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The inability to screen exhibition swine for influenza A virus using body temperature

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Summary

Agricultural fairs create an unconventional animal-human interface that has been associated with swine-to-human transmission of influenza A virus (IAV) in recent years. Early detection of IAV-infected pigs at agricultural fairs would allow veterinarians to better protect swine and human health during these swine exhibitions. The present study assessed the use of swine body temperature measurement, recorded by infrared and rectal thermometers, as a practical method to detect IAV-infected swine at agricultural fairs. In our first objective, infrared thermometers were used to record the body surface temperature of 1,092 pigs at the time of IAV nasal swab collection at the end of the exhibition period of 55 agricultural fairs. IAV was recovered from 212 (19.4%) pigs and the difference in mean infrared body temperature measurement of IAV-positive and negative pigs was 0.83°C. In a second objective, snout wipes were collected from 1,948 pigs immediately prior to the unloading of the animals at a single large swine exhibition. Concurrent to the snout wipe collection, owners took the rectal temperatures of his/her pigs. In this case, 47 (2.4%) pigs tested positive for IAV before they entered the swine barn. The mean rectal temperatures differed by only 0.19°C between IAV-positive and negative pigs. The low prevalence of IAV among the pigs upon entry to the fair in the second objective provides evidence that limiting intra-species spread of IAV during the fairs will likely have significant impacts on the zoonotic transmission. However, in both objectives, the high degree of similarity in the body temperature measurements between the IAV-positive and negative pigs made it impossible to set a diagnostically meaningful cut point to differentiate IAV status of the individual animals. Unfortunately, body temperature measurement cannot be used to accurately screen exhibition swine for IAV.

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Sources and manufacturers

Keywords

influenza A virus; swine; public health; livestock; exhibits; detection

Introduction

Influenza A is a zoonotic disease threatening economies and public health worldwide. Swine are important hosts in the ecology and epidemiology of influenza A virus (IAV) and periodic bi-directional transmission of IAV between pigs and people occurs worldwide (Myers *et al.*, 2007, Nelson *et al.*, 2012, Nelson & Vincent, 2015). The role of swine as a source of IAVs that impact human health has received considerable public attention following the pandemic caused by influenza A(H1N1)pdm09 in 2009 (Garten *et al.*, 2009) and the 343 H3N2 variant IAV cases occurring in 2011–2014 (CDC, 2015). In the United States, there are generally limited opportunities for large populations of pigs and humans to intersect with each other; however, live animal markets and agricultural fairs are unique swine-human interfaces that have been associated with the recent variant influenza cases (Jhung *et al.*, 2013, Wong *et al.*, 2012, Choi *et al.*, 2014). In contrast to the smaller human populations visiting live animal markets, agricultural fairs in North America attract nearly 150 million attendees each year (International Association of Fairs and Expositions, personal communication, 2011), providing non-swine producers direct contact with pigs and the IAVs they might carry and vice versa. Additionally, the structure of many agricultural fairs results in co-housing multi-source pigs in a single location for multiple days, a time period sufficient for the spread and amplification of IAV through the swine population. These conditions make agricultural fairs suitable for zoonotic IAV transmission.

In an effort to protect both swine and human health, animal and public health officials have been working together to mitigate the threat of IAV at agricultural fairs. Recommendations to livestock exhibition organizers included precluding ill pigs from entering the fair and the immediate removal of pigs exhibiting influenza-like-illness during the fair (Officials & Veterinarians, 2014). Unfortunately, subclinical IAV infections are common among exhibition swine (Bowman *et al.*, 2012, Gray *et al.*, 2012) and visual examination of the pigs is unlikely to detect IAV-infected animals in a timely fashion. In addition to nasal discharge, coughing, anorexia, and lethargy, fever is a classic clinical sign of IAV infections in swine (Van Reeth *et al.*, 2012), a fact that led animal health officials to hypothesize that body temperature could be used to screen pigs for IAV at agricultural fairs.

The normal rectal temperature of finishing pigs is approximately 38.8°C whereas the temperature of IAV-infected pigs has been typically characterized to fall within the range of 40.5–41.5°C (Van Reeth *et al.*, 2012). While rectal thermometers are the gold standard method for body temperature measurement, this method can be time consuming and stressful for the animal. In an attempt to overcome these limitations, infrared thermometer use has been suggested as a practical, non-invasive method for body temperature measurement in swine (Traulsen *et al.*, 2010). Thus, the objective of the present study was to assess the use of swine body temperature measurement, as recorded by infrared and rectal

thermometers, as a practical method to screen for IAV infections in swine at agricultural fairs.

