BIOAEROSOL SAMPLING FOR AIRBORNE RESPIRATORY VIRUSES IN AN EXPERIMENTAL MEDICINE PIG HANDLING FACILITY, SINGAPORE

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Abstract. A number of recent reports have documented likely swine-to-human virus transmission in swine facilities. During the month of January 2016, weekly bioaerosol and pig oral secretion samplings were performed in a pig handling facility to assess the possible occupational exposure to swine influenza A virus and adenovirus. During the 4 weeks, a total of 35 specimens were collected from multiple pig pens within the animal facility. One bioaerosol sample and five pig oral secretion samples were found positive for porcine adenovirus and further sequencing data revealed two different porcine adenoviruses. None of the samples showed evidence for influenza A virus by molecular assays. While swine adenoviruses are not thought to infect man, their detections suggests that bioaerosol sampling may be a non-invasive approach to detecting emergent zoonotic pathogens in agricultural industries.

Keywords: respiratory virus, bioaerosol sampling, pig handling facility, molecular assay

INTRODUCTION

The emergence of novel zoonotic pathogens has recently caused considerable morbidity and public health alarm. Outbreaks such as Severe Acute Respiratory Syndrome (SARS) in 2002 (Peiris *et al*, 2003), pandemic H1N1pdm09 influenza virus in 2009 (Novel Swine-Origin Influenza *et al*, 2009), and Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012 (Zaki *et al*, 2012) have reminded policy makers of the importance of conducting novel pathogen surveillance among animals. In particular, there is considerable evidence that such surveillance should be conducted among livestock populations as modern high density livestock production techniques may accelerate novel agent generation (Klous *et al*, 2016). One such precedent event is the pandemic swine influenza that likely emerged from industrialized swine farms in Mexico in 2009 (Garten *et al*, 2009). Numerous other reports have

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documented zoonotic infections among individuals with intense exposure to animals (Baker and Gray, 2009; McDaniel *et al*, 2014) as well as their transmission of human pathogens to the animals they care for (Messenger *et al*, 2014). In particular, studies have shown a number of swine workers to have a significant risk of infection with various swine-related pathogens (Olsen *et al*, 2002; Gray *et al*, 2007; Uddin Khan *et al*, 2013).

Airborne microorganisms are typically present in the air we breathe and are potential causes of infectious diseases in animals and humans. Influenza viruses and adenoviruses are among the respiratory pathogens that can be transmitted among humans by airborne routes (Wan *et al*, 2012; Lindsley *et al*, 2016). A recent study showed influenza virus could be detected in air lasting up to 20 days during outbreaks in pig barns (Neira *et al*, 2016). Evidence is mounting that swine infected with influenza could produce aerosolized virus particles capable of infecting man (Corzo *et al*, 2013; Zhang *et al*, 2013; Anderson *et al*, 2016; O'Brien and Nonnenmann, 2016). Corzo *et al* (2013) also reported detecting influenza A antibodies to swine-origin influenza viruses in turkey flocks that were reared in premises nearby to pig farms, suggesting a possible aerosol route of transmission of swine influenza.

In this pilot study, we aimed at assessing the occupational risk for exposure to influenza A virus and adenovirus through environmental bioaerosol samplings in a pig handling facility in Singapore.

MATERIALS AND METHODS

Temperature and relative humidity measurement

Air temperature and relative humidity were measured in the pig handling facility (a naturally ventilated environment) using a HOBO U12 Data Logger (Onset Computer, Pocasset, MA). The measurement was set to take readings at a 1-minute interval throughout the whole bioaerosol sampling period.

Liquid impingement air sampler

We followed previously published bioaerosol sampling techniques (Anderson *et al*, 2016). A SKC BioSampler (SKC, Eighty Four, PA) was calibrated before each sampling to permit a total flow rate of approximately 8 liters/minute for a 30 minutes collection period (equivalent to a collection of approximately 240 liters of air). The set-up of the BioSampler's inlet height was adjusted accordingly at each of the 3 designated sampling sites (breeder sows; nursing piglets with sows; and weaners and starters) in the pig facility so as to approximate the level of a pig snout. A 15 ml sterile PBS pre-mixed with 0.5% w/v BSA fraction V was used as the impinger liquid for aerosol sample collection. After the 30 minutes sampling, the impinger liquid was transferred into 50 ml tubes. Samples were transported on ice to the laboratory and preserved at -80°C until processing.

