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Prevalence of Influenza A Virus in Exhibition Swine during Arrival at Agricultural Fairs

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Summary

The exhibition swine at agricultural fairs provides a critical human–swine interface that allows for the bidirectional transmission of influenza A virus (IAV). Previous IAV surveillance at the end of fairs has resulted in frequent detection of IAV-infected swine; little is known, however, about the frequency with which swine arrive at fairs already infected with IAV. We investigated the IAV prevalence among exhibition swine entering fairs to better understand the epidemiology of IAV in this unique human–swine interface. In 2014, snout wipes were collected from 3547 swine during the first day of nine agricultural exhibitions in Indiana and Ohio. Samples were screened for IAV using rRT-PCR and positive samples were inoculated into cultured cells for virus isolation. The overall IAV prevalence detected among swine arriving at exhibitions was 5.3% (188/3547) via rRT-PCR and 1.5% (53/3547) via virus isolation, with IAV being detected and recovered from swine at 5 of the 9 exhibitions. Within the fairs with IAV-positive swine, the individual exhibition IAV prevalence ranged from 0.2% (1/523) to 34.4% (144/419) using rRT-PCR and 0.2% (1/523) to 10.3% (43/419) with virus isolation. Single IAV subtypes were detected at three of the fairs but subtype diversity was detected among the pigs at two fairs as both H1N1 and H3N2 were recovered from incoming swine. At two of the exhibitions, a temporal relationship was observed between the order of the individual swine in sampling and the associated IAV rRT-PCR results, indicating the fomite transmission of IAV through common contact surfaces may occur. With the knowledge that a small proportion of swine arrive at fairs shedding IAV, resources should be directed towards preventive strategies focused on limiting transmission during fairs to protect swine and humans during exhibitions.

Keywords

Swine; influenza A virus; virus shedding; exhibits; prevalence; livestock

Introduction

Influenza A virus (IAV) is an endemic pathogen in swine populations around the world (Vincent et al., 2008; Liang et al., 2014; Simon et al., 2014). Swine actively infected with IAV may display clinical signs characterized by loss of appetite, lethargy, dyspnoea, fever,

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

nasal discharges and coughing (Vincent et al., 2008; Van Reeth et al., 2012). Additionally swine can be subclinically infected with IAV, which complicates detection and diagnosis (Bowman et al., 2012; Gray et al., 2012; Van Reeth et al., 2012).Transmission of IAV in swine has been documented to occur via multiple routes, such as direct contact between animals, through contact with IAV contaminated fomites and via airborne transmission. Direct contact is the most effective route of transmission for IAV; however, fomite and airborne transmission continue to provide an alternative route for IAV into swine populations (Allerson et al., 2013; Corzo et al., 2013a).

Swine are a potential source of novel IAV for the human population, as swine have demonstrated the capability to serve as 'mixing vessels' in which multiple IAVs can reassort (Scholtissek, 1990; Ma et al., 2009). When humans are infected with an IAV that typically circulates in the swine population, the infection is classified as 'variant' IAV (WHO, 2014). Zoonotic movement of IAV between swine and people has been documented globally and most is commonly associated with swine–human interfaces such as agricultural fairs, live animal markets, abattoirs and swine farms (Shinde et al., 2009; Van Reeth and Nicoll, 2009; Jhung et al., 2013; Perera et al., 2013; Choi et al., 2014).

The largest outbreaks of swine-to-human transmission of IAV have occurred at agricultural fairs and transmission events have been well documented in the United States (Myers et al., 2007; Jhung et al., 2013). In 1988, a woman contracted variant IAV and subsequently died after attending a county fair where ill swine had been reported (Wells et al., 1991). Subclinically infected swine can contribute to zoonotic IAV transmission, as documented in 2009 when a 12-year-old boy contracted variant H3N2 IAV after petting visually healthy swine at a county fair in Kansas (Cox et al., 2011). In 2011, multiple cases of variant IAV were associated with exposure to swine at an agricultural fair in Pennsylvania (Wong et al., 2012). The number of variant IAV cases in humans spiked in 2012, with 306 cases of variant H3N2 IAV (Jhung et al., 2013). With the advent of sequencing technology, many of these cases can be directly classified as swine lineage IAV through molecular epidemiology and linked to swine exposure at agricultural fairs (Bowman et al., 2014). These cases illustrate that infection with variant IAV can result in human hospitalizations and in some cases death. Every swine-to-human IAV transmission event is concerning from a pandemic preparedness standpoint; thus, controlling IAV at agricultural fairs is critical to protecting public health.

