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Protection of human influenza vaccines against a reassortant swine influenza virus of pandemic H1N1 origin using a pig model

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ABSTRACT

Since the pandemic H1N1 emergence in 2009 (pdmH1N1), many reassortant pdmH1N1 viruses emerged and found circulating in the pig population worldwide. Currently, commercial human subunit vaccines are used commonly to prevent the influenza symptom based on the WHO recommendation. In case of current reassortant swine influenza viruses transmitting from pigs to humans, the efficacy of current human influenza vaccines is of interest. In this study, influenza A negative pigs were vaccinated with selected commercial human subunit vaccines and challenged with rH3N2. All sera were tested with both HI and SN assays using four representative viruses from the surveillance data in 2012 (enH1N1, pdmH1N1, rH1N2 and rH3N2). The results showed no significant differences in clinical signs and macroscopic and microscopic findings among groups. However, all pig sera from vaccinated groups had protective HI titers to the enH1N1, pdmH1N1 and rH1N2 at 21 DPV onward and had protective SN titers only to pdmH1N1and rH1N2 at 21 DPV onward. SN test results appeared more specific than those of HI tests. All tested sera had no cross-reactivity against the rH3N2. Both studied human subunit vaccines failed to protect and to stop viral shedding with no evidence of serological reaction against rH3N2. SIV surveillance is essential for monitoring a novel SIV emergence potentially for zoonosis.

1. Introduction

In April 2009, a pandemic H1N1 influenza virus (pdmH1N1) emerged, rapidly spread and caused the pandemic scheme worldwide (Bai et al., 2011; Nelson et al., 2012). The virus was suspected circulating in the pig population prior to transmit back to humans (Forrest and Webster, 2010; Peiris et al., 2009). The pdmH1N1 virus genome contained genes from the triple reassortant internal gene (TRIG) viruses found circulating in North America (Zhou et al., 1999) and the Avian-like Eurasian swine H1N1 virus, circulating in Asia and Europe (Arias et al., 2009; Forrest and Webster, 2010). Later, pdmH1N1 was found firstly in the pig population in Canada (Smith et al., 2009). The pdmH1N1-infected pigs showed asymptomatic to mild respiratory signs (Sreta et al., 2010). In early 2010, the first reassortant of pdmH1N1 in pig was reported in Hong Kong (Vijaykrishna et al., 2010) and later found in many areas around the world. In Thailand, the first reassortant virus having its internal gene called the TRIG cassette from pdmH1N1and obtained N1 gene form an endemic Thai swine lineage (Kitikoon et al., 2011a). Recently, the surveillance data of Thai swine influenza viruses found three types of reassortant viruses derived from the pdmH1N1 (rH1N1, rH1N2 and rH3N2) viruses (Charoenvisal et al., 2013a). These data suggested that, after the introduction of the pdmH1N1 to the Thai swine population, Thai SIV status

Abbreviations: pdmH1N1, pandemic H1N1 2009 virus; WHO, World Health Organization; DPV, days post-vaccination; HI, hemagglutination inhibition test; SN, serum neutralization test; enH1N1, endemic H1N1 swine influenza virus; rH1N2, reassortant H1N2 swine influenza virus; rH3N2, reassortant H3N2 swine influenza virus; SIVs, swine influenza viruses; TRIG, triple reassortant internal gene virus; HA, hemagglutination; NA, neuraminidase; LAV, live attenuated vaccine; VAERD, vaccine-associated enhanced respiratory disease; $TCID_{50}$, 50% tissue culture infective dose; MDCK, Madin-Darby canine kidney cell line; ml, milliliter; °C, degree Celsius; ELISA, enzyme-linked immunosorbent assay; MEM, minimal essential media; DPI, days post-infection; RDE, receptor destroying enzyme; RBCs, red blood cells; NP, nucleoprotein; RT-PCR, reverse transcriptase polymerase chain reaction.

