



Exposure of domestic swine to influenza A viruses in Ghana suggests unidirectional, reverse zoonotic transmission at the human–animal interface

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Abstract

Influenza A viruses (IAVs) have both zoonotic and anthroponotic potential and are of public and veterinary importance. Swine are intermediate hosts and ‘mixing vessels’ for generating reassortants, progenies of which may harbour pandemic propensity. Swine handlers are at the highest risk of becoming infected with IAVs from swine but there is little information on the ecology of IAVs at the human–animal interface in Africa. We analysed and characterized nasal and throat swabs from swine and farmers respectively, for IAVs using RT-qPCR, from swine farms in the Ashanti region, Ghana. Sera were also analysed for IAVs antibodies and serotyped using ELISA and HI assays. IAV was detected in 1.4% ($n = 17/1,200$) and 2.0% ($n = 2/99$) of swine and farmers samples, respectively. Viral subtypes H3N2 and H1N1pdm09 were found in human samples. All virus-positive swine samples were subtyped as H1N1pdm09 phylogenetically clustering closely with H1N1pdm09 that circulated among humans during the study period. Phenotypic markers that confer sensitivity to Oseltamivir were found. Serological prevalence of IAVs in swine and farmers by ELISA was 3.2% ($n = 38/1,200$) and 18.2% ($n = 18/99$), respectively. Human H1N1pdm09 and H3N2 antibodies were found in both swine and farmers sera. Indigenous swine influenza A viruses and/or antibodies were not detected in swine or farmers samples. Majority (98%, $n = 147/150$) of farmers reported of not wearing surgical mask and few (4%, $n = 6$) reported to wear gloves when working. Most ($n = 74$, 87.7%) farmers reported of working on the farm when experiencing influenza-like illness. Poor husbandry and biosafety practices of farmers could facilitate virus transmission across the human–swine interface. Farmers should be educated on the importance of good farm practices to mitigate influenza transmission at the human–animal interface.

KEYWORDS

humans, Influenza A virus, phylogeny, prevalence, swine

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1 | INTRODUCTION

Influenza A viruses (IAVs) are members of the Orthomyxoviridae family with eight gene segments that code for at least 10 proteins. Two viral surface glycoproteins, haemagglutinin (HA) and neuraminidase (NA) are used to classify the virus into subtypes (Hoffmann, Hoffmann, Henritzi, Beer, & Harder, 2016). Many avian and several mammalian species are susceptible to IAV infections with varying degrees of morbidity and mortality. Among humans, annual influenza morbidity and associated mortality are estimated at 3–5 million and 250,000–500,000, respectively (Talla Nzussou et al., 2017). In swine, IAV infections cause mild respiratory disease which may predispose to opportunistic bacterial infections causing reduced weight gain during the fattening period and leading to substantial losses in pork production. Fever-induced abortions and infertilities in sows may add to economic losses (Harder et al., 2013).

Several host restriction barriers limit spread and transmission of avian and mammalian viruses. Such barriers include recognition of different sialic acid (SA) receptors, α -2,3 and α -2,6 expressed on host cell surfaces of avian and human respiratory epithelia, respectively, to initiate infections. Inter-species transmission can occasionally occur, particularly in species such as quails and swine which carry both types of sialic receptors (Yang et al., 2016). The segmented genome of the virus enables reassortment (eventually leading to antigenic shift) when a cell is simultaneously infected with more than one IAV leading to progenies with mixed parental segments. Accumulating point mutations (eventually leading to antigenic drift) are based on error-prone genome replication. Both processes increase genetic diversity fostering also viral adaptation to new hosts. Ultimately, this may result in the emergence of novel variants with zoonotic and even pandemic propensities (Taubenberger & Kash, 2010).

Swine play an important role in the ecology and evolution of IAVs. They can be naturally infected with IAVs of both avian and human origin because they express both SA receptor types in their respiratory tract, making them possible intermediate hosts and appropriate 'mixing vessels' for generating reassortants (Ito et al., 1998; Yang et al., 2016). Therefore, enzootic swine influenza A viruses (swIAVs) are not only of veterinary, but also of potential public health concerns. In addition to economic losses to farmers, swIAVs may sporadically be transmitted to humans causing sub-clinical or mild respiratory symptoms indistinguishable from that of seasonal influenza, but can harbour pandemic propensity as well (Corzo et al., 2013; Epperson et al., 2013; Kong et al., 2015; Shinde et al., 2009). Enzootic swIAVs of subtypes H1N1, H1N2 and H3N2 of different origins (avian or human) and genetic constellations circulate at variable frequencies in swine populations worldwide with the occasional emergence of other subtypes restricted temporally and geographically (Corzo et al., 2013; Harder et al., 2013; Zhu et al., 2011). For instance, H1N1 that circulates in European swine is a purely avian-derived virus transmitted into the swine population in 1979, while in North America, several H1N1 virus