The North American exhibition swine industry is a diverse mixture of culture, education and business. Swine are frequently exhibited at agricultural exhibitions as part of educational projects to expand youth knowledge about agricultural practices. Additionally, swine can be shown in exhibitions open to any age competitor that occur throughout the year across the United States. For 2015, the National Swine Registry estimated that 1 million swine were involved in the United States exhibition swine industry (National Swine Registry, personal communication). Exhibition swine are a relatively small part of the national swine industry as a whole, representing an estimated 1.5% of the total swine population in the United States (USDA, 2012); however, exhibition swine represents the most common direct interface with a large number of humans, outside of the owners and caretakers, when commingled for the competition events.

Surveillance conducted from 2009 to 2011 identified one or more IAV-infected pig(s) at the end of approximately one quarter of the agricultural fairs tested in Ohio (Bowman et al., 2012). Of the fairs with infected swine, the average frequency of virus isolation was 62.9% (Bowman et al., 2012), demonstrating that a high proportion of the pigs at those exhibitions were actively shedding IAV at the end of the 5- to 7-day fairs. Overall, Bowman et al. (2012) found an IAV prevalence of 14.4% among exhibition swine at the end of fairs, higher than the ≤5% IAV prevalence typical of commercial swine herds (Corzo et al., 2013b). While it seems likely that IAV is amplifying through swine populations at livestock exhibitions, little is known about IAV prevalence in swine arriving at exhibitions before swine have commingled and IAV spread through the population. Previously, one study found a large variation in incoming prevalence estimates across two study sites, ranging from 0% to 19% via rRT-PCR testing (Gray et al., 2012). However, only a small proportion of the swine population was tested. A pilot study by Bowman et al. (in press) found the prevalence of IAV at one state fair to be 2.4% among the incoming swine. The objective of this study was to estimate the prevalence of IAV in exhibition swine as they enter fairs as a prelude to understanding the transmission of IAV at the fair with an ultimate goal of preventing intrafair transmission.

Materials and Methods

Enrolment of fairs

Nine agricultural fairs (labelled A through I) that previously enrolled in an Ohio State University IAV surveillance program were recruited for this study. Exhibitions were selected based on willingness to participate in the program, previous history of IAV in their exhibition swine, the number of swine that were historically exhibited at the fair and the date of exhibition that allowed for proper sampling. Due to the need for multiple sample collectors in the field during the fair season and a self-imposed 3-day downtime between fairs for investigators to minimize risk of transmitting IAV between exhibitions, fairs were selected to minimize overlap in sampling dates. The nine fairs where sampling was conducted occurred in July and August of 2014, with four fairs sampled in Ohio and five sampled in Indiana.

Sampling of swine

Swine were sampled at the exhibitions at the first time point that swine could be individually identified. This sampling occurred either on the trailer before unloading (Exhibition A), in the pen prior to weighing (exhibitions D and E), or in a chute as swine were moved individually through a narrow series of gates and weighed on a scale (exhibitions B, C, F, G, H and I). Study team members targeted all swine entering Exhibition B through I for sample collection. At Exhibition A, sample size was intentionally restricted in an effort to expedite sampling because swine were sampled on the trailers during arrival to the fair. For each participating trailer at Exhibition A, study team members were instructed to sample no more than two swine that were easily accessible without entering the trailer. Sampling at all exhibitions was performed via the snout wipe method as previously described (Edwards et al., 2014). Vials were stored at −70°C until testing was completed. The Ohio State

University Institutional Animal Care and Use Committee approved sampling of animals in this study (protocol no. 2009A0134-R1).

