, sera from 78 swine-exposed participants were collected from two large scale commercial farms in the central-eastern region of Thailand. Subjects were farm owners ($n = 2$), pig handlers ($n = 52$), veterinarians ($n = 8$), farm cleaners ($n = 8$) and people working in the farm office ($n = 8$) with their age ranging from 18 to 59 years (50% males and 50% females). Sixty negative control subjects with no history of swine exposure (verified by personal interview) were voluntarily recruited from the Blood centre and hospital in the central-eastern region of Thailand. The non-swine-exposed control sera were obtained from 50% males and 50% females with their age ranging from 19 to 60 years. During the time of investigation all subjects were healthy with no influenza-like illness. The study had been approved by the institutional review board (137/2007record#400/49).

2.1.2. Swine population

Eighty-five pig serum samples were collected from farms A and B, 46 and 39 samples, respectively. Both farms maintained an open housing system. Swine serum was randomly sampled cross-sectionally from different age groups including gilts, sows, weaning and growing pigs.

2.2. Influenza viruses for hemagglutination-inhibition (HI) test

Influenza viruses used as HI test antigens included two swine and human influenza viruses each. The swine viruses, H1N1 (A/Swine/Thailand/CU-CB1/06; swH1N1) and H1N2 (A/Swine/Thailand/CU-CHK4/09; swH1N2) were isolated from the lungs of pigs during an outbreak of widespread illness among nursery pigs from farm A and B, respectively. Both isolates were propagated in MDCK cells as described previously (Kitikoon et al., 2006). The human viruses, Thai seasonal H1N1 (A/Thailand/CU41/06; huH1N1] and pandemic H1N1 2009 (A/Nonthaburi/102/09; pH1N1), accession numbers EU021246 and GQ150342, respectively were kindly provided by Professor Y. Poovorawan (Chulalongkorn University, Thailand).

2.3. Hemagglutination-inhibition (HI) test

Swine and human serum samples were pretreated with 20% kaolin and receptor-destroying enzyme (Denka Seiken Co., Ltd., Tokyo, Japan) and influenza-specific antibody detection was performed with a standard HI assay (Yoon et al., 2004). Serum-only controls along with positive and negative serum controls (from influenza A seronegative rabbits hyperimmunized with swH1N1, swH1N2 and pH1N1 HI test antigens) were included with each set of samples tested. HI assays on swH1N1, swH1N2 and huH1N1 viruses were performed using 0.5% chicken RBC in phosphate buffered saline (PBS). Assays on pH1N1 virus were performed with 0.5% turkey RBC in PBS. Samples with HI titers ≥ 40 were considered positive evidence to previous exposure (Olsen et al., 2002).

2.4. HA1 gene analysis of the Thai swine H1 viruses

Viral RNA was extracted from swH1N1 and swH1N2 using the NucleoSpin Extract Viral RNA Kit (Macherey-Nagel, Düren, Germany) and cDNAs were synthesized using universal primer (Uni12 primer 5′-AGCAAAAGCAGG-3′) and Omniscript RT kit (Qiagen, USA) followed by hemagglutinin (HA) gene amplification as described previously (Hoffmann et al., 2001). Complete HA gene sequencing was carried out by 1st BASE Company and analyzed by Bioedit Sequence Alignment Editor V.7.0.5.3. Sequences were submitted to Genbank [accession numbers, GU454848 (swH1N1) and GU454849 (swH1N2)]. The phylogenetic tree was constructed using MEGA4 with neighbor-joining method and 1000 bootstrap replicates (Saitou and Nei, 1987).

2.5. Statistical analysis

The differences between each group's HI geometric mean titers were measured using Wilcoxon/Kruskal–Wallis test or Rank sum test (JMP 5.1 Software, SAS Institute, Cary, NC). HI test results were also evaluated as dichotomous outcomes with HI titers ≥ 40 considered to be previously exposed to virus antigen. The association of occupational risks was then examined for statistical significance by Chi-square or two-sided Fisher's exact analyses. Analysis was performed using Open Source Epidemiologic Statistics for Public Health, Version 2.3 (Dean et al., 2009).

3. Results

3.1. Influenza virus antibodies in human population

Fifty and 92% of swine-exposed workers from farm A and B, respectively had developed antibodies against the