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Bioaerosol Sampling in Modern Agriculture: A Novel Approach for Emerging Pathogen Surveillance?

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Background. Modern agricultural practices create environmental conditions conducive to the emergence of novel pathogens. Current surveillance efforts to assess the burden of emerging pathogens in animal production facilities in China are sparse. In Guangdong Province pig farms, we compared bioaerosol surveillance for influenza A virus to surveillance in oral pig secretions and environmental swab specimens.

Methods. During the 2014 summer and fall/winter seasons, we used 3 sampling techniques to study 5 swine farms weekly for influenza A virus. Samples were molecularly tested for influenza A virus, and positive specimens were further characterized with culture. Risk factors for influenza A virus positivity for each sample type were assessed.

Results. Seventy-one of 354 samples (20.1%) were positive for influenza A virus RNA by real-time reverse-transcription polymerase chain reaction analysis. Influenza A virus positivity in bioaerosol samples was a statistically significant predictor for influenza A virus positivity in pig oral secretion and environmental swab samples. Temperature of <20°C was a significant predictor of influenza A virus positivity in bioaerosol samples.

Discussions. Climatic factors and routine animal husbandry practices may increase the risk of human exposure to aerosolized influenza A viruses in swine farms. Data suggest that bioaerosol sampling in pig barns may be a noninvasive and efficient means to conduct surveillance for novel influenza viruses.

Keywords. One Health; bioaerosol; influenza A virus; swine; China; modern agriculture; emerging pathogens.

Modern agricultural production systems produce some of the safest and least expensive meat products the world has ever known. However, the large scale of these farms provides opportunities for some pathogens to be enzootic, which could lead to the emergence and spread of novel pathogens [1–3]. The likelihood of an emergence event occurring in such settings is thought to be particularly high for influenza A viruses [4].

Influenza A viruses are a major cause of morbidity and mortality among human and animal populations worldwide [5, 6]. Numerous studies have been conducted to better understand influenza A viral ecology, particularly conditions that may increase the propensity for influenza A viruses to reassort in animals and cross-over to human populations. There is strong evidence documenting that swine are important for the genetic evolution and potential emergence of novel influenza A viruses [7–12].

To keep up with increasing pork demand, the pork industry is shifting to the modern agricultural practice of rearing pigs in larger, more-efficient concentrated animal feeding operations. This is perhaps most notable in China, which has seen the largest increase in domestic pork production and consumption in the past 10 years. There is concern that such a move to larger production facilities, without increases in biosecurity measures, will create environments more conducive for the mixing and generation of novel pathogens [13], which puts workers and their family members at increased risk of infection.

Given that current surveillance methods to detect zoonotic influenza A virus among swine are invasive and require extensive resources to operate, production managers may be hesitant to adopt them. Additionally, there are economic barriers to the transparent monitoring of swine herds. Alternative methods that embrace a One Health approach and incorporate human, animal, and environmental testing strategies could be a way to overcome these challenges, but few of these methods have been developed and evaluated.

One technology that has potential for the noninvasive detection of influenza A viruses in swine production facilities is bioaerosol sampling. While recent studies of various bioaerosol sampling devices have shown some promise in overcoming the inherent challenges of low detection efficiency for different swine viruses [14–17], bioaerosol sampling data for influenza A

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virus in operational swine production facilities are sparse, and it seems no such studies have been attempted in China.

The goal of this study was to use a One Health approach (human, animal, and environmental sampling) to pilot a novel bioaerosol sampling technique to study 5 swine farms in Guangdong Province, China, for influenza A virus during 2 seasonal periods. Risk factors associated with a greater rate of molecular detection were also assessed. We hypothesized that if this bioaerosol sampling technique could be successfully piloted in Chinese swine farms, it could be readily adapted to larger and more diverse animal production settings.

METHODS

Study Design

Two institutional review boards (at Sun Yat-sen University and the University of Florida) and the Zhongshan Center for Disease Control and Prevention (CDC) approved this study. In 2014, 5 swine farms located in Zhongshan, China, were selected and sampled weekly for 2 weeks during the summer and weekly for 4 weeks during the fall/winter, using bioaerosol, pig oral secretion, and environmental swab sampling techniques (Figure 1). Criteria for farm selection included ease of access by vehicle, number of pigs produced, and proximity to the Zhongshan CDC, to maintain specimen cold chain. Upon enrollment of a farm, each owner or manager was asked to complete an enrollment questionnaire assessing various descriptive details of their facility. Responses were kept confidential, to reduce possible reporting bias.

