Influenza A viruses are likely highly prevalent in South African swine farms

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Influenza A viruses are likely highly prevalent in South African swine farms

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Abstract

Growth in pork production during the last decade in South Africa has escalated the risk of zoonotic pathogen emergence. This cross-sectional study was conducted to evaluate evidence for transmission of influenza A virus between pigs and swine workers. Between February and October 2018, samples from swine workers and pigs were collected from three farms in KwaZulu-Natal Province, South Africa. Workers nasal washes and serum samples, and swine oral secretion samples (rope sampling method) were studied for evidence of swine influenza A virus infection using molecular and serological methods. Among 84 human nasal washes and 51 swine oral secretion specimens, 44 (52.4%) and 6 (11.8%) had molecular evidence of influenza A virus. Microneutralization assays with enrolled workers' sera against swine H1N1 and H3N2 viruses revealed a high prevalence of elevated antibodies. Multivariate risk factor analysis showed that male workers from the age-group quartile 23–32 years, who self-reported a recent history of exposure to someone with influenza disease and seldom use of personal protective equipment were at highest risk of molecular detection of influenza A virus. These pilot study data suggest that influenza A viruses are likely highly prevalent in South African swine farms. South Africa would benefit from periodic surveillance for novel influenza viruses in swine farms as well as education and seasonal influenza vaccine programmes for swine workers.

KEYWORDS
epidemiology, influenza A virus, one health, swine influenza, zoonoses

1 | INTRODUCTION

Evidence continues to mount that pigs may play important roles in amplifying and generating novel viruses, some of which are zoonotic (Krueger & Gray, 2013; Mena et al., 2016; Pepin et al., 2021; Pickering et al., 2021; Smith et al., 2011; Wardeh et al., 2021). With their close association to humans, other mammals and birds, pigs are not only key reservoirs for respiratory viruses but they also may serve as a host for novel virus generation (W. Ma et al., 2009; Wardeh et al., 2021). Numerous epidemiological studies have found evidence that occupational exposure to pigs is an important risk factor for swine influenza A virus infection among swine workers (Borkenhagen et al., 2020; M. Ma et al., 2015; M.-J. Ma et al., 2018). For instance, it is recognized that multiple novel influenza A viruses have emerged from pigs and pigs were at least associated with the new influenza A virus strains causing the 1918 (H1N1),1957 (H2N2),1968 (H3N2) and 2009 (H1N1) pandemics (Crosby 2003; Easterday, 2003; Krueger & Gray, 2013; Scholtissek et al., 1978). Nonetheless, surveillance for emerging and re-emerging viruses among pigs remains sparse, especially in Africa.

During the past 10 years, pork production in South Africa has increased on average 3.5 percent per year (USDA Foreign Agricultural Service, 2017). As swine farming industrializes, dense populations of pigs and humans remain in close proximity and this may escalate the risk of zoonotic virus transmission. The shortage of veterinary and agricultural services in South Africa, as well as swine farmers' limited knowledge regarding zoonotic pathogen control, may increase the risk of virus spillover between pigs and swine workers (Gcumisa et al., 2016). Little is known about influenza A and other enzootic influenza viruses in South African swine farms. In this research project, we sought to increase knowledge regarding influenza A virus. The overall goal of the study was to aid South African public health and animal health officials by providing insight regarding the prevalence of influenza A viruses in pigs, swine workers and possible zoonotic transmission.

2 | METHODS

2.1 | Sample collection

Study participants were recruited from swine farms in KwaZulu Natal Province, South Africa, using informed, written consent. Blood samples, nasal wash samples and questionnaire data were obtained from swine workers ≥18 years of age. For the nasal wash procedure, each participant was asked to tilt their head back and hold their breath while a researcher used a sterile syringe to flush one nostril with 3 mL of sterile water. The participant was then asked to express the return fluid in a sterile collection cup. Participants were also asked to complete a brief questionnaire regarding their demographics, medical history, exposure to animals, work environment and their use of personal protective equipment. In addition to human samples, swine oral secretion samples were collected per a previously reported procedure (Bailey et al., 2018; Prickett et al., 2008). Briefly, 100% cotton rope was wet with sterile water, fixed to a rod and held by a researcher at the height of the pigs' heads. The number of pigs in each enclosure were estimated to have ranged from 1 to 25. Multiple pigs were allowed to chew the rope for up to 5 min. Once the rope was saturated with oral fluids, it was manually wrung out inside a sterile plastic biohazard bag. Oral fluids were transferred to a sterile collection tube and preserved at −80°C.