Personal PTFE filter-based impactor sampler

A filter cassette holder loaded with Polytetrafluoroethylene (PTFE) filter (0.3 μm pore, 37 mm) was used with AirChek TOUCH Single High Flow Pump (SKC, Eighty Four, PA). The set-up of the filter cassette holder was adjusted accordingly at each of the 2 designated sampling sites (breeder sows; weaners and starters) in the pig facility so as to approximate the level of a pig snout. The pump was set to permit a flow rate of 5 liters/minute for 120 minutes sampling period to allow approximately 600 liters of air collection.

After 120 minutes sampling, the PTFE filter was removed and scraped with flocked swabs pre-wetted with PBS-0.5%w/v BSA fraction V. After that the swab were resuspended into 15 ml of PBS with 0.5% w/v BSA fraction V and preserved at -80ºC until molecular testing.

Sample processing for collected bioaerosol sampling media

The collected 15 ml of PBS with 0.5% w/v BSA fraction V media was concentrated to 500 µl by ultrafiltration using Amicon Ultra 15 filter units (Merck, Darmstadt, Germany), aliquot and preserved at -80ºC until molecular testing.

Rope sampling to collect pig oral secretions

Each week pig oral secretion specimens were collected at the 3 designated sites (breeder sows; nursing piglets with sows; and weaners and starters) using an unbleached 100% cotton rope pre-wet with 0.5% W/V sugar water. The rope was hung near the pen gate to allow pigs to chew on it for approximately 20 minutes. After that the pig oral fluids from the chewed rope was then expressed into a sterile sampling bag and transported on ice to the laboratory for preservation at -80ºC until molecular testing.

Molecular assays

The presence of respiratory viruses in collected samples was determined by real-time qPCR screening for influenza A matrix gene (World Health Organization influenza A virus primers; WHO, 2009) and gel-based screening PCR for mastadenovirus hexon gene (Sibley *et al*, 2011). Positive PCR amplicons for adenovirus hexon are purified from excised gel bands and sent to sequencing company (AITbiotech, Singapore) for Sanger sequencing.

Phylogenetic analysis

In order to identify the phylogenetic relationships of our six novel adenoviruses, full-length of 43 hexon sequences were downloaded from GenBank, representing five different genera of the family Adenoviridae: *Mastadenovirus, Aviadenovirus, Atadenovirus, Siadenovirus* and *Ichtadenovirus*. Sequence alignment was performed using MAFFT v.7 (Katoh and Standley, 2013) as implemented in Geneious R 9.0.3 (Biomatters, Auckland, New Zealand). Maximum likelihood phylogeny was reconstructed using 1,000 bootstrap replicates in RaxML v8.0.14 (Stamatakis, 2014). The phylogeny was then rooted with the white sturgeon ichtadenovirus A from a fish species *Acipenser transmontanus* (AJ495768). The six newly generated adenovirus sequences were deposited in GenBank, with the accession numbers 0000000-0000000.

RESULTS

Collection of the bioaerosol and pig oral secretion samples

Over the four weeks sampling period, a total of 12 liquid impingement air samplings, 8 PTFE filter-based impactor air samplings and 15 rope samplings were conducted. The pig handling facility is a natural ventilated enclosure that holding approximately 60 to 80 pigs in three different pen sections: breeder sows; nursing piglets with sows; and weaners and starters. Specific places in these pens were selected to perform weekly air samplings for targeted respiratory viruses. Air temperature and relative humidity (RH) measured during the whole sampling period showed a temperature range of 30.51 - 33.73°C and RH range of 59.73 - 80.04% (Table 1).

Screening of porcine adenoviruses in bioaerosol and pig oral secretion samples

Among the total 35 samplings taken, six samples (one bioaerosol sample from

Table 1
Summary of the 4-week bioaerosol and pig oral secretion samplings in a pig handling facility.