Laboratory processing

Original samples were quickly thawed and RNA was extracted using a laboratory-modified protocol for a 100 μL sample extraction using the Mag-Bind® Viral DNA/RNA 96 Kit (Omega Bio-tek Inc., Norcross, GA, USA) and a MagMAX™ Express 96 Magnetic Particle Processor (Applied Biosystems, Foster City, CA, USA) (AM1836_DW_100_V2 program). The modified protocol used 120 μL TNA lysis buffer, 140 μL isopropanol, 4 μL Carrier RNA, 2 μL internal positive control template and 7 μL proteinase K per reaction well. Additionally there were two washes with 200 μL VHB buffer and two washes with 200 μL SPR buffer. RNA was eluted into 50 μL nuclease-free water. Sample RNA was screened via a one-step real-time reverse transcription–polymerase chain reaction (rRT-PCR) for IAV (VetMAX-Gold SIV Detection Kit; Life Technologies, Austin, TX, USA). Original samples were once again frozen at −70°C while rRT-PCR was being performed and analysed. Any samples demonstrating a cycle threshold (C_t) value ≤ 35 were considered rRT-PCR positive for IAV and were treated with 120 μg amphotericin, 5.000 mg gentamicin sulphate and 1.625 mg kanamycin sulphate. Samples were vortexed and inoculated into 4 wells of a 24-well plate with monolayers of serum-free-adapted Madin-Darby canine kidney (MDCK) cells (Bowman et al., 2013). Cells were observed daily for 72 h post-inoculation for cytopathic effects (CPE). Upon harvest, cell culture supernatant was tested for hemagglutinating activity using 0.5% turkey erythrocytes (Hierholzer et al., 1969). Samples demonstrating CPE and/or hemagglutination were tested via a rapid strip test for the p56 nucleoprotein of IAV (FluDETECT Avian Influenza Virus Type A Antigen Test Kit, Synbiotics Corporation, San Diego, CA, USA). If the sample had an initial C_t value ≤ 30 and IAV was not isolated during the first passage in MDCK cells, a second passage was attempted (Zhang and Gauger, 2014). Recovered isolates were subtyped with a multiplex hemagglutinin and neuraminidase rRT-PCR assay (Life Technologies, Carlsbad, CA, USA). Matrix gene lineage was determined to be either the North American swine triple reassortant lineage or influenza A(H1N1)pdm09 virus lineage through a multiplex rRT-PCR (Harmon et al., 2010). Viral isolation data were used to determine the incoming prevalence of IAV because rRT-PCR does not differentiate between residual RNA and active virus, whereas virus isolation demonstrates that infectious IAV was recovered from the snout of the pig during sampling.

Data analysis

Cluster analysis of the rRT-PCR-positive data from exhibitions B and C was performed using principle component analysis (STATA version 11.1, StataCorp, College Station, TX, USA). This was preformed when a temporal relationship was observed in the data and used to determine whether there were clusters present in the sampling order of the pigs in relation to the C_t value from the rRT-PCR screening for IAV.

Results

A total of 3547 samples were collected from the 5462 swine in attendance at the nine exhibitions. Of the samples collected, 188 (5.3%) were IAV positive using rRT-PCR and viable IAV was recovered from 53 (1.5%) (Table 1). Within exhibitions A through E, IAV prevalence, as determined by virus isolation, ranged from 0.2% to 10.3%. No IAV was detected in the samples collected from the swine at exhibitions F, G, H or I. Overall, IAV isolates were recovered from 28.2% of the samples identified as rRT-PCR positive but there was a wide range in isolation success from rRT-PCR-positive samples between fairs. For Exhibition E, 100% of the rRT-PCR-positive samples yielded an IAV isolate, whereas at Exhibition C, only one IAV isolate was recovered from the 16 rRT-PCR-positive samples (6.2%). Forty-seven (88.3%) of the 53 isolates were recovered during the first passage and the remaining 6 isolates were recovered through a second passage.

The 53 IAV isolates were subtyped as H1N1 ($n = 23$), H3N2 ($n = 28$) and mixed with both H1/H3 N1/N2 subtypes ($n = 2$) (Table 2). All IAV isolates contained the influenza A(H1N1)pdm09 lineage matrix gene. In Exhibition A, both H1N1 ($n = 2$) and H3N2 ($n = 4$) subtypes were recovered. Similarly, H1N1 ($n = 18$), H3N2 ($n = 23$) and mixed subtype isolates ($n = 2$) were found at Exhibition B. Only one IAV subtype per exhibition was detected among the swine entering exhibitions C-E (Table 2).

