Transmission of influenza A viruses at the human-animal interface in Ghana

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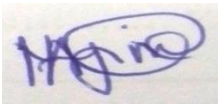
Dedication

This work is dedicated to my husband and children and all women who make the effort to combine family with higher academic excellence. It is extra difficult but totally achievable.

Eidesstattliche Versicherung

Declaration on oath

Hiermit erkläre ich as Eides statt, dass ich die vorliegende Dissertationsschrift selbst verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt habe
I hereby declare, on oath, that I have written the present dissertation by my own and have not used other than acknowledged resources and aids.



(Hamburg, 2020)

Zusammenfassung

Humane Infektionen mit Krankheiten tierischen Ursprungs sind global im Anstieg und Viren machen einen großen Bestandteil dieser Infektionen aus. Influenza A Viren (IAVs) rufen eine beträchtliche Krankheitslast und Sterblichkeit hervor mit gelegentlichen Pandemien beim Menschen bzw. Panzootien bei Tieren. Darüber hinaus hat das Virus das Potential, zoonotische und anthroponotische Infektionen hervorzurufen. Damit sind zirkulierende Viren an der Schnittstelle von Menschen und Tieren ein Anliegen des Veterinär- sowie des öffentlichen Gesundheitswesens, das einen One Health Ansatz für eine effiziente Kontrolle erforderlich macht.

In Ghana sind die Forschung sowie die Überwachungsprogramme von Influenza krankenhausbasiert und primär auf Kindern mit Influenza-ähnlichen Krankheiten oder akuter schwerer Atemwegserkrankungen fokussiert/ausgelegt. Überwachung von Tieren, insbesondere Geflügel, findet anlassorientiert, vermehrt während Ausbrüchen mit hochpathogenen Vogelgrippeviren (HPAIV) statt. Überwachung von Schweinen, die 'mixing vessels'/'Mischgefäße' zur Generierung von reassortierten Influenzaviren mit zoonotischen und pandemischen Eigenschaften sind, ist so gut wie nicht vorhanden. Landwirte sind einem hohen Risiko ausgesetzt, sich mit einem zoonotischen Influenzavirus zu infizieren, aber es gibt in Ghana kein Influenza-Überwachungsprogramm an der Schnittstelle von Menschen und Tieren. Diese Querschnittsstudie wurde durchgeführt, um grundlegende Informationen zur Influenzaübertragung an der Schnittstelle zwischen Landwirten und Tieren in Ghanas Ashanti Region zu erlangen. Die Ashanti Region ist die bevölkerungsreichste Region Ghanas, in der auch große Bestände von Geflügel und Schweinen gehalten werden. Die Studie untersucht ebenfalls die Tierhaltungspraktiken von Landwirten in der Region in Bezug auf das Risiko zoonotischer Infektionen.

Vom Geflügel wurden Tupfer aus Trachea und Kloake sowie Blut entnommen, von den Landwirten wurden Rachenabstriche durchgeführt und Blut entnommen, und von Schweinen wurden Nasenabstriche gemacht und Blut entnommen. Darüber hinaus wurde mit den Landwirten ein Fragebogen ausgefüllt. Alle Tupferproben wurden auf menschliche und tierische IAVs mithilfe von PCR untersucht und durch Sequenzierung charakterisiert. Serum wurde per ELISA auf Antikörper von Menschen, Vögeln und Schweinen untersucht und durch Hämagglutinationshemmungstest serotypisiert.

AIV wurde bei Geflügel mit einer Prävalenz von 0.2% nachgewiesen, mit einer höheren Detektion in Tupfern aus der Kloake (0.3%) als Tupfern aus der Trachea (0.1%). AIV

Antikörper wurden nicht nachgewiesen. Geflügelzüchter waren mit saisonalen IAVs und H1N1pdm09 infiziert, nachgewiesen mit einer Prävalenz von je 2% und 10.8%. Weder AIV noch Antikörper zu Vogel H5 und H7 wurden in Geflügelzüchtern nachgewiesen. Schweinezüchter waren nicht mit Schweinegrippeviren infiziert sondern mit saisonalen IAVs, H1N1pdm09 und H3N2, festgestellt mit einer Prävalenz von 2%. Serologisch wurden ebenfalls H1N1pdm09 und H3N2 Antikörper in Schweinezüchtern mit einer totalen Prävalenz von 18.2% nachgewiesen.

Schweine in der Region waren mit H1N1pdm09 infiziert, nachgewiesen mit einer Prävalenz von 1.4%. Phylogenetisch gruppierte sich das identifizierte Virus eng mit ähnlichen Viren, die beim Menschen in Ghana zirkulierten. Hohe Titer von humanen H1N1pdm09 und H3N2 Antikörpern wurden auch in Schweineserum nachgewiesen.

Die meisten Landwirte hatten eine Schulausbildung. Geflügelzüchter hatten ein gutes Wissen über Geflügelkrankheiten, aber nicht über Geflügelzoonosen. Ein hohes Bildungsniveau und lange Arbeitserfahrung verbesserten das Wissen der Landwirte hinsichtlich Geflügelkrankheiten und -zoonosen. Sowohl Geflügel- als auch Schweinezüchter waren sich der Möglichkeit bewusst, sich über Ihre Tiere mit deren Erregern anzustecken und waren sich auch mehrerer Biosicherheitsmaßnahmen bewusst, die das Risiko einer Zoonose minimieren könnten. Nichtsdestotrotz wurde eine fehlende Umsetzung dieser Maßnahmen wahrgenommen.

Infektionen von Schweinen mit humanen Influenzavieren geben Anlass zur Besorgnis über das mögliche Aufkommen von reassortierten Influenzaviren und erfordern eine kontinuierliche Überwachung an der Schnittstelle von Schweinen und Menschen. Schweinezüchter sollten geimpft werden um Influenza-Anthroponosen in der Ashanti Region zu verhindern.

Eine zukünftige Studie über die Gründe für die beobachtete Umsetzungslücke wird dabei helfen, passende Kontrollmaßnahmen zu entwickeln um das Risiko der Landwirte durch zoonotische Influenzainfektionen in der Ashanti Region zu minimieren.

Summary

Human infections with diseases of animal origins are on a global increase and viruses have been a major component of such new infections. Influenza A viruses (IAVs) causes substantial morbidity and mortality with occasional pandemics and panzootics in humans and animals respectively. In addition the virus has zoonotic and anthroponotic potential. Therefore, circulating viruses at the human-animal interface a veterinary and public health concern and requires an OneHealth approach in effective control.

In Ghana, influenza surveillance programmes and research are hospital-based and focused primarily on children with influenza-like illness and acute or severe acute respiratory infections. Surveillance in animals particularly poultry, is *adhoc*, intensified during outbreaks with high pathogenic avian influenza virus (HPAIV). Surveillance in swine, which are 'mixing vessels' for generating influenza reassortants with zoonotic and pandemic propensities are nearly non-existent. Farmers are at high-risk of becoming infected with zoonotic influenza viruses but there is no influenza surveillance programme at the human-animal interface in Ghana. The present cross-sectional study was undertaken to provide baseline information on influenza transmission at the farmer-animal interface in the Ashanti region of Ghana. The Ashanti region is the most populated region of Ghana and also large amounts of poultry and swine are kept there. The study further investigates the animal husbandry practices of animal farmers in the region as a risk of zoonotic infections.

From poultry, tracheal and cloacal swabs and blood were collected, nasal swabs and blood were collected from swine and throat swabs and blood were collected from poultry and swine farmers. from poultry kept intensively on farms. Throat swab and blood were collected from the farmers. Additionally, questionnaire was administered to the farmers. All swabs were analysed for human and animal IAVs using PCR and characterised by sequencing. Sera were analysed for human, avian and swine influenza antibodies by ELISA and serotyped by haemagglutination inhibition assay.

AIV was detected in poultry at a prevalence of 0.2% with higher detection in cloacal (0.3%) compared with tracheal (0.1%) swab. AIV antibodies were not detected. Poultry farmers were infected with and exposed to seasonal IAV H1N1pdm09, detected at a prevalence of 2% and 10.8% respectively. Neither AIV nor antibodies to avian H5 and H7 were detected in poultry farmers. Swine farmers were not infected with or exposed to swine IAV but were infected with seasonal IAVs, H1N1pdm09 and H3N2, detected at a prevalence of 2%. Serologically,

H1N1pdm09 and H3N2 antibodies were also detected in farmers sera at an overall seroprevalence of 18.2%.

Swine in the region were infected with H1N1pdm09, detected at a prevalence of 1.4%. Phylogenetically, the identified virus clustered closely with similar viruses that circulated in humans in Ghana. High titres of human H1N1pdm09 and H3N2 antibodies were also detected in swine sera.

Most animal farmers were formally educated. Poultry farmers had good knowledge about poultry diseases but not poultry zoonoses. A high educational level and longer work experience improved farmers' knowledge of poultry diseases and poultry zoonoses. Both poultry and swine farmers were aware of the possibility of becoming infected with pathogens from their animals and were also aware of several biosecurity and biosafety practices that could minimise their risk of zoonoses. However, an implementation gap was observed. Infections of swine with human influenza viruses raises concerns for the possible emergence of influenza reassortant viruses and calls for continued surveillance at the swine-human interface. Swine farmers should be vaccinated against influenza to reduce influenza anthroponoses in the Ashanti region. A further study to understand the reasons for the observed implementation gap will aid in the development of suitable control measures to minimise farmers' risk of animal influenza infections in the Ashanti region.

Abbreviations

ID	Infectious disease
ELISA	Enzyme-Linked Immunosorbent Assay (ELISA)
PCR	Polymerase Chain Reaction
CDC	Centre for Disease Control
WHO	World Health Organization
OIE	World Organisation for Animal Health
PI	Post infections
GDP	Gross Domestic Product
SSA	Sub-Saharan Africa
PI	Post infections
DOC	Day old chick
AIV	Avian influenza virus
IAV	Influenza A virus
LPAIV	Low pathogenic avian influenza virus
HPAIV	High pathogenic avian influenza virus
swIAV	swine influenza A virus
ASR	Ashanti region
GSS	Ghana Statistical Service
ECOWAS	Economic Community of West African States
LBM	Live bird market
LMICS	Low and middle income countries

amino acids

A	Alanine
C	Cysteine
D	Aspartic acid
E	Glutamic acid
F	Phenylalanine
G	Glycine
H	Histidine
I	Isoleucine
K	Lysine
L	Leucine
M	Methionine
N	Asparagine
Q	Glutamine
R	Arginine
S	Serine
T	Threonine
V	Valine
Y	Tyrosine

1.0 Introduction to the study

1.1 Study background

Infectious diseases (IDs) continue to pose a substantial threat to global public health and animals are a major source of these infections. According to the World Health Organisation (WHO), two-thirds of all newly emerged and re-emerged infectious diseases are of animal origin (WHO, 2011). The increase in zoonoses is directly linked to the increasing demand of livestock and livestock products by the human population which have led to modification of agriculture and land use; increasing urbanization, trading of live animals, climate change and globalisation. Traditional animal production systems have been modified from small animal numbers raised in free range and/or semi-intensive to large animal populations under confinement, creating an opportunity for a large pool of animal pathogens to be formed during an infection. (Zinsstag et al., 2007). Zoonotic diseases are of veterinary, public health, and economic importance. In animals, infections reduce animal growth and productivity. Mortality may be substantial. Sick animals such as donkeys may not be able to plough lands for crop cultivation and infected animals or their meat may be discarded or sold at a cheaper price reducing net profit of the producers. In humans, infections may increase hospital visit, hospitalisation and home stay, affecting the individual's ability to work and generate income. In the worst case, infections can lead to death.

The impact of zoonotic diseases is largely felt by low and middle income countries (LMICs) which characteristically have poor animal and health care surveillance systems. Among developing countries, 30% of livestock farmers reside in sub-Saharan Africa (SSA) where food insecurities and poverty levels are high (Thornton et al., 2002). In the region, livestock are cash reserves and source of income for the poor. In rural communities it is common to find animals scavenging for food and water. In urban and peri-urban communities where human populations are high, total (intensive)- and partial (semi-intensive)- confinement of relatively large numbers of animals are practiced. Here, farmers practice long standing traditional animal care methods in accordance with the culture of the people or combines it with modern methods of farming. The proximity of these farmed animals to human settlement increases the risk of human infections with zoonoses. Consequently, zoonotic

diseases such as brucellosis and bovine tuberculosis occurs at high prevalence in SSA and requires urgent control to reduce disease burden in the sub-region (Mangesho et al., 2017).

Influenza A virus (IAV) causes the disease influenza, commonly called the flu. Wild birds are the natural reservoirs and harbour several viral subtypes. Many avian and mammalian species are susceptible to IAVs. In humans, influenza is an acute self-limiting febrile illness resulting from infection of the respiratory tract. The virus is transmitted through inhalation of virus-containing-aerosols produced by an infected person through coughing, talking or sneezing. The virus can also spread through touching of surfaces such as virus-contaminated door handles and hands of infected person and subsequently touching ones face or mouth. People of all ages can be infected but incidence and disease severity are higher among children under 5 years, the elderly (≥ 65 years), pregnant women, and persons with certain chronic conditions such as lung cancer and immunosuppressive conditions . Persons infected with the virus show symptoms 1-4 days (average 2 days) post infection (PI) and continuously shed viral particles up to 5-7 days PI. Disease symptoms include sudden onset of fever, headache, cough, sneeze, nasal congestion, sore throat, body pains, runny nose and general weakness. Annually about 3-5 million severe illness and about 650,000 deaths occur worldwide (WHO, 2018). In temperate regions, annual epidemic peaks coincide with the cold winter months of November-February in the northern hemisphere and May-October in southern hemisphere. In the tropics and subtropics, the disease occurs throughout the year with multiple peaks often coinciding with the rainy season (Hirve et al., 2016). Occassionally new viral strains emerge in naïve human populations to cause pandemic.

Farm animals such as domestic poultry and swine are also susceptible to IAV infections. In these animals, infections cause poor growth, decrease egg production (poultry) and increased mortality resulting in substantial economic losses. Infections threaten the livelihood and food security of the poor who depend on these animals for their sustenance. IAVs exhibit host restriction barriers but this is not rigid and inter-species transmission can ocassionally occur. Swine in particular can naturally be infected with influenza of both avian and human origin and are ideal ‘mixing vessels’ for generating reassortant viruses. Swine and avian IAV also have zoonotic and pandemic potentials. Several human infections with swine and avian origin IAVs have been reported around the globe. Although the majority have been mild or asymtomatic, others have been severe requiring hospitalisation and even death (Freidl et al., 2014). Since 2003, 861 laboratory confirmed human infections with avian influenza virus (AIV) H5N1 have been reported from 17 countries across the world including Nigeria,

Djibouti and Egypt, in Africa. Of these 455 people have died (fatality rate 52.8%) with the majority of deaths reported from Egypt (WHO, 2020a). In 2009, influenza subtype H1N1pdm09 of swine origin emerged to cause human pandemic killing over 18,000 people in the first year of circulation (Dawood et al., 2012). Between 2011 and 2019, 430 human infections witih swine influenza subtype H3N2 was reported in the USA, with some associated deaths (CDC, 2020).

The incidence of human infections with animal IAVs is notably high among individuals in direct contact with infected animals (dead or alive) and/or contaminated environment such as farms and live animals or 'wet' markets (Monamele et al., 2019). This places livestock farmers at a high risk of becoming infected with influenza of animal origins. In Egypt where AIV H5N1 is endemic in the poultry population, signifcantly higher prevalence of H5N1 antibodies (2.0%) have been detected in poultry-exposed individuals compared with non-exposed individuals (0%) (Gomaa et al., 2015). In China, Zhou and colleagues observed 11.2% seroprevalence of european avian-like H1N1 swine antibodies among swine farm residents in southern China (Zhou et al., 2014).