Impacts

- Swine in Ghana are infected and exposed to human-type influenza A viruses, H1N1pdm09 and H3N2 but not swine influenza A viruses (swIAVs).
- Swine workers are infected with and exposed to human seasonal IAVs but not swine IAVs.
- Poor biosafety practices of farmers are likely to facilitate virus transmission across the human–swine interface. This calls for implementation of suitable preventive practices such as enforcement of sick leave when farmers are experiencing influenza-like illness and wearing of surgical mask when working to prevent inter-species transmission of IAVs.

lineages of different origins are circulating (Henritzi et al., 2016). The transmission of the 2009 human pandemic IAV (H1N1pdm09) in a reverse zoonotic mode from human into swine populations has further led to the emergence of a plethora of unique reassortants in swine, some of which have been characterized to have zoonotic and prepandemic propensity (Fobian et al., 2015; Henritzi et al., 2016).

Swine populations in Africa constitute just 2.9% of the global swine population but are important sources of livelihood, employment and animal protein for many people (FAOSTAT, 2008). The animals are kept often at household backyards, roaming freely in rural communities. In urban and peri-urban communities where human populations are comparatively dense, relatively large numbers of swine are reared under semi-intensive and/or intensive systems with low or minimal biosecurity, creating a suitable environment for bi-directional transmission of IAVs at the swine–human interface and increasing the risk of zoonotic infections particularly for occupationally exposed individuals (Kirunda et al., 2014; Larison et al., 2014). Despite the zoonotic and prepandemic propensity of some swIAVs, knowledge of circulating viral subtypes in swine populations and exposed human individuals is scanty and even non-existent in many African countries (Meseko, Olaleye, Capua, & Cattoli, 2014). Quite recently, human influenza viruses have been detected in swine and symptomatic swine handlers in Ghana (Adeola, Olugasa, & Emikpe, 2015, 2016; Adeola, Olugasa, Emikpe, & Folitse, 2019), but there is no information on enzootic swIAVs and the molecular properties of these viruses in swine populations in the country. Since such information is important in developing control measures to minimize public health threats and further contribute to a better panzootic and pandemic preparedness, we conducted a cross-sectional study to identify and genetically characterize IAVs circulating between swine and swine farmers (hereafter referred to as farmers) in Ghana and further assessed farmers' attitude and husbandry practices that could facilitate inter-species transmission at the human–swine interface.

2 | MATERIALS AND METHOD

2.1 | Ethical consideration

Ethical approval for the study was obtained from the ethics committees of the Council for Scientific and Industrial Research (RPN 001/CSIR-IACUC/2016), Ghana and Ärztekammer Hamburg (PV5296), German.

2.2 | Study area and design

The study was carried out in the Ashanti region of Ghana, which lies in the forest belt of the southern part of the country and covers 10.2% of the national land area of 238,539 km². The region has a bimodal rainfall made up of major and minor rainy seasons and a dry season that is characterized by lower temperature and low humidity. The average annual rainfall is about 1,270 mm, and the average daily temperature is 27°C. The Ashanti region has the highest human and pig population (19.4% and 11.1%, respectively, of national total) in the country (Ghana Statistical Service, 2013, Nyanteng, Takyi, Lawford, Acheamfuor, Nii, & Tawiah, 2013). A list of farmers in the region was obtained from the Veterinary office of the Ministry of Food and Agriculture. A visit was made to the farms with the help of relevant district Agriculture officers and the study explained to the farmers. The snowball technique was also employed to include farmers who were not on the list. Eligibility criteria for enrolling a farm were the availability of weaners and/or growers on the farm at the time of visit and willingness of farmer to allow samples to be taken from the animals. For farms that met the above criteria, an informed consent was obtained from the farmer prior to sample collection.

For farms that were enrolled, individuals present on the farm and additionally regularly perform any swine-related activity on the farm such as cleaning the stys, serving feed and water, assisting in treating sick swine or slaughtering of swine, were invited to participate in a structured interview questionnaire. Farmers were again invited to provide biological samples (blood and throat swab) for laboratory analysis. Informed consent was obtained from each participant for the above purposes.

Sample collection for this active cross-sectional study was conducted between April–July 2016 (major rainy season) and December 2016–February 2017 (dry season). All farms were visited only once during the entire study period. The sample size was estimated using Epi-Tools (<http://epitools.ausvet.com.au>) assuming an influenza prevalence of 10% for swine and a confidence of 0.95 (Adeola et al., 2015).

