Preventive Veterinary Medicine 153 (2018) 24–29

Contents lists available at ScienceDirect

Preventive Veterinary Medicine

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

Detection of influenza A virus from agricultural fair environment: Air and surfaces

Sarah E. Lauterbach[a], Courtney M. Wright[a], Michele M. Zentkovich[a], Sarah W. Nelson[a], Joshua N. Lorbach[a], Nola T. Bliss[a,1], Jacqueline M. Nolting[a], Raymond M. Pierson[b], Maria D. King[c], Andrew S. Bowman[a,*]

[a] The Ohio State University, Department of Veterinary Preventive Medicine, 1920 Coffey Road, Columbus, OH, 43201, USA
[b] Northrop Grumman ES Homeland Defense Group, 7055 Troy Hill Drive S#300, Elkridge, MD, 21075, USA
[c] Texas A&M University, Department of Biological and Agricultural Engineering, 333 Spence Street, MS 2117, College Station, TX, 77843, USA

ARTICLE INFO

Keywords:
Air microbiology
Zoonoses
Fomites
Transmission
Swine
Public health

ABSTRACT

Agricultural fairs facilitate an environment conducive to the spread of influenza A virus with large numbers of pigs from various different locales comingling for several days (5–8 days). Fairs are also associated with zoonotic transmission of influenza A virus as humans have unrestricted contact with potentially infected swine throughout the fair's duration. Since 2005, the Centers for Disease Control and Prevention has reported 468 cases of variant influenza A virus, with most cases having had exposure to swine at agricultural fairs. Many mechanisms have been proposed as potential direct and indirect routes of transmission that may be enhancing intra- and inter-species transmission of influenza A virus at fairs. This study examines airborne respiratory droplets and portable animal-care items as potential routes of transmission that may be contributing to enhanced viral spread throughout the swine barn and the resulting variant cases of influenza A. Air samples were taken from inside swine barns at 25 fairs between the years 2013 and 2014. Influenza A virus was detected molecularly in 11 of 59 (18.6%) air samples, representing 4 of the 25 fairs. Viable H1N1 virus, matching virus recovered from swine at the fair, was recovered from the air at one fair in 2013. During the summer of 2016, 75 of 400 (18.8%) surface samples tested positive for molecular presence of influenza A virus and represented 10 of 20 fairs. Seven viral isolates collected from four fairs were recovered from the surfaces. Whole genome sequences of the viruses recovered from the surfaces are > 99% identical to the viruses recovered from individual pigs at each respective fair. The detection and recovery of influenza A virus from both the air and surfaces found within the swine barn at agricultural fairs provide evidence for potential viral transmission through these routes, which may contribute to both intra- and inter-species transmission, threatening public health. These findings reinforce the need for new and improved mitigation strategies at agricultural fairs in order to reduce the risk to animal and public health.

1. Introduction

Influenza A viruses (IAV) are a highly diverse group of single stranded RNA viruses that infect a wide variety of species including birds, pigs and humans, with wild waterfowl considered as the primary reservoir (Webster et al., 1992). With a single stranded, segmented RNA viral genome, IAVs are susceptible to very high rates of genetic mutations that occur rapidly as the virus replicates, and easily undergo genetic reassortment when two strains co-infect the same host (Webster et al., 1992; Reid and Taubenberger, 2003; Yoon et al., 2014). Due to the ability of IAVs to infect multiple host species and evolve rapidly, there is great risk for zoonotic transmission, which has been documented at various interfaces (Alexander and Brown, 2000; Bowman et al., 2012b; Wong et al., 2012; Epperson et al., 2013; Gao et al., 2013; Jhung et al., 2013; Bowman et al., 2014a). At the bird-human interface, Asian lineages H5N1and H7N9 continue to infect humans in China, where cases are often associated with poultry contact at live-bird markets (Abdel-Ghafar et al., 2008; Zhou et al., 2017). Similarly, at the