Poultry and swine farming are major agricultural activities in Ghana. Backyard and commercial farming of these animals are sometimes the sole source of livelihood for many individuals and families and offers employment for a substantial proportion of women and youth. The poultry sector has experienced outbreaks of AIV H5N1 with zoonotic potentials in 2007 and multiple outbreaks starting from 2015 through to 2018. Both outbreaks caused the loss of several thousands of birds, eggs and destruction of poultry feed resulting in high revenue loss to farmers and collapse of certain poultry establishments (Asante et al., 2016). The maiden outbreak initated surveillance in poultry in certain parts of the country and identified a high risk of infections of farmers with zoonotic AIVs (Agbenohevi et al., 2015; Burimuah et al., 2016; Odoom et al., 2012). In swine, human seasonal IAVs of subytpe H3N2 and H1N1pdm09 have been detected at high prevalence of 4-10%, in Ghana indicating high rate of viral transmission from humans to swine and an equally high zoonotic risk of farmers to viruses that may be circulating in swine (Adeola, Olugasa, & Emikpe, 2016; Adeola, Olugasa, & Emikpe, 2015). The high level of anthroponoses in swine in the country is a public health concern since reassortants with zoonotic and pandemic propensities could emerge in the swine population.

It is therefore important that influenza surveillance in animals (poultry and swine) and their farmers are regularly undertaken in the country for early viral detection and early detection of new viral introductions into populations. Surveillance will further provide knowledge on viral genetic changes and zoonotic potentials of circulating viruses. Coupled with risk assessment of husbandry practices, surveillance can provide baseline data that can be used to develop suitable measures to mitigate infections in animals and farmers, minimise zoonotic risk of farmers and further contribute to pre-pandemic preparedness of the country.

1.2 Study aim and objectives

The study aimed to provide baseline information on the transmission of IAVs between farmers and their livestock (poultry and swine) at the farm level in the Ashanti region of Ghana.

The specific objectives were to:

1. Determine the prevalence of IAV circulating in poultry and animal-handlers at poultry farms in the Ashanti region of Ghana
2. Analyse the husbandry practices and awareness of zoonotic diseases to evaluate the disease risk of poultry farmers in the Ashanti region of Ghana
3. Identify and genetically characterise IAVs circulating in swine and animal-handlers on pig farms in the Ashanti region of Ghana

1.3 Study site

Ghana is a lower-middle income country located in SSA within latitude 4° 44'N and 11° 11'N and longitude 3 ° 11' W and 1° 11'E. The country shares geographical boundaries with three other West African countries: in the north with Burkina Faso, the East with Togo and the west with Cote d'Ivoire. The Gulf of Guinea, which is part of the Atlantic Ocean, borders the south of the country at a stretch of 550 km (figure 1). The total land size is 238,533 km^2. The country is divided into 16 administrative regions and has a multi ethnic population of 28 million, with annual growth rate of 2.3%. Agriculture is a major economic activity in the country, offering employment for about 44.7% of the labour force and contributing about 54% of the country's Gross Domestic Product (GDP). Livestock rearing is a major component of the Agriculture sector and contributes to about 7% of GDP. It plays a central role in the socio-economic, food security and maintenance of livelihood of many individuals.

Commercial poultry production is a vibrant enterprise in the country. It is categorised into small-scale (<5,000 birds) (which includes backyard farms), medium-scale (5,000-10,000 birds) and large-scale producers (>10,000) (FAO, 2014; Ghana Ministry of Food and Agriculture, 2015). The medium- and small- scale categories together make up 80% of the producers in the country and depend on hatcheries (both local and foreign) and feed mills for their day old chicks (DOCs) and feed respectively. Ghana imports large quantities of DOCs mostly from Netherlands and Belgium. In 2018, approximately 7.6 million DOCs comprising of about 512,000 broiler and 7.1 million layers were imported (Boschloo, 2020). The large-scale producers (comprising 20% of producers) mostly operate their own feed-mills; some maintain their own hatchery and parent stock. Unlike the large-scale producers, small- and medium-scale producers practice minimal biosecurity. This sometimes allows wild birds and rodents to gain access to the farms predisposing the poultry to diseases such as AIV. There is also frequent outbreaks of diseases in this sector impacting negatively on productivity and profit margins of farmers (FAO, 2014). Swine production is steadily increasing in the country due to increase demand of pork and pork-derived products by Ghanaians. Small-scale commercial- (holding 20-100 pigs) and backyard- (holding <20 pigs) operators largely drive the growth of the industry and accounts for 95% of production. Imported breeds; Danish large white, Landrace and crosses thereof, dominate the sector (Banson et al., 2018). Ghana has five main agroecological zones: rain forest, deciduous forest, transitional zone, coastal savannah and northern savannah. The climate is tropical and annual average temperature ranges from 26.1°C to 28.9°C. The southern part has two rainy seasons (bimodal rainfall system), a major rainy season from March to July and a minor rainy season from September to October. In the north, only one rainy season exist (mono-modal rainfall) occurring from July to September (Ghana Ministry of Food and Agriculture, 2015). Ghana actively participates in international trade as well as trading with other West African countries under the framework of the Economic Community of West African States (ECOWAS).

The study was carried out in the Ashanti region (ASR) of Ghana, which is located in the forest belt of the southern part of the country. It is the third largest region in the country, covering 10.2% of the country's total land size (figure 1). The region has the highest human population (19.4% of national population as at September, 2010). The predominant language in the region is 'Asante Twi'. The rainfall is bimodal and the average daily temperature is 27°C. ASR has a vibrant livestock sector (11% of the country's total) comprising mostly of poultry, swine, sheep, goats and cattle (Nyanteng et al., 2013). The region is the second

largest poultry producer holding 28% of the country's total poultry population, and the highest swine producer (11.1%) in the country (FAO, 2014; Ghana Statistical Service, 2013; Nyanteng et al., 2013).

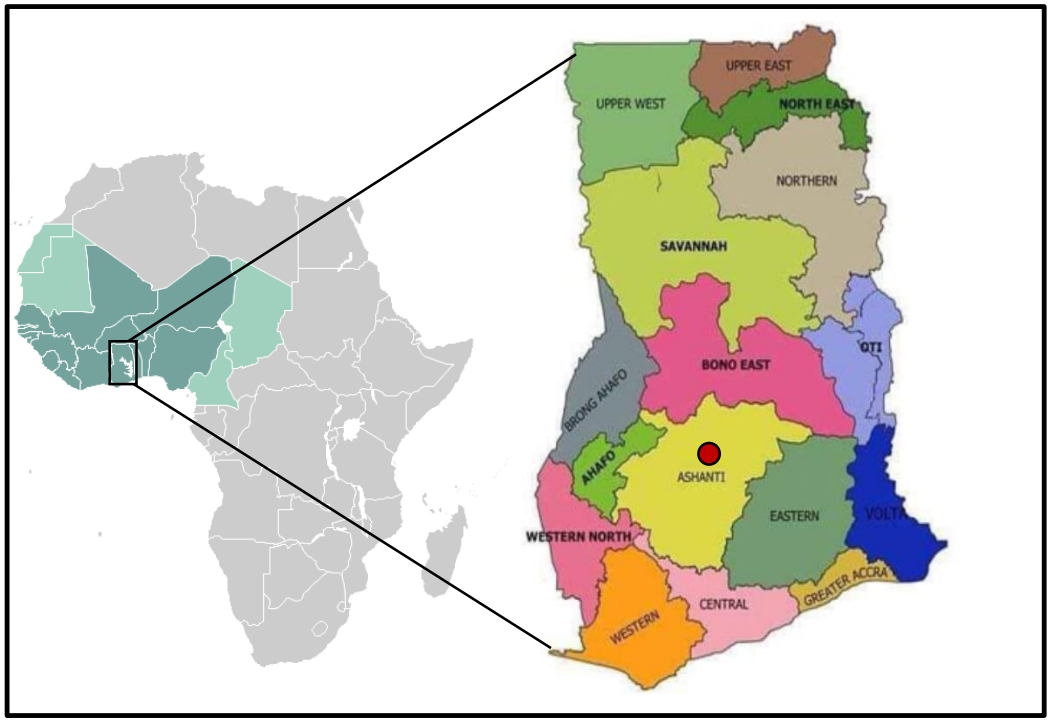


Figure 1. Map of Africa highlighting Ghana
● Study site
Source: Google images modified

2.0 Influenza A Viruses (IAVs)

2.1 Virus classification

Influenza A viruses (IAVs) are enveloped viruses of the family *Orthomyxoviridae*. Members of this family are characterised with segmented, single stranded RNA genome with negative polarity (complementary to the mRNA). The family consist of six additional genera: *Influenza B, C* and *D, Thogotovirus, Isavirus* and *Quaranfilvirus*. *Influenza B* viruses are pathogens of humans; *Influenza C*, human and pig; *Influenza D*, pig and cattle; *Thogotovirus*, human and livestock; *Isavirus*, fish, and *Quaranjavirus*, birds and humans. Among the genera of Influenzas, there is the exchange of gene segments (reassortment) within a particular type but reassortments between different types do not occur. IAV affects many species causing high morbidity and mortality (Kim, Webster, & Webby, 2018; Su, Fu, Li, Kerlin, & Veit, 2017; Suarez, 2017).

2.2 Virus composition

IAVs have eight (8) distinct gene segments that code for at least 10 proteins. These proteins are classified into structural proteins and non-structural proteins that also contain the internal proteins. The structural proteins are composed of the three surface proteins; haemagglutinin (HA), neuraminidase (NA) and membrane ion channel 2 (M2) proteins. The internal protein consists of nucleoprotein (NP), matrix protein (M1) and the polymerase complex (PC), which is made up of polymerase basic protein 1 (PB1), polymerase basic protein 2 (PB2), and polymerase acidic protein (PA). The non-structural proteins consist of non-structural protein 1 (NS1) (produced in host cell) and non-structural protein 2 (NS2) (nuclear export protein, NEP) (found in both host cell and virion). In addition to these, IAVs produce many accessory proteins from splicing and alternative reading frames of transcription (Shaw & Palese, 2013)

Table 1. Summary of the gene segments, proteins and protein functions of IAV

RNA segment	Length (nt)	Protein	Length (aa)	Function
1	2341	PB2	759	subunit of RNA polymerase, cap recognition
2	2341	PB1-F2*	87	pro-apoptotic activity
		PB1	757	subunit of RNA-polymerase, endonuclease, elongation
3	2233	PA	716	subunit of RNA-polymerase, protease
4	1778	HA	566	surface glycoprotein, receptor binding, fusion
5	1565	NP	498	RNA binding, synthesis and nuclear import
6	1413	NA	454	surface glycoprotein, neuraminidase, virus release
7	1027	M1	252	matrix protein, nuclear export, budding
		M2**	97	ion channel membrane protein, assembly
8	890	NS1	230	multi-functional protein, IFN-antagonist
		NS2**	121	nuclear export of vRNPs

* Encoded by alternate open reading frame of the PB1 mRNA

** Encoded by spliced mRNA

Adapted from (Shaw & Palese, 2013)

2.3 Virus structure

Morphologically, IAVs are variable in size, existing from roughly spherical forms of diameter 80-120 nm, to filamentous forms of several micrometres. The matured virus is enveloped with a lipid membrane that is derived from the host cell plasma membrane. The structural proteins are embedded in the lipid membrane. The HA is a trimer projecting as spikes on the membrane surface. The NA is a tetramer that forms a globular structure outspreading from the membrane and the M2 protein is a small protein that transverses the

membrane. The HA is the most abundant surface protein existing in a ratio of 4HA: 1NA and 10^1-10^2 HA: 1M2. The NP, PC and viral RNA segment form the viral ribonucleoprotein (vRNP) complex. The M1 protein is located beneath the envelope and forms a bridge between the lipid membrane and vRNP (Suarez, 2017) (Figure 2).

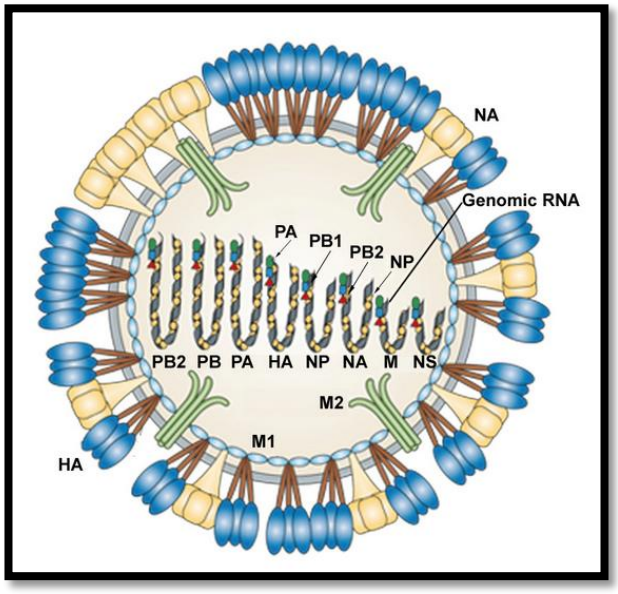


Figure 2: Structure of IAV
Adapted from (Karlsson Hedestam et al., 2008)

2.4 Virus nomenclature

IAVs are named according to certain specific features that contribute to providing consistent information to the scientific community. These features are (i) the antigenic type of the influenza virus (ii) the host species from which the virus was isolated from (which is left out if the virus is from a human source) (iii) the geographical origin of the virus such as the city, state or country (iv) the laboratory identification number (v) the year of isolation and (vi) for influenza A viruses, the HA and NA subtypes in parenthesis. For example an influenza virus isolated from chickens in a laboratory in Ghana in 2015, would be named as A/chicken/Ghana/42/2015 (H5N1) (Suarez, 2017).

2.5 Virus life cycle

2.5.1 Virus attachment and entry into host cell cytoplasm

Viral infection is initiated upon successful attachment of viral HA protein to specific sialic acid (SA) receptors located on the host cell surface. The viral particle is endocytosed. H^+ ions

are pumped into the virion through the M2 ion channel. This lowers the pH in the endocytosed virion causing conformational changes in the HA. The fusion domain of the HA is activated resulting in the fusion of the viral membrane with the endosomal membrane. The viral RNA (vRNA)-polymerase complex is then released into the cytoplasm through an open pore in the endosome and actively imported into the nucleus (Hussain, Galvin, Haw, Nutsford, & Husain, 2017) (Figure 3). The adamantane drugs (amantadine and rimantadine) were used for treatment of IAVs for several decades. These antivirals bind to the M2 channel pore blocking the influx of H^+ ions and the fusion process. Since the late 1980s, increased amantadine resistance due to M2 mutations S31N, L26F, V27A, and A30T were increasingly detected in circulating seasonal IAVs as well as some zoonotic AIVs. Therefore, the use of adamantane drugs have been discontinued as a treatment drug for influenza infections (Hussain et al., 2017).

2.5.2 Viral genome replication in the nucleus and protein translation

In the nucleus, the negative-sense viral RNA is transcribed into positive-sense mRNA by the PC. The virus requires a 5' capped primer to begin this process. The viral PB2 steals the primer from the host mRNA in a process called cap-snatching. The virus also uses proteins of the host cell such as the RNA polymerase II in the transcription process. The positive sense viral mRNA moves into the cytoplasm of the host cell where it is translated into proteins using the host cellular machinery. Some of the positive sense viral mRNA in the nucleus are used as template to produce more negative sense vRNA, which also migrates into the cytoplasm (Suarez, 2017) (Figure 3).

2.5.3 Virus assembly, packaging and release

The HA, NA and some of the M2 proteins, in the cytoplasm enters the endoplasmic reticulum of the host cell where they are folded and glycosylated (an enzymatic reaction where glycans are attached to proteins). The integral membrane proteins, the vRNP complex and each of the eight gene segments are assembled at the apex of the plasma membrane. Each gene segment has at its 5' and 3' end, a 13 and 12 nucleotides, respectively that are extremely conserved and serve as important packaging signals. RNA packaging is however inefficient and about 90% of viruses do not package all eight gene segments, leading to loss of infectivity of such viruses. Some also package multiple gene segment which impact on the phenotype of the virus. The NA protein enzymatically removes SA from the HA to prevent self-binding and aggregation of virus on the host cell surface. The new virus buds off to infect naive cells

spreading the infection (Suarez, 2017). The neuraminidase inhibitors (NAI), oseltamivir, zanamivir, peramivir and laninamivir are antiviral drugs currently used in treating IAV infections. They are SA or transition state analogues that compete with cell surface SA for binding to viral NA, and thus inhibits the enzymatic reaction and release of the newly formed virus. Amino acid substitutions E119G/A/V/D, I222V, H274Y, R292K, and N294S,that confer resistance to these NAIs have been globally observed at increasing frequencies since the early 2000s. By 2008, 15% of the H1N1 isolates in circulation were resistant to oseltamivir and a remarkable 90% in the 2008-2009 influenza season (Hussain et al., 2017).