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has changed due to the emergence of several novel reassortant viruses. Since the viruses are able to survive and circulate in the swine population, transmission to the human population or other species in the future is of concern (Kong et al., 2015; Nelson and Vincent, 2015).

Pig is one of good animal models used for human influenza A study based on the similarity of the sialic acid (SA) receptors expression in human respiratory tract and the immune response to the influenza A infection (Radigan et al., 2015; Rajao and Vincent, 2015). SIVs are of public health concern since the pandemic H1N1 2009 emergence (Arias et al., 2009). Recently, swine H3N2 virus was found in humans in 2012 in the US (Bowman et al., 2014). Additionally, serological evidence supported that SIV-infection was found in humans (H1N1 and H1N2), particularly, in Thai swine workers (Kitikoon et al., 2011b), despite most SIVs circulating in human and swine population were genetically distant. These findings indicated that interspecies transmission of SIVs between pigs and humans might occur.

Recently, many types of vaccines are used for influenza protection. (Houser and Subbarao, 2015; Webster and Govorkova, 2014). The live attenuate virus vaccine has the high efficiency to protect the homologous virus infection. However, awareness of the virus reverse virulence or the vaccine-associated enhanced respiratory disease (VAERD) is of concern when having heterologous infection (Gauger et al., 2014; Houser and Subbarao, 2015; Rajao et al., 2014a). Killed virus vaccines are safe but having low efficiency to induce the immunity to the virus especially for the mucosal immunity or cell mediated immunity (Houser and Subbarao, 2015; Rajao et al., 2014b). The commercial subunit vaccine, composing of viral surface antigen HA and NA, is commonly used worldwide (Hannoun, 2013) and this particular vaccine is much safer and has its efficacy to induce the immunity to homologous viruses and provide partially cross protection to other viruses (Reisinger et al., 2009). The strains of the vaccine viruses are annually changed between Northern and Southern hemisphere based on the World Health Organization (WHO) recommendation. Immunity induces by vaccination or previous exposure to influenza infection usually could not protect the heterologous infection. However, previous study demonstrated that prior infection could partially induce cross protection including HA specific antibody, viral neutralization antibody and HA antibody-dependent cell-mediated cytotoxicity (ADCC) to heterologous strain.

Interestingly, the 2014 surveillance data of Thai SIV revealed many reassortant viruses of pdmH1N1 origin in both H1 (Fig. 1a) and H3 (Fig. 1b) subtypes having genetical differences from the virus vaccines. It should be noted that the predominant reassortant H3N2 (rH3N2), the reassortant between human origin virus (H3, N2) and the rest of the genes from pdmH1N1 was prevailingly found during 2012–2014 in central Thailand (Nonthabenjawan et al., 2015) (Fig. 1b). Protection of human influenza vaccines against the major reassortant SIV was conducted using a pig model to elucidate the preliminary data and the yielded results would benefit the human influenza vaccine development against the potentially zoonotic SIVs.

2. Materials and methods

2.1. Viruses

Four of Thai swine influenza isolates were selected from the surveillance data in 2012 (Charoenvisal et al., 2013a). A/swine/Thailand/CU-PS73/2010 (endemic Thai swine influenza virus: enH1N1 from 2010), A/swine/Thailand/CU-PL63/2010 (pandemic H1N1 influenza virus: pdmH1N1 from 2010), A/swine/Thailand/CU-CT43/2011 (reassortant H1N2 influenza virus: rH1N2 from 2011, having H1 closely related to the pdmH1N1 and N2 obtained from the human origin virus) and A/swine/Thailand/CU-CG45/2011 (reassortant H3N2 influenza virus: rH3N2 from 2011–14) were shown in Fig. 1 (square). All of those viruses were propagated in 9-day-old embryonic chicken eggs and harvested after 72 h post-infection. The infectivity of stock viruses was determined in Madin-Darby canine kidney (MDCK) cells according to standard