2.3 | Nasal swab and blood collection from swine

On each farm, swine aged 6–24 weeks were identified by convenience sampling. For farms with population up to 20, all animals were sampled and for those with higher population (>20), the number sampled was determined to achieve an estimated prevalence of 10%. A flock swab (Copan Group) was inserted 2–3 inches into the back of one nostril

while being rotated in a clockwise manner to obtain epithelial cells. Swab was removed, inserted into the other nostril and the process repeated. Swab was placed in a 1 ml viral transport medium (VTM) previously prepared according to the protocol of (Eisfeld, Neumann, & Kawaoka, 2014). Tubes were immediately placed in a cool box containing ice. Blood (2 ml) was drawn from the medial caudal vein of each swine after nasal swabbing, using a sterile syringe and needle, into a vacutainer without anti-coagulant and placed on racks. All animals were apparent healthy at the time of sampling and none had been vaccinated against IAV as this is not a practice in Ghana.

2.4 | Throat swab and blood collection from farmers

The tongue of the farmer was depressed with a depressor and a flock swab (Copan Group) used to swab vigorously (about four times) the posterior of the pharynx to collect epithelial cells. Swab was immediately put in 1 ml VTM and placed on ice. Venous blood (2 ml) was collected from the farmer into a vacutainer without anti-coagulant. None of the farmers had been vaccinated against IAVs. All tubes were labelled with identification codes generated prior to sampling.

2.5 | Questionnaire administration

A questionnaire with open- and close-ended questions was administered to the farmers whose animals were sampled irrespective of whether the farmers offered test samples themselves or not. The interview was done face-to-face in English and where necessary the local dialect 'Twi' was used (which was translated into English for analytical purposes). The questionnaire included sections on worker demographics, swine husbandry practices, farmers' attitude to work when sick and knowledge of swine zoonoses.

2.6 | Sample transport, initial laboratory processing and storage

All samples were transported to Kumasi Centre for Collaborative Research in Tropical Medicine (KCCR), Kumasi, Ghana within 5 hr of collection. Swabs were transported on ice and blood on racks at ambient temperature. Upon arrival in the laboratory, swabs were immediately stored at –80°C until further analysis. Sera were centrifuged at 730 g for 15 min to separate the red blood cells. Sera were individually harvested into pre-labelled centrifuge tubes and stored at –20°C until needed.

2.7 | Initial molecular screening of samples using conventional PCR

Human and animal samples were processed in different institutions (Animal Research Institute, for animal samples, and KCCR for

human samples), but following the same procedure. Swine swabs were pooled in maximum of fives according to farms. Human swabs were pooled in twos or fives. RNA was isolated from all pools using QIAamp viral RNA mini kit (Qiagen). The manufacturer's instructions were followed. RNA was eluted in 50 μ l of Qiagen AVE buffer and stored at -70°C . All extracted RNAs were tested for matrix (M)-specific gene common to all IAVs in one-step reverse transcriptase (RT), RT-PCR (Superscript III one-step RT-PCR kit; Invitrogen) or two-step RT-PCR. In the two-step process, cDNA was synthesized with random hexamers using the RevertAid First strand cDNA synthesis kit (Thermo Scientific) and following the manufacturer's instructions. From the cDNA, 5 μ l was used as a template for the PCR. Primers and protocol used were according to (Eisfeld et al., 2014). All PCR products were resolved on a 1.5% agarose gel stained with ethidium bromide. Appropriate controls were added to each batch of extraction and PCR to check for possible cross contamination.

2.8 | Identification of individual positive samples by real-time RT-PCR (Germany)

Individual samples that constituted an M-specific-positive-pool were selected and shipped on dry ice to Germany (Bernhard Nocht Institute for Tropical Medicine and the Friedrich Loeffler Institute) for further analysis. RNA was isolated from all samples individually using the Qiagen viral RNA kit (Qiagen). To evaluate the efficiency of RNA isolation and inhibition-free transcription and amplification of viral RNA, 3 μ l of In-type internal control (IC) RNA (Qiagen) was added to each sample prior to isolation. RNA was eluted in 30 μ l of elution buffer. Isolated RNAs were tested for IAV matrix-specific gene fragments by a generic real-time RT-PCR using the AgPath-ID One-Step RT-PCR kit (Applied biosystems) in a 25 μ l reaction volume. Samples with Cq values <40 were considered positive. Primers, probes and protocols used were as described elsewhere (Harder et al., 2013).