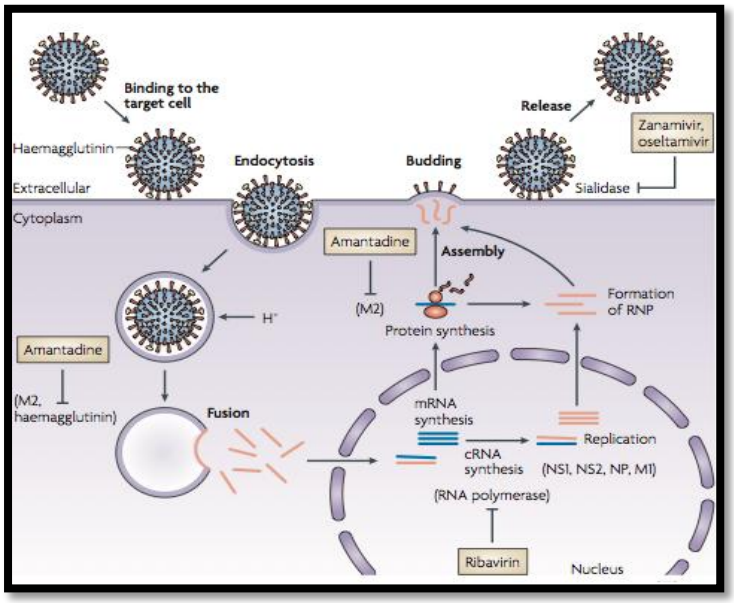


Figure 3. Schematic diagram of IAV replication cycle

Adapted from Min & Subbarao (2010)

2.6 IAV subtype classification and host range

IAVs are classified into subtypes based on the two surface glycoproteins, haemagglutinin (HA) and neuraminidase (NA), which function antagonistically in viral attachment to host cell and viral release from host cell. Up until now 18 different HA (1-18) and 11 different NA (1-11) in various combinations have been identified (Su et al., 2017). IAVs infect a wide range of host including humans, swine, horses, seals and several avian species. Wild aquatic birds mostly of the orders *Anseriforms (such as dabbling ducks and geese)* and *Charadriiformes* (such as gulls, shorebirds and terns) are the natural host and primary reservoir of all known IAVs except H17N10 and H18N11 that have been detected in bats (de Graaf & Fouchier, 2014; França, Stallknecht, & Howerth, 2013). In the natural host, the virus evolves slowly, mutations often do not confer selective advantage for the virus and the virus

maintains a constant balance with the host. Most of the internal genes are highly conserved at the amino acid (aa) level but there is greater genetic diversity in the HA and NA genes. About 25% of the amino acids are conserved between the 16HAs and about 20-63% divergence occurs between subtypes (Suarez, 2017). There is a cycle of infection within the wild bird populations and between these birds, poultry and mammals. Most wild birds are migratory in nature. As they move around the globe, they spill viruses over to poultry and other terrestrial animals. Most of these transmissions are however short-lived, but occasionally some may evolve to become adapted to specific hosts. The adapted virus transmits easily and frequently between individuals of the same species but can occasionally cross the species barrier to infect other species (Figure 4) (Swayne, Suarez & Sims, 2013).

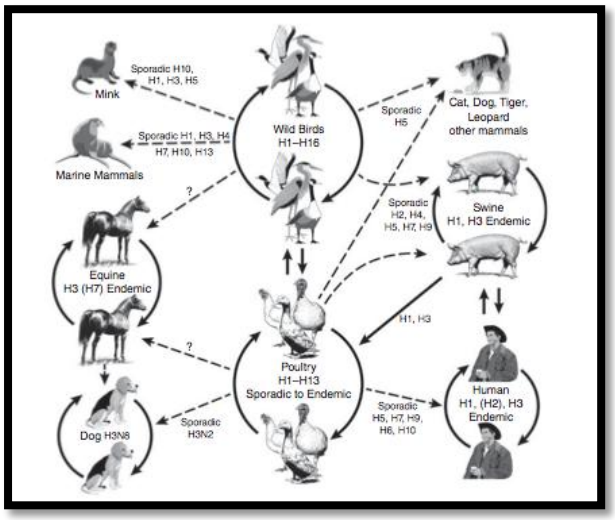


Figure 4. Schematic diagram of IAV host range and circulating subtypes in different hosts.

Adapted from Suarez, 2017

2.7 Host receptor specificity

IAVs recognise and bind to specific SA receptors on host cell surfaces to begin infection. The type, distribution and level of expression of these receptors determine the cells, tissues and host that the virus may infect as well as the pathogenic potential of the virus. The receptor-binding cavity of the virus is made up of amino acid sequences located at the globular head of the matured viral HA glycoproteins. This binding cavity recognises and strongly binds to α-2, 3 and α-2, 6 linked SA receptors on host cell membranes. AIVs preferentially binds to α -2, 3- linked SA receptors that are expressed in epithelial tissues of the intestines and respiratory tract of birds (França et al., 2013) while human-adapted IAVs preferentially binds to α-2, 6 – linked SA receptors expressed by ciliated cells and mucous producing cells of the upper respiratory tract. In the lower respiratory tract of humans, high levels of α -2, 3- linked SA

receptors are further expressed by ciliated epithelia cells of the alveoli and can support AIVs infections. Swine express high levels of both α -2,3 and α -2,6 linked SA receptors in their respiratory tract (de Graaf & Fouchier, 2014). This host restriction barrier is however not absolute. Certain amino acid substitutions have been described to confer changes of receptor binding preferences of some AIVs. For example, amino acid substitutions in the HA such as Asp-94-Asn, Ser-133-Ala (in avian H5) and Gln-226-Leu and Gly-228-Ser (in avian H2 and H3) increase the binding affinity of these avian viruses to human-type receptors (de Graaf & Fouchier, 2014; Mertens et al., 2013).

2.8 Introduction to antigenic drift and shift

After an inoculation, either by natural infection or vaccination, antibodies are produced against several proteins of the virus but only antibodies developed against the surface glycoproteins are protective. The HA is the most immunogenic part of the virus producing antibodies that can protect the individual against clinical signs and death (Kim et al., 2018). The HA head-specific antibodies interfere with the attachment of the virus to the host cell surface and neutralise the infection. The amino acid divergence between the different HA subtypes account for differences in immune response to infections such that antibodies produced against one subtype, does not provide protection for another subtype. IAVs undergo rapid evolution as a strategy to provide antigenic changes in susceptible populations. This allows the virus to escape from the immune response produced by the host in previous infections. The evolution further helps the virus to adapt to new hosts and increase its infectivity and pathogenicity (Suarez, 2017). IAVs use two mechanisms to achieve evolutionary diversity. These are antigenic drift and antigenic shift.

2.8.1 Antigenic drifts-epidemics

The RNA polymerase of IAVs lacks a proof-reading ability and therefore introduces errors during mRNA transcription. This results in point mutations in gene segments, some of which may confer loss-of-fitness to the virus. Gradual accumulation of point mutations in the antigenic epitope of the HA and to a lesser extent NA, causes variations in viral antigenicity and offers the virus an escape from the pre-existing immunity of the host (Kim et al., 2018). The selection of immune-escape strains is influenced by high selection pressure of the host's immune response, contributed by previous viral infections and vaccination. Antigenic drift occurs frequently in human-adapted IAVs and is responsible for the seasonal influenza epidemics, which drives annual vaccination in some parts of the world such as Europe and

America and to a lesser extent Africa. Vaccination helps in preventing infections and reducing disease severity. Each year the WHO reviews and makes recommendations on the composition of the influenza vaccine by tracking the genetic changes of the virus during the epidemic season in temperate regions. An expert committee meets twice a year, in February for the upcoming Northern hemisphere's influenza season vaccine and September for the Southern hemisphere influenza season vaccine. Seasonal influenza vaccines are composed of the two circulating IAV subtypes, H1N1 and H3N2 and one or two of the lineages of influenza B virus (Yamagata or Victoria lineage) (Hirve et al., 2016). In the tropics and sub-tropics there is all-year round infections and multiple peaks often coinciding with the rainy season. In Africa, there is increased influenza activity from October-December in the North, April to June in the South and throughout the year in countries near the equator in SSA. The composition and timing of the vaccine complicates vaccination in these areas. Nevertheless, there has been an increase in influenza vaccine use (of both northern and southern hemisphere composition) in Africa in the past decade often targeting pregnant women, young children, healthcare workers, the elderly, and persons with underlying medical conditions in countries like Ghana, Egypt, South Africa and Zambia (Hirve et al., 2016).

In domestic animals such as swine, antigenic drift is less frequent. The selective pressure on the virus is reduced because large sections of matured animals are frequently removed from the population for slaughter and replaced with immunologically immature ones who are susceptible to the previously circulated virus. Nonetheless, due to increasing use of vaccines in animals, antigenic drifts is also increasing in certain animal-adapted IAVs, contributing to the emergence of vaccine escape mutant variant (Rahn, Hoffmann, Harder, & Beer, 2015).

2.8.2 Antigenic shift- pandemics

Antigenic shift occurs when a new IAV (mostly HA and/or NA) emerges in a host population (humans and animals alike).. Antigenic shift can arise by the mechanism of exchange of gene segments (reassortment) when a single cell is co-infected with at least two different influenza subtypes. During viral assemblage, the gene segments from both subtypes can be packaged together, leading to the emergence of progenies with mixed parental heritage and new HA and/or NA (Henritzi et al., 2016). An entirely new virus may also be transmitted directly from animals such as birds to the human population or indirectly through intermediate host such as swine. Because the host population has little to no protective immunity against the newly introduced virus, severe influenza outbreaks can occur and may accelerate to the

global level of a pandemic (Kim et al., 2018). Antigenic shift rarely occurs but when it does the effect is very dramatic.

2.9 Influenza pandemics

Influenza pandemics have occurred repeatedly although infrequently in human populations. Since 1918 four major pandemics involving viruses from animal origins have occurred. The 1918 (H1N1) Spanish flu was of avian origin. The 1957 (H2N2) Asian flu, was a descendent of Spanish flu that acquired HA; NA and PB1 gene segment from an avian virus. The 1968 (H3N2) Hong Kong flu, was a descendant of H2N2 that had HA and PB1 from avian origin and the 2009 (H1N1pdm09) virus contained gene segments from avian, human and swine origins (Kim et al., 2018). Each pandemic has been associated with high morbidity and mortality. Among the four, the Spanish flu has been the most devastating associated with about 500 million infections and 50 million deaths worldwide (de Graaf & Fouchier, 2014). A key feature of influenza pandemic viruses is that as the population attain pandemic herd immunity, the virus circulates as seasonal influenza undergoing antigenic drift to cause annual epidemic. In some instances, it replaces or co-circulates with a previous virus in the human population. Since 2010, H1N1pdm09 has replaced the H1N1 of 1918 and co-circulates with H3N2 to cause seasonal influenza epidemics (Al Khatib, Al Thani, Gallouzi, & Yassine, 2019).

3.0 Avian influenza viruses (AIVs)

3.1 AIVs in domestic poultry

Domestic poultry such as chickens and turkeys are under constant threat of becoming infected with avian influenza viruses (AIVs) from wild birds. Infected birds shed viruses in large quantities in their faeces and respiratory secretions. Viruses are transmitted among birds largely along the faecal-oral route, inhalation of the virus in droplets or contact with infected mucous membrane. Live bird markets (LBM) where different species of birds are sold presents a source of AIV infections for poultry. In addition, lakes, rivers and other water bodies used as sources of drinking water for poultry may be contaminated with faeces from migratory birds that stopover at these sites, serving as additional source of infection.

Poultry infected with AIV produce syndromes ranging from asymptomatic infections through respiratory diseases to systemic infections. In egg laying poultry, there is drop in egg

production. Morbidity is often high (up to100%) but mortality varies. The severity and outcome of infection depends on among other factors the pathogenicity of the virus involved in the infection. Based on their pathogenicity in chickens, AIVs are classified into low pathogenic avian influenza virus (LPAIV) and high pathogenic avian influenza virus (HPAIV) (Böttcher-Friebertshäuser, Klenk, & Garten, 2013)

3.2 High pathogenic avian influenza virus (HPAIV)

HPAIV denotes any AIV that causes at least 75% mortality in four-eight-week old chickens when infected intravenously or has an intravenous pathogenicity index (IVPI) greater than 1.2 in six-week old chickens or possess multiple basic amino acid motif at the cleavage site of the HA molecule (HA0) (Abolnik, Strydom, Rauff, Wandrag, & Petty, 2019). HPAIVs cause systemic infections in poultry. After successful replication, the precursor HA produced (HA0) remains non-infectious until it is cleaved into HA1 and HA2 intracellularly or extracellularly (Böttcher-Friebertshäuser et al., 2013). Cleavage is important for the activation of the fusion domain to enable viral uncoating step of replication to occur. Cleavage is an enzymatic activity performed by proteases. In HPAIV, the cleavage site of the virus contain multiple arginine and lysine residues, generated through recombination, insertions or mutations (Bertran et al., 2018). The multiple basic amino acid motifs are recognised and cleaved by furin and pro protein convertase 6 (PC6) proteases abundantly expressed intracellularly by several host cells, enabling the virus to replicate in several organs such as brain, kidney, pancrease, lungs etc, leading to systemic infections. The cleavage of HPAIVs occurs during viral assembly making the virus infectious immediately after release (Steinhauer, 1999; Stieneke-Gröber et al., 1992). HPAIV infections are associated with high mortality (up to 100%) in poultry and infections are notifiable to the OIE. Up until now, all HPAIV detected have been of H5 and H7 subtypes (Böttcher-Friebertshäuser et al., 2013). HPAIV outbreaks are of significant importance to poultry producers around the world mainly due to severe social and economic implications associated with its infections. In outbreak situations aggressive and rigorous control methods involving movement control, depopulation/culling with compensation, disinfection and proper carcass disposal are rolled out to stamp out the disease and minimise spread within and beyond the affected area and country. Trade restrictions both locally and internationally may be imposed on affected region or nation. This affects the income of producers and other stakeholders along the value chain and ultimately the economy of the country. In the USA, an outbreak of HPAIV in

2014-2015 caused the loss of more than 50 million chickens and turkeys (death due to both disease and culling). More than 50 countries including Canada, China, Mexico and South Korea closed their markets to USA products either completely or restrictive to outbreak state and county. Federal cost of depopulation, cleaning and disinfection were estimated at $ 879 million and compensation for bird lost was estimated at $200 million dollars (Ramos & MacLachlan, 2017). In 2013, HPAIV H5N1 outbreak in Nepal caused the loss of 1.7 million poultry and a domestic revenue loss of $9 million (McLeod & Hinrichs, 2017). Between 2003-2006, the Asian HPAIV H5N1 is estimated to have caused a global loss of more than 400 million poultry and revenue of $20 billion and has since 2012 become enzootic in poultry in China, Vietnam, Indonesia, Bangladesh, India and Egypt (CDC, 2018; FAO, 2012) HPAIVs do not normally circulate in the natural reservoir but are thought to arise primarily from LPAIVs.

3.3 Low pathogenic avian influenza virus (LPAIV)

Wild birds often carry the low pathogenic forms of AIV transmitting it occasionally to domestic poultry to cause subclinical infections, mild respiratory diseases, depression and drop in egg production. Associated mortality is often low but the presence of other pathogens such as Infectious Bronchitis virus (IBV) can exacerbate disease outcome (Awuni et al., 2019; Bertran et al., 2018). LPAIVs have single arginine (rarely lysine) amino acid residue at their HA cleavage site. This is recognised and cleaved by extracellular trypsin and trypsin-like proteases, produced by cells in the respiratory and intestinal tracts and thus limit replication and infections to these sites (Böttcher-Friebertshäuser et al., 2013).