Materials and Methods

Infrared thermometers

The body temperatures of exhibition swine were recorded in conjunction with ongoing active IAV surveillance at agricultural fairs during the summer of 2013. Fairs in Ohio and Indiana were recruited for IAV surveillance based on the number exhibition swine expected to attend in 2013 and the organizing committee's willingness to participate. A sample size of 20 pigs per fair was targeted for the collection of nasal swabs and body temperature via infrared thermometer on the last day of each participating fair. The targeted sample size per fair was intended to provide 95% probability of detecting IAV for 15% prevalence among the swine. Nasal swab specimens were collected from selected pigs based on convenience, but while making certain that sampling was distributed across all areas of each animal exhibit. Individual nasal swabs were placed in vials containing 1.8 ml viral transport medium and then frozen at -70°C until testing commenced. Handheld infrared thermometers^a with a fixed emissivity of 0.97 were used for the study. At the time of swabbing, the temperature of the pigs were recorded by holding the infrared thermometer approximately 30 cm from skin surface and pointing it at the caudal side of one pinna, aiming at the point where the base of ear meets the body. Mean temperature of the IAV-positive and negative pigs were compared using a cluster-adjusted t-test.

Rectal thermometers

In July 2013, we attempted to collect snout wipes from all pigs entering a single large swine exhibition where exhibitors were required by the exhibition organizers to provide animal health officials with the rectal temperature of their pig(s) prior to unloading the animals from trailers. Upon arrival at the fairgrounds, snout wipe samples were collected from the pigs while still on their respective transportation vehicles in a manner as previously described (Edwards *et al.*, 2014). Snout wipes were placed in vials containing 5 ml viral transport medium and frozen at -70°C until testing was initiated. Concurrent to IAV sampling, exhibitors took the rectal temperature of their pigs using a provided quick read digital thermometer^b. If the rectal temperature was $\geq 40.5^{\circ}\text{C}$, the animal was rechecked using a glass thermometer following a 30 minute wait period. Student's t-test was used to compare the mean rectal temperatures of the pigs grouped by IAV test result (Herrin, 2012).

Influenza A virus testing

Nasal swab and snout wipe samples were screened for IAV with rRT-PCR^c and virus isolation was performed on rRT-PCR-positive samples using procedures described previously (Bowman *et al.*, 2013a). A pig was considered positive if viable IAV was recovered via virus isolation procedures.

^aModel:IRFS; ThermoWorks, Lindon, UT, USA

^bCVS Digital Flexible Tip Thermometer; CVS Health Woonsocket, RI, USA

^cVetMAX-Gold SIV Detection Kit; Applied Biosystems, Austin, TX, USA

Animal use

The Institutional Animal Care and Use Committee of The Ohio State University approved protocol number 2009A0134 for the use of animals in this study.

Results

Infrared thermometers

In total, 1,092 pigs were sampled across 55 agricultural fairs ($\bar{x}$ =19.85 pigs per fair, range=17–20). IAV was recovered from a total 212 (19.4%) pigs from 21 of the 55 (38.2%) fairs. Within fairs with IAV positive pigs, the estimated IAV prevalence ranged from 5% (1/20) to 90% (18/20). The mean temperature recorded for the IAV-positive pigs was 34.38°C (SEM=0.18), whereas the mean temperature for the 880 IAV-negative pigs was 33.55°C (SEM=0.10). After adjusting for the clustering of the data, the difference in means, 0.83°C, was not statistically significant ($p=0.30$). The median temperature for IAV-positive and negative pigs was 34.38°C and 33.83°C respectively (Figure 1). Individual examination of the 21 fairs where there were positive and negative pigs revealed no statistically significant differences in recorded temperature except for one fair where the temperature of IAV-positive pigs averaged 1.25°C lower than the IAV-negative pigs ($p=0.03$). We were unable to determine a biologically meaningful temperature cut point that could be used to differentiate between IAV-positive and negative pigs.

Rectal thermometers

Snout wipes were collected from 1,948 of the 2,104 pigs (92.3%) entering the fair. IAV was recovered from 47 pigs and the mean rectal temperature of the IAV-positive pigs was 38.84°C (SEM=0.08). The mean rectal temperature of the 1,901-IAV negative pigs was 38.65°C (SEM=0.01). While the 0.19°C difference in means was statistically significant ($p=.049$) because of the large sample size, there was no practical significance as the difference in rectal temperatures between IAV-positive and negative pigs were clinically indistinguishable (Figure 2).

Discussion

We were unable to accurately identify IAV-infected swine using either infrared or rectal thermometers. Elevations in body temperature can be expected with many infectious diseases and as expected, the average body temperature of the IAV-infected pigs was higher than the IAV negative pigs in both aspects of this study. However, the magnitude of those differences was not large enough to set diagnostically meaningful cut points. Our data indicate that body temperature measurement cannot be used to screen exhibition swine for IAV.

While we were hopeful that infrared thermometers would be a useful screening tool for veterinarians and other officials inspecting pigs entering fairs, we were not surprised by the results of the study. Swine regulate their body temperature through altering the rate of cutaneous blood flow. German researchers reported data that the back of the ear would be a suitable location for infrared thermography in swine, but they also concluded infrared body

temperature measurements were probably more useful for longitudinal monitoring of the same animal over time (Schmidt *et al.*, 2013). Our subjective impression was that environmental conditions (ambient temperature, air movement, sunlight, barn and pen configuration, stocking density, etc.) have substantial detrimental effects on the reliability of infrared thermometry use in swine; this concurs with the findings of other investigators (Wendt *et al.*, 1997, Dewulf *et al.*, 2003). In addition, blind screening of pigs for IAV during an exhibition with rectal thermometers, while not specifically evaluated in the present study, appears to be of limited utility based on the results of the rectal thermography trial reported herein.