2.9 | HA and NA subtyping by real-time RT-PCR

All M-gene-specific RNA-positive samples (human and swine) were subjected to two different multiplex real-time RT-PCRs for enzootic European swIAVs and seasonal IAVs. The tetraplex HA assay targeted H1av, swH3, seasonal pre 2009 H1hu and H1pdm. The combined tetraplex HA/NA assay detected H3hu, N1av, N2, and N1pdm. Primers and protocols were as described elsewhere (Henritzi et al., 2016).

2.10 | Amplification, sequencing and phylogenetic analyses of selected swIAVs

Eight swIAV positive samples with Cq values less than 25 were selected for amplification and genome sequencing. The HA and NA

of all eight samples were fully sequenced. Due to low volumes of samples, the six 'internal' gene segments of three samples were not sequenced. The polymerase basic 1 (PB1) segment of two other samples was also not sequenced for the same reason. The one-step SuperScript III amplification kit was used for the amplification and sequencing according to the manufacturer's instructions. For the full-length amplification of HA, NA, nucleoprotein (NP), matrix (M) and non-structural (NS) genes, primers described by Hoffmann, Stech, Guan, Webster, and Perez (2001) were used. For polymerase basic 1 (PB1), polymerase basic 2 (PB2) and polymerase acidic (PA) gene segments, primers described by Li et al. (2007) were used. All amplicons were separated by agarose gel electrophoresis and purified with Qiagen gel purification kit. Purified products were used for Sanger sequencing in both forward and reserve directions using the BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems Inc) according to the manufacturer's instructions. Sequence searches of the HA and NA were performed using Basic Local Alignment Search Tool in the NCBI database (<https://blast.ncbi.nlm.nih.gov>). Sequences were aligned using the online multiple sequence alignment program, MAFFT version 7 (<https://mafft.cbrc.jp/alignment/software>). Phylogenetic analyses of full-length HA gene sequences were performed by maximum likelihood analyses (IQTree software v. 1.6.).

2.11 | Serological analysis

Sera from swine and farmers were evaluated for antibodies against influenza A anti-NP protein by the generic ID-Screen IA antibody competitive multispecies ELISA kit (IDvet; 91% and 87% sensitivity and specificity for antibodies against the NP of the human pandemic H1pdm IAV, Tse et al., 2012) and the Serion classic influenza A IgG ELISA kit (Institut Virion/Serion; 92.3% and 90.1% sensitivity and specificity for detection of human antibodies in serum against conserved NP of IAVs according to the kit manual (update #123.17). The manufacturer's instructions were followed for testing and data interpretation. ELISA positive sera were serotyped by haemagglutination inhibition (HI) assay using reference European swine influenza viruses A/swine/R1738/Germany/2010 (H1avN1av) and A/swine/Germany/R96/2011(H3N2) and human influenza viruses A/Germany/R26/2010 (H1N1pdm) and A/Germany/R72/2013 (H3N2).

2.12 | Statistical analysis

Frequency and percentages were calculated for categorical variables. Medians and interquartile ranges were computed for continuous variables. Point prevalence of IAVs with their 95% confidence interval (CI) was separately calculated as a proportion of positive samples detected in swabs or sera in swine or farmers. Data were analysed with Stata (Version 14; StataCorp).

3 | RESULTS

3.1 | Farm and farmer characteristics

Eighty-seven swine farms were visited during the study period. Of these, nearly equal numbers (45 vs. 42) of farms were visited in the rain and dry season, respectively. Swine were exclusively confined in pens largely built with blocks or wood and roofed with metal sheets or palm branches. Depending on the type and number of swine on a farm, pens for weaners, growers, sows, sows with litter and boar were physically separated. The number of pens per farm ranged from one to more than three. Swine production was largely small-scale (defined here as a population of ≤ 200 swine per farm) with the majority ($n = 54$, 62.07%) having 50–200 swine. Most farms ($n = 61$, 70.11%) sold matured swine (growers, sows or boars) of any quantity alive at the farm gate. An appreciable number of farms ($n = 26$, 29.2%) however used both live sales and slaughtering options to sell their matured animals. Of the farms that offered slaughtering services, nearly all slaughtering ($n = 25$, 96.15%) was performed at the farm premises. Farm workers carried out slaughtering on majority ($n = 24$, 93.31%) of these farms. The number of workers on the farm varied from one to more than three with most farms ($n = 51$, 58.6%) having 2–3 persons (Table 1).

One hundred and fifty farmers answered the questionnaire. The majority ($n = 131$, 87.33%) were male. The median age was 36.5 years (IQR = 25–45 years). Most farmers ($n = 140$, 93.33%) were formally educated and almost half ($n = 65$, 43.33%) had 'Junior high' as the highest level of education. Farmers had worked from less than 1 year to more than 5 years on the farms with about half of them ($n = 76$, 50.67%) working 1–5 years on the present farm (Table 2).