LPAIV outbreaks are common in poultry than HPAIV and in some countries such as Mexico, Bangladesh, China and Egypt, certain subtypes such as H9N2 have become endemic in poultry populations. Despite the high frequency of occurrence, outbreaks of LPAIVs often do not attract the rigorous control response of compulsory depopulation mounted by countries during HPAIV outbreaks. LPAIV outbreaks are also not notifiable to the OIE except when it involves subtypes H5 and H7 subtypes. Nevertheless when allowed to continuously circulate LPAIV mostly of H5 and H7 subtype, can mutate to high forms to cause severe disease. In Europe the most severe AIV epidemic in poultry occurred in Italy in 1999-2000. The epidemic began as LPAIV outbreak on a single farm in North Eastern part of the country where 65% of commercial poultry is found. Stamping out was not initiated and 199 outbreaks in turkeys, chickens and guinea fowl flocks occurred. The extended circulation of the virus

caused the LPAIV to mutate to HPAIV resulting in 413 more outbreaks and a great loss to the industry (Capua et al., 2002). Continuous circulation of LPAIV without control further increases the probability of exchange of gene segments with other circulating IAVs of avian, swine or human origin. The resulting reassortant may harbour unpredictable phenotypic characteristics of panzootic and/or pandemic potentials. For instance in Egypt HPAIV H5N2 with increased virulence potentials in mammals was detected in poultry as a reassortant between endemic LPAIV H9N2 and endemic HPAIV H5N8 circulating in Egypt (Hagag et al., 2019). In 2012, an HPAIV H5N1 reassortant with a PB2 of LPAIV H9N2 emerged in Asia causing epidemic in Asia, Europe, Middle East and Africa (Monne et al., 2015; Naguib et al., 2015).

4.0 AIV Epidemiology

4.1 AIV infections in wild birds and domestic poultry in Africa

Africa harbours a variety of different species of wild birds that migrate within and between countries along well-defined migratory pathways. During the winter seasons in temperate regions, large numbers of Eurasian breeding birds migrate to overwinter in Africa. According to Hahn and co-workers, about 2.1 billion birds migrate from Europe to Africa each year (Hahn, Bauer, & Liechti, 2009). During migration, these palearctic birds make stopovers at water bodies along their migratory pathway to rest, refuel, mate and co-mingle with the Afrotropical birds, facilitating the exchange of pathogens including AIV between these bird species. In a 20 month longitudinal surveillance of AIV, in waterfowls in Zimbabwe, AIV prevalence of 1.3 to 22.3% was observed (Caron et al., 2011). In SSA where an estimated 5.4 million waterfowl from Eurasia congregate annually, AIV surveillance in waterfowls across 12 countries showed viral circulation in eight countries at an overall prevalence of 3.5% (Gaidet et al., 2007). In these studies, AIV was detected throughout the year and even during periods when palearctic birds were not in circulation, suggesting that the virus persist in Afrotropical birds. Several AIVs including rare HA/NA combinations, have been identified in wild bird populations in Africa and have included LPAIV H5N3, H11N9 and H12N5 (in Mali), H8N4 (in Ethiopia) and H1N1 (in Senegal) (Gaidet et al., 2007); H3N8, H4N6, H6N2, H9N1 and H11N9 (in Zambia) (Simulundu et al., 2011). In South Africa, LPAIV of subtype H1N8, H5N2, H3N8, H4N8, and H5N1 have been detected in many wild bird populations (Abolnik et al., 2010). In Egypt where the Mediterranean-Black Sea and East Africa-West

Asia flyways overlap with the more regional Rift Valley-Red Sea flyway, LPAIV of all HA subtypes except H8, H12, H14-16 in various NA combinations and HPAIV H5N1 and H5N8 have been identified in wild bird populations (Naguib et al., 2019). In Tunisia HPAIV H5N8 was detected as the cause of death of 30 wild birds in the Ichkeul National park (Malek Zrelli, 2016).

In Africa, free-range poultry are kept in nearly every home in rural communities. In the urban and peri-urban communities however, small to medium scale backyard farms dominate the poultry sector where the relevant market exists. Whiles free-range poultry scavenge for food and water, commercial poultry are kept indoors and provided with feed and water. Most commercial poultry farms in Africa implement very low biosecurity measures on farms exposing poultry to frequent infections from other animals including wild birds (FAO, 2014).
South Africa is the world's leading producer of farmed ostrich, which is considered by the OIE as domestic poultry. Wild birds from Europe overwinter in Southern Africa and mingle with indigenous *Anseriformes* that often have contact with farmed ostriches. Consequently, outbreaks of AIVs of both LPAIV and HPAIV subtypes have occurred in ostriches in the country with increased incidence. From 1991-2002, LPAIV H7N1, H5N9, H9N2, H6N8 and H10N1 were isolated from ostriches in South Africa (Abolnik et al., 2010). In 2004, 2006 and 2011, three unrelated HPAIV H5N2 were claimed to have been introduced by wild birds causing severe outbreaks in ostrich farms in the Eastern and Western Cape Province of South Africa resulting in imposition of trade restrictions and a significant socio-economic consequences. From 2012-2014 LPAIV H5N2, H7N1 and H7N7 have been associated with more than 70 outbreaks in ostriches in the country (Abolnik et al., 2016). In chickens, LPAIV H6N2, a reassortant of ostrich-isolated H6N8 and H9N2, has been involved in outbreaks in layers and broiler breeder chickens causing asymptomatic to severe respiratory disease, 5-60% drop in egg production, and mortality soaring up to about 37% in South Africa. The persistence and widespread of the virus required South Africa to include H6N2 in the AIV control programme in the country. (Abolnik et al., 2019). In Kenya, surveillance at LBM detected AIV prevalence of 0.8% among chickens but no subtype was reported (Munyua et al., 2012), but recent studies have isolated and identified LPAIV H9N2 with virulence and mammalian adaptation markers from poultry at LBM in the country (Kariithi et al., 2020) .
In Egypt, HPAIV H5N1, LPAIV H9N2 and HPAIV H5N8 have become endemic in poultry populations since 2006, 2010 and 2016 respectively. The co-circulation of these three

subtypes in domestic poultry makes Egypt a potential 'epicenter' for generating reassortant AIV in Africa. However although wild birds in Egypt harbour a wide variety of AIV subtypes, surveillance in poultry has largely focused on detection of H5, H7 and H9 viruses limiting information on other circulating viruses as well as changes in the internal genes of these viruses in the poultry population (Naguib et al., 2019). In 2006, Libya reported of outbreaks of G1 lineage of LPAIV H9N2 (one of the four poultry-adapted H9N2 and one of the two lineages capable of infecting humans) in poultry for the first time in Africa (Kariithi et al., 2020). The virus circulated extensively in the North African countries of Tunisia (2010-2012), Egypt (2011 to present), Morocco (2016), Algeria (2017) and Libya (again in 2013) and migrated southwards in 2017 causing outbreaks in west African countries: Burkina Faso, Ghana and Nigeria. In Ghana, concomitant infections with IBV led to an increase in mortality rate of about 25% among layer chickens (Awuni et al., 2019; Kariithi et al., 2020). In a broad surveillances study in three West African countries i.e Cote d'Ivoire, Benin and Togo, AIV antigen and/or antibodies were not detected in backyard poultry in two years despite a previous outbreak of HPAIV H5N1 in these countries, showing that the virus or any other AIV for that matter has not become endemic in these countries (Couacy-Hymann et al., 2012).

In 2006, Africa recorded its first outbreak of Asia HPAIV H5N1 in poultry in Nigeria. The virus spread rapidly on the continent causing outbreaks in 10 additional countries (Burkina Faso, Cameroon, Cote d'Ivoire, Djibouti, Egypt, Niger, Sudan, Benin, Ghana, and Togo) (Fasanmi, Odetokun, Balogun, & Fasina, 2017). The impact of the outbreak (2006-2008) was huge on the poultry sector and a real threat to livelihood in many countries. In Nigeria, infections were recorded in 25 of the 37 states. About 160 million poultry died and/or culled and an estimated $5.4 million dollar compensation was paid to farmers (FAO, 2015). In Ghana, outbreaks occurred in three regions, Greater Accra, Volta and Brong Ahafo. More than 40,000 birds were lost and the total cost of disease prevention and control was estimated at $4.3 million (Akunzule, Koney, & Tiongco, 2009). The source of HPAIV H5N1 in Africa has never been confirmed. Wild birds were hypothesised to have introduced the virus onto the continent but trading of live poultry between African countries and uncontrolled movement of birds within and between countries may have contributed to viral spread on the continent. In 2014, a second wave of HPAIV H5N1 in Africa occurred with Libya recording her first HPAIV outbreak most likely from introduction from Egypt where the virus was endemic. Reinfections in Nigeria in late 2014 however, ushered Africa into another epidemic.

In SSA, outbreaks occurred in Burkina Faso, Cote d'Ivoire, Ghana, Togo, Cameroon and Niger assuming endemicity in Nigeria. HPAI H5N8 viruses have since 2014 affected the poultry population in Africa with devastating impact. In sub-Saharan Africa, outbreaks have occurred in at least eight countries; Cameroon, Congo, Namibia, Niger, Nigeria, South Africa, Uganda and Zimbabwe. In Nigeria, HPAIV H5N6 has additionally been identified in poultry with no spread yet to neighbouring countries (FAO 2020).

4.2 AIV infections in humans

AIVs of both low and high pathogenicity can be transmitted to humans. However, for AIV to cause infections in humans, the virus must attach to the human host cell, be endocytosed and replicate efficiently. The receptor-binding specificity of IAVs is a major determinant for the host tropism of the virus, which enables inter- species transmission. The virus has to overcome the host restriction barrier of the HA to attach to cells in the upper respiratory tract. Certain amino acid substitutions in the HA of avian virus have been identified to confer an increase binding affinity of AIVs to human-like receptors and have included T156A, S133A, Q222L, D94N and G244S among others. Some of these amino acid substitutions (e.g. T156A and G244S) additionally enhances airborne transmission of AIV in mammals (Mertens et al., 2013). The bronchioles and alveoli expresses alpha 2,3 SA and can thus support the attachment of AIV without prior mutation. Such infections are associated with severe pneumonia and sometimes death. The polymerase complex of IAV is responsible for transcription and replication of the viral genome inside the nucleus of an infected cell. It has been shown that polymerases of AIVs have reduced activity in mammalian cells. But certain amino acid substitutions have been found to contribute to the rescue of this lost function of AIVs in mammalian cells. The most extensively described mutation in the polymerase complex is E627K in PB2, where at position 672 glutamic acid (E) is replaced by lysine (K) and D701N (Mertens et al., 2013). Both E627K and D701N mutations are usually not detected in combination in isolates, an observation suggesting that both signatures play a similar role and that their simultaneous occurrence in the protein would hinder their function.

Globally, human infections with AIV have largely occurred among individuals who are in direct contact with infected poultry and/or contaminated environment. The transmission often ends with the affected individual although in some cases transmission have or suspected to have occurred among individuals in a cluster. But sustained human-to-human transmission

have not occurred. Human infections with AIV are associated with a range of mild symptoms of conjuctivitis, respiratory distress, fever, cough, bronchitis, running nose, vomiting, sore throat and headache but can become severe and progress to even death (Freidl et al., 2014).
In Canada, LP and HP H7 were isolated from two individuals (one per person) who were exposed to poultry infected with HPAIV H7N3 in British Columbia. Although the virus was succesfully isolated and confirmed, antibodies using haemagglutintion and microneutrilization assays were not detected (Tweed et al., 2004). In the Netherlands, Koompmans and co-workers reported 89 human infections with H7N7 during an H7N7 outbreak in poultry in 2003. Affected individuals were mostly veterinarians, farmers and their families, cullers and medical personnels (Koopmans et al., 2004). In Taiwan LPAIV H6N1 was isolated from a 20 year old female witih influenza-like illness (Wei et al., 2013) From 2014 to June 2020, 24-laboratory confirmed cases of H5N6 and seven deaths has been reported in China (WHO, 2020b). In 1998, the first human infection with LPAIV H9N2 was reported in Hong Kong. Since then more than 40 human infections and one death has occurred mostly in China, Hong Kong and Bangladesh with recent infections in a 14-month old child in Omar (Almayahi et al., 2020). In Australia, H10N7 was detected in two abattoir workers after exposure to infected poultry (Arzey et al., 2012). Since 2013, H7N9 has caused 1,568 laboratory-confirmed human infections in the western pacific region. The first human case of HPAIV H5N1 occurred in Hong Kong during a poultry oubreak in 1997. From 2003 to May 2020, 861 human infections with H5N1 have occurred globally. Infections have been reported from Asia, Middle East, Europe and Africa, totalling 17 countries. More than half (455) of the affected individuals have died (WHO, 2020b).

In Africa, reports of human infections with AIV have been mostly to H5, H7 and H9 subtypes. Gobally, Egypt has recorded the most of human infections with HPAI H5N1 at a record high of 359 out of the global total of 861. The case fatality rate in Egypt has been 33.4% (WHO, 2020b). In a longitudinal surveillance study of AIV infections among poultry and non-poultry exposed individuals in Egypt, significantly high levels of H5N1 antibodies were detected among poultry exposed persons compared with non-exposed persons (2.1% verses 0.0%). In that same study, the authors also detected antibodies to H9N2 at a high seroprevalence of 5.9% to 7.5% in the poultry exposed individuals (Gomaa et al., 2015). In Cameroon, H5N1 and H7N9 viral antigens were not detected in 633 individuals on poultry farms and LBM where H5N1 outbreak were recorded but a longitudinal sero-surveillance detected a 1.5% prevalence of H5N1 antibodies among these same workers (Monamele et al.,

2019). H5N1 antibodies were however not detected in 294 individuals who were exposed to H5N1 infected birds at poultry farms and LBM during the maiden outbreak of H5N1 in Kano, Nigeria (Ortiz et al., 2007) but surveillance at the human-animal interface among 316 adult poultry workers from 2008-2010, indicated elevated titres to AIV H9N2, H11N1, H5N2 and H5N1 at a seroprevalence of 1.3%, 0.9%, 0.3% and 0.3% respectively (Okoye et al., 2013).

5.0 Swine influenza A viruses (swIAVs)

5.1 Influenza in swine

Influenza was recognised as a disease in swine around the occurrence of 1918 Spanish flu pandemic and subsequently isolated and characterised in 1930 (Vincent, Ma, Lager, Janke, & Richt, 2008). The virus is introduced into a swineherd often by an infected pig. Infected animals shed the virus in nasal secretions and disseminated through droplets or aerosols. Transmission between swine in a herd is primarily through the nasopharyngeal route via nose-to-nose contact or direct contact of mucus (Neira et al., 2018). The incubation period is 1-3 days. Affected swine begin to shed the virus 24 hours after infection and continues until 7-10 days PI. Swine usually self-recover 4-7 days PI. The outcome of infection is dependent on host, viral and environmental factor. In naïve animals, the virus spread rapidly. Herd morbidity is often high (up to 100%) (Vincent et al., 2008). Mortality is usually low (1-4%) and often results from secondary bacterial infections or co-infection with other pathogens such as *Haemophilus parasuis* or porcine reproductive and respiratory syndrome virus (PRRSV). Most infections are subclinical but an estimated 25-30% of affected animals may become symptomatic. In outbreak situations, 80-100% clinical signs may be observed. Clinical symptoms exhibited include fever, respiratory distress, coughing, sneezing, nasal discharge, difficulty in breathing and lethargy, most of which may be self-limiting (Kyriakis et al., 2018). IAV infections in swine are of great importance to swine producers around the world. In the USA, the prevalence among swine is estimated to be between 3.6% and 4.6% (Corzo et al., 2013; Kyriakis et al., 2018). In piglets and growers, mild respiratory forms of the disease often occur in benign forms. Infections reduce the feed intake of animals, increasing the time spent to attain appreciable market weight on the farm. In sows, infections may cause abortions and infertility disorders affecting the projected herd size and profit margins of producers (Harder et al., 2013). The economic losses due to influenza infections

can be substantial and annual losses in the USA are estimated at $360 million to $1 billion (Kyriakis et al., 2018).

Swine play a very important role in the ecology of IAVs. They express high levels of α 2, 3- and α 2, 6- SA receptors in the epithelia cells of their upper respiratory tract and can be infected with IAVs of both avian and mammalian/human origins. Swine are often regarded as 'mixing vessels' of IAVs. Simultaneous infections of swine with different IAVs may lead to the emergence of reassortants that may harbour different phenotypic characteristics. Avian and human IAVs are sporadically transmitted to swine populations but only few become established. Historically, all IAVs that have caused major human pandemic (except the Asian flu, H2N2 of 1958) have seeded in swine populations where they become adapted and further evolved into lineages different from what circulates in the human population. Three major IAV subtypes, H1N1, H1N2 and H3N2 are endemic in the global swine population. However, the origin and genetic constellations of these enzootic swine IAVs (swIAVs) differ across different regions of the world (Harder et al., 2013). Occasionally, other subtypes emerge in certain swine populations, but these often do not become established and disappear after brief circulation. The introduction of the H1N1pdm09 into swine populations has dramatically complicated the influenza situation in swine around the world.