* Corresponding author.
E-mail addresses: lauterbach.7@osu.edu (S.E. Lauterbach), wright.1798@osu.edu (C.M. Wright), zentkomm@mail.uc.edu (M.M. Zentkovich), nelson.514@osu.edu (S.W. Nelson), lorbach.5@osu.edu (J.N. Lorbach), bliss@battelle.org (N.T. Bliss), nolting.4@osu.edu (J.M. Nolting), raymond.pierson@ngc.com (R.M. Pierson), mdking@tamu.edu (M.D. King), bowman.214@osu.edu (A.S. Bowman).
[1] Battelle Memorial Institute, 505 King Avenue, Columbus, OH, 43201, USA.

https://doi.org/10.1016/j.prevetmed.2018.02.019
Received 9 January 2018; Received in revised form 24 February 2018; Accepted 27 February 2018

swine-human interface, variant IAVs, which typically circulate in swine but are also found in humans, continue to cause illness in humans after contact with infected swine (Bowman et al., 2017). Though human-to-human transmission of most variant IAVs is typically limited, sporadic intra-species transmission of novel and variant IAVs between humans can facilitate new and devastating pandemics that significantly impact public health (Van Reeth, 2007). Accordingly, zoonotic transmission of IAVs emerging from animal reservoirs warrants a high level of concern.

Swine are recognized as important mixing vessels, where novel reassortant strains form often silently under sub-clinical infection (Ma et al., 2008). Exhibition pigs are an important niche of the swine population that is often overlooked in respect to IAVs. Agricultural fairs frequently contain large numbers of exhibition pigs from various different locales held in close proximity, creating an environment that facilitates viral spread and the rapid transmission of IAV. Active surveillance in swine at agricultural fairs has shown that with only 1.5% of the pigs entering the exhibition with active infection (Bliss et al., 2016), greater than 60% of the pig population will have active infection by termination of the fair, five to eight days later (Bowman et al., 2012a). Agricultural fairs also create a significant interface between swine and humans that is conducive to bidirectional transmission of IAVs because comingling between species is extensive. High prevalence of IAV positive pigs at fairs threatens public health since young exhibitors and the general public have direct contact with swine, where zoonotic transmission has been known to occur (Bowman et al., 2012b; Wong et al., 2012; Bowman et al., 2014a; Bowman et al., 2017). As of December 2017, 468 variant cases have been reported by the Centers for Disease Control and Prevention since 2005, with most cases associated with direct or indirect exposure to swine at agricultural fairs (CDC, 2017).

Close contact, length of fair (i.e. > 72 h), and certain fair-specific practices have all been suggested as possible risk factors contributing to this dramatic increase of IAV positive pigs by the end of the fair by enhancing direct and indirect contact (Bliss et al., 2016; Lauterbach et al., 2017). Here, we attempt to identify other aspects of the agricultural fair environment that might be contributing to increased viral spread and potential zoonotic transmission. Previous studies report the ability of IAVs to be transmitted and cause intra-species infection through airborne respiratory droplets (Munster et al., 2009; Herfst et al., 2012; Bertran et al., 2017), as well as the significance of fomites in the indirect spread of infection (Bean et al., 1982; Boone and Gerba, 2005; Allerson et al., 2013). The present study investigates the potential of IAVs to contaminate the air and portable animal-care items (i.e. waterers and feeders) found in the swine barn during agricultural fairs.