Sampling Site Selection

Five sampling sites on each farm with varying types of pig herds (weaning pigs, sows, growers, and finishers) were selected, denoted with an identification number, and sampled weekly for 2 weeks during the summer and for 4 weeks during fall/winter. Bioaerosol sampling was performed concomitantly with pig oral secretion and environmental swab sampling.

Bioaerosol Sampling

Bioaerosol sampling was conducted using BioSamplers (SKC, Eighty Four, Pennsylvania; catalog number 225-9595) operated with 220-volt BioLite sampling pumps (SKC; catalog number 228-9610) [18]. In-line vapor traps (SKC; catalog number 225-22-01) were used to protect the pumps against moisture. Pumps were warmed up by running them for 5 minutes prior to sampling, then samplers were filled with 15 mL of commercial-made sterile phosphate-buffered saline with 0.5% (w/v) bovine serum albumin fraction V (BSA) powder, which was kept in an insulated cooler with ice packs until use. The BioSamplers were operated at a flow rate of 8 L/minute for 30 minutes,

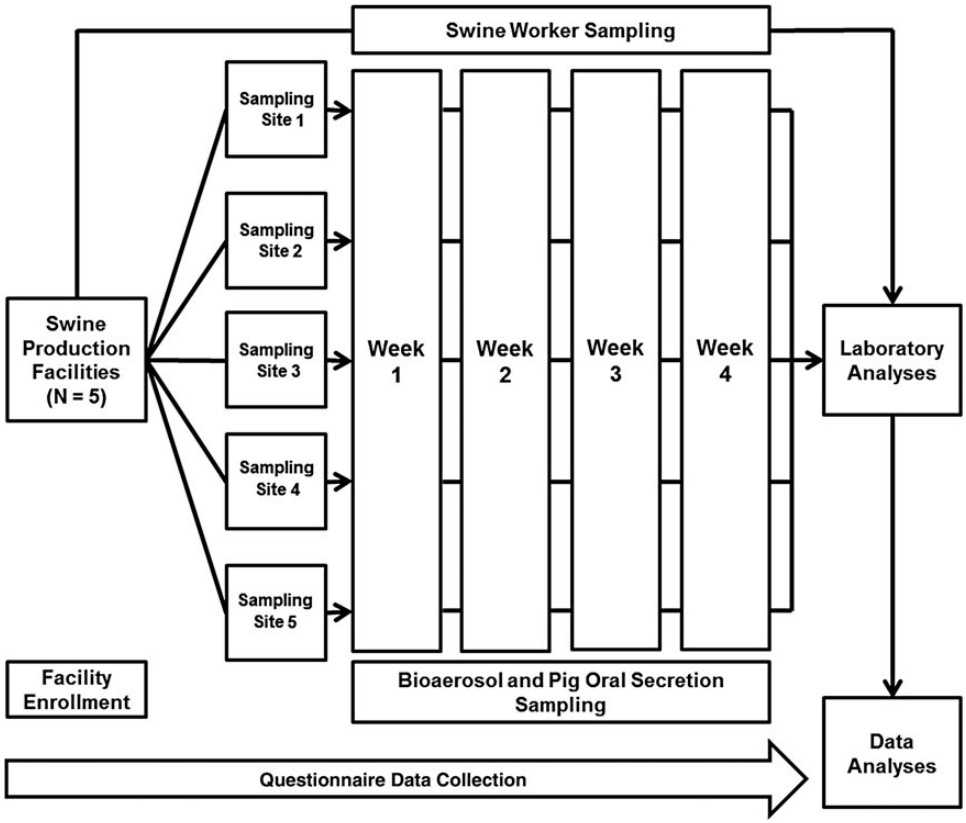

Figure 1. Schematic of human, animal, and environmental sampling strategy at 5 swine farms in Guangdong Province, China.

allowing for the controlled sampling of approximately 240 L of air per site. Once the sampling period was completed, the pump was shut off, the BioSampler was disconnected, and sample medium was aseptically transferred from the SKC BioSampler collection vessel into a sterile 15-mL conical tube, which was immediately placed in an insulated cooler with ice packs.

Samplers were disinfected after each use at a test site by using a 2.5% bleach solution and were rinsed using sterile water before they were moved to a new sampling location. All samplers were autoclaved for disinfection under pressure (100 kPa) at 121°C for 60 minutes at the end of each sampling day. The 3 BioSampler components (inlet, outlet, and collection vessel) remained matched throughout the study period; no pieces were exchanged between individual BioSampler units.

Hanging Rope Sampling

A hanging rope method to capture pig oral secretions was used as previously described [19–21]. Briefly, 3-strand braided unbleached 100% cotton ropes with 1.6 cm diameter were placed in pig pens that were <5 m to the bioaerosol sampling sites. During bioaerosol sampling, ropes were hung approximately 40 cm above the floor for 20–30 minutes, during which time the pigs could chew them to the point of oral secretion saturation. At the conclusion of the sampling time, oral fluids were aseptically extracted from the rope by manually expressing the wet portion of the rope into a sterile zip-lock plastic specimen bag, which was thereafter placed in an insulated cooler with ice packs.