5.1.1 swIAVs in North America

North American countries, USA, Canada and Mexico, are among the top ten pork producing countries in the world. In 2013, a swine head count in USA and Canada estimated swine population in these countries at 66 and 12.7 million respectively (Vincent, Lewis & Webby, 2017). There is trading of live animals between these countries. In addition, export of swine from USA to Mexico, China and Russia also occurs. Classical swine H1N1 (cH1N1) virus, similar to the Spanish flu pandemic virus of 1918 (H1N1) circulated in a stable manner in North American swine population for nearly 70 years until the late 1990s. In 1998 a triple reassortant, trH3N2, emerged changing ecology of swIAV in North America. The gene segments of trH3N2 were derived from cH1N1 (NP, M, and NS gene segments), human seasonal H3N2 (HA, NA, and PB1 gene segments) and avian (PB2, and PA gene segments) IAVs (Vincent et al., 2014). After emergence, trH3N2 reassorted with cH1N1 to give new lineages of H1N1 and H1N2. Most of the reassortment event occurred between the H1 and/or N1 segments only. The internal genes were maintained and came to be called 'triple

reassortant internal gene (TRIG) cassette' (Vincent et al., 2008). In the early 2000s, H1 viruses with HA or NA or both gene segments derived from human seasonal IAVs (hu-H1N1, huH1N2) were detected in swine populations in North America, possibly resulting from reassortment event(s) between human seasonal IAV (H1N1) and the new H1N1 and H1N2 swine lineages. The internal genes of these human- derived viruses harboured the TRIG cassette. The co-circulation of human- and cH1N1-derived HA viruses led to a phylogenetic cluster within North American swine population. Viruses with H1 genes similar to that of human seasonal IAV are designated as δ and those similar to cH1N1 are designated α, β, and γ (Vincent et al., 2014). swIAVs that have become established in North America have been characterised to have the TRIG cassette but varied HA and NA and therefore suggests that the TRIG cassette allows multiple HA and NA types, which may confer a selective advantage for swIAVs. These H1N1, H1N2 and H3N2 subtypes co-circulate in swine population in USA and Canada but at different frequencies. In an active surveillance in Midwestern swine population in USA, the prevalence of H1N1, H1N2 and H3N2 were reported to be 18.0%, 16.0% and 7.6% respectively (Corzo et al., 2013).

5.1.2 swIAV in Europe

In 2013, the European Union (EU) reported swine population of 147 million heads, with Germany and Spain recording 27 and 25 million respectively (Vincent et al., 2017). In European swine population, cH1N1 circulated solely until 1979 when a whole avian H1N1 virus was introduced into swine population. The avian-like H1N1 (avH1N1) became established and replaced the cH1N1 (Harder et al., 2013). In the 1970s, descendants of the 1968 pandemic virus, H3N2, emerged in European swine populations. In 1984, reassortants of this human-like virus and avH1N1 emerged with the new virus (huH3N2) acquiring the internal genes of the avH1N1 and became endemic in European swine. In 1994 a novel H1N2 virus emerged in swine population in UK and subsequently spread to other EU countries. The HA of this virus (huH1N2) was similar to H1N1 virus that circulated among humans in 1980, the NA was of swine H3N2 virus origin and the internal genes of avH1N1. These three lineages avH1N1, huH3N2 and huH1N2 that share common internal genes have co-circulate at different frequencies in different countries in European swine. Reassortants of these endemic viruses also occur with some such as rH1avN2 assuming endemic status in the Danish swine population (Harder et al., 2013; Simon et al., 2014; Van Reeth et al., 2008). To expand knowledge of swIAVs in Europe, the European commission initiated a coordinated

surveillance of swIAVs in several member states from the year 2000 under the programme, European surveillance network for influenza in pigs (ESNIP). Under the ESNIP2, 169 IAV were isolated from swine. avH1N1 was the most frequent lineage detected at a prevalence of 48%. huH3N2 and huH1N2 constituted 0.8% and 28% of isolates respectively. All three subtypes were detected in swine populations in Belgium, Italy and Spain but huH3N2 was not detected in the UK and France (Kyriakis et al., 2011). Within the ESNIP3 (conducted from 2010-2013), avH1N1 dominated again at a prevalence of 53.6% and was detected in all 16 countries. Like ESNIP2, huH3N2 was the least detected (9.1%), circulating mainly in high swine producing countries of Germany, Spain, Italy and the Netherlands. huH1N2 was the second highest and identified at a prevalence of 13% (Simon et al., 2014).

5.1.3 swIAV in Asia

Asia is the largest swine-producing region in the world with about 588 million heads. With an estimated 475 million heads, China is the world's leading swine holder. Intercontinental trading with Europe and North America and incursions of human seasonal IAVs have influenced the ecology of swIAV in Asia and generated unique reassortants. In southern China, cH1N1, European or Eurasian avian-like swine (H3N2 and H1N1), and viruses with TRIG cassette circulate in swine populations. Avian H5N1 and H9N2 have also been detected. In Japan, the classical lineage H1 and human seasonal lineage H3 viruses circulate in swine. cH1N1, H1N2, Eurasian H1N1, H3N2 and TRIG viruses of H1N2 were detected in swine slaughtered in Hong Kong from 1998 to 2010 (Vijaykrishna et al., 2011; Vincent et al., 2017).

5.1.4 Diversity in swIAV-emergence of H1N1pdm09

In early April of 2009, a novel IAV, H1N1pdm09, emerged to cause human infections in Mexico. The virus spread rapidly among humans across the globe to attain a pandemic status. Phylogenetic analysis of the virus indicated that the H1N1pdm09 was a triple reassortant virus between human, avian and swine IAVs. The PB2 and PA gene segments were of avian origin; PB1, from human H3N2 lineage,; HA, NP and NS from classical swine H1N1 lineage, and the NA and M from Eurasian avH1N1 lineage (Garten et al., 2009). Although the geographic origin and host species was not immediately confirmed, Mena and co, recently identified the precursor virus in swine in Mexico, a country that was not previously labelled as an influenza epicentre (Mena et al., 2016). Shortly after emergence in humans

(late April, 2009), the virus was detected in swine farm in Alberta, Canada (Howden et al., 2009) and has since been detected in the global swine populations including countries where swIAV have not been reported (Deng et al., 2012; Forberg, Hauge, Gjerset, Hungnes, & Kilander, 2013). The bidirectional transmission of H1N1pdm09 has globally influenced the ecology of swIAVs. H1N1pdm09 has become endemic in several swine populations co-circulating and reassorting with endemic swIAVs. In Argentina, IAVs with HA and NA from human H3N2 and all internal genes from H1N1pdm09 were isolated from swine in 2011 (Vincent et al., 2017). In the USA; influenza surveillance from 2009-2011, detected a 14.5% prevalence of H1N1pdm09 in swine (Corzo et al., 2013). In Europe under ESNIP3 H1N1pdm09 and its reassortants with enzootic swIAVs were detected at a prevalence of 16.8% making these variants the second commonly detected IAVs in European swine population (Simon et al., 2014). A reassortant, rH1N2, with a N2 gene from European swine H3N2 and all seven genes from H1N1pdm09 was detected in swine herds in German and Denmark in 2011 and 2012 respectively and has recently been shown to transmit efficiently among ferrets by direct contact, confirming the zoonotic potential of these reassortant (Fobian et al., 2015; Starick et al., 2012). Quite recently surveillance in swine in China has identified a triple reassortant Eurasian avian-like (EA) H1N1 virus, named G4 that has since 2016 predominated the swine population. Genetically G4 has been characterised to have HA and NA gene segment from EA H1N1, M and vRNP gene segment from H1N1pdm09 and NS internal-derived TRIG cassette. G4 has been shown to replicate in human epithelia cells and transmits efficiently via aerosols in ferrets (Sun et al., 2020) .

5.1.5 swIAV in Africa

The population of swine in Africa is low compared with that in other continents. In 2013, the swine population in Africa stood at 35.6 million whiles that of Europe and Asia stood at 147 and 588 million (FAO, A-I and I, 2017). Despite the relatively small population, swine play significant roles in the livelihood of people in Africa. They are important sources of food and nutritional security as well as source of income generation. The swine population in Africa is largely concentrated in SSA of which the majority (40.3%) are found in West Africa. The populations in Eastern, Central and Southern Africa are estimated at 35.6, 19.7 and 5.1% respectively (FAO, A-I and I, 2017).

Prior to 2009, there was very little information of influenza in swine in SSA. In Nigeria anti-influenza A antibodies were reported in swine populations in the country (Adeola, Adeniji, &

Olugasa, 2010; Aiki-Raji, Oyadele, Ayoade & Fagbohun, 2004; Olaleye, Omilabu, Baba, & Fagbami, 1990) but a similar observation was not made in Zambia (Stafford, Stafford, Paton, & Gamble, 1992). The Nigeria studies therefore confirmed the exposure of swine in SSA to IAV infections. After the emergence of the H1N1pdm09 and its transmission into swine a substantial number of studies of IAV infections in swine were initiated in many African countries. These studies have mostly been carried out in Nigeria, Kenya, Cameroon and Ghana, where at least two studies each have been reported. The study population have largely been apparently healthy swine kept under the intensive system as well as swine presented at slaughterhouses with varied housing systems. These studies have largely been cross-sectional and both serological and molecular methods have been used to estimate the prevalence of infection in swine in the different countries (Table 2).

Using PCR, the prevalence of IAVs in swine in SSA have ranged from a low of 0% in countries such as Cote d'Ivoire and Benin to a high of about 33% in Nigeria (Table 3). The real time PCR method has been the most frequently used laboratory method. In Uganda the prevalence of IAV in swine was estimated at 1.4% but the subtype(s) was not determined. For countries such as Kenya, Cameroon and Togo, H1N1pdm09 has been the only IAV subtype detected in swine population by PCR. For Nigeria other subtypes besides H1N1pdm09 have been reported. During the second wave of HPAIV H5N1 in Africa, the virus successfully crossed the host-species barrier to infect swine population in Nigeria (Meseko et al., 2018) (Table 3). Unlike Europe and North America, very few studies mostly in Nigeria, Kenya, Cameroon and Togo have fully or partially sequenced the individual gene segments of the virus. In such studies the gene segments have been identified to be highly homologous to the human seasonal influenza virus (Table 3).

ELISA, HA and immunohistochemistry, have been used to estimate the seroprevalence of IAV in swine in SSA but the ELISA method has largely been utilised particularly in Kenya, Uganda and Cameroon. Serological prevalence of IAV in swine in the sub-region has ranged from 4%, detected in Nigeria and Ghana (using antigen capture ELISA) to a high of 90% in Nigeria (when HI assay was used). In Mozambique where immunohistochemistry was used, a prevalence of 84% was reported (Table 4).

Sera from swine in SSA have reacted positively to influenza viral antigens of human, swine and avian sources. The American triple reassortant H3N2 and H1N1 were detected in Kenya at a prevalence of 21% and 15% respectively. Antibodies to European avH1avN1 were also

detected at a prevalence of 16% and 6.1% in swine in Nigeria and Cameroon respectively. H1N1pdm09 antibodies have been detected in all the countries i.e. Kenya, Nigeria, Ghana, Uganda and Cameroon that have performed serotyping (Table 5).

The enhanced influenza surveillance in SSA has demonstrated that although the population of swine in the sub-region may be significantly less compared with that in Europe, Asia and America, they are infected and/or have been exposed to IAVs of multiple sources including human, avian and swine origins.

Table 2. Descriptive studies of IAV infections in swine in SSA (Up to January, 2020)

Study #	Reference	Country	Design, data period(s)	Sampling method	Study population, Sampling location	Health status of sampled pigs	Sample type	Number of samples collected	Laboratory method used
1	(Adeola, Olugasa, Emikpe, & Folitse, 2019)	Ghana	Cross-sectional during the 2013-2014 and 2014-2015 early influenza seasons (Jan to March)	Two-stage random sampling	Pigs intensively kept at commercial piggery farm and pigs brought to Kumasi Abattoir for slaughtering	Apparently healthy	Nasal swabs	132 nasal swabs	PCR
2	(Meseko, Heidari, Odaibo, & Olaleye, 2019)	Nigeria	Cohort study (sentinel surveillance at monthly intervals), July 2010 to June 2012	Not specified	Multi-complex commercial piggery in a peri-urban pig estate in Lagos Nigeria	Pigs with upper respiratory signs	Nasal swabs	227 swabs	PCR
3	(Osoro, Lidechi, Marwanga, et al., 2019)	Kenya	Cross-sectional, September 2013 and April 2014 and at follow-up visits 12 to 14 weeks after enrolment	Systematic random sampling	Pigs at households in two sub-counties in Kiambu county	Not specified	Nasal swab; Blood	2066 nasal swab; 1990 blood, sample size calculation performed	ELISA, PCR
4	(Osoro, Lidechi, Nyaundi, et al., 2019)	Kenya	Cross-sectional, 2013-2014	Targeted/consecutive sampling	3 slaughterhouses in Kiambu, Kisumu and 5Siaya counties in Kenya	Not specified	Nasal swabs; blood	1082 swabs; 1128 blood, sample size calculation performed	ELISA, PCR
5	(Munyua et al., 2018)	Kenya	Cross-sectional, May 2010 and every three months between August 2011-December 2012	Convenience sampling	Households in Asembo and Kibera and slaughter house facility located on the outskirts of Nairobi	Not specified	Nasal and bronchial swabs; Blood	1491nasal swabs; 1084 blood,	ELISA, HI, PCR
6	(Dione et al., 2018)	Uganda	Cross-sectional, Date not specified	Random sampling	Free range, intensive and tethered pigs in Lira and Masaka district	Not specified	Blood	522 serum	ELISA

7	(Laisse et al., 2018)	Mozambique	Cross-sectional, Dec 2014-Feb 2015 and Dec 2015-Feb 2016	Consecutive sampling	Slaughterhouse in Matola City, Maputo Province, Southern Mozambique	Pigs with pneumonia	Lungs of pigs with pneumonia	38 lung tissue	Immunohistochemistry
8	(Meseko et al., 2018)	Nigeria	Cross-sectional, 2013, December 2015-February 2016	Not specified	Jos central abattoir in Plataeu state and Enugu slaughter slab	Apparently healthy pigs	Tracheal swabs; blood	129 tracheal swabs, 500 sera	ELISA, HI and PCR
9	(Adeola, Olugasa, & Emikpe, 2017)	Nigeria	Cross-sectional during the 2013-2014 and 2014-2015 early influenza seasons (Dec to March)	Simple random sampling during first and purposive sampling during second influenza season	Pigs brought to Ibadan abattoir and pigs kept at nine piggeries, Ibadan, Oyo State, South West Nigeria	Apparently healthy pigs	Nasal swabs	218 nasal swabs, Sample size calculation performed	PCR
10	(Adeola et al., 2016)	Nigeria and Ghana	Cross-sectional, January to February 2014	Stratified random sampling	Pigs intensively kept at University teaching and research farm, University of Ibadan and pigs brought to Municipal Abattoir, Bodija, both in Nigeria and pigs brought to Kumasi Abattoir in Kumasi, Ashanti Region Ghana	Apparently healthy pigs	Nasal swabs	75 pigs in Ibadan; 50 pigs in Ghana, Sample size calculation performed	ELISA
11	(Snoeck et al., 2015)	Nigeria and Cameroon	Cross-sectional within cohort, September – October 2009, January – April 2012	Random sampling within cohort	Large and small scale farms in North west province in Cameroon and South western states in Nigeria And Pigs at point of slaughter	Apparently healthy pigs except 5 animals with nasal discharge, rhinitis, sneezing and/or weakness	Blood	1088 blood	Virus micro-neutralisation assay
12	(Ducatez, Awoume, & Webby, 2015)	Togo	Cross-sectional, October 2012 - January 2014	Not specified	Slaughterhouse near Lomé in Togo	Apparently healthy pigs	Nasal swabs, 325	325 nasal swabs	PCR