TABLE 1 Characteristics of swine farms

Parameter	N (%)
Herd size	
<50	21 (24.14)
50–200	54 (62.07)
>200	12 (13.79)
Number of employees	
1	14 (16.09)
2–3	51 (58.62)
>3	22 (25.29)
Method of selling matured swine	
Live at farm gate	61 (70.11)
Slaughter	1 (1.15)
Live and slaughter	25 (28.74)
Place of slaughter	
On-farm	25 (96.15)
Abattoir	1 (3.85)
Slaughter personnel on farm	
Farm worker	24 (96.00)
Casual worker	1 (4.00)

Almost all farmers ($n = 143$, 95.33%) changed their clothes to dedicated farm clothing before attending to swine on the farm. Few ($n = 6$, 4.0%) farmers wore gloves and nearly all ($n = 147$, 98.0%) did not wear a surgical mask when working. All farmers washed their hands after attending to the animals (Table 2). More than half ($n = 81$, 54.00%) of the farmers reported of experiencing symptoms of ILI (ILI, is here defined as fever (body temperature higher than 37°C), cough, sore throat, headache and weakness) at least eight weeks before but not at the time of sampling. Farmers reported of often experiencing ILI in nearly equal proportions during the rain or dry season ($n = 33$, 40.7% vs. $n = 29$, 35.8%, respectively) with an appreciable proportion ($n = 19$, 23.5%) experiencing ILI equally in both seasons. Majority ($n = 74$, 87.7%) of farmers reported of working on the swine farm when experiencing ILI.

TABLE 2 Demographics and safety practices of farmers

Variable	N (%)
Sex	
Male	131 (87.33)
Female	19 (12.67)
Age (years)	
<29	46 (30.67)
29–39	39 (26.00)
40–49	36 (24.00)
50–59	23 (15.33)
60–69	5 (3.33)
≥ 70	1 (0.67)
Education	
No formal education	10 (6.67)
Primary	15 (10.00)
Junior secondary	65 (43.33)
Senior secondary	28 (18.67)
Tertiary	32 (21.33)
Length of stay on present farm	
<1 year	21 (14.00)
1–5 years	76 (50.67)
>5 years	53 (35.33)
Wearing of dedicated farm clothing	
Yes	143 (95.33)
No	7 (4.67)
Wearing of gloves	
Yes	6 (4.00)
No	144 (96.00)
Wearing of nose mask	
Yes	3 (2.00)
No	147 (98.00)
Washing of hands after farm work	
Yes	150 (100.00)
No	0 (0.00)

More than half ($n = 78$, 52.00%) of farmers were aware that they could become infected with diseases from swine but the majority ($n = 54$, 69.2%) could not correctly name any disease. swIAVs were the only pathogen mentioned by a substantial proportion ($n = 24$, 30.8%) of farmers as swine zoonosis. More than half ($n = 57$, 73.08%) of the farmers who were aware of possibly becoming infected with pathogens from swine correctly named at least one on-farm attitude or practice that could contribute to minimizing farmer's risk of zoonotic infections on farms. In total, farmers identified ten different measures that can be broadly distinguished into two categories: (a) improvement of farm and personal hygiene, and (b) usage of personal protective equipment (PPE) when working on the farm. The most frequently mentioned preventive measure was the wearing of a surgical mask. The practices of washing hands after farm work and properly disposing of dead swine were among the preventive measures that were least often mentioned (Figure 1).

3.2 | Serological and virological examination of farmers' samples

Throat swabs and blood were analysed for IAV and antibodies respectively, from 99 (66.0%) asymptomatic farmers from 44 (50.5%) farms during the two seasons. Two swabs collected from two farmers from different farms in the rainy season tested positive for IAV. Human seasonal influenza viral subtypes H3N2 and H1N1pdm09 were each detected. IAV antibodies were detected in 18 of 99 human sera in both seasons. Antibodies to both human H1N1pdm09 and H3N2 were detected in the farmers' sera with positive titres ranging from ≥ 40 to $\geq 1,280$. RNA of swIAVs by RT-qPCR and/or swIAV-specific antibodies by HI, respectively, was not detected in

the farmers' samples. The overall viral prevalence of IAV by real-time RT-PCR in farmers was 2.0% (95% CI = 0.0–4.8) and overall seroprevalence by ELISA was 18.2% (95% CI = 11.1–27.2).