2.2 | RNA extraction and molecular detection

RNA was extracted from human nasal washes and swine oral secretion samples using the QIAamp Viral RNA Mini Kit (QIAGEN, Inc., Valencia, CA) per manufacturer instructions. Human original specimens and swine RNA samples were sent to the Duke One Health laboratory for further study. Real-time reverse transcription polymerase chain reaction (rRT-PCR) was performed with the SuperScript® III Platinum One-Step qRT-PCR System with Platinum® Taq DNA Polymerase (Thermo Fisher Scientific, Inc., Waltham, MA) to identify influenza A matrix gene positives per the assay published by the World Health Organization from the Centers for Disease Control and Prevention (World Health Organization, 2014). Samples with cycle threshold (Ct) values <38 were considered positive.

Human specimens positive for influenza A were further studied at the Duke One Health laboratory using a conventional two-step RT-PCR assay that amplified the hemagglutinin (HA) and neuraminidase (NA) genes. First, reverse transcription was conducted using a universal primer (U12) and SuperScript® III reverse transcriptase (Thermo Fisher Scientific, Inc., Waltham, MA) to convert the viral RNA to cDNA. Next, the cDNA was amplified using HA and NA specific primers and Platinum® Taq DNA Polymerase (Thermo Fisher Scientific, Inc., Waltham, MA) (Hoffmann et al., 2001). These HA and NA segments were submitted to Eton Bioscience Inc. (Research Triangle Park, NC) for partial genome sequencing. The resultant partial sequences were then studied with the Basic Local Alignment Search Tool (BLAST) available at the National Center for Biotechnology Information (NCBI) (http://blast.ncbi.nlm.nih.gov) and matches with greater than 95% identity were recorded. Sequences were aligned and phylogenetic analysis was performed using the neighbour joining method tool of Geneious Prime 2021.1.1 (Biomatters Ltd., CA).

Influenza A positive specimens were also studied with the FluChip-8G Influenza A+B Insight Assay (InDevR Inc., Boulder, CO) per manufacturer's instruction (Bailey et al., 2021; Taylor et al., 2019). Full length influenza A gene segments were amplified with the provided multiplexed RT-PCR primers. The microarray was then labelled and imaged using the fluorescence-based FluChip-8G Imaging System.

2.3 | Influenza virus isolation

Original swine oral secretion samples were sent to the Ecole Nationale Vétérinaire de Toulouse, France where culture in MDCK cells was

attempted (two passages) according to the WHO influenza virus propagation protocol (World Health Organization, 2011).

2.4 | Microneutralization assays

Human sera were studied with microneutralization assays (MN) against two swine influenza viruses, A/SW/Iowa/73(H1N1) and A/SW/TX/1/98(H3N2) at the Duke One Health Laboratory. The laboratory team followed WHO-recommended microneutralization (MN) assay methods (World Health Organization, 2011). These strains were selected based upon the availability of viruses and their similarity to other viruses in the region. Briefly, serial twofold dilutions of duplicates of participants' heat inactivated sera were mixed with swine influenza viruses (H1N1 and H2N3) diluted to 100 × 50% tissue culture infectious dose ($TCID_{50}$). After 1 h of incubation at 37°C with 5% CO_2, MDCK cells were added at 2×10^5 to each well of a 96-well plate. After 22 h of incubation at 37°C with 5% CO_2, antibody responses against the swine viruses were measured by direct enzyme-linked immunosorbent assay (ELISA) using mouse anti-influenza virus A nucleoprotein (NP) monoclonal antibody MAB8251 Clone A1, A3 blend (MilliporeSigma, Burlington, MA) as the primary staining antibody and goat anti-mouse IgG horseradish peroxidae (HRP) conjugated (catalog number 474-1802, Kirkegaard & Perry Laboratories, Gaithersburg, MD) as the secondary staining antibody. Absorbance was determined using a spectrophotometer, and the 50% virus neutralization (NT) titre of each serum was determined. Neutralizing antibody titres were defined as the reciprocal of the highest dilution of serum samples that achieved at least 50% neutralization. Serum MN titre ≥1:20 was used as cut-off for indicating past infection (Olafsdottir et al., 2018; Nhat et al., 2017).

2.5 | Statistical analyses

To assess potential risk factors for cross-species transmission of influenza A virus between pigs and swine workers, standard descriptive, bivariate, and multivariate analyses were conducted. Chi-squared and Fisher Exact tests yielded odds ratio (OR) with 95% confidence intervals (CI). Potential risk factors with a p-value < .1 were fitted into a saturated multivariate logistic regression model and further examined with stepwise elimination. Analyses were performed using STATA version 16.0 (StataCorp, College Station, TX).

2.6 | Ethical approval

This study received ethical approval from the University of KwaZulu-Natal Biomedical Research Ethics Committee (BREC) (BE632/17) and Animal Research Ethics Committee (AREC 071/017), and the Duke University Health System Institutional Review Board (Pro00089731). The field sampling protocols, sample collection from animals, and the research were conducted in compliance with Section 20 of the Animal Diseases Act of 1984 (Act No 35 of 1984) and were approved by the South African Department of Agriculture, Forestry and Fisheries (Section 20 approval Reference number 12/11/1/5 granted to Prof. M.E. El Zowalaty).