At the two fairs where swine were sampled in a chute and swine tested positive for IAV (exhibitions B and C), a pattern in rRT-PCR C_t values for IAV was observed at both fairs. As illustrated in Fig. 1a and b, samples with a low rRT-PCR C_t value were often followed by samples with similarly low C_t values that would gradually increase over subsequent samples (time) until the next low C_t spike. Principle component analysis found three distinct clusters of rRT-PCR-positive samples in Exhibition B and two clusters were observed at Exhibition C (Supporting Information). This pattern was not observed at exhibitions D and E, fairs where IAV-positive swine were detected but the swine were not sampled in a chute (Fig. 1c).

Discussion

With viable IAV being recovered from only 1.5% of the swine entering the sampled fairs, findings of the present study highlight the potential to, and importance of, limiting the intraspecies spread of IAV during swine exhibitions. As swine are co-housed for several days during an exhibition, viruses arriving at the beginning of the fair have ample time to spread through the swine population. Surveillance for IAV in swine conducted at the end of exhibitions indicates that when IAV is among the swine at a given fair, viable IAV is typically recovered from 60% to 70% of the pigs (Bowman et al., 2012, 2014). This demonstrates a remarkable increase in IAV prevalence among swine between entry to the exhibitions and the end of exhibition. This rapid spread with in the population is expected as the basic reproductive rate for IAV in unvaccinated swine has been estimated at 10.66 (Romagosa et al., 2011). Certainly the risk to public health in these settings increases as the IAV prevalence among the exhibition swine increases during the fair, a notion supported by the timing of variant IAV detection in relation to the implicated fair (i.e. when variant IAV cases are associated with fairs, they are almost always detected at the end of the fair or

in the days immediately following the conclusion of the fair). Strategies to maintain a very low prevalence of IAV within an exhibition site, thus preventing the apparent amplification of IAV during fairs, have the potential to reduce the threat to public and swine health. Strategies such as shortening the swine exhibition period and encouraging IAV vaccination have been suggested (Bowman et al., 2014; Officials & Veterinarians, 2014). However, vaccine effectiveness is challenged by the rapid evolution of IAV and strains circulating in the field strains may quickly differ from strains used in vaccine production. Additionally, vaccination has been shown to eliminate clinical signs of IAV in swine, without blocking infection and pathogen transmission (Loving et al., 2013). In these cases of subclinical infections and mismatched strains, vaccination of the pigs likely does not completely prevent swine–human transmission of IAV.

Subclinical infections of IAV occur in pigs and have been detected at 83.3% of agricultural fairs with IAV-infected swine (Bowman et al., 2012). This presents an additional challenge as IAV-infected swine cannot be identified by clinical signs during entry. Attempts to use infrared and rectal thermometers have also been unsuccessful for screening pigs of IAV (Bowman et al., in press). Ultimately, the IAV status of the swine can only be determined by diagnostic testing. Given the extensive spread of IAV within the population of swine at an exhibition, a viable method to detect the small percentage of IAV-positive pigs at entry and prevent their entry to the exhibition site would be ideal. However, this option is currently an unrealistic proposition because the labour, cost and time required to perform diagnostic testing are beyond the capacities of most exhibitions. Mitigation strategies to avert swine-to-swine transmission of IAV during the fair will likely decrease the total IAV burden in swine barns at fairs and reduce the risk of zoonotic IAV transmission.

For the present study, the ideal time point for sampling was on the trailer prior to unloading, as was performed at Exhibition A, as the swine were not yet exposed to the exhibition's animals or environment. Given logistic complications, this approach was not feasible at the other exhibitions. The maximum time that swine were at Exhibition B through I prior to sampling was 24 h. Swine at exhibitions D and E were sampled in their pens after unloading but prior to weighing. The swine at the remaining exhibitions were sampled in the chute during weighing. The clustering of IAV positive exhibition swine at exhibitions B and C observed in Fig. 1a and b are similar; however, the amount of viable IAV recovered differed greatly between these two exhibitions. At Exhibition B, 34.8% of the samples were rRT-PCR positive for IAV, and 43 IAV isolates were recovered. In the case of Exhibition C, 4.5% of the samples were rRT-PCR positive for IAV, and one isolate was recovered.