Environmental Swab Sampling

Environmental swab samples were collected at each sampling site during fall and winter only, by wiping approximately 10-cm^2 areas of different hard surfaces (railings, food troughs, weaning boxes, and gate handles) located <1 m from the BioSampler with a cotton-tipped polystyrene swab. Swabs were then placed into a sterile collection tube containing 3 mL of universal transport medium and placed on ice packs.

Data Collection

Temperature and relative humidity data were collected at each sampling site using HOBO temperature and relative humidity detectors (Onset HOBO Data Loggers, Bourne, Massachusetts; catalog numbers U12-013 and UA-002-64). Collections occurred at 1-minute intervals for 5 minutes at a random time during each bioaerosol collection period. Information regarding the type and number of pigs at each collection site was also recorded.

Swine Worker Enrollment

Human subject enrollment has been previously published [22]. Briefly, study subjects from each swine production facility were recruited through face-to-face interactions with study personnel during farm visits. After consent, participants were asked to complete a questionnaire and to permit collection of a 5-mL blood specimen. Human sera samples were assessed using the hemagglutination inhibition assay for detection of antibodies against circulating human H1N1 and H3N2 influenza A viruses and swine H1N1 and H3N2 influenza A viruses.

Sample Processing

All samples were transported at the end of each sampling day to the Zhonghsan CDC viral laboratory. Pig oral secretion and environmental swab specimens were aliquoted into sterile cryovials and then stored at −80°C until laboratory analysis was conducted. Bioaerosol samples were concentrated using Amicon Ultra-15 Centrifugal Filter Units with Ultracel-100 membranes (Merck, Darmstadt, Germany) at $4000 \times g$ for 20 minutes, separated into 3 equal aliquots (0.2 mL–0.5 mL each), and stored at −80°C. All aliquots were labeled with the unique specimen number, facility identifier, pen location, and date.

Laboratory Analyses

Bioaerosol, pig oral secretion, and environmental swab samples were thawed, and total nucleic acid was extracted using the QIAxtractor (Qiagen, Venlo, the Netherlands). Extracted viral RNA was then assessed with real-time reverse-transcription polymerase chain reaction (rRT-PCR). using the Takara One-step rRT-PCR kit (Takara Bio, Otsu, Japan) with World Health Organization influenza A virus primers (forward: 5′-GACCRATCCTGTCACCTCTGAC-3′; reverse: 5′-AGGGC ATTYTGGACAAAKCGTCTA-3′) and probe (5′-FAM-TGC AGTCCTCGCTCACTGGGCACG-BHQ1-3′) on an ABI7500 real-time platform (Thermo Fisher Scientific, Waltham, Massachusetts) [23]. Original, unthawed aliquots of viral RNA extracted from positive samples were carried by hand on dry ice to the Beijing Institute of Microbiology and Epidemiology, where swine influenza A virus subtyping was performed by using previously published conventional RT-PCR protocols [24].

Data Analysis

A 2 sample *t* test was used to compare the means of temperature and relative humidity for each sampling period. A multivariate modeling strategy was used to identify risk factors for influenza A virus positivity. First, bivariate χ^2 tests of independence or the Fisher exact test was used to examine the strength of association for potential risk factors for influenza A virus among bioaerosol, pig oral secretion, and environmental swab samples. Variables determined by bivariate analyses to be statistically associated with positivity ($P < .25$) were then entered into a multivariate logistic regression model. A backward elimination strategy was performed, and predictors with a P value of <.05 were retained in the final models. Collinearity was tested using bivariate χ^2 tests, and Hosmer-Lemeshow χ^2 analysis for goodness of fit was performed to determine how well the model fit the data. Statistical analysis was performed using Stata 14.0 (StataCorp, College Station, Texas).

RESULTS

Swine Production Facility Enrollment

Five swine production facilities were enrolled in southern Guangdong Province, close to Zhongshan (Figure 2). Farms ranged in production size (from 2000 to 12 000 pigs), reported animal death rates (from 0.2% to 7.0%), and number of workers (from 7 to 150), with 181 males and 79 females employed. There were also variations in barn types, fecal management practices, and biosecurity protocols (Table 1).