Sample ID	Sample type	Site	Temp (°C)	RH (%)	AdV	FluA
SEMC1-week1	BioSampler A	breeder sows	33	59.73	-	-
SEMC2-week1	BioSampler B	nursing piglets with sows	31.39	70.93	-	-
SEMC3-week1	BioSampler C	weaners and starters	30.51	80.04	-	-
SEMC4-week1	Personal Sampler A	breeder sows	31.61	70.46	-	-
SEMC5-week1	Personal Sampler B	weaners and starters	31.5	71.28	-	-
SEMC6-week1	Rope sample A	breeder sows	-	-	-	-
SEMC7-week1	Rope sample B	piglets	-	-	-	-
SEMC8-week1	Rope sample C	weaners and starters	-	-	+	-
SEMC9-week1	Rope sample D	weaners and starters	-	-	+	-
SEMC10-week2	BioSampler A	breeder sows	30.92	69.9	-	-
SEMC11-week2	BioSampler B	nursing piglets with sows	31.72	66.41	-	-
SEMC12-week2	BioSampler C	weaners and starters	31.41	67.11	-	-
SEMC13-week2	Personal Sampler A	breeder sows	31.33	67.96	-	-
SEMC14-week2	Personal Sampler B	weaners and starters	31.3	68.13	+	-
SEMC15-week2	Rope sample A	breeder sows	-	-	-	-
SEMC16-week2	Rope sample B	piglets	-	-	-	-
SEMC17-week2	Rope sample C	weaners and starters	-	-	-	-
SEMC18-week2	Rope sample D	weaners and starters	-	-	+	-
SEMC19-week3	BioSampler A	breeder sows	33.73	60.51	-	-
SEMC20-week3	BioSampler B	nursing piglets with sows	33.33	62.32	-	-
SEMC21-week3	BioSampler C	weaners and starters	32.97	63.13	-	-
SEMC22-week3	Personal Sampler A	breeder sows	33.25	62.39	-	-
SEMC23-week3	Personal Sampler B	weaners and starters	33.27	62.33	-	-
SEMC24-week3	Rope sample A	breeder sows	-	-	-	-
SEMC25-week3	Rope sample B	breeder sows	-	-	-	-
SEMC26-week3	Rope sample C	weaners and starters	-	-	-	-
SEMC27-week3	Rope sample D	weaners and starters	-	-	+	-
SEMC28-week4	BioSampler A	breeder sows	31.15	66.52	-	-
SEMC29-week4	BioSampler B	nursing piglets with sows	31.87	62.75	-	-
SEMC30-week4	BioSampler C	weaners and starters	31.58	63.34	-	-
SEMC31-week4	Personal Sampler A	breeder sows	31.42	64.49	-	-
SEMC32-week4	Personal Sampler B	weaners and starters	31.34	64.46	-	-
SEMC33-week4	Rope sample A	breeder sows	-	-	-	-
SEMC34-week4	Rope sample B	weaners and starters	-	-	+	-
SEMC35-week4	Rope sample C	weaners and starters	-	-	-	-

Temp, average termperature; RH, average relative humidity; AdV, adenovirus; Flu a, influenza A virus.

personal PTFE filter-based sampler and five pig oral secretion samples) were positive by PCR for porcine adenoviruses (Table 1). Based on BLASTN (http://blast.ncbi.nlm.nih.gov), there were two different types of porcine adenoviruses detected in this study. Rope samples SEMC 8, 9 and 27 were most similar (≥ 90%