Temporal clustering of positive swine within fairs was expected because swine from the same farm, predicted to have similar IAV exposure, would arrive at the exhibition together, be placed in related pens, and moved together through the chute. However, the large proportion of samples testing IAV positive with rRT-PCR and the unusual trailing off of C_t values observed at exhibitions B and C (Fig. 1a and b) was not anticipated. The most likely explanation for this trend is swine snout contamination during corralling activities. In this scenario, an IAV-infected pig deposits virus via oral and/or nasal secretions onto swine contact surfaces (i.e. gating, hurdle boards, scale walls, etc.) as it moves through the chute. Subsequent swine moving through the chute likely contact the contaminated surface

(s) and acquire IAV on their snouts, thus testing positive for IAV. Over time, each pig passing through the chute following the truly infected pig likely removes some IAV from the contaminated surface(s). Therefore, IAV concentration on the surfaces, and thus the snouts contacting the contaminated surface(s), would be expected to diminish over time. Although genomic sequencing is needed to fully assess IAV isolate identities, the temporal clustering of identically subtyped isolates, as observed at Exhibition B (Fig. 1a), provides additional support for this hypothesis. One would expect a more dispersed distribution of subtypes across the sampling if the 43 IAV-positive swine at Exhibition B were in fact infected prior to arrival at the exhibition. We hypothesize that moving swine through a chute has the potential to expedite pathogen transmission during the exhibition because this practice allows many swine to be rapidly exposed to a variety of pathogens within hours of arrival. This deposition of IAV onto the snouts of swine during corralling likely results in greater IAV exposure for the swine population than if the virus had to spread pig-to-pig via direct contact. IAV can be transmitted between swine via fomites (Allerson et al., 2013); thus, it is possible that common contact surfaces within the chute (i.e. gates, handing equipment, walls, scale, etc.) could facilitate IAV spread. Corralling activities of swine, and similar situations during the exhibition, may be critical points for limiting IAV transmission at fairs. Future work is needed to assess IAV contamination of chutes and similar surfaces at fairs. Supporting these findings is the recent study by Choi et al. (2015), where IAV was recovered from railings at live animal markets, demonstrating that IAV isolates can be recovered from gates and railing areas that have high contact with swine. Attention to reducing contamination of these surfaces (e.g. frequent disinfection) may be warranted.

Other studies investigating IAV prevalence among incoming exhibition swine found discrepancies similar to what was observed between fairs in the present study. During 2008–2009, Gray et al. (2012) conducted a surveillance study at fairs in Minnesota and South Dakota. No IAV was detected in the swine sampled at the Minnesota fair in 2008; however, in 2009, 19.3% of the swine at the same Minnesota fair and 2.2% of the swine at a South Dakota fair were PCR positive for IAV. The lack of IAV found in 2008, during the study by Gray, can be explained by the year-to-year variability of IAV within fairs as previously demonstrated (Bowman et al., 2012). This study provided an insightful preliminary look at IAV among swine at exhibitions; however, the sample size was relatively small and timing of sample collection relative to arrival was loosely defined making on-sight IAV transmission prior to sampling possible. In a 2013 pilot study, Bowman et al. (in press) recovered IAV isolates from 2.4% of samples collected from swine on trailers prior to unloading at Exhibition A.

There are some limitations with the prevalence established during the present study. It was necessary, due to time and funding restraints, to screen samples with rRT-PCR before inoculating them for virus isolation. Therefore, all samples were subject to two freeze-thaw cycles prior to a virus recovery attempt. Freeze-thaw cycles have been associated with decreased IAV infectivity, and thus, the frequency of virus isolation in the present study is likely lower than if samples had been directly inoculated without refreezing during rRT-PCR screening (Greiff et al., 1954). Additionally, the snout wipe method used in the current study is less sensitive than the gold standard nasal swabs (Edwards et al., 2014). Snout wipes were chosen because they are non-invasive, do not require restraint and take less

time to administer than nasal swabs. In the present study, exhibitors were very concerned about stress on their animal, even momentary stress; therefore, snout wipes were chosen to maintain a level of acceptance from the exhibitor to the sampling process. It is important to note that snout wipes are taken from the surface of the pig's snout and may represent more of a conglomerated sample of the pig and its environment when compared with a gold standard nasal swab that requires pig restraint. The possible cross-contamination observed at exhibitions B and C creates the potential that the individual animal IAV prevalence was overestimated in the present study. Based on the IAV subtypes identified at Exhibition C and the results of the cluster analyses, we suspect at least three swine had active IAV infections during the time of sampling at Exhibition C.