3 | RESULTS

Between 22 February and 16 October 2018, a total of 87 swine workers were enrolled and 51 swine oral secretion samples were collected in this pilot study from three South African swine farms (Figure 1). Enrolled farmworkers were mostly male (70.2%, $n = 59$) and were mainly enrolled from farm A (61.9%, $n = 52$). The age of the workers ranged between 20 and 74 years, with a mean (SD) of 35.78 (13.06) years (Supplemental Table 1). The majority of the swine workers (96.6%) reported that they had not received the season influenza vaccine during the last 12 months. Of the 51 swine rope oral secretion samples, 33 were from Farm A (64.7%), 10 from Farm B (19.6%) and 8 from Farm C (15.7%). The pigs were between 3 and 364 weeks of age with a mean (SD) of 27.45 (7.63) weeks. The main swine type was finisher or grower, 43% of the total number.

3.1 | Influenza A virus surveillance in farmworkers

A total of 84 human nasal wash and 78 human sera samples were collected from swine workers in this study. Of these, 44 human nasal washes screened positive for influenza A virus (Figure 2). Cycle Threshold (Ct) values of these positive samples ranged between 32.03 and 37.75. The mean (SD) Ct was 35.08 (1.48). Culturing of influenza viruses in MDCK cells was attempted at Duke University according to the WHO influenza virus propagation protocol (World Health Organization, 2011). No sample yielded a live virus. However, viral sequences of HA and NA genes were successfully obtained from eight nasal washes using partial genome sequencing. Two samples had 97.9% and 94.6% identity with swine influenza A H1N1 virus. The other six samples matched different strains of human influenza A H1N1 virus, with identity percentages that ranged between 92% and 100% (Table 1). A phylogenetic tree of nine closely related influenza A H1N1 virus sequences downloaded from GenBank (https://www.ncbi.nlm.nih.gov/genbank/), and the viral sequences obtained from human nasal washes and swine oral secretions in the current study is illustrated in Figure 3.

Demographic outcomes significantly associated with influenza A virus positivity included biological sex of male (OR, 2.9; 95% CI [1.1, 7.5]), age quartile 25–32 years (OR 7.7; 95% CI [1.8, 32.2]), and living with less than or equal to four cohabitants in the household (OR, 3.4; 95% CI [1.1, 10.2]). Participants with a history of respiratory illness in the last 12 months had higher odds of screening positive for influenza A virus (OR, 2.6; CI [1.1, 6.3]). Similarly, having a household member or a co-worker with history of influenza A virus infection was significantly associated with higher odds of positive influenza A virus rRT-PCR (OR, 3.7; 95% CI [1.5, 9.4] and OR, 3.0; 95 % CI [1.1, 8.6], respectively). Finally, the use of cloth gloves while working with animals during the