13	(Adeola et al., 2015)	Nigeria and Ghana	Cross-sectional, January to March 2014	Stratified random sampling	Pigs intensively kept at University teaching and research farm University of Ibadan and pigs brought to Municipal Abattoir, Bodija, both in Nigeria and pigs brought to Kumasi Abattoir in Kumasi, Ashanti Region Ghana	Apparently healthy pigs	Nasal swabs	75 swabs in Nigeria; 50 pigs in Ghana	ELISA
14	(Kirunda et al., 2014)	Uganda	Cross-sectional, October 2009 - June 2011	Consecutive sampling at markets and random sampling at farm households	Central, Eastern, Northern, Western Uganda, markets and farm households in the 1.5km surroundings of respective market. Pigs were from extensive, semi-intensive and intensive system	Apparently healthy pigs	Nasal swabs; blood	511 nasal swabs, 417 sera , sample size calculation performed	PCR, ELISA, HI
15	(Larison et al., 2014)	Cameroon	Cross-sectional, December 2009-August 2012	Not specified	12 sites in West, Central, North and Extreme North, village compounds and surrounding farms. Pigs raised under intensive and free range	Not specified	Nasal swabs and sera	325 swabs, sera not stated	PCR, ELISA , HI
16	(Meseko, Odaibo, & Olaleye, 2014)	Nigeria	Cohort study (sentinel surveillance at monthly intervals	Not specified	Peri-urban pig estate in Lagos Nigeria	Case definition of fever, cough and respiratory distress in pigs	Nasal swabs	227 nasal swabs	PCR , HI
17	(Couacy-Hymann et al., 2012)	Côte d'Ivoire, Benin	Cross-sectional, November 2008 – December 2010,	Not specified	Côte d'Ivoire: Abidjan, Middle-Comoé, South-Comoé Benin: slaughterhouses in Parakou, Borgou Province	Not specified	Nasal swabs; Blood	1610 swabs; 457 serum	PCR , ELISA, HI
18	(Njabo et al., 2012)	Cameroon	Cross-sectional, December 2009-April 2010	Random sampling	11 herds in villages and farms in two Cameroonian regions (Centre and North)	Some pigs with mild respiratory symptoms	Nasal swab; Blood	104 nasal swab; 98 blood	PCR, HI

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Table 3. Molecular prevalence of IAV in swine in SSA (Up to January, 2020)

Study #	Country	Molecular method	# samples analysed	# positives detected	Molecular prevalence (95%CI)	Subtype (s) identified	Subtype prevalence (95% CI)	Advanced laboratory method performed		Phylogenetic relationship of segment(s)	References
								Viral isolation (VI) method	Genome sequencing		
1	Nigeria	Real time RT-PCR	227	31	13.7%(9.2-18.1)	H1N1pdm09	7.9%(4.4-11.4)	VI in 8–10 day old specific antibody negative chicken embryonated eggs	Full genome sequencing of all eight segments	Virus showed high homology to H1N1pdm09 that circulated in humans in Nigeria, Cameroon, Ghana and USA	(Meseko et al., 2019)
2	Ghana	RT-PCR	132	16	12.1% (6.6-17.7)	H1N1pdm09 and	9.8%(4.8-14.9)	NA	M gene sequenced	M gene sequences are highly homologous to M genes of H1N1pdm09 of humans	(Adeola et al., 2019)
						H3N2	2.3%(0.0-4.8)	Not stated	Not stated	Not stated	
3	Kenya	RT-PCR	1128	5	0.4%(0.1-0.8)	H1N1pdm09	0.4%(0.1-0.8)	NA	NA	NA	(Osoro, Lidechi, Nyaundi, et al., 2019)
4	Kenya	RT-PCR	2066	0	0% (0.0-0.0)	NA	NA	NA	NA	NA	(Osoro, Lidechi, Marwanga, et al., 2019)
5	Kenya	Real-time RT-PCR	1491	11	0.7% (0.4 – 1.3)	H1N1pdm09	0.5%(0.2-0.9)	VI in MDCK cells	Full genome sequencing of all eight segments of four randomly selected isolates	Virus showed high homology to H1N1pdm09 that circulated in humans in Kenya in 2011	(PMunyua et al., 2018)
6	Nigeria	Real-time RT-PCR	129	43	33.3% (25.2 - 41.5)	H5N1 clade 2.3.2.1 c	22/25	NA	Sequencing of the HAII fragment	Virus showed high homology to avian H5N1 isolated from chickens in Nigeria, Asia and other African countries	(Meseko et al., 2018)
7	Nigeria	One-step RT-PCR	218	24	11.0%(6.9-15.2)	H1N1pdm09 and	8.7%(0.5-12.5)	NA	M gene sequenced	M gene sequences highly homologous to sequences of H1N1pdm09 strains which circulated in humans from 2011 to 2013	(Adeola et al., 2017)
						H3N2	2.3%(0.3-4.3)				
8	Togo	Real-time RT-PCR	325	8 /65 positive pools	Pool prevalence can be calculated	H1N1pdm09	8/65 pools	VI in MDCK cells	Full genome sequencing of all eight segments	Virus showed high homology to H1N1pdm09 that circulated in humans in in the region and worldwide in 2012–2013	Ducatez, Awoume, & Webby, 2015)
9	Nigeria	Real-time	227	31	13.7%(9.2-18.1)	H1N1pdm09	7.9%(4.4-	VI in in 8–10	NA	NA	(Meseko et al.,

		RT-PCR					11.4)	day old specific antibody negative chicken embryonated eggs			2014)
10	Cameroon	Real-time RT-PCR	Not stated	2	NA	H1N1pdm09	Denominator not stated	VI in MDCK cells and SPF embryonated chicken eggs.	Full genome sequencing of all eight segments	NA	(Larison et al., 2014)
11	Uganda	Real-time RT-PCR and RT-PCR	511	7	1.4%(0.4-2.4)	NA	NA	NA	NA	NA	(Kirunda et al., 2014)
12	Cameroon	Real-time RT-PCR	104	2	1.9%(0.0-4.6)	H1N1pdm09	1.9%(0.0-4.6)	VI in MDCK cells and SPF embryonated chicken eggs.	Full genome sequencing of HA, partial sequencing of remaining seven	Virus showed high homology to H1N1pdm09 that circulated in humans	(Njabo et al., 2012)
13	Côte d'Ivoire,	Two-step RT-PCR	497	0	0%(0.0-0.0)	NA	NA	NA	NA	NA	Couacy-Hymann et al., 2012)
	Benin	One – step RT-PCR	1112	0	0%(0.0-0.0)						

Table compiled by Matilda Ayim-Akonor and Eva Lorenz, BNITM, January, 2020

Table 4. Serological prevalence of IAV in swine in SSA (Up to January, 2020)

Study #	Serological method	Country	Number of positives detected /total number of samples analysed	Serological prevalence (95% CI)	References
1	ELISA	Kenya	230/1990	11.6%(10.2-13.0)	(Osoro, Lidechi, Marwanga, et al., 2019)
2	ELISA	Kenya	214/1082	19.8%(17.4-22.2)	(Osoro, Lidechi, Nyaundi, et al., 2019)
3	ELISA	Kenya	172/1084	15.9%(13.7-18.0)	(Munyua et al., 2018)
4	ELISA	Uganda	26/522	5.0% (3.1-6.8)	(Dione et al., 2018)
5	ELISA	Nigeria	222/500	44.4% (40.0-48.8)	(Clement Meseko et al., 2018)
6	ELISA	Nigeria and Ghana	5/125	4.0% (0.6-7.4)	(Adeola et al., 2016)
7	ELISA	Nigeria and Ghana	11/125	8.8% (3.8-13.8)	(Adeola et al., 2015)
8	ELISA	Uganda	19/417	4.6% (2.6-6.6)	(Kirunda et al., 2014)
9	ELISA	Cameroon	Not stated	NA	(Larison et al., 2014)
10	ELISA	Cote d'Ivoire	16/457 weakly positive	Not conclusive	(Couacy-Hymann et al., 2012)
11	ELISA	Cameroon	27/98	27.6%(18.7-36.4)	(Njabo et al., 2012)
12	HI	Nigeria	82/91	90.1% (84.0-96.2)	(Adeola et al., 2010)
13	HI	Nigeria	7/50	14.0% (4.4-23.6)	(Adeola, Adeniji, & Olugasa, 2009)
14	Immunohistochemistry	Mozambique	32/38	84.2%(72.6-95.8)	(Laisse et al., 2018)
15	VN	Cameroon			(Snoeck et al., 2015)

Table compiled by Matilda Ayim-Akonor and Eva Lorenz, BNITM

Table 5. IAV serotypes detected in swine in SSA(Up to January, 2020)

Study #	Serological method	Country	Antigens/antibody used	Positive titre level	Number of positives detected /total number of samples analysed	Serotype prevalence (95% CI)	Reference
1	HI	Kenya	A/California/04/2009(H1N1)	≥ 80	123/172	71.5% (64.8-78.3)	(Munyua et al., 2018)
			A/Swine/Texas/4199-2/98 triple-reassortant (H3N2) (antigen)		36/172	20.9% (14.9-27.0)	
			A/Swine/Iowa/15/30 (H1N1) (antigen)		25/172	14.5% (9.3-19.8)	
2	ELISA	Nigeria	H5- ELISA	-	42/500	8.4% (6.0 -10.8)	(Meseko et al., 2018)
3	HI	Nigeria	A/Dubai/AR/3435/15(H5N1 2.3.2.1c)	≥ 160	Purposive selection of 6 positives for testing	NA	(Meseko et al., 2018)
			A/swine/Germany/R26/2011 (H1N1pdm09)	≥320	Purposive selection of 14 NP positive/H5 negative for testing	NA	
4	ELISA	Nigeria	A/Brisbane/10/2007 (H3N2) virus	-	3/75	4.0% (0.0-8.4)	(Adeola et al., 2016)
		Ghana			2/50	4.0% (0.0 – 9.4)	
5	ELISA	Nigeria	A/California/04/2009 (H1N1) virus	-	6/75	8.0% (1.9-14.1)	(Adeola et al., 2015)
		Ghana			5/50	10% (1.7-18.3)	
6	Virus neutralisation	Nigeria		≥ 40			(Snoeck et al., 2015)
			A/swine/Belgium/1/98 H1N1		140/891	15.7% (13.3-18.1)	
			A/swine/Iowa/H04YS2/04 H1N1		276/891	31% (27.9-34.0)	
			A/swine/Ontario/33853/05 H3N2		15/264	5.7% (2.9-8.5)	
			A/Luxembourg/572/2008 H1N1		3/264	1.1% (0.0-2.4)	
			A/Luxembourg/01/2005 H3N2		6/264	2.3% (0.5-4.1)	
			A/Luxembourg/46/2009 H1N1		603/891	67.7% (64.6-70.7)	
		Cameroon	A/swine/Belgium/1/98 H1N1		12/197	6.1% (2.8-9.4)	
			A/swine/Iowa/H04YS2/04 H1N1		25/197	12.7% (8.0-17.3)	
			A/Luxembourg/46/2009 H1N1		48/197	24.4% (18.4-30.4)	
7	HI	Uganda	H1	Log ₂4	19/417	4.6% (2.6-6.6)	(Kirunda et al., 2014)
8	HI	Cameroon	A/California/04/2009 (H1N1)	≥ 40	1/total number of sera not stated	NA	(Larison et al., 2014)
			A/Sw/Italy/716/06 (H3N2)		1/ total number of sera not stated	NA	
9	HI	Cameroon	A/Sw/Italy/716/06 (H3N2)		27/98	27.6% (18.7-36.4)	
			A/Sw/Italy/4660-3/09 (H1N2)		12/98	12.2% (5.8-18.7)	

			A/Sw/Italy/5766-15/09 (H1N1)	≥ 20	27/98	27.6% (18.7-36.4)	Njabo et al., 2012)
			A/Italy/3983/2009 (seasonal H1N1)		28*/98	28.6% (19.6-37.5)	
			A/California/04/2009 (H1N1)		27/98	27.6 (27.6% (18.7-36.4)	
10	HI	Nigeria	A/Brisbane/10/2007 (H3N2)	≥ 40	47/91	51.6% (41.4-61.9)	(Adeola et al., 2010)
			A/Brisbane/59/2007 (H1N1)		73/91	80.2% (72.0-88.4)	
11	HI	Nigeria	A (H1N1)	≥ 40	4/50	8.0% (0.5-15.5)	(Adeola et al., 2009)
			A (H3N2)		2/50	4.0% (0.0-9.4)	
12	HI	Nigeria	A/H1N1	Not stated	101/107	94.4% (90.0-98.8)	(Aiki-Raji, Oyadele, Ayoade, Fagbohun, 2004)

Table compiled by Matilda Ayim-Akonor and Eva Lorenz, BNITM

6.0 Human infections with swIAVs

swIAVs do not only cause economic losses to swine producers but also have the potential to be transmitted to humans to cause disease which can sometimes be fatal. The relative risk of re-introduction of swine-adapted IAVs into the human population with the potential to cause human pandemic makes circulating swIAVs a public health threat. Clinical symptoms exhibited by individuals infected with swIAVs are typical of human seasonal influenza infections and are likely to be misdiagnosed or ignored in poor resource settings as well as countries where surveillance is limited in the general human population. Globally, reports of human infections with swIAV have been low and mostly reported from North America, Europe and Asia. Between 1958-2005, 50 cases of apparent zoonotic swIAV infections were globally reported. An increased risk of infections were detected among individuals who were exposed to swine and accounted for 61% of all infections among civilians (K. P. Myers, Olsen, & Gray, 2007). In 1976, cH1N1 caused severe respiratory illness in 13 soldiers at Fort Dix, New Jersey, USA of which one died. Among the 13 individuals, the virus was successfully isolated in five persons while antibodies were detected in the other eight individuals. In an epidemiological investigation, a total of 230 people were identified to have been exposed to the cH1N1 (Gaydos, Top, Hodder, & Russell, 2006). In a cross-sectional sero-epidemiological study performed in the USA between 2002-2004, elevated antibody titres against cH1N1 and H1N2 were detected in swine farmers, veterinarians and meat-processors, indicating previous exposures to these viruses (Kendall P Myers et al., 2006).

Quite recently in the USA, there has been increasing number of reports of human infections, hospitalisation and even death with a novel swIAV designated H3N2v, that was detected in USA swine in 2010. The virus is a reassortant between the endemic North American trH3N2 (seven genes) and H1N1pdm09 (M gene) (Vincent & Nicola., 2017). Between 2010 and 2019, 430 human infections with this virus occurred in the USA. Some infections were associated with severe illness requiring hospitalisation and even death among individuals with general influenza high-risk predisposing factors such as age and/or chronic medical condition like asthma, diabetes, heart disease, weakened immune systems among others(CDC, 2020). Like other swine-human IAV transmissions, direct and indirect exposure to swine has been a risk factor of human infections. In some instances limited transmission from close contact with infected persons has occurred in clusters but sustained human-to-

human transmission has not been detected. Most infected individuals have been children often below 18 years. Between August 2011 and April 2012, 92% of all H3N2v infections occurred in children with a median age of 4 years (Epperson et al., 2013). In Ontario, Canada, the human-classical swine-avian triple reassortant virus, H3N2, was isolated from a swine worker who developed influenza-like illness(ILI) 2-3 days after swine in his farm developed similar symptoms (Olsen et al., 2006). In Europe, avH1N1 was antigenically and genetically identified as the cause of severe viral pneumonia in two persons in the Netherlands. Genetic analysis of the viral gene segments did not indicate prior reassortment in swine and demonstrated the possibility of the virus to cause severe disease in humans (Rimmelzwaan et al., 2001). In Germany, a seroprevalence study conducted from December 2007 to April 2009, showed high exposure of swine farmers and Veterinarians in Thuringia to other two swine IAVs endemic in Europe. In that study, the prevalence of H1N2 was found to range from 4.3% (among swine farmers) to 9.1% (among veterinarians) and that of H3N2 ranged from 8.7% (among swine farmers) to 22.7% (among veterinarians) (Krumbholz et al., 2010). In China the European avH1N1 virus was isolated from a 3 year old boy during routine seasonal influenza surveillance (Wang et al., 2013). In a larger serological study involving 546 swine farm residents in southern China, avH1N1 antibodies was detected at a prevalence of 11.2% (Zhou et al., 2014). Ma and co-workers also recently reported that swine farmers in China have elevated antibodies against H3N2, a unique double-reassortant between cH1N1 and human H3N2 that circulated in the 2000s and circulates in swine populations in China (Ma et al., 2015). In China a serological surveillance among swine workers showed a 10.4% seropositivity to the newly emerged swIAV, G4 (Sun et al., 2020)

7.0 Publications

7.1 Molecular and serological prevalence of influenza A viruses in poultry and poultry farmers in the Ashanti region, Ghana

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ARTICLE

Molecular and serological prevalence of influenza A viruses in poultry and poultry farmers in the Ashanti region, Ghana

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ABSTRACT

For an analysis of the prevalence of influenza A viruses (IAVs) circulating in chickens and their farmers in the Ashanti region, Ghana, we examined 2,400 trachea and cloaca swabs (chickens) and 102 oropharyngeal swabs (farmers) by qRT-PCR. Sera from 1,200 (chickens) and 102 (farmers) were analysed for IAV antibodies by ELISA and haemagglutination inhibition (HI). Avian influenza virus (AIV) was detected in 0.2% (n = 5) of chickens but not farmers. Virus detection was more pronounced in the cloacal (n = 4, 0.3%) than in tracheal swabs (n = 1, 0.1%). AIV antibodies were not detected in chickens. Two farmers (2.0%) tested positive to human seasonal IAV H1N1pdm09. Sixteen (15.7%) farmers tested seropositive to IAV of which 68.8% (n = 11) were due to H1N1pdm09-specific antibodies. AIV H5- or H7-specific antibodies were not detected in the farmers. Questionnaire evaluation indicated the rare usage of basic personal protective equipment by farmers when handling poultry. In light of previous outbreaks of zoonotic AIV in poultry in Ghana the open human-animal interface raises concern from a OneHealth perspective and calls for continued targeted surveillance.