Other potential bias in our prevalence estimate include the following: fair type, proximity to other swine shows, differences in county exhibition swine industry, weather conditions and the intrinsic differences between fair management. Eight of the agricultural fairs sampled were local county fairs, and one exhibition was a larger regional fair. The majority of the pigs attending the regional fair would have attended a county fair prior to the sampling in this study and thus may have had a previous exposure to IAV. Similarly, pigs raised in counties with multiple swine shows could have attended exhibitions prior to this study's sampling and experienced previous IAV exposure. Some counties are also known to have a rich tradition of exhibition swine, with many families travelling to multiple swine shows throughout the year. While the weather condition between the nine fairs was not identical, it was similar, as all fairs were sampled in a 2-month period of July and August.

In conclusion, the current project demonstrated that a small number of swine arrived at exhibition actively infected with IAV. However, the estimated prevalence of IAV at individual fairs ranged widely and may in part be due to the slight difference in sampling procedures used at each fair. In particular, sampling that occurred in chutes resulted in a far greater prevalence of IAV when compared with sampling in pen or on a trailer. We hypothesize this to be the result of IAV contamination in the chute. If true, fomite transmission of IAV may occur whenever swine are moved through the barn such as during weighing, washing, walking to and from the show ring and at times when animals encounter contaminated sites. These activities could be heightened areas for IAV transmission between swine, and thus, potential time points and locations to target for controlling swine-to-human zoonosis. Increasing our knowledge about IAV introductions into the agricultural fair settings is important for controlling animal-to-animal IAV spread, which will in turn limit swine-to-human IAV transmission. These data provide a critical first step towards mitigation strategies that will limit zoonotic transmission and improve public health.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Impacts

- The frequency of influenza A virus isolation from exhibition swine arriving to fairs in the Midwestern United States was low at 1.49% (53/3547).
- Intra-exhibition pig movement and corralling activities, which are typical fair management practices, likely enhance pathogen transmission during exhibitions.
- Due to the relatively low overall prevalence of influenza A virus in swine at the beginning of fairs, focus should be placed on mitigating influenza A virus spread during swine exhibitions rather than attempting to completely preclude entry of influenza A virus-infected swine.