Sample Collection

In total, 145 bioaerosol samples, 114 pig oral secretion samples, and 95 environmental swab samples were collected. Fifty bioaerosol samples and 28 pig oral secretion samples were collected in the summer sampling period (14–24 July 2014) and 95 bioaerosol samples, 86 pig oral secretion samples, and 95 environmental swab samples were collected in the fall and winter sampling period (10–31 December 2014). Environmental swab sampling was added after the summer at the request of the Chinese CDC, as it is routinely used as part of the Chinese CDC's regular surveillance programs. Sera samples were collected from 130 swine workers at the 5 swine farms, in addition to 115 control subjects in the nearby city of Guangzhou.

Influenza A Virus Positivity, Subtyping, and Serology

A total of 71 of 354 samples (20.1%) were confirmed positive for influenza A virus RNA by rRT-PCR. By season, 7 of 28 pig oral secretion samples (25.0%) and none of the bioaerosol samples collected during the summer and 9 of 95 bioaerosol samples (9.5%), 16 of 86 pig oral secretion samples (18.6%), and 39 of 95 environmental swab samples (41.1%) collected during the fall and winter were positive for influenza A virus RNA (Figure 3). The highest rate of influenza A virus molecular positivity detected among bioaerosol samples was in week 2 of the fall sampling season, with 6 of 25 samples (24%) collected that week testing positive for influenza A virus RNA. Of the 71 samples positive for influenza A virus RNA by rRT-PCR, 9 (14.8%) were subtypable: 7 were identified to be swine influenza A virus (6 pig oral secretion samples and 1 environmental swab sample) and 2 were identified as swine influenza A virus (pig oral section samples only). Twenty-three of 130 swine-exposed workers (18.0%) and 8 of 115 controls (7%) were seropositive for antibody against swine H3N2 influenza A virus [22].

Temperature and Relative Humidity

There was a statistically significant difference ($P < .001$) in the mean temperature for collected bioaerosol samples that tested positive for influenza A virus RNA (17.8°C; 95% confidence