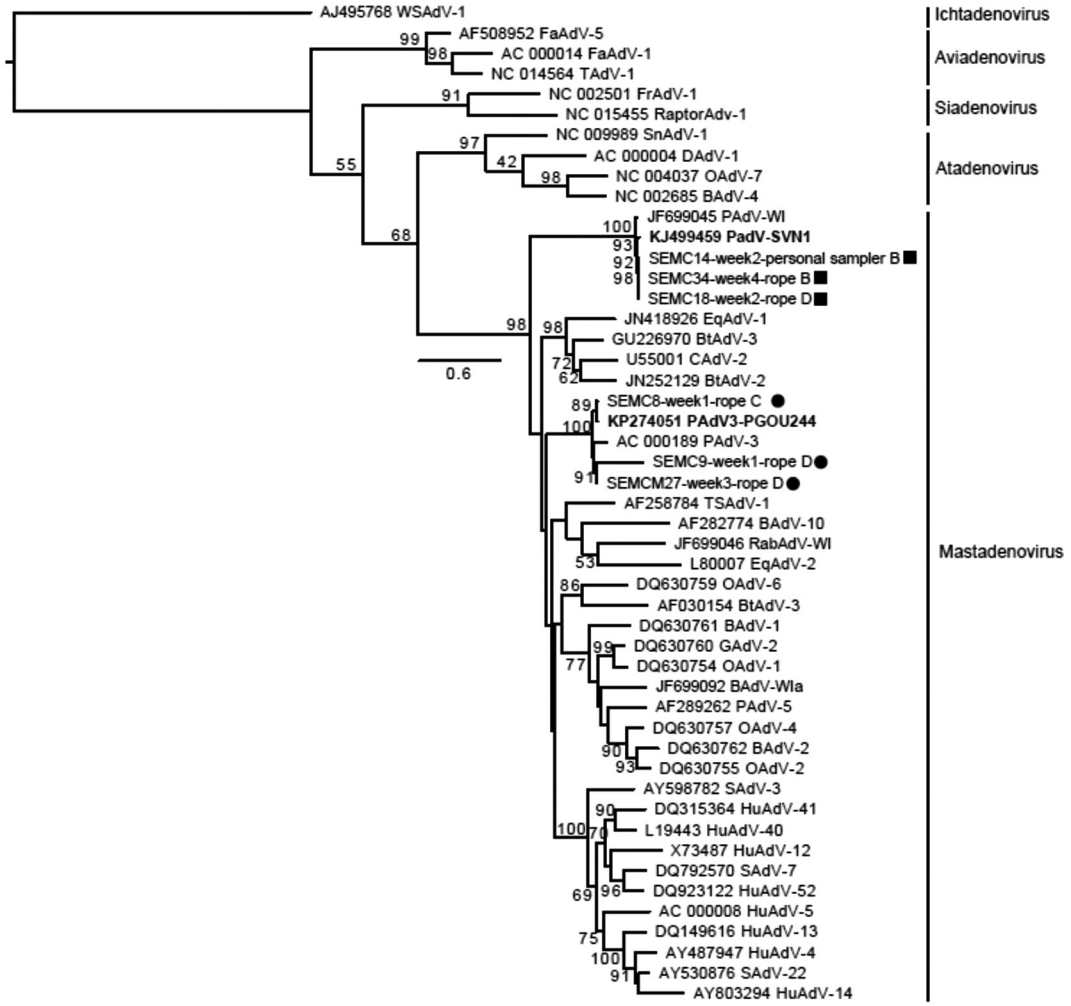

Fig 1–Phylogenetic tree of the hexon gene of the adenoviruses inferred from maximum likelihood method. Bootstrap values greater than 50% are indicated at the nodes. Black circles denote novel adenoviruses that are well-nested within the prototype porcine adenovirus 3 (PAdV-3) lineage, whereas black squares represent novel adenoviruses that are closely related with recently discovered porcine adenoviruses (PAdV-SVN1 and PAdV-WI).

nucleotide identity) to porcine adenovirus 3 isolate PGOU244/Cote d'Ivoire/2012. Bioaerosol sample collected from personal PTFE filter-based sampler, SEMC 14, and rope samples SMEC 18 and 34 were most similar (≥ 98% nucleotide identity) to a most recently discovered novel porcine adenovirus isolate PadV-SVN1 discovered in porcine urothelial cells isolated from urinary bladders of domestic pigs (Jerman *et al*, 2014). Our phylogenetic analysis further revealed the novel porcine adenoviruses were separated into two distinct monophyletic lineages: the adenoviruses from the SEMC 14, 18 and 34 samples were found to be closely related with two porcine adenovirus strains PadV-SVN1 (Jerman *et al*, 2014) and PAdv-WI (Sibley

et al, 2011) that are recently detected in 2009 and 2012, respectively. In contrast, the adenoviruses from the SEMC 8, 9 and 27 samples formed a strongly supported clade with porcine adenovirus 3 (BS=100%). This consistently suggests that porcine is capable of harboring genetically diverse groups of adenoviruses.

Screening of influenza virus in bioaerosol and rope samples

None of the 35 samples were positive for influenza A viruses (Table 1).

DISCUSSION

In this study, using a PTFE filter-based air sampler during a 2-hour sampling period and the rope sampling strategy, we detected porcine adenoviruses from both bioaerosol and pig oral secretion samples. Porcine adenovirus is considered a low grade pathogen that is also detected in healthy pigs (Horak and Leedom Larson, 2016; Karlsson *et al*, 2016). Transmission of porcine adenovirus includes fecal-oral (Sanford and Hoover, 1983) and possibly inhalation (Hirahara *et al*, 1990). Although our data is limited to analysing the association between the positivity of porcine adenovirus in ambient air and in pig oral fluids, the findings suggest the presence of adenovirus aerosolization and multiple molecular types in this pig handling facility.

In contrast to our previous studies in China and other researchers' reports in the United States (Ma *et al*, 2015; Anderson *et al*, 2016; Neira *et al*, 2016), our results were remarkable in finding no evidence of influenza A virus. The present study was conducted in an experimental animal research facility that reared pigs from high health status breeder pigs with known genetic background for medical surgery training. There is no potential exchange of pigs from sources outside the facility. It is also a natural ventilated facility with low-density pig production that would have contributed to the result we observed in this study.

As swine adenoviruses are not thought to infect man, our detections of swine adenovirus in both an aerosol sample and in pig oral secretions suggests that bioaerosol sampling could serve as a non-invasive tool in detecting respiratory animal viruses in agribusinesses. Knowing that such bioaerosol sampling has been previously used to detect a number of other swine respiratory viruses in intensive farming, perhaps it should be considered as a non-invasive, early warning method for the emergence of novel swine pathogens. It could serve in a One Health way as both as a biosecurity tool for the swine production industry and an occupational biosafety tool for the protection of swine workers.

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