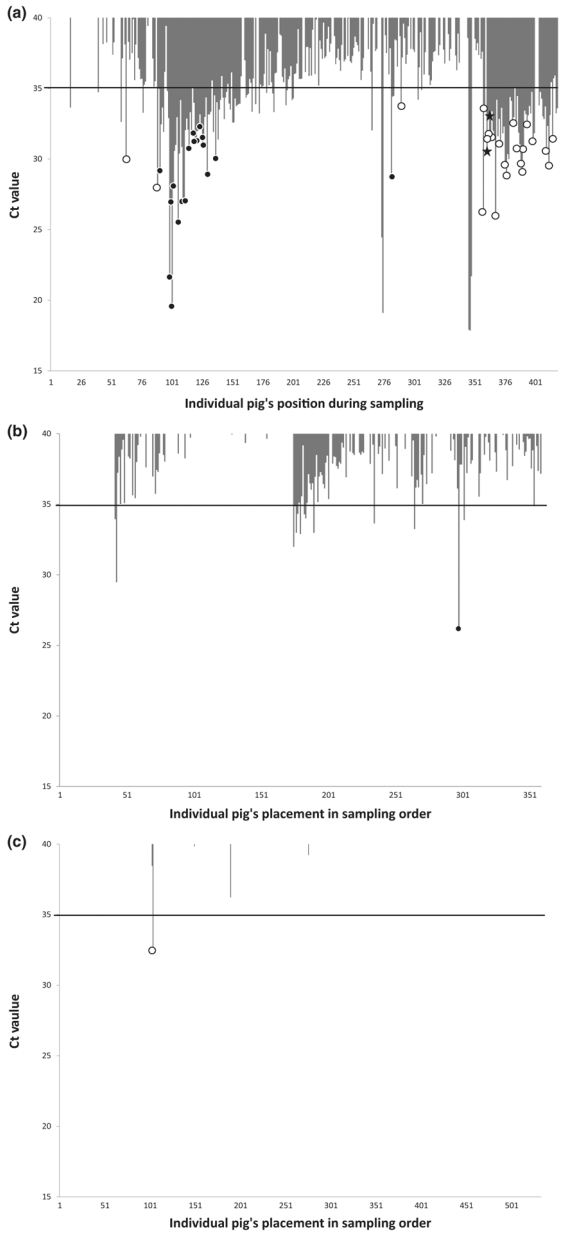

Fig. 1.

Temporal relationship of sampling order and influenza A virus among swine entering exhibitions. The horizontal axis represents the relative order of individual swine as they were sampled. In Panel a (Exhibition B) and Panel b (Exhibition C), swine were sampled as they moved through a narrow passage and a series of gates, collectively known as a ‘chute’, for arrival process. Panel c (Exhibition E) swine were sampled in pen prior to being moved through a chute. The snout wipe sample collected was screened for influenza A virus via rRT-PCR. The C_t value determined for each individual pig is displayed on the vertical axis. The black line at 35 indicates the cut point for a positive rRT-PCR sample. Closed black circles indicate recovery of an isolate with H1N1 subtype, open circles indicate recovery of an isolate with H3N2 subtype, and black stars indicate a mixed isolate with H1/H3 and

N1/N2 subtypes. Note that there appears to be a temporal relationship between the order of the individual pig in the samples and the C_t value displayed in the collected sample.

Table 1.

Sampling results for influenza A virus in swine at the beginning of nine agricultural fairs, 2014. The nine agriculture exhibitions where snout wipes were collected from swine in 2014 are displayed. The *sampling location* column indicates whether the snout wipes were collected on the trailer prior to unloading, in the pig's pen prior to weighing or in the chute during weighing. No. *swine tested* shows the number of samples per fair that were screened for influenza A virus (IAV) via rRT-PCR. The number of samples that were rRT-PCR positive ($C_t \leq 35$) for IAV and the number of IAV isolates recovered from cell culture are listed

Fair	Sampling location	No. of swine exhibited at fair	No. (%) swine tested	No. (%) rRT-PCR positive	No. (%) virus isolation positive
Exhibition A	Trailer	2149	382 (17.8)	21 (5.5)	6 (1.6)
Exhibition B	Chute	424	419 (98.8)	144 (34.4)	43 (10.3)
Exhibition C	Chute	377	359 (95.2)	16 (4.4)	1 (0.3)
Exhibition D	Pen	465	445 (95.7)	6 (1.4)	2 (0.4)
Exhibition E	Pen	523	523 (100.0)	1 (0.2)	1 (0.2)
Exhibition F	Chute	367	367 (100.0)	0	–
Exhibition G	Chute	274	274 (100.0)	0	–
Exhibition H	Chute	597	492 (82.4)	0	–
Exhibition I	Chute	286	286 (100.0)	0	–
Total		5462	3547 (64.9)	188 (5.3)	53 (1.5)

Table 2.

Influenza A virus subtypes recovered from incoming swine at agricultural fairs, 2014. Surveillance for influenza A virus at nine agricultural fairs in 2014 was conducted on swine during their arrival to the exhibition. The five agricultural exhibitions where influenza A virus isolates were recovered via cell culture are displayed by their hemagglutinin and neuraminidase subtype

Fair	No. (%) H1N1 IAV	No. (%) H3N2 IAV	No. (%) mixed subtype IAV, H1/H3 and N1/N2
Exhibition A	2 (33.33)	4 (66.67)	–
Exhibition B	18 (41.86)	23 (53.50)	2 (4.65)
Exhibition C	1 (100)	–	–
Exhibition D	2 (100)	–	–
Exhibition E	–	1 (100)	–
Total	23 (43.40)	28 (52.83)	2 (3.77)