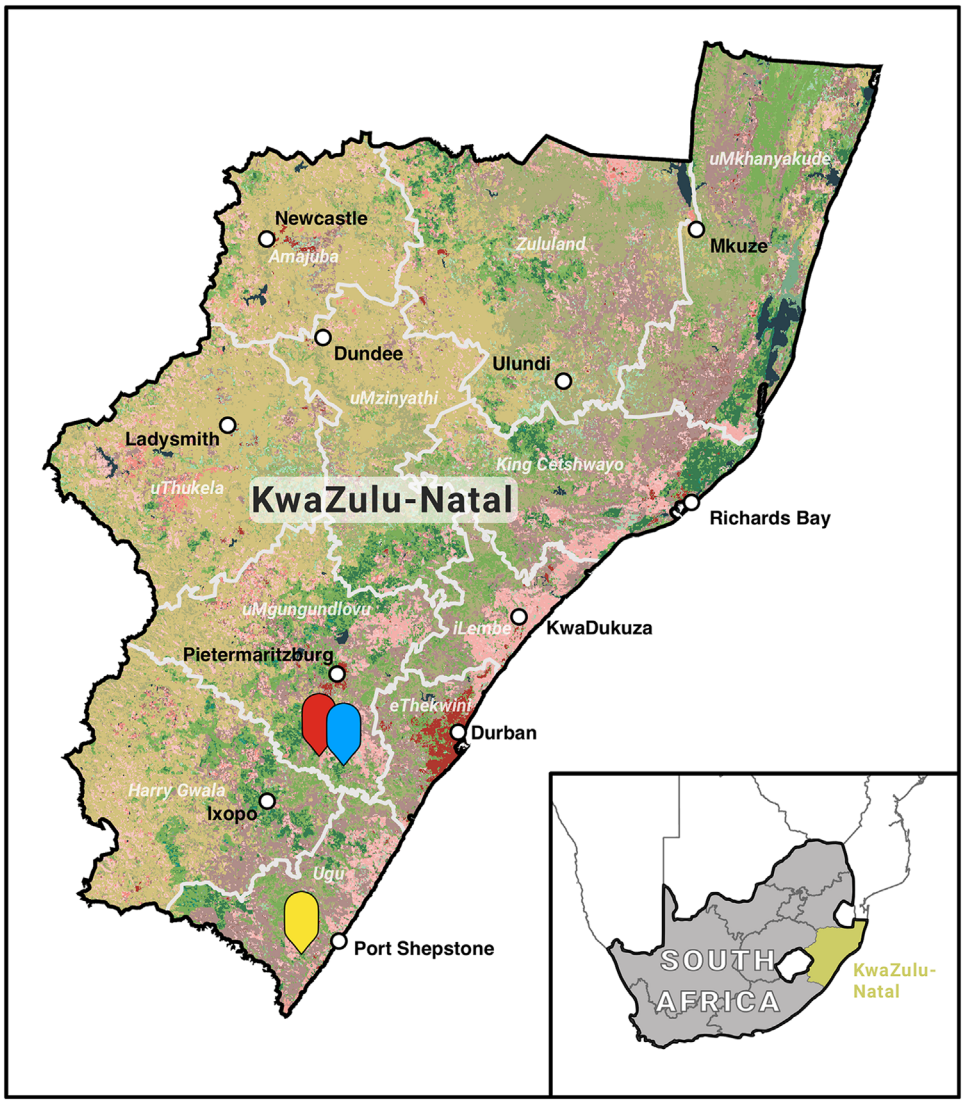

FIGURE 1 Locations of the farms where samples of the present study were collected in KwaZulu Natal Province, South Africa between February–October 2018. Map was generated using the software ArcGIS Pro (v2.7, ESRI, Redlands, CA, USA)

last 12 months demonstrated a protective effect against influenza A virus positivity (OR 0.3; CI, [0.1, 0.9]). Stepwise, backward elimination logistic regression was conducted with these variables. Adjusted odds ratios revealed strong association between molecular detection of influenza A virus and the sex of male, age group (25–32), having a household member with history of respiratory infection in the last 12 months, and infrequent use of gloves. The full results of the bivariate and multivariate risk factors assessments are shown in Table 2.

Microneutralization assays revealed that 23 swine workers (29%) had elevated MN titres (≥1:20) against swine influenza virus (H1N1) and all but one worker (98.7%) had elevated MN titres ≥1:20 against swine influenza virus (H3N2) (Figure 4). Geometric mean antibody titre against swine H1N1 was 10.95, and the geometric mean against swine H3N2 was 66.70.

Multivariate regression analysis revealed that factors significantly associated with elevated H1N1 MN titres ($p < .05$) included the age-group 25–32 year (adjusted OR,11.2; 95% CI, [1.0, 60.2]), having a co-worker with history of respiratory illness (adjusted OR, 17.5; 95% CI [1.1, 270.0]) and working in swine farms for 5 years or more (adjusted OR, 22.0; 95% CI [2.7, 177.2]). Bivariate and multivariate risk factor analysis results are shown in Table 3.

3.2 | Influenza A virus surveillance in pigs

A total 51 swine oral secretions were collected from these farms. Among these, six samples screened positive for influenza A virus (11.8%). Five of these samples were from Farm A and one was from Farm B. Of the six positive samples, three were successfully identified with the FluChip-8G assay and had an agreement that exceeded 95% with H1N1 influenza A virus. In addition, sequences of HA and NA genes were successfully obtained from three of the six positive swine oral secretions. BLAST results showed that these isolates are closely related to human influenza A H1N1 and swine influenza A H1N1 with