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Introduction

Worldwide, influenza A viruses (IAVs) are important veterinary and public health pathogens causing substantial morbidity and mortality in varying species including humans and poultry [1,2]. The viruses are facing host restriction barriers, but interspecies transmission with variable sequelae can occur: (i) abortive infection, (ii) productive infection associated or not with clinical disease, (iii) adaptation to new host species with secondary virus transmission. For avian influenza viruses (AIVs) high pathogenic (HP) and low pathogenic (LP) phenotypes have been described and both can harbour zoonotic propensity. The impact of HPAIVs on livelihood and food security especially of low-income countries can be immense due to the highly lethal course of disease especially in gallinaceous poultry [1]. Outbreaks of LPAIVs in gallinaceous poultry do not necessarily receive control responses in contrast to HPAIV outbreaks. However, when allowed to continuously circulate in gallinaceous poultry, LPAIV of subtypes H5 and H7 can mutate to notifiable HPAIV; other subtypes may reassort with other IAVs of avian, porcine or human origin to generate strains with extended zoonotic and even human pandemic potential [3–5]. Sporadic human infections with AIVs have been reported worldwide with higher incidences among individuals in direct contact with infected poultry, contaminated poultry products and/or poultry environment [6–9]. There has been a growing interest in AIV infections in Africa following the introduction of HPAIV H5N1 in gallinaceous poultry in 2006 [1], contributing to the identification of different AIV subtypes with known and unknown zoonotic propensities in birds on the continent [5,10–14]. Simultaneously, evidence of AIV infections, exposures and death among humans in regular contact with poultry on the continent have also increased [9,15,16].

In Ghana, outbreaks of zoonotic HPAIV H5N1 (clade 2.2 and 2.3.2.1c) in poultry have been reported with no human deaths [9,17]. Studies focusing on active infection after the first outbreak (in 2007), recognised an increased risk of zoonotic transmission due to poor implementation of biosecurity and biosafety practices among poultry handlers [18–20].

The Ashanti region is the second-largest commercial poultry-producing region in Ghana. An HPAIV H5N1 outbreak was recorded in the region only during the second HPAI outbreak in the country in 2015. The region is a hub for trading live poultry and/or poultry products to other parts of the country and to neighbouring countries. Little is known about AIV

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infections in commercial poultry and much less of poultry handlers within the area. AIV was not detected in surveillance carried out in commercial poultry before the first H5N1 outbreak in the country, and in backyard poultry in military barracks in the region after the first outbreak [20]. We performed a cross-sectional study to determine the prevalence of IA viruses in commercial chickens and their farmers within the Ashanti region of Ghana. This will contribute to our understanding of influenza at the human-animal interface in the region and aid to develop IAV control strategies to prevent infections in poultry and humans.

Materials and methods

Ethics and sampling

Ethical approval was obtained from the Council for Scientific and Industrial Research (RPN 001/CSIR-IACUC/2016), Ghana, and Ärztekammer Hamburg (PV5296), Germany. Between April 2016 to February 2017 tracheal and cloacal swabs and blood samples (2 mL) were collected from 1,200 clinically healthy chickens raised exclusively in-house on 76 commercial chicken farms in the Ashanti region. An oropharyngeal swab and a blood sample (2 mL) were obtained from 102 farmers from 39 of these farms. None of the farmers had symptoms suggestive of any respiratory illness at the time of sampling. Swabs were collected into viral transport medium [21] and transported on ice to the laboratory. Questionnaires were used to collect relevant farm and farmer data.

Laboratory analysis

RNA was isolated from swabs (QIAamp viral RNA mini kit, Germany) and tested for influenza A Matrix-specific gene by qRT-PCR [22]. All positive samples were subjected to direct subtyping of all AIV subtypes [23,24]. Additionally, human samples were tested for seasonal influenza viruses of subtypes H1 and H3 by qRT-PCR [24]. Viral isolation in embryonated chicken eggs and MDCK cells of positive samples was attempted. ELISA was used to test sera for IAV antibodies (IDEXX AI MultiS-Screen kit, chicken; Serion IgG ELISA kit, human). Hemagglutination inhibition (HI) assay was used to test ELISA positive sera for avian H5 and H7 and seasonal H1 antibodies (A/tky/England/647/1977 (H7N7); A/Teal/England 7394-2805/2006 (H5N3); source: European Reference Laboratory for Avian Influenza, Weybridge, UK, and H1N1pdm in-house control strain of FLI, A/Germany/R26/2010 (H1N1pdm)). Frequency and percentages were computed for categorical variables. Median and interquartile range (IQR) were computed for continuous variables. The point prevalence along with the 95% confidence interval (CI) was estimated. Data were analysed with STATA 14.

Results

Influenza a prevalence on poultry holdings

Based on questionnaire analyses, most farms (n = 55, 72.4%) had up to 5,000 chickens and the majority (n = 72, 94.7%) kept only layers. Majority of farms (n = 69, 90.8%) reported at least one episode of respiratory infection among the chickens between 3 weeks to 4 months prior to sampling. Nearly all farms (n = 75, 98.7%) retailed their spent layers live, and table eggs at the farm gate. Vaccination against AIV is not practiced in Ghana. AIV was detected in 0.2% (n = 5/2400, 95% CI = 0.19–0.23) of chicken swabs. Viral RNA was detected on 5.3% (n = 4/76) of farms. Four out of five of AIV positive samples were of cloacal origin (Table 1). All positives were detected in layers. The quantitation cycle (Cq) value of all positives ranged from 35 to 38 indicating a very low virus load. The direct subtyping attempt was unsuccessful. Viral isolation attempts failed. AIV antibodies were not detected in any of the 1,200 chicken sera (Table 1).

Influenza a prevalence among chicken farmers

The median age of farmers was 25 years (IQR = 22.0–35.0) and most (n = 74, 72.5%) had worked at the present farm for more than 1 year. Only 2 (2.0%) reported to wear a surgical face mask and none reported to wear gloves when working.

IAV RNA was detected in two swabs from humans. Both were subtyped as H1N1pdm09. Sixteen farmers had IAV antibodies. AIV H5- and H7-specific antibodies were not detected. Antibodies to H1N1pdm09 were detected in 10.8% (11/102) of total sera analyzed (Table 2), and formed 68.8% (11/16) of seropositive samples. All AIV positive farms had a farmer who tested positive to either H1N1pdm09 virus or antibody.

Table 1. Molecular and serological prevalence of AIV detected in chickens.

Sample	Number analysed	No. of positive detected	Prevalence (95% CI)
Cloacal swab	1200	4	0.33 (0.30–0.36)
Tracheal swab	1200	1	0.08 (0.06–0.10)
Serum	1200	0	0

Table 2. Molecular and serological prevalence of IAV detected in farmers.

				Influenza sub-/sero-type identified (%)		
Sample	Number analysed	No. positive	Prevalence (CI)	H5	H7	H1N1pdm09
Oropharyngeal swab	102	2	2.0 (1.70–2.22)	0(0)	0(0)	2(2.0)
Serum	102	16	15.7 (8.15–22.0)	0(0)	0(0)	11(10.8)*

*HI titres ≥ 40

Discussion

The study could not find evidence for endemic circulation of AIV in apparently healthy commercial chickens raised exclusively in-house on farms in the Ashanti region shortly before and during the study period. This is highlighted by the lack of AIV antibodies in any of the chickens examined; following an AIV infection antibodies in layer chickens are expected to be detectable at least 4–6 months after recovery. Thus, past episodes of respiratory disease in layers, as reported by farmers, are most likely unrelated to AIV infections. However, very few cases of active shedding of clinically healthy chickens mostly through faeces were detected. This suggests rare sporadic infection with LPAIV. Subtyping of these viruses was precluded by the very low virus load present in the samples. Previous reports from Ghana likewise did not detect active AIV infection in healthy poultry [18–20] and a low prevalence was reported from Kenya [25].

In several African countries, in contrast, LPAIV alone or in co-infections with other avian pathogens have caused high morbidity, drop in egg production and mortality [26–28]. Interestingly, LPAIV H9N2 in co-infection with infectious bronchitis virus (IBV) caused a significant drop in egg production and high mortality on several layer farms in the Ashanti region a few months after the current study had been finalised. The current study suggests that this virus has not previously circulated in the farms visited but likely was recently introduced into the chicken population, highlighting the consequences of low biosafety measures on farms [29]. Unrestricted moving of AIV-infected live chickens between farms and markets may have played a key role in spreading LPAIV in the country and increase public health risks [30]. The origin of the H9N2 virus later on detected remained unclear but the close phylogenetic relationship to viruses circulating endemically in poultry in several North African countries suggested transboundary i ncursions related to poultry trade [29]. Therefore, raising biosafety standards on poultry farms would be a basic precondition to limit economic losses due to infectious diseases. Controlling trade-related transports of live poultry may further aid in reducing the risk of viral spread. This would be particularly important in case zoonotic AIVs are encountered. Interestingly, the H9N2 viruses causing the reported incursion into Ghana are members of the zoonotic G1 lineage that previously caused human infections in Egypt [31].

Members of the Asian HPAIV H5 lineage with mammalian receptor affinity caused sporadic outbreaks in chicken farms in the Ashanti region, in 2015, 2016 and 2018 [32,33]. The rapid response of the veterinary services of Ghana significantly reduced viral spread and possible contact of farmers with the virus. The absence of H5- and H7-specific antibodies in the farmers despite frequent and long contact to poultry rules out infection with these zoonotic pathogens [7]. In contrast, infections, acute and past, with seasonal human IAV subtype H1N1 was detected. In Nigeria, Cameroon, and Egypt where H5 and H7 antibodies have been detected in poultry workers, the corresponding avian viruses were observed to have circulated for longer periods and affected more poultry holdings, increasing the net exposure risk of poultry workers with possibly infected poultry [15,16,34]. Nevertheless, farmers' compliance with certain basic biosafety practices were largely poor as noted previously in other parts of the country [18–20] and therefore the risk of exposure to zoonotic AIVs such as LPAIV H9N2 [29] and other non-viral avian pathogens remains high. Co-circulation of IAVs in farmers and their chickens increases the risk of generating reassortants. Regular surveillance of IAVs at the human-animal interface in poultry production for early detection and effective control of these emerging zoonotic and potentially pandemic IAVs would be highly desirable.

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Disclosure statement

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References

[1] Fasanmi OG, Odetokun IA, Balogun FA, et al. Public health concerns of highly pathogenic avian influenza H5N1 endemicity in Africa. 2017. Veterinary World, 10(10):1194–1204.

[2] Iuliano AD, Roguski KM, Chang HH, et al. Estimates of global seasonal influenza-associated respiratory mortality: a modelling study. Lancet. [Internet]. 2018 Mar 31 [cited 2018 May 4];391(10127):1285–1300. Available from http://www.ncbi.nlm.nih.gov/pubmed/29248255

[3] Castrucci MR, Donatelli I, Sidoli L, et al. Genetic reassortment between avian and human influenza a viruses in. Pigs I Virology. [Internet]. 1993 Mar 1 [cited 2019 Nov 5];193(1):503–506. Available from https://www.sciencedirect.com/science/article/abs/pii/S0042682283711554

[4] Hagag NM, Erfan AM, El-Husseiny M, et al. Isolation of a novel reassortant highly pathogenic avian influenza (H5N2) Virus in Egypt. Viruses. [Internet]. 2019 Jun 18 cited 2019 Aug 30;11(6):565. Available from http://www.ncbi.nlm.nih.gov/pubmed/31216712

[5] Naguib MM, Verhagen JH, Samy A, et al. Avian influenza viruses at the wild-domestic bird interface in Egypt. Infect Ecol Epidemiol. [Internet]. 2019 [cited 2019 Aug 3];9(1):1575687. Available from http://www.ncbi.nlm.nih.gov/pubmed/30815236

[6] Puzelli S, Rizzo C, Fabiani C, et al. Influenza A(H7N7) virus among poultry workers, Italy, 2013. Emerg Infect Dis. [Internet]. 2016 [cited 2019 Aug 27];22(8):1512–1513. Available from http://www.ncbi.nlm.nih.gov/pubmed/27434025

[7] Gao R, Cao B, Hu Y, et al. Human infection with a novel avian-origin influenza A (H7N9) Virus. N Engl J Med. [Internet]. 2013 May 16 [cited 2018 May 4];368(20):1888–1897. Available from http://www.nejm.org/doi/10.1056/NEJMoa1304459

[8] Zhou L, Liao Q, Dong L, et al. Risk factors for human illness with avian influenza A (H5N1) virus infection in China. J Infect Dis. [Internet]. 2009 Jun 15 [cited 2019 Aug 21];199(12):1726–1734. Available from http://www.ncbi.nlm.nih.gov/pubmed/19416076

[9] World Health Organization. Cases deaths [Internet]. 2019. 2019 [cited 2019 Aug 28]. p. 3. Available from: https://www.who.int/influenza/human_animal_interface/2019_06_24_tableH5N1.pdf?ua=1

[10] Coker T, Meseko C, Odaibo G, et al. Circulation of the low pathogenic avian influenza subtype H5N2 virus in ducks at a live bird market in Ibadan, Nigeria. Infect Dis Poverty. [Internet]. 2014 [cited 2019 Aug 7];3(1):38. Available from http://www.ncbi.nlm.nih.gov/pubmed/25671118

[11] Zecchin B, Minoungou G, Fusaro A, et al. Influenza A (H9N2) Virus, Burkina Faso. Emerg Infect Dis. [Internet]. 2017 Dec [cited 2019 Aug 7];23(12):2118–2119. Available from http://www.ncbi.nlm.nih.gov/pubmed/28980894

[12] FULLER TL, DUCATEZ MF, NJABO KY, et al. Avian influenza surveillance in Central and West Africa, 2010–2014. Epidemiol Infect. [Internet]. 2015 Jul 22 [cited 2019 Aug 27];143(10):2205–2212. Available from http://www.ncbi.nlm.nih.gov/pubmed/25530320

[13] Abolnik C, Olivier A, Reynolds C, et al. Susceptibility and status of avian influenza in ostriches. Avian Dis. [Internet]. 2016 Feb 10 [cited 2019 Aug 7];60(1s):286. Available from http://www.ncbi.nlm.nih.gov/pubmed/27309069

[14] Couacy-hymann E, Kouakou VA, Aplogan GL, et al. Surveillance for infl uenza viruses in poultry and swine, West Africa. Emerg Infect Dis. 2012;18(9):1446–1452.