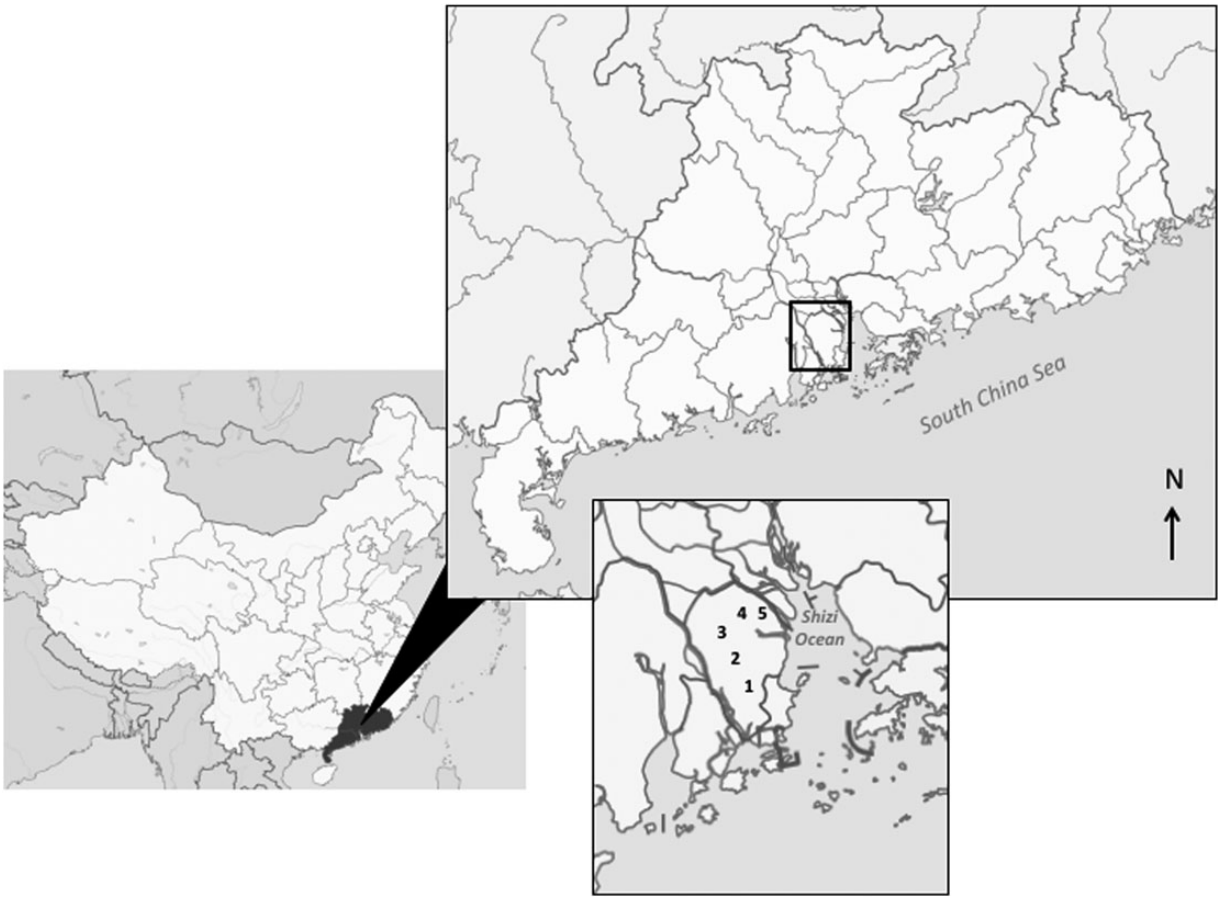

Figure 2. Map of geographical locations where 5 swine farms (denoted 1–5) were selected and sampled in Guangdong Province, China.

Table 1. Descriptive Variables, by Farm

Descriptive Variables	Overall	Farm 1	Farm 2	Farm 3	Farm 4	Farm 5
Worker sex, no. (%)						
Male	181 (69.6)	98 (65.3)	64 (80.0)	10 (66.7)	4 (57.1)	5 (62.5)
Female	79 (30.1)	52 (34.7)	16 (20.0)	5 (33.3)	3 (42.9)	3 (37.5)
Total	260 (100)	150 (100)	80 (100)	15 (100)	7 (100)	8 (100)
Animals on site, by size, maximum no. (%)						
Weaning	50 000 (50.0)	29 000 (48.3)	14 000 (50.0)	5 000 (50.0)	800 (40.0)	1200 (40.0)
Growing	30 700 (30.7)	19 000 (31.7)	8000 (28.6)	2200 (28.0)	600 (30.0)	900 (30.0)
Finishing	16 500 (16.5)	10 000 (16.7)	4000 (14.3)	2000 (20.0)	200 (10.0)	300 (10.0)
Sows	5800 (5.8)	2000 (3.3)	2000 (7.1)	800 (8.0)	400 (20.0)	600 (20.0)
Total	103 000 (100)	60 000 (100)	28 000 (100)	10 000 (100)	2000 (100)	3000 (100)
Animals on site, no., mean	32 000	10 000	12 000	5000	2000	3000
Reported monthly animal death rate, %, mean	2.4	7.0	1.7	1.6	0.2	1.6

interval [CI], 15.2°C–20.3°C) as compared to those that tested negative (26.8°C; 95% CI, 25.7°C–27.9°C), as well as the mean temperature for collected environmental swab samples that tested positive (20.6°C; 95% CI, 19.6°C–21.7°C) as compared to those that tested negative (22.7°C; 95% CI, 21.9°C–23.5°C). There was no significant difference ($P = .483$) in the average temperatures among collected pig oral secretion samples or in the relative humidity among any sample type.

Bivariate and Multivariate Analysis

Important bivariate predictors for influenza A virus positivity included concomitant sampling, production facility, pig type, temperature, and relative humidity (Tables 2 and 3). Using pig oral secretion influenza A virus positivity as the outcome, bioaerosol influenza A virus positivity (odds ratio [OR], 13.4; 95% CI, 2.9–62.3) remained a statistically significant predictor. Use of environmental swab influenza A virus positivity as the outcome revealed that bioaerosol influenza A virus positivity (OR, 10.1; 95% CI, 1.1–92.2) and farm 3 (OR, 10.1, 95% CI, 2.6–39.7) remained statistically significant predictors. Last, use of bioaerosol influenza A positivity as the outcome showed that pig oral secretion influenza A virus positivity (OR, 16.4; 95% CI, 2.4–112.5), environmental swab influenza A virus positivity (OR, 7.9; 95% CI, 1.7–89.1), and a temperature between 14.0°C and 19.9°C (OR, 16.2; 95% CI, 2.4–112.5) remained statistically significant predictors. No collinearity problems were detected, and Hosmer-Lemeshow χ^2 analysis for goodness of fit indicated that predictors sufficiently described the data.