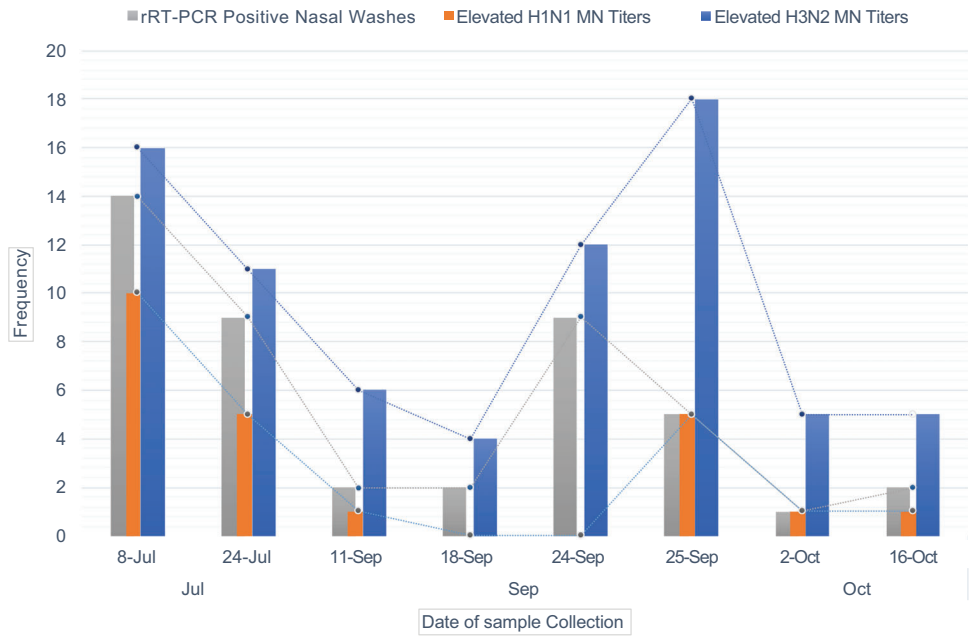

FIGURE 2 Results of molecular and serological detection of influenza A virus by date of sample collection from participants in South African swine farms upon enrolment. Abbreviations: rRT-PCR, real-time reverse transcription polymerase chain reaction; H1N1 MN titres, microneutralization antibody titres against swine influenza virus A/SW/Iowa/73(H1N1); H3N2 MN titres, microneutralization antibody titres against swine influenza virus A/SW/TX/1/98(H3N2)

TABLE 1 Results of successfully sequenced specimens positive by Influenza A virus RT-PCR assay (Hoffmann et al., 2001)

Sample ID	Source	Type	Sequence alignment result	GenBank accession number[a]	Identity score (%)
H002	Human	Nasal wash	Swine Influenza A virus H1N1 strain A/swine/Thailand/CB228/2010	KC859096.1	97.87
H003	Human	Nasal wash	Swine Influenza A virus H1N1 strain A/swine/Steinberg/21495/2015	MK367337.1	94.62
H008	Human	Nasal wash	Human Influenza A virus H1N1 strain A/Singapore/SS004/2010	CY067194.1	92.68
H014	Human	Nasal wash	Human Influenza A virus H1N1 strain A/Helsinki/Vi1/2009	JQ409128.1	100.00
H015	Human	Nasal wash	Human Influenza A virus H1N1 strain A/Ontario/0188466/2012	KC456609.1	100.00
H016	Human	Nasal wash	Human Influenza A virus H1N1 strain A/Helsinki/Vi1/2009	JQ409128.1	100.00
H019	Human	Nasal wash	Human Influenza A virus H1N1 strain A/Helsinki/Vi1/2009	JQ409128.1	100.00
H050	Human	Nasal wash	Human Influenza A virus H1N1 strain A/Awb/NIV25611/2010	CY075916.1	99.78
S057	Swine	Oral secretion	Human Influenza A virus H1N1 strain A/Saudi Arabia/108/2015	MK246073.1	98.07
S063	Swine	Oral secretion	Swine Influenza A virus H1N1 strain A/swine/Rengo/VN1401-95/2014	MF098856.1	100.00
S064	Swine	Oral secretion	Swine Influenza A virus H1N1 strain A/Ontario/0188466/2012	MG856208.1	100.00

[a]Recorded GenBank accession numbers were those with the highest identity scores and query coverage.