3.3 | Serological and virological examination of swine samples

Influenza A viruses antibodies were detected in 38 of 1,200 (3.2%) swine sera analysed. Although few sera reacted with European swIAVs, the majority of sera revealed HI antibody titres against human-like H3 and H1N1pdm09 viruses with titres ranging from ≥ 40 to $\geq 1,280$ (Table S1).

A total of 1,200 nasal swabs and 1,200 sera were each collected from apparent healthy swine from the 87 swine farms during the two seasons. Half of the samples were collected in the rainy season and the other half in the dry season. A proportion of 1.4% ($n = 17$) of the swine swabs collected was finally confirmed to be positive for IAV by real-time RT-PCR. These positives were detected in 8.0% (7/87) of the farms. The number of viral RNA-positive swabs varied between the two seasons. A substantial proportion of 2.0% (12/600) samples collected in the rainy season ($n = 5/45$, 11.1% of farms) and 0.8% (5/600) of dry season samples ($n = 2/42$, 4.8% of farms) tested positive in the M-specific generic IAV RT-qPCR (Table 3). Viral detection in the rain season was more than twice as high as in the dry season (Prevalence ratio = 2.5, 95% CI = 0.89–7.05).

All the 17 swine IAVs were subtyped as H1N1pdm09. A query blast of sequenced samples returned similar viruses that circulated in humans in Africa, Europe and North America in 2016 and 2017 as well as from swine in other parts of the world. The HA of all the swine origin H1N1pdm09 from our study was assigned to clade 1A.3.3.2

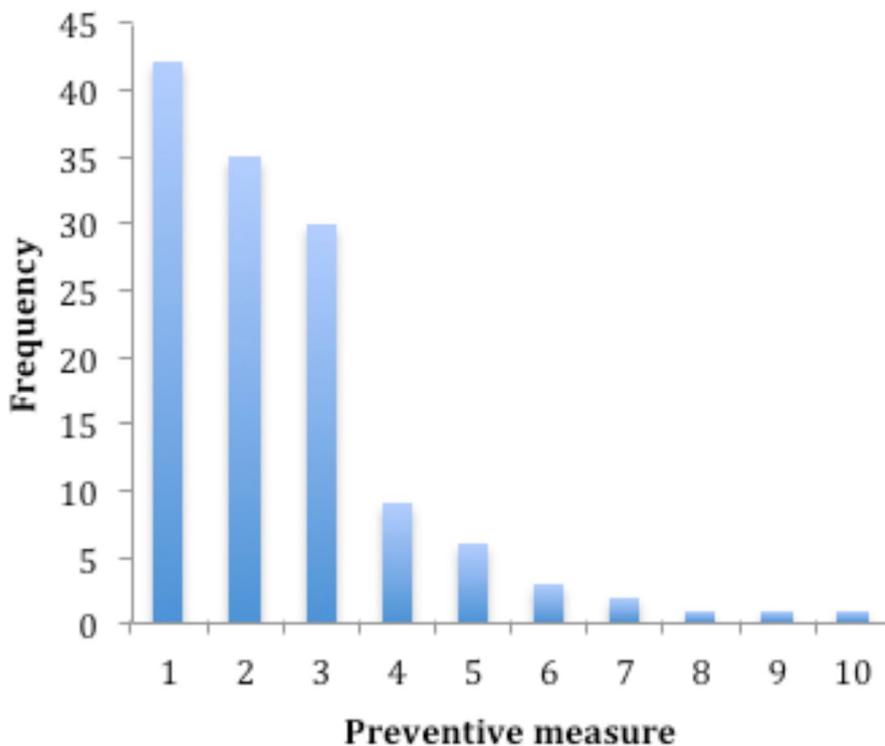


FIGURE 1 Preventive measures against swine zoonoses as mentioned by farmers. Key: 1 = Wear surgical mask, 2 = Wear protective footwear, 3 = Wear gloves, 4 = Wear dedicated farm clothing when working, 5 = Stay away from swine when sick, 6 = Bath after farm work, 7 = Wear goggles, 8 = Wash farm clothing regularly, 9 = Wash hands after farm work, 10 = Proper burial of dead swine [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 3 Molecular prevalence of IAV detected in swine and swine farms

Season	Influenza A virus detected in swine nasal swabs by real-time RT-PCR		
	No. of positives/total samples tested	Prevalence % (95% CI)	No. of positive farms/total farms
Rain	12/600	2.0 (0.9–3.1)	5/45
Dry	5/600	0.8 (0.1–1.6)	2/42
Total	17/1,200	1.4 (0.7–2.1)	7/87

according to the global swine H1 clade classification scheme (Zhang et al., 2017). By topology, the swine origin H1N1pdm09 viruses clustered into three sub-groups. Each of the subgroup held at least one H1N1pdm09 viral sequence obtained from a human host in Ghana in 2016 and 2017. Interestingly, all the swine viruses from the present study were also closely related to some human and swine origin H1N1pdm09 viruses from Europe and America deposited in the Global initiative on sharing all influenza data (GISAID) but highly distant from all other H1N1pdm09 viruses identified in swine in Africa deposited in GISAID as at September, 2019 (Figure 2).