DISCUSSION

In this study, we used a novel bioaerosol sampling method to assess the burden of aerosolized influenza A virus during 2 seasonal periods in 5 swine farms located in Guangdong Province

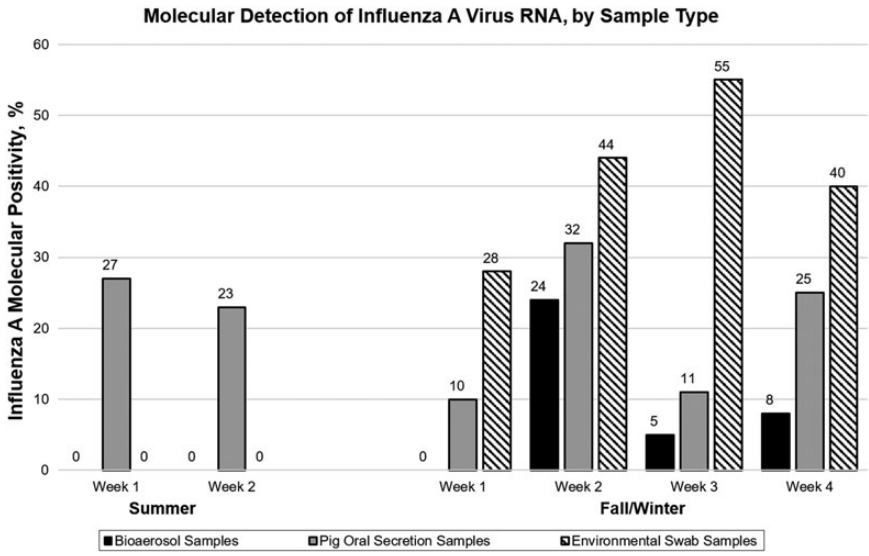

Figure 3. Detection of influenza A virus–positive samples among bioaerosol, pig oral secretion, and environmental swab samples obtained during 2 weeks in the summer and 4 weeks in the fall/winter.

Table 2. Unadjusted and Adjusted Odds Ratios (ORs) for Risk Factors Associated With Influenza A Virus Positivity in Pig Oral Secretion Samples and Environmental Swab Samples by Molecular Analysis

	Pig Oral Secretion Samples				Environmental Swab Samples			
Risk Factor	Analyzed, No.	Positive, No. (%)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	Analyzed, No.	Positive, No. (%)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
Season								
Summer	28	7 (25.0)	NA	NA	. . .	. . .	NA	NA
Fall/winter	86	16 (18.6)	NA	NA	95	39 (41.1)	NA	NA
Bioaerosol								
Positive	9	6 (66.7)	10.4 (2.4–45.5)	13.4 (2.9–62.3)	9	8 (88.9)	14.2 (1.7–118.8)	10.1 (1.1–92.2)
Negative	105	17 (16.2)	Reference	Reference	86	31 (36.1)	Reference	Reference
Pig oral secretion								
Positive	NA	NA	NA	NA	16	10 (62.5)	2.5 (.8–7.7)	. . .
Negative	NA	NA	NA	NA	70	28 (40.0)	Reference	. . .
Environmental swab								
Positive	38	10 (26.3)	2.5 (.8–7.7)	. . .	NA	NA	NA	NA
Negative	48	6 (12.5)	Reference	. . .	NA	NA	NA	NA
Farm								
Farm 1	21	3 (14.3)	. . .	. . .	20	7 (35.0)	. . .	. . .
Farm 2	20	2 (10.0)	0.4 (.1–1.8)	. . .	20	1 (5.0)	0.1 (.01–.4)	. . .
Farm 3	26	6 (23.1)	. . .	. . .	20	17 (85.0)	13.7 (3.6–51.3)	10.1 (2.6–39.7)
Farm 4	23	5 (21.7)	. . .	. . .	20	8 (40.0)	. . .	. . .
Farm 5	24	7 (29.2)	1.9 (.7–5.3)	. . .	15	6 (40.0)	. . .	. . .
Pig type								
Sow	9	1 (11.1)	. . .	. . .	8	2 (25.0)	. . .	. . .
Sow/weaning	68	14 (20.6)	. . .	. . .	58	22 (37.9)	. . .	. . .
Weaning	14	2 (14.3)	. . .	. . .	12	10 (83.3)	9.3 (1.9–45.4)	. . .
Growing	15	6 (40.0)	3.2 (1.0–10.2)	. . .	9	5 (55.6)	. . .	. . .
Finishing	8	0 (0.0)	. . .	. . .	8	0 (0.0)	. . .	. . .
Temperature, °C								
14.0–19.9	33	8 (24.2)	. . .	. . .	34	21 (61.8)	3.9 (1.6–9.3)	. . .
20.0–22.9	23	4 (17.4)	. . .	. . .	24	7 (29.2)	0.5 (.2–1.4)	. . .
23.0–25.9	26	3 (11.5)	0.4 (.1–1.6)	. . .	29	10 (34.5)	. . .	. . .
26.0–33.9	21	6 (28.6)	. . .	. . .	8	1 (12.5)	0.2 (.02–1.6)	. . .
34.0–37.0	11	2 (18.2)	. . .	. . .	0	0 (0.0)	. . .	. . .
Relative humidity, %								
29.0–43.9	26	5 (19.2)	. . .	. . .	29	9 (31.0)	0.5 (.2–1.4)	. . .
44.0–56.9	27	5 (18.5)	. . .	. . .	28	14 (50.0)	. . .	. . .
57.0–62.9	19	2 (10.5)	. . .	. . .	14	5 (35.7)	. . .	. . .
63.0–65.9	18	7 (38.9)	3.2 (1.1–9.5)	. . .	9	3 (33.3)	. . .	. . .
66.0–77.0	24	4 (16.7)	. . .	. . .	15	8 (53.3)	. . .	. . .