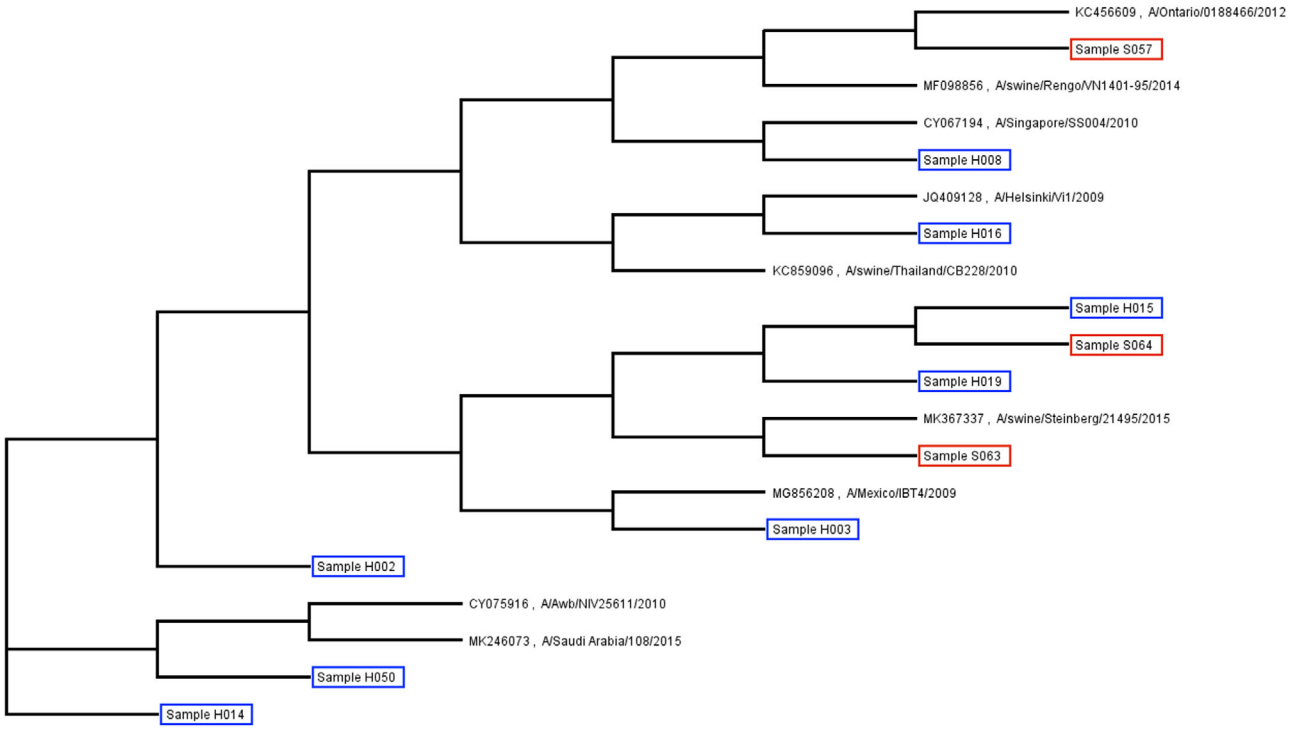

FIGURE 3 Phylogenetic analysis of nine strains of influenza A viruses available in GenBank (accession numbers: KC859096.1 (A/swine/Thailand/CB228/2010), MK367337.1 (A/swine/Steinberg/21495/2015), CY067194.1 (A/Singapore/SS004/2010), JQ409128.1 (A/Helsinki/Vi1/2009), KC456609.1 (A/Ontario/0188466/2012), CY075916.1 (A/Awb/NIV25611/2010), MK246073.1 (A/Saudi Arabia/108/2015), MF098856.1 (A/swine/Rengo/VN1401-95/2014), MG856208.1(A/Ontario/0188466/2012)). Analysed human samples are highlighted in blue (H002, H003, H008, H014, H015, H016, H019 and H050). Analysed swine samples are highlighted in red (S057, S063 and S064)

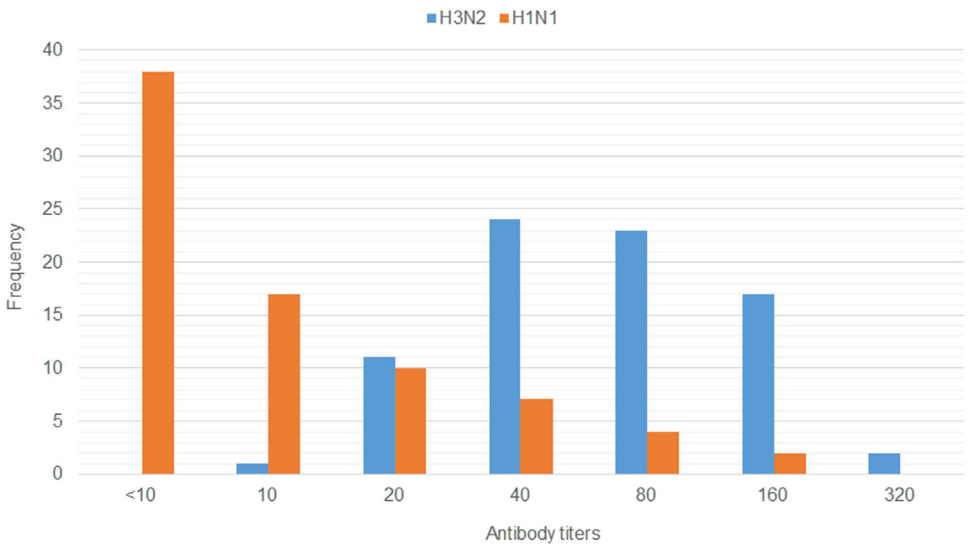

FIGURE 4 Titres of serum neutralizing antibodies against swine influenza viruses H1N1 and H3N2. Abbreviations: H1N1, titres of neutralizing antibodies against swine influenza virus A/SW/Iowa/73(H1N1); H3N2, titres of neutralizing antibodies against swine influenza virus A/SW/TX/1/98(H3N2)

TABLE 2 Bivariate and multivariate examination of potential risk factors for molecular detection of influenza A virus in swine workers' nasal washes