For three samples, the full genome of H1N1pdm09 was established. All segments were derived from human H1pdm IAV as shown by a very high homology (>99.5%). The N1pdm09 neuraminidases from our study had phenotypic markers that conferred sensitivity to the antiviral Oseltamivir. Other markers of internal gene segments suggested decreased virulence and replication in mice (Table S2). Unique amino acid substitutions with unknown functions were found at position 256D and 591R in PB2 and at position 357T in PA.

4 | DISCUSSION

We investigated IAVs circulating in swine and swine farmers in a major pork-producing region in Ghana. The cross-sectional active surveillance showed that IAV was shed in nasal secretions of a minority (1.4%) of apparent healthy weaners and growers kept on farms in the region during the period of study. Viral spread in swineherds in the region is wide as 23% (20 of 87) of the farms had at least one sample positive for viral antigen or specific antibody indicating current or previous infections, respectively. Viral subtype H1N1pdm09 and corresponding antibodies and human-like H3-specific antibody were detected in swine. An analysis of the full genome of the H1N1pdm09 isolates from swine indicated that the virus was highly similar to H1N1pdm09 that circulated among humans in Ghana between 2016 and 2017. Viral isolation attempts failed. This prevented us from having enough material to sequence all gene segments of influenza positives identified from swine in the study. Swine in the region were thus infected with human-like influenza A viruses. No enzootic swIAVs known to circulate in swine in other parts of the world were detected. Farmers were infected and exposed to seasonal human IAVs (H1N1pdm09 and H3N2) but not to viruses of swine origin. All influenza positive farms (virus or antibody) had at least a farmer who tested positive for IAV by either PCR or serology.

Four human, influenza pandemics have been observed globally since 1918, each of which has caused substantial morbidity and mortality with the 1918 Spanish flu causing more than 50 million deaths worldwide. Animals, particularly birds and swine, have played key roles in the evolution and introduction of these pandemic viruses into human populations. Characteristically after a pandemic, the virus in question assumes circulation in humans as seasonal influenza causing annual (seasonal) epidemics in the temperate regions of the northern and southern hemisphere. With the exception of the so-called Asian H2N2 IAV, all pandemic viruses found their way into swine populations by reverse zoonotic transmission where they evolved into lineages different from that of humans. The high potential of swine as a 'mixing-vessel' for generating IAV reassortants was confirmed in 2009 by the emergence of the H1N1pdm09 virus which carries essential genetic elements derived from IAV of avian, swine and human origin (Meseko, Heidari, Odaibo, & Olaleye, 2019). This virus now co-circulates with H3N2 as seasonal IAV in the general human population.

Since the emergence of this virus, swine populations across the world including countries such as Australia, Norway or Iceland that were previously free of swIAVs have reported of swine infections with H1N1pdm09 due to reverse zoonotic transmissions, showing high susceptibility of swine to this pandemic virus (Deng et al., 2012; Forberg, Hauge, Gjerset, Hungnes, & Kilander, 2013). The first report of swine infections with H1N1pdm09 originated from Canada; few weeks after, it was detected in humans. In Europe, H1N1pdm09 and its reassortants (with endemic swIAVs) constituted up to 17%, of all the IAVs that were detected in European swine populations between 2010 and 2013 (Simon et al., 2014).

The H1N1pdm09 was the only viral subtype detected in the Ghanaian swine populations studied here in both the rainy and dry season with a statistically insignificant increase of detection in the rainy season. Previous exposure to human-like H3 was also detected (serologically), showing that human seasonal IAV has been transmitted to swine in Ghana throughout the year. The infection of swine with H1N1pdm09 appears to be due to introductions of the virus from humans to swine. However, the available data do not allow us to define the time point of virus introduction, and, in principle, (limited) lateral spread from swine to swine cannot be ruled out. Swine in the region are not vaccinated against IAV, and therefore, the IAV antibodies detected are not vaccine-derived. Phylogenetic topologies of swine and human-derived H1pdm HA sequences corroborated this finding (Figure 2) indicating at least one human-derived sequence closer to the root of three distinct