Abbreviations: CI, confidence interval; NA, not applicable.

and compared these results to concomitant animal, environmental, and human sample testing.

Swine influenza A viruses, particularly H1N1 and H3N2 subtypes, are highly prevalent and persistent among swine herds worldwide [10]. Swine influenza A virus circulation among pigs has also been documented in China [25–29]. Historically, classical H1N1 swine influenza A virus was the most prevalent circulating subtype of influenza A virus in pigs throughout China, until it was supplanted by European or Eurasian avian-like H3N2 swine influenza A virus and triple-reassortant H1N1 swine influenza A virus, beginning in the 2000s [29, 30]. After 2009, pandemic H1N1 influenza A virus has been readily detected among swine populations in China, as well as H3N2 swine influenza A viruses containing pandemic H1N1–origin gene segments [29, 31, 32]. These phylogenetic transitions underscore the complexity of influenza A virus ecology in swine populations in China.

Current thinking is that circulation of swine influenza viruses occurs year-round with outbreaks or increases in cases more likely during the late fall and early winter months, when temperatures begin to decrease [33, 34]. It is most likely that the year-round maintenance of influenza A viruses in swine production facilities is influenced by the continual introduction of susceptible pigs into the farms, by movement of pigs between farms, and by human behaviors that violate biosecurity

Table 3. Unadjusted and Adjusted Odds Ratios (ORs) for Risk Factors Associated With Influenza A Virus Positivity in Bioaerosol Samples by Molecular Analysis

	Bioaerosol Samples			
Risk Factor	Analyzed, No.	Positive, No. (%)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
Season				
Summer	50	0 (0.0)	. . .	. . .
Fall/winter	95	9 (9.5)	. . .	. . .
Pig oral secretion				
Positive	23	6 (26.1)	10.4 (2.4–45.5)	16.4 (2.4–112.5)
Negative	91	3 (3.3)	Reference	Reference
Environmental swab				
Positive	39	8 (20.5)	14.2 (1.7–118.8)	7.9 (1.7–89.1)
Negative	56	1 (1.8)	Reference	Reference
Farm				
Farm 1	30	0 (0.0)	3.4 (.8–13.5)	. . .
Farm 2	30	2 (6.7)	. . .	. . .
Farm 3	30	4 (13.3)	. . .	. . .
Farm 4	30	3 (10.0)	. . .	. . .
Farm 5	25	0 (0.0)	. . .	. . .
Pig type				
Sow	12	0 (0.0)	. . .	. . .
Sow/weaning	88	7 (8.0)	. . .	. . .
Weaning	18	2 (11.1)	. . .	. . .
Growing	15	0 (0.0)	. . .	. . .
Finishing	12	0 (0.0)	. . .	. . .
Temperature, °C				
14.0–19.9 (Q1)	34	8 (23.5)	33.8 (4.05–282.6)	16.2 (2.4–112.5)
20.0–22.9 (Q2)	24	0 (0.0)	. . .	. . .
23.0–25.9 (Q3)	29	1 (3.5)	. . .	. . .
26.0–33.9 (Q4)	29	0 (0.0)	. . .	. . .
34.0–37.0 (Q5)	29	0 (0.0	. . .	. . .
Relative humidity, %				
29.0–43.9 (Q1)	2	2 (6.9)	. . .	. . .
44.0–56.9 (Q2)	2	2 (6.9)	. . .	. . .
57.0–62.9 (Q3)	1	1 (3.5)	. . .	. . .
63.0–65.9 (Q4)	0	0 (0.0)	. . .	. . .
66.0–77.0 (Q5)	4	4 (13.8)	. . .	. . .

Abbreviation: CI, confidence interval.

precautions [35]. Despite biosecurity measures, pigs reared in concentrated animal feeding operations are in such close contact that dissemination of viruses throughout herds can be rapid and difficult to contain [14, 36, 37].