Risk Factor	Number IAV Positive (%)	Unadjusted odds ratio (95% CI)	Adjusted odds ratio (95% CI)
Sex			
Male	35 (60.3)	2.9 [1.1, 7.5])	10.6 [2.4, 80.8]
Female	9 (34.6)	Ref.	Ref.
Age in years (quartiles)			
20–24	8 (40.0)	1.1 [0.3, 4.2]	2.5 [0.5, 13.9]
25–32	18 (81.8)	7.7 [1.8, 32.2]	14.1 [2.5, 29.6]
33–43	10 (52.6)	1.9 [0.5, 7.0]	4.7 [0.9, 23.6]
44–74	7 (36.8)	Ref.	Ref.
Number of cohabitants in household (not including self)			
<5	18 (60.0)	3.4 [1.1, 10.2]	-
35	8 (30.8)	Ref.	
In the last 12 months did you develop a respiratory illness?[a]			
Yes	25 (64.1)	2.6 [1.1, 6.3]	-
No	18 (40.9)	Ref.	
In the last 12 months has anyone in your household developed a respiratory illness?[a]			
Yes	28 (66.7)	3.7 [1.5, 9.4]	7.1 [1.9, 26.8]
No	13 (35.1)	Ref.	Ref.
In the last 12 months has anyone at your work developed a respiratory illness?[a]			
Yes	18 (64.3)	3.0 [1.1, 8.6]	-
No	13 (37.1)	Ref.	
Have you worn cloth gloves while working with animals in the last 30 days?			
Yes	5 (29.4)	Ref.	Ref.
No	39 (58.2)	3.3 [1.1, 10.6]	7.2 [1.3, 41.1]
Enrolment site number			
A	25 (51.0)	2.9 [0.9, 9.3]	-
B	14 (87.5)	19.6 [3.2, 118.5]	
C	5 (26.3)	Ref.	

Note: Influenza A virus infection (IAV) was detected by real-time reverse transcription polymerase chain reaction (rRT-PCR).
Data are number (%) of subjects, unless otherwise indicated. Two-sided Chi-square tests or Fisher's Exact Test were used for dichotomous data analysis.
Ref. = Referent Group
[a]Respiratory illness was defined as fever and cough or sore throat.

identity scores between 98.1% and 100%. Sequences were included in the phylogenetic analysis with the human samples and illustrated in Figure 3.

4 | DISCUSSION

In this study, we conducted molecular and serological surveillance for influenza A virus in pigs and swine workers. Our aim was to assess evidence of cross-species transmission of influenza A viruses between swine workers and pigs in South African swine farms using a One Health approach. Similar to previous studies conducted among swine workers, our results demonstrated considerable molecular and serological evidence that swine workers are at increased risk of infection with swine influenza viruses (Awosanya et al., 2013; Borkenhagen et al., 2020; Gray et al., 2007; M. Ma et al., 2015;M.-J. Ma et al., 2018).

All except one swine worker enrolled in our study had elevated MN titres for H3N2. However, only three out of 87 swine workers reported receiving a seasonal influenza vaccination. This suggests that the high sero-prevalence of H3N2 was due to live influenza virus exposure before the time of sample collection. A study by Awosanya et al. (2013) in Lagos, Nigeria, reported a similar finding.

A study by Olsen et al., 2002) found that age ≥50 years was associated with higher swine influenza virus seropositivity. Lopez-Robles and others also reported that elderly workers were more likely to have elevated antibody titres (López-Robles et al., 2012). Our risk factors analysis indicated that young workers (25–32 years) with history

TABLE 3 Bivariate and multivariate examination of potential factors associated with elevated swine influenza virus H1N1 microneutralization titres

Risk Factor	Titre <1:20 *n* = 55	Titre ≥1:20 *n* = 23	Unadjusted odds ratio (95% CI)	Adjusted odds ratio (95% CI)
Gender				
Female	16	9	Ref.	-
	29.1%	39.1%		
Male	39	14	0.6	
	70.9%	60.9%	[0.2, 1.8]	
Age				
20–24	18	1	Ref.	Ref.
	32.7%	4.4%		
25–32	11	10	16.4	11.2
	20.0%	43.5%	[1.8,145.9]	[1.0, 184.5]
33–43	11	7	11.5	2.2
	20.0%	30.4%	[1.2, 106.0]	[0.3, 19.8]
44–74	13	4	5.5	Omitted[a]
	23.6%	17.4%	[0.6, 55.5]	
History of respiratory illness in the last 12 months [b]				
No	28	10	Ref.	-
	50.9%	43.5%		
Yes	27	12	1.2	-
	49.1%	52.2%	[0.5, 3.4]	
Household member with a history of respiratory illness in the last 12 months [b]				
No	24	8	Ref.	-
	43.6%	34.78%		
Yes	29	13	1.3	-
	52.7%	56.5%	[0.4, 3.8]	
Co-worker with a history of respiratory illness in the last 12 months[b]				
No	24	7	Ref.	Ref.
	43.6%	30.4%		
Yes	14	13	3.2	17.5
	25.5%	56.5%	[1.0, 9.9]	[1.1, 270.0]
Years of work as a swine farm worker				
<1 year	17	3	Ref.	Ref.
	30.9%	13.0%		
1–4 years	17	5	1.7	6.2 [0.3, 122.4]
	30.9%	21.7%	[0.3, 8.1]	
≥ 5 years	9	9	5.7	22.0
	16.4%	39.1%	[1.2, 26.3]	[2.7, 177.2]
Influenza A rRT-PCR result				
Negative	28	6	Ref.	-
	50.9%	26.1%		
Positive	25	16	3.0	-
	45.45%	69.6%	[1.0, 8.8]	