2. Materials and methods

2.1. Air sample collection

In summer 2013, air sampling was performed with a liquid cyclonic collector (Midwest Micro-Tek, Brookings, SD, USA) with an air sampling flow rate of 400 L/min as previously described (Corzo et al., 2013) using brain and heart infusion broth as the collection media. Disinfection was performed by rinsing the collection vessel with water followed by contact of 70% absolute alcohol for one minute and another water rinse. A sterile swab was used to wipe the collection vessel after disinfection as a means of disinfection control. In summer 2014, air sampling was performed with a battery operated, portable wetted wall cyclonic collector prototype, the viable bioaerosol collector (VBAC), developed at the Aerosol Technology Laboratory at Texas A&M (College Station, TX, USA) and manufactured at Northrop Grumman Inc., (Falls Church, VA, USA). For the VBAC, the cut point (the particle size where the collection efficiency is 50%) of the aerosol-to-hydrosol efficiency curve is 1.2 μm AD (Aerodynamic Diameter), and the average collection efficiency for single cells and clusters of bacterial particles is 86% over a size range of 1–8.6 (McFarland et al., 2010) while maintaining the culturability of the collected microorganisms (King and McFarland, 2012). The droplets carrying the viral particles cover a wide size range from 0.5 μm–10 μm (Alonso et al., 2015), resulting in efficient collection of the virus by the collector. In the sampling event, the VBAC was operated at an air sampling flow rate of 100 L/min and a collection liquid inflow rate of 100 μL/min to concentrate the airborne particles present in the air by a factor of up to 10^6 in sterile, DNA/RNA free water (collection media). To clean the –wetted wall cyclonic collector units between sample collections, 100 μL of fresh 10% bleach was squirted into the wetted wall cyclonic collector inlet three times within a 2-min period, followed by 100 μL of isopropanol five times into the collector. Finally, 100 μL aliquots of sterile water were sprayed five times consecutively into the wetted wall cyclonic collector inlet. The collector was operated with sterile collection liquid for 20 min prior to each sample collection to prevent particle carryover and as a means of disinfection control. Two different validated air samplers were available for and used during this study, but are not meant to compared to each other. During collection, both air samplers were placed at selected sites throughout the swine barn conveniently and unitrudingly located 3–4 feet from the ground above the pig holding pens on the last day of each fair. The liquid cyclonic collector was allowed to run for 30 min for each sample and the WWC ran for 15 min for each sample. Collection media was recovered from each air sampler after each sampling event, placed into 15 mL Falcon tubes (Becton Dickinson Labware, Franklin Lakes, NJ, USA), and was frozen (–80 °C) until testing. Twenty-five fairs (Fairs A–D in 2013 and Fairs E-X in 2014) across Ohio and Indiana, USA were chosen for sampling and a total of 59 samples were collected: 14 in 2013 and 45 in 2014.

2.2. Surface sample collection

In summer 2016, surfaces of five common, portable animal-care items used by swine exhibitors that can be readily found at exhibitions were chosen for sampling at twenty individual agricultural fairs across Ohio and Indiana, USA. Surface types included tack box (utility box for holding animal-care supplies), sort panel (rectangular panel used to guide animals when walking freely), feeder (feed holder for animals to eat from; most often a bowl or trough), waterer (water holder for animal to drink; most often a nipple waterer or bowl), and chair (chairs found in barns often include cloth bag chairs, folding chairs, and plastic picnic chairs). Surfaces were either in direct contact with the pigs, where a pig could potentially touch the surface with its snout (e.g. feeder in a pen with a pig), or not in direct contact with the pigs, where no pigs were able to contact the surface due to physical restrictions (e.g. sort panel across the aisle from pens). Four of each surface type were sampled at each fair for a total of 20 samples per fair; 400 samples total. If less than four of any surface type was available for testing, other portable animal-care item surfaces (e.g. bucket, broom) commonly used by swine exhibitors and found in the barn were chosen at the discretion of the field sampler. Each surface selected for testing was wiped with a 2” × 2” sterile cotton gauze wipe (Convidien LLC, Mansfield, MA, USA), with the maximum possible amount of exposed surface area (i.e. area exposed to pigs) wiped on each surface (varied for each surface). Individual wipes were placed in vials containing 5 mL of viral transport media prepared from brain and heart infusion broth and frozen (–80 °C) until testing. Each sampled surface was deemed to be either in direct contact or not in direct contact with swine at the time of sampling. The materials (e.g. plastic, metal, etc.) of which the surface was made of were also recorded. Logistic regression was performed in STATA (StataCorp LLC, College Station, TX, USA) to compare molecular IAV detection differences on different surface materials.

2.3. Laboratory testing methods

All samples were tested for presence of IAV by RNA extraction (Mag-Bind Viral DNA/RNA 96 Kit; Omega Bio-tek Inc., Norcross, GA, USA) (Bliss et al., 2016) and real-time reverse transcription-polymerase