Our results suggest that viral shedding in pigs occurs in both seasons in China but that detection of aerosolized virus is largely dependent on other factors, such as animal husbandry practices (eg, ventilation and barn enclosure) and/or climatic factors. We speculate that it is very likely that virus aerosols were present during the summer, but because of higher relative humidity, particles may have developed a larger droplet nuclei radius, causing them to settle more rapidly to the ground and not be detected by the BioSampler. Alternatively, the combination of higher temperatures and lower humidity could have resulted in convection currents that dispersed aerosolized particles, diluting the overall concentration and reducing the sampling sensitivity. It is also possible that the aerosolized particles were too small to detect using the SKC Biosampler, as previous studies have shown the detection efficiency of submicron (<1 μm) aerosolized particles to be reduced [38]. Interestingly, multivariate modeling for influenza A virus RNA positivity in bioaerosol samples resulted in a significant OR for temperatures between 14°C and 19.9°C (OR, 16.2; 95% CI, 2.4–112.5), but it did not identify a statistically significant association with relative humidity.

During week 2 of the fall sampling season, temperatures drastically decreased owing to a passing cold front. Rates of influenza A virus RNA detection also markedly increased. Many of the barns in which sampling was performed were closed or covered with plastic to maintain a more comfortable environment for the pigs inside. Fans in many of the sampled barns were also turned off, likely reducing the amount of air being circulated

and/or ventilated. These animal husbandry practices, though routine, may have created a higher concentration of viral aerosols in the enclosed swine barns, resulting in the higher rates of influenza A virus molecular detection observed, confounding our multivariate models. It is also possible that aerosolized virus was not being diluted or blown away by air currents, making them easier to detect.

Studies have shown that the propensity by which a virus is aerosolized and suspended in the air is greatly influenced by both temperature and relative humidity [39–41]. In a controlled study by Lowen et al, using a guinea pig model, virus transmission via aerosolization was optimal at temperatures of ≤20°C with a relative humidity of 20%–40% or 60%–80% [42]. These results were later validated by Yang et al [43]. Despite these findings, there still remains little consensus regarding the exact mechanisms by which temperature and relative humidity influence aerosol generation and transmission. Our results seem to be consistent with these previously conducted controlled studies and suggest a relationship between aerosolization and climatic factors on the virus particle level. Additional research, ideally in a controlled setting, would be useful to further explore these associations.

The use of bioaerosol sampling technology to conduct targeted routine surveillance for viruses in environments posing a greater risk of aerosolization is gaining traction. Several research groups have incorporated bioaerosol sampling methods into their routine surveillance studies, which have been conducted in clinics and various hospital settings [44–46]. Bioaerosol sampling was more recently used as part of a study that identified a novel swine-origin influenza A virus likely circulating between animals and humans in a live animal market [17]. It was also used to identify broad dissemination of influenza A virus RNA downwind from poultry farms [47]. It seems that with access to basic PCR testing capabilities, bioaerosol sampling can be readily applied to a variety of surveillance scenarios, with the capability of detecting pathogens before they infect new human or animal hosts.

There were several limitations in this study. Given that our sampling periods were fairly short, it is likely we missed some viral shedding, particularly in the summer months, resulting in the underestimation of the viral aerosol burden during the summer. We were unable to isolate virus from molecularly positive samples, so it is not clear whether influenza A virus RNA detected in our samples using RT-PCR alone were from viable viruses. It is possible that the virus may have been inactivated by ambient conditions (eg, exposure to UV light or desiccation) or through the sample collection process. Finally, owing to limited resources, we were not able to perform full-genome sequencing on the influenza A virus–positive samples that were nontypable by the swine influenza A viral subtyping assay. This step would be important for the detection of known human and avian influenza virus subtypes, in addition to the identification of novel influenza virus strains.

Overall, study data revealed considerable detection of influenza A virus, with bioaerosol samples having a higher rate of detection during the fall and winter seasons. A temperature of <20°C was a strong predictor for detection of influenza A virus RNA in bioaerosol samples. While we are not able to directly associate aerosol exposure to human infection by using cross-sectional human sampling, considering our findings and the serological evidence that the swine workers do have elevated levels of antibodies to H3N2 swine influenza A virus [22], it seems very likely that aerosol transmission is an important route of exposure of workers, particularly during periods of decreased temperature, and that routine animal husbandry practices used in some farms may unintentionally compound this risk. It seems plausible that essentially noninvasive bioaerosol sampling techniques might be used in swine confinement facilities to detect and characterize swine influenza viruses. It is also likely that bioaerosol sampling technology could be easily adapted to other animal production industries, such as those involving poultry and cattle. Such sampling would be an important strategic addition to our surveillance programs for the emergence of novel influenza viruses and other dangerous pathogens. Longer-term, multiyear prospective studies that use a One Health approach should be performed to further explore these relationships and establish baseline epidemiological data for the circulation of influenza A viruses in these important ecological settings.

Notes

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