(Continues)

TABLE 3 (Continued)

Risk Factor	Titre <1:20 $n = 55$	Titre ≥1:20 $n = 23$	Unadjusted odds ratio (95% CI)	Adjusted odds ratio (95% CI)
Use of cloth or leather gloves while working with animals				
No	41	21	3.6	-
	74.6%	91.3%	[0.7, 17.0]	
Yes	14	2	Ref.	-
	25.5%	8.7%		

Note: Microneutralization assay (MN) was performed against swine influenza virus A/SW/Iowa/73(H1N1). Neutralizing antibody titres were defined as the reciprocal of the highest dilution of serum samples that achieved at least 50% neutralization. Serum MN titre ≥1:20 was used as cut-off for indicating past infection.

Data are number (%) of subjects, unless otherwise indicated. Two-sided Chi-square tests or Fisher's Exact Test were used for dichotomous data analysis. Ref. = Referent Group.

[a]Category was omitted as data were too sparse for the model to converge.

[b]Respiratory illness was defined as fever and cough or sore throat.

of influenza A virus infection in the work or household environment within the last 12 months and more than 5 years of swine work were at higher risk of both molecular and serological detection of influenza A virus. Bivariate analysis of age and usage of gloves showed that age group (25–32 years) was significantly associated with decreased use of gloves when handling animals ($p < .018$). This may explain the reason, in our study, younger workers were more likely to have evidence of exposure to influenza A virus.

Use of gloves and personal protective equipment (PPE) have been previously reported to reduce the risk of zoonotic influenza A virus infection (Morgan et al., 2009; Ramirez et al., 2006). Our study found sparse use of PPE by swine workers. This highlights the need for targeted educational programmes or work to encourage the use of PPE to increase worker safety and farm biosecurity.

Our study lacks detailed virus characterization data, which may have provided more insight into the circulating virus strains. Our attempt to isolate live influenza A virus in cell culture was unsuccessful, which could be attributed to the low viral load in our samples. In a study evaluating different isolation methods for avian influenza, Moresco et al. (2012) found that samples with Ct values between 29.4 and 37 had a low virus isolation rate in MDCK (31.5%).

Additionally, our analysis was limited by the small sample size and the lack of comparison to control subjects. However, since our risk factors analysis is relevant and supported by previous studies, this limitation does not seem to have significantly impacted our study.

Overall, our findings demonstrate the need for periodic surveillance for influenza viruses and other possible zoonotic viruses among swine workers and their pigs. Researchers have argued that surveillance at the human–animal nexus is a more efficient and a less expensive approach to identify future pre-pandemic threats (Bailey et al., 2018; Gray & Abdelgadir, 2021). Based upon these findings, we urge public health officials in South Africa to conduct such surveillance. We also recommending that public health officials offer seasonal influenza vaccines to swine workers as well as educational programmes to help them better understand how to prevent zoonotic influenza transmission.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

AUTHORS CONTRIBUTIONS

GCG and MEEZ conceived and designed the study. MEEZ, and LKB collected samples and data. MEEZ facilitated, coordinated and

supervised the study in the farms. MEEZ collected and entered participant and farm data. AA, MEEZ, LKB and ESB conducted the laboratory assays. MFD attempted to culture and characterize influenza A viruses. AA, LKB conducted the data analysis. AA, GCG, LKB and MEEZ wrote the manuscript. GCG, MEEZ, and AA provided technical expertise and guided manuscript development. All the authors reviewed the final version of the manuscript and agreed to its submission.

DISCLAIMER

No other person has any role in the study concept, study design, data collection, experimental work, data analysis, data interpretation or the decision to publish. Any use of commercial names, commercial diagnostic products or firm names is for descriptive purposes only and does not imply endorsement by the funding agencies, the National Institutes of Health or the United States Government. Any opinions, findings and conclusion or recommendations expressed in this material are those of the authors and do not necessarily reflect the view of the National Institutes of Health or the United States Government.

PARTICIPANTS CONSENT STATEMENT

This study received ethical approval from the University of KwaZulu-Natal Biomedical Research Ethics Committee (BREC) (BE632/17) and the Duke University Health System Institutional Review Board (Pro00089731). Informed consent was obtained from each participant.

DATA AVAILABILITY STATEMENT

Additional data supporting study findings are available in the supplementary material. Sequence data generated in the present study were deposited in Genbank, National Library of Medicine, NCBI under accession numbers MZ803005, MZ803006, and MZ853188. In addition, sequences were deposited in Global initiative on sharing all influenza data (GISAID; https://www.gisaid.org/) under accession numbers EPI1886087, EPI1886088, EPI1886089, EPI1886090, EPI1886091, EPI1886092, EPI1886093, EPI1886094, EPI1886095, EPI1886096, EPI1886097, and EPI1886098.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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