The mean rectal temperature of the of IAV-positive pigs was 38.84°C; this is considered a normal temperature of healthy pigs and these data further highlight the potential for subclinical IAV infection in swine. While unlikely to alter the overall findings of this project, the use of snout wipes instead of nasal swabs, which are the current gold standard, may have introduced misclassification bias into the study by incorrectly identifying the IAV status of individual animals. It is important to note that the swine rectal temperatures were collected on trailers following transportation to the fairgrounds on a hot summer day. Depending on the distance traveled and the wait-time to enter the fairgrounds, exhibitors could have been actively cooling their pigs for hours by misting them with water and providing the pigs with cool drinking water and/or ice. Prior to this exhibition, animal health officials announced that pigs with a rectal temperature $\geq 40.5^{\circ}\text{C}$ would not be allowed entry to the fair. Given the environmental temperature, varied wait-times, and travel distances, pigs that had a rectal temperature $\geq 40.5^{\circ}\text{C}$ were given 30 minutes to cool down and then rechecked using a glass thermometer. Ultimately, no pigs were excluded from entering the fairgrounds on the basis of rectal temperature, but we feel that the public announcement prior to the fair regarding temperature checks likely deterred exhibitors from bringing clinically ill pigs to the event.

Identification of IAV-infected swine requires direct detection of the pathogen. Timely detection of IAV is needed at agricultural fairs, which makes submission to a diagnostic laboratory impractical. Commercially available rapid immunoassays, such as enzyme-linked immunosorbent assay (ELISA)-based tests kits, are useful for detecting IAV antigen in clinical samples from symptomatic humans (Hurt *et al.*, 2007, Storch, 2003, Uyeki *et al.*, 2009, Fedorko *et al.*, 2006). In the United States a licensed rapid on-farm test for IAV in swine^d is now available. Published trials using rapid IAV detection kits in swine have been limited to date; however, one small study from Mexico found good agreement between virus isolation and a commercial rapid-immunomigration test (Nava *et al.*, 2013). It is important to note that the kit is intended to assess swine herd IAV status where clinical signs of influenza are present, not individual animals without signs of disease.

Detection of 2.4% IAV prevalence among swine entering the fair was an interesting ancillary finding from the rectal thermometer study. The scientific community currently has little knowledge about IAV activity among exhibition swine at the beginning of agricultural fairs because the majority of the IAV surveillance at agricultural fairs has occurred at the

^dFlu DETECT[®] Swine; Zoetis, Florham Park, NJ, USA

end of the exhibition period, when it is common to find >75% of the pigs infected when IAV is detected at the fair. (Bowman *et al.*, 2013b, Bowman *et al.*, 2014). The working assumption has been that a small number of pigs enter the fair infected with IAV and the virus subsequently spreads within the comingled population of pigs during the course of the fair or exhibition. In a sampling of 102 pigs across three pig shows, Gray *et al.* estimated an IAV prevalence of 12% among exhibition swine during the opening days of each show. Unlike the study by Gray *et al.*, the pigs in the present study were sampled before they were unloaded and exposed to other swine and people. Snout wipes were used in this objective for ease of collection and pig owner acceptance, but the slightly lower sensitivity of this method, 83% sensitivity when compared to nasal swabs (Edwards et al., 2014), likely means our 2.4% prevalence estimate was slightly lower than the true prevalence in this population. Although our results for this objective are limited to a single fair, the large sample size provides additional evidence that IAV prevalence among exhibition swine is low at the start of the fair. While further studies are needed to more accurately estimate IAV prevalence in exhibition swine upon arrival at agricultural fairs, steps to minimize intra-species transmission will likely have positive impacts on swine and human health.

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Impacts

- Preventing and controlling influenza A virus infections in swine at agricultural fairs is important for public and animal health.
- Swine body thermometry, both infrared and rectal, failed as screening tests to identify pigs potentially infected with influenza A virus at agricultural fairs.
- Direct detection methods are needed to accurately diagnose influenza A virus in exhibition swine populations, especially since subclinical infections are a common occurrence.

Figure 1.

Box-plot diagram showing the body temperature, as recorded with an infrared thermometer, of 1,092 exhibition swine grouped by influenza A virus (IAV) test result. The IAV-negative pigs (n=880; median: 33.83 °C, and range: 20.66–40.77 °C) and IAV-positive pigs (n=212; median: 34.38 °C, and range: 24.38–40.0 °C) had very similar temperatures and a diagnostically relevant cut-off could not be established to differentiate between the IAV-positive and negative pigs.

Figure 2.

Box-plot diagram showing the rectal temperature of 1,948 exhibition swine upon entry to one agricultural fair grouped by influenza A virus (IAV) test result. The rectal temperatures of IAV-negative pigs (n=1901; median: 38.72 °C, and range: 34.94–42.06 °C) were practically indistinguishable from the IAV-positive pigs (n=47; median: 38.78 °C, and range: 37.61–40.28 °C).