[15] Gomaa MR, Kandeil A, Kayed AS, et al. Serological evidence of human infection with avian influenza A H7virus in Egyptian poultry growers. PLoS One. Internet]. 2016;11(6):1–10. Available from http://dx.doi.org/10.1371/journal.pone.0155294

[16] Monamele CG, Karlsson EA, Vernet M-A, et al. Evidence of exposure and human seroconversion during an outbreak of avian influenza A(H5N1) among poultry in Cameroon. Emerg Microbes Infect. [Internet]. 2019 [cited 2019 Aug 28];8(1):186–196. Available from http://www.ncbi.nlm.nih.gov/pubmed/30866772

[17] Tassoni L, Fusaro A, Milani A, et al. Genetically different highly pathogenic avian influenza A(H5N1) viruses

in West Africa, 2015. Emerg Infect Dis. [Internet]. 2016 [cited 2019 Sep 30];22(12):2132–2136. Available from http://www.ncbi.nlm.nih.gov/pubmed/27389972

[18] Agbenohevi PG, Odoom JK, Bel-Nono S, et al. Biosecurity measures to reduce influenza infections in military barracks in Ghana. BMC Res Notes. 2015;8(1):1–8.

[19] Burimuah V, Ampofo WK, Awumbila B, et al. The evaluation of domestic ducks as potential reservoir of avian influenza virus in post Hpai H5n1 outbreak area, sunyani municipality, brong ahafo region Of Ghana. African J Infect Dis. [Internet]. 2016 cited 2019 Aug 28;10(2):134–145. Available from http://www.ncbi.nlm.nih.gov/pubmed/28480449

[20] Odoom JK, Bel-Nono S, Rodgers D, et al. Troop education and avian influenza surveillance in military barracks in Ghana, 2011. BMC Public Health. [Internet]. 2012;12:957.Available from http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3534292&tool=pmcentrez&rendertype=abstract

[21] Eisfeld AJ, Neumann G, Kawaoka Y. Influenza A virus isolation, culture and identification. Nat Protoc. [Internet]. 2014;9(11):2663–2681.

[22] Hoffmann B, Depner K, Schirrmeier H, et al. A universal heterologous internal control system for duplex real-time RT-PCR assays used in a detection system for pestiviruses. J Virol Methods. [Internet]. 2006 Sep [cited 2018 Oct 29];136(1–2):200–209. Available from http://www.ncbi.nlm.nih.gov/pubmed/16806503

[23] Hoffmann B, Hoffmann D, Henritzi D, et al. Riems influenza a typing array (RITA): an RT-qPCR-based low density array for subtyping avian and mammalian influenza a viruses. Sci Rep. [Internet]. 2016;6 (February):27211. Available from http://www.ncbi.nlm.nih.gov/pubmed/27256976%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC4891686

[24] Henritzi D, Zhao N, Starick E, et al. Rapid detection and subtyping of European swine influenza viruses in porcine clinical samples by haemagglutinin- and neuraminidase-specific tetra- and triplex real-time RT-PCRs. Influenza Other Respi Viruses. [Internet]. 2016 [cited 2018 Jul 5];10(6):504–517. Available from http://www.ncbi.nlm.nih.gov/pubmed/27397600

[25] Munyua P, Onyango C, Mwasi L, et al. Identification and characterization of influenza A viruses in selected domestic animals in Kenya, 2010-2012. PLoS One. [Internet]. 2018 [cited 2019 Oct 1];13(2):e0192721. Available from http://www.ncbi.nlm.nih.gov/pubmed/29425232

[26] Sid H, Benachour K, Rautenschlein S. Co-infection with multiple respiratory pathogens contributes to increased mortality rates in algerian poultry flocks. Avian Dis. [Internet]. 2015 Sep [cited 2019 Sep 30];59(3):440–446. Available from http://www.ncbi.nlm.nih.gov/pubmed/26478165

[27] Kammon A, Heidari A, Dayhum A, et al. Characterization of avian influenza and newcastle disease viruses from poultry in Libya. Avian Dis. [Internet]. 2015 Sep [cited 2019 Sep 30];59(3):422–430. Available from http://www.ncbi.nlm.nih.gov/pubmed/26478162

[28] El Houadfi M, Fellahi S, Nassik S, et al. First outbreaks and phylogenetic analyses of avian influenza H9N2 viruses isolated from poultry flocks in Morocco. Virol J. [Internet]. 2016 [cited 2019 Sep 30];13 (1):140. Available from http://www.ncbi.nlm.nih.gov/pubmed/27527708

[29] Awuni JA, Bianco A, Dogbey OJ, et al. Avian influenza H9N2 subtype in Ghana: virus characterization and evidence of co-infection. Avian Pathol. 2019;1–7. DOI:10.1080/03079457.2019.1624687

[30] Mensah-bonsu A, Rich KM Ghana ' s Poultry Sector Value Chains and the Impacts of HPAI.2010;(Oct)

[31] Gomaa MR, Kayed AS, Elabd MA, et al. Avian influenza A(H5N1) and A(H9N2) seroprevalence and risk factors for infection among Egyptians: a prospective, controlled seroepidemiological study. J Infect Dis. [Internet]. 2015 May 1 [cited 2019 Sep 30];211 (9):1399–1407. Available from http://www.ncbi.nlm.nih.gov/pubmed/25355942

[32] Asante IA, Bertram S, Awuni J, et al. Highly pathogenic avian influenza A(H5N1) virus among poultry, Ghana, 2015. Emerg Infect Dis. 2016;22 (12):2209–2211.

[33] Services-Ghana DKMKG (Veterinary. Follow-up report No. 34 (Final report) Outbreak details [Internet]. Vol. 34, Oie, UPDATE ON HIGHLY PATHOGENIC AVIAN INFLUENZA IN ANIMALS. 2017 [cited 2019 Sep 30]. Available from: https://www.oie.int/wahis_2/temp/reports/en_fup_0000021647_20161124_170217.pdf

[34] Okoye J, Eze D, Krueger WS, et al. Serologic evidence of avian influenza virus infections among Nigerian agricultural workers. J Med Virol. [Internet. 2013 Apr [cited 2019 Aug 28];85(4):670–676. Available from http://www.ncbi.nlm.nih.gov/pubmed/23400898

7.2 Understanding attitude, practices and knowledge of zoonotic infectious disease risks among poultry farmers in Ghana

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ORIGINAL ARTICLE

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Understanding attitude, practices and knowledge of zoonotic infectious disease risks among poultry farmers in Ghana

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Abstract

Zoonotic infectious diseases (ZIDs) are increasing globally, and livestock farmers in low- and middle-income countries are at particularly high risk. An evaluation of farmer's behaviour on farms can be used to identify the risk factors and to develop tailored control strategies. This study documents the knowledge of zoonotic poultry diseases (ZPD) among 152 poultry farm workers (respondents) from 76 farms in the Ashanti region of Ghana and assessed their on-farm attitude and practices that increase their risk to exposure of ZPD. The median age of respondents was 29 years, 91.4% (*n* = 139) had a formal education, and 80.9% (*n* = 123) had worked on the farm for more than 1 year. The majority of farms (*n* = 69, 90.8%) had multiple flocks and 27.6% (*n* = 21) kept other animals, of which 57.1% (*n* = 12) were pigs. The majority of respondents had good knowledge about poultry diseases but not about ZPD. A higher level of education and longer work experience improved respondents' knowledge of poultry and ZPD. Although respondents identified the wearing of personal protective equipment (PPE) as a major ZPD preventive measure, the majority did not put that knowledge into practice. Most farms (71.1%, *n* = 54) had no footbath and 55.3% (*n* = 42) deposited farm-waste on the farm. While 97.4% (*n* = 148) of respondents washed their hands after working, only 48.7% (*n* = 74) wore protective footwear, 2.7% (*n* = 4) wore overalls, 2% (*n* = 3) wore nose masks and none (*n* = 0) wore gloves. The husbandry practices and attitude of farmers expose them to pathogens on the farm and increase their risk of becoming infected with ZPD in the sub-region. The results from this study could be used to promote human health among farm workers in Ghana.

KEYWORDS
attitudes, farmers, Ghana, health knowledge, practices, zoonoses

1 | INTRODUCTION

The global prevalence of human infectious diseases remains high and zoonotic infectious diseases (ZIDs) form the highest percentage, accounting for 61% of all known infectious diseases, and 75% of emerging infectious diseases (WHO, 2011). ZIDs are also of agricultural and economic importance, as they impact animal health, reduce productivity, affect income and food security of farm products. The

revenue loss from the imposition of trade restrictions, low patronage by consumers and increased marketing costs to regain consumer confidence may impede economic growth of countries, where ZIDs are common in farmed livestock (Halliday et al., 2015; McDermott & Arimi, 2002; WHO, 2006). There has been a growing demand for animal products in many urban and peri-urban communities in low- and middle-income countries (LMIC) due to increasing rural–urban migration, and changes in socio-cultural and socio-economic status. This has contributed to intensification of livestock production in densely populated areas, thus increasing the risk of human infections with zoonotic pathogens (Thornton, 2010; Zinsstag et al., 2007). Livestock farmers remain at high risk of acquiring ZIDs due to their proximity and frequent contact with the animals and their environment.

Thirty percent of livestock farmers from developing countries live in sub-Saharan Africa (Thornton et al., 2002). Their husbandry practices are often based on traditional knowledge and skills inherited from their ancestors, which may be fused with modern methods of livestock keeping. Such practices may differ between and within countries even for the same species and therefore the potential risk of becoming infected with ZIDs may differ (FAO, 2009; Mangesho et al., 2017). Poultry production is a major component of the livestock sector in Ghana and contributes substantially to the animal protein source and food security. Several poultry diseases with both zoonotic and non-zoonotic potential characterise the industry (Andoh et al., 2016; Ayim-Akonor, Obiri-Danso, Toah-Akonor, & Sellers, 2018; FAO, 2014). Three outbreaks of highly pathogenic avian influenza virus (HPAIV) H5 have affected the Ghanaian poultry industry in the last 12 years (Asante et al., 2016; OIE, 2019). Although no human infections occurred, the risk to poultry farmers remains high; 16 countries worldwide have recorded human infections with a 53% fatality rate and contact with infected poultry or the environment was identified as transmission pathways (WHO, 2019).

In this study, we aimed to assess the knowledge level of poultry farmers regarding zoonotic poultry disease (ZPD) and further evaluate their on-farm attitude and practices that increase their risk of becoming infected with ZPD. The information will provide baseline data to develop practical control methods to reduce zoonotic transmission among poultry farmers in sub-Saharan Africa.

2 | MATERIALS AND METHODS

2.1 | Study area

The study was performed in the Ashanti region of Ghana from April 2016 to February 2017. The Ashanti region is located in the forest belt of the country. It is the third largest region covering 10.2% of the total country land size. The region has the highest human population (19.4% of national population). The Ashanti region is the second largest poultry-producing region, holding 28% of the total poultry population in Ghana. Breeders, layers (egg-type) and broilers (meat-type) form the bulk of poultry kept by farmers, with layer birds dominating the sector (FAO, 2014; Ghana Ministry of Food & Agriculture, 2015; Nyanteng et al., 2013).

2.2 | Ethical consideration

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

2.3 | Farm selection

Members of the regional poultry farmer association were contacted personally or through a mobile phone call. The study was explained to the farmers and, where informed consent was provided, farms were visited to conduct interviews. Famers were asked whether they knew colleagues who were not members of the poultry farmer association, and these were considered as potential study participants (snow-ball sampling). Questionnaires were administered only on farms if birds were present and at least one farm worker worked at the time of visit.

2.4 | Questionnaire administration

A questionnaire with open-ended and close-ended questions was used. Questionnaires were administered in English and in the local language 'Twi' (responses were translated into English for analysis). The questionnaire included sections on farm characteristics, farm husbandry practices, demographics, biosecurity practices, knowledge of poultry diseases, awareness of zoonotic diseases and self-protection from zoonotic diseases.

2.5 | Data entry and analysis

Medians and interquartile ranges were computed for continuous variables, and the frequency and percentages were computed for categorical variables. Data on age, education and length of employment on the farm were dichotomised to calculate association measures. A dichotomised knowledge level score of poultry diseases (good or poor) was developed based on the respondents' ability to name at least one correct visible clinical sign indicating animal disease and being able to name at least one poultry disease and its corresponding clinical signs. A dichotomised knowledge level score of ZPDs (good or poor) was developed based on the respondents' awareness of becoming infected with certain diseases of poultry, correctly naming at least one zoonotic poultry disease, and mentioning at least one method to protect against zoonotic poultry disease. Risk ratios (RR), with their corresponding 95% confidence intervals (CI), were calculated to estimate associations between the dichotomised scores and individual characteristics. A binomial regression model, with a log-link function, was fitted to calculate multivariate models. Backward elimination was applied to select the final models. All analyses were conducted using the statistical program Stata (Version 14, StataCorp).

3 | RESULTS

3.1 | Farm characteristics

In all, 76 poultry farms in the study region were visited during the sampling period. Seven farms were reported to have one flock (9.2%), 38 (50%) to have two or three flocks and 31 (40.8%) to have more than three flocks. The total number of birds on the farms varied from 50 to more than 10,000. In all, 55 farms had less than 5,000 birds (72.4%), 13 (17.1%) had 5,000–10,000 birds and eight (10.5%) had more than 10,000 birds. Most farms (*n* = 55, 72.4%) kept only chickens. Some farms (*n* = 21, 27.6%) additionally kept other animals, predominantly pigs (*n* = 12, 57.1%), ruminants (*n* = 7, 33.3%) and others such as free-range chicken, ducks, guinea fowl and turkeys. The majority of farms (*n* = 65, 85.5%) prepared their animal feed at local feed mills.

Different preventive measures against poultry diseases were in place. Few farms (*n* = 22, 28.9%) had footbaths, of which 12 (54.5%) treated with fresh disinfectants weekly and 10 (45.5%) applied fresh disinfectants occasionally. Water troughs were washed daily on all farms. Wood shavings were used as bedding materials by nearly all farms (*n* = 74, 97.4%) and were changed occasionally (*n* = 28, 36.8%) or at the end of the production cycle (*n* = 48, 63.2%). Nearly half of the farms (*n* = 34, 44.7%) disposed their farm waste outside the farm premises. All farms (*n* = 76, 100%) vaccinated their poultry against Newcastle disease virus (NDV) and infectious bursal disease virus (causing a disease also known as Gumboro). In addition, layer and breeder farms (*n* = 68, 89.5%) vaccinated against fowl poxvirus. Different personnel carried out vaccination and treatment of sick birds. Veterinarians administered vaccines on half of the farms (*n* = 39, 51.3%), farm personnel on 34 (44.7%) farms and both veterinarian and farm personnel on fewer (*n* = 3, 3.9%) farms. Veterinarians treated sick animals on the majority (*n* = 47, 61.8%) of farms; some farms (*n* = 21, 27.6%) self-medicated and the minority (*n* = 8, 10.5%) practiced both. Almost all farms (*n* = 75, 98.7%) sold their birds live at the farm gate. Most farms (*n* = 45, 59.2%) had two or three employees.

3.2 | Demographics

A total of 152 respondents participated in the study. Of these, 131 (86.2%) were males. The majority (*n* = 65, 42.8%) were 20–29 years with a median age of 29 years (IQR = 23.0–41.6). Most respondents (*n* = 57, 37.5%) had middle-school level education and few (*n* = 13, 8.6%) had non-formal education. Almost half (*n* = 75, 49.3%) of respondents had worked on their farm for 1–5 years (Table 1).

3.3 | Safety and hygiene practices of respondents on the farm

Respondents reported on various safety and hygiene practices that they perform routinely on their farms. Most respondents (*n* = 148, 97.4%) changed their clothes before starting work on the farm. Few respondents (*n* = 4, 2.7%) wore 'overalls' and nearly half (*n* = 74, 48.7%) wore footwear that covers the entire foot. Most respondents (*n* = 151, 99.3%) changed their footwear before leaving the farm. The majority of respondents (*n* = 149, 98%) did not wear nose masks and none (*n* = 152, 100%) wore gloves when working on the farm. However, nearly all respondents (*n* = 148, 97.4%) washed their hands before leaving the farm (Table 2).

3.4 | Knowledge of poultry diseases

Most respondents (*n* = 132, 86.8%) could identify when their birds were sick. Respondents used clinical signs exhibited by their chickens to determine their health status. Common clinical signs reported comprised the following: greenish diarrhoea, weakness, loss of appetite, trachea rales, cough, sneeze, drop in egg production, bloody spots in faeces, pox