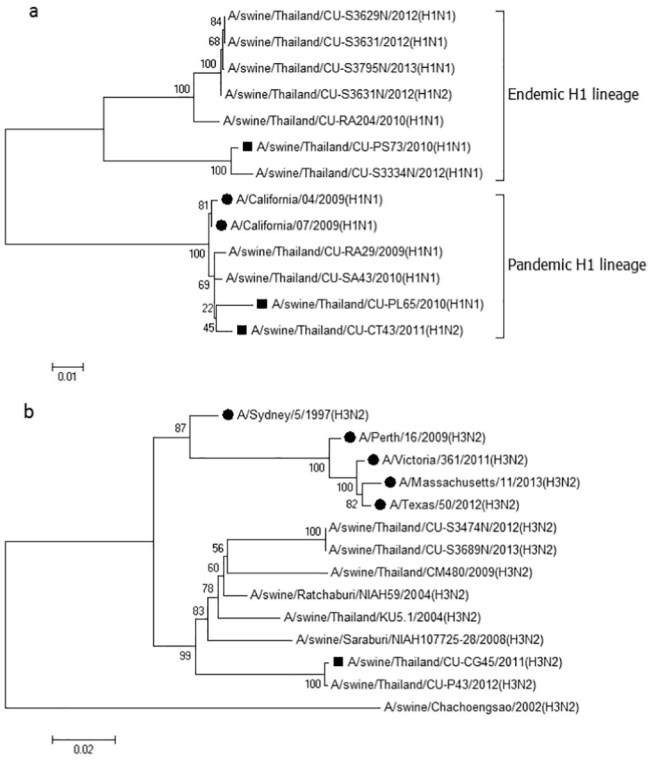

Fig. 1. Phylogenetic analysis of H1 subtype (a). Phylogenetic analysis of H3 subtype (b). The phylogenetic tree was constructed with the neighbor-joining algorithm and the Kimura-2 parameter model applied to 1000 replications of bootstrap percentage. Node label shows the bootstrap percentage, the circle and square symbols shows the human vaccine strains and the representative viruses in this experiment, respectively.

procedures routinely performed at the Chulalongkorn University-Veterinary Diagnostic Laboratory (CU-VDL) (Sreta et al., 2009) and stored at −80 °C until used. The 50% tissue culture infectious dose ($TCID_{50}$) was calculated by Reed and Muench method (Reed, 1938). All experiments involving live viruses were conducted under biosafety containment level 2 (BSL-2).

2.2. Vaccines

Two commercially available human subunit influenza vaccines 2014/2015 were used in this study (vaccine A and B) (Fig. 1). Vaccines A (Influvac®, Abbott Biologicals B.V., Olst, Netherland: Batch No. E08) and Vaccine B (Agripal™S1, Novartis Vaccine and Diagnostics S.r.l., Italy: Lot 131501C). Both studied vaccines are composed of A/California/7/2009 (H1N1) as the pdm09-derived strain, A/Victoria/361/2011 (H3N2) as the seasonal human influenza H3N2 and B/Massachusetts/2/2012 as the influenza B virus. Both vaccines were stored at 4 °C until used.

2.3. Animals and experimental design

Twenty 3-week-old cross-bred pigs were obtained from a negative influenza A virus herd. One week prior to the experiment, nasal swabs were tested for the absence of influenza A virus. Similarly, sera were tested with a commercial ELISA and a routine diagnostic HI test at CU-VDL (Sreta et al., 2013).

All pigs were firstly divided into 3 groups at the beginning (group 1: $N = 8$ and group 2 and group 3: $N = 6$). Those pigs in group 2 and group 3 were intramuscularly vaccinated (injection site: left Hamstring with "1/2" sterile needles) with a single dose of vaccine A and B, respectively. Pigs in group 1 served as a negative control group were injected with the 0.5 ml of normal saline solution. All experimental pigs were observed daily on clinical signs and nasal swabs and serum samples