FIGURE 2 Phylogenetic relationship between HAs of H1N1pdm09 viruses identified in swine population in Ghana in our study (red), H1N1 from humans in Ghana (blue and green) and H1N1 from humans and swine from Africa (other than Ghana), Europe and North America (black) [Colour figure can be viewed at wileyonlinelibrary.com]

sub-groups holding swIAV H1pdm. Furthermore, these swine H1N1pdm09 clustered distantly from other H1N1pdm09 detected in swine in other African countries. The rearing of swine in urban and peri-urban communities where human populations are equally high could facilitate transmission of human viruses to swine. Additionally, farmers who are frequently in direct contact with the animals barely wore surgical mask while working and do not absent themselves from swine when experiencing ILI. These poor biosecurity practices of farmers possibly contribute to an increase in transmission of viruses from farmers to swine. In the event of emergence of zoonotic pathogens from swine, the same practices will increase farmers' risk of infection.

Our serological prevalence of IAV in swine was low compared with that reported earlier in the same region (Adeola et al., 2015, 2016). This difference could be the smaller sample size (50 and 132) investigated by the previous authors compared with the larger sample size (1,200) used in the present study. In addition, swine influenza

isolates from Germany had to be used for the HI rather than isolates from swine in the region. This was because such isolate was none existent in the country. The use of German rather than Ghana isolates could have reduced the sensitivity of the HI test leading to an underestimation of the rate of HI seropositives. Previous studies also focused on swine slaughtered at the abattoir which only few farmers in the region accessed and therefore infected swine could have come from fewer farms in the region compared with the larger farm coverage of this study which may have established the true prevalence of the infection in the region at greater reliability. The viral prevalence in swine compares favourably with the 0.7% reported in Kenya (Munyua et al., 2018) but is higher than that reported in Togo, Benin and Cote d'Ivoire in studies of similar sample sizes (Couacy-Hymann et al., 2012).

Reports from other African countries such as Nigeria, Togo, Kenya and Cameroon also indicated that H1N1pdm09 circulated in several swine populations raised under extensive,

semi-intensive and intensive systems of production and is often the only subtype identified (Ducatez, Awoume, & Webby, 2015; Meseko, Odaibo, & Olaleye, 2014; Njabo et al., 2012; Osoro et al., 2019). The dominance of this highly transmissible human influenza virus in swine populations in Ghana and other African countries is of major public health concern. In Nigeria where H1N1pdm09 was detected to circulate among swine sub-clinically, highly pathogenic avian influenza H5N1 has also been identified in swine (Meseko et al., 2018).

Human influenza studies in Ghana have largely focused on children presenting with ILI, acute and/or severe acute respiratory infections at hospitals with increased detection in the rainy season (Hogan et al., 2017; Jones et al., 2016). We show here that a small proportion of apparent healthy adults on farms in the study region is actively infected with influenza viruses with higher detection rate in the rainy season and at viral prevalence similar (2.1%) to that reported among asymptomatic adults (Annan et al., 2015).

The co-circulation of H1N1pdm09, H5N1 and other human-like IAV such as H3N2 in swine and of H9N2 avian influenza viruses in poultry (Awuni et al., 2019; Ayim-Akonor, May, Ralf, Harder, & Mertens, 2019) increases the possibility of generating reassortant viruses in swine in the region. The emergence of such a reassortant with zoonotic propensity may pose significant health risk particularly to the swine farmers. Intra- and inter-regional trade of live animals could also facilitate the dissemination of such virus to the general human population in the region. H1N1pdm09 has been circulating in many swine populations in Africa at least since July 2010 (Meseko et al., 2019) but genetic analysis of these viruses, both in our study and that from other African countries such as Togo, Kenya and Cameroon (Ducatez et al., 2015; Munyua et al., 2018; Njabo et al., 2012), indicate no reassortment with endemic swIAVs as seen in Europe and America (Corzo et al., 2013; Harder et al., 2013; Simon et al., 2014). Thus, there appears to be apparently little if any presence of endemic swIAVs in many African countries compared with countries in Europe, America and Asia. The low swine population density coupled with the different systems of raising swine in Africa (mainly extensive and semi-intensive) may largely account for this observation. Nevertheless, as more African countries intensify swine production, as a means of increasing animal protein sources, such swIAVs and their reassortants with human-like influenza viruses may become important pathogens in the region.

Swine farmers in the region were aware of basic biosafety measures that they could put in place to reduce their risk of zoonotic infections including swine influenza, but their actual husbandry and biosafety practices remain below acceptable levels. There is the need to continuously educate farmers on the public and veterinary importance of influenza at the human–animal interface. There is also the need to consider the possibility of introducing influenza vaccination among farmers especially in the rainy season as a measure to reduce reverse zoonotic transmission in the region, as influenza vaccination is not routinely administered in the general human or animal population in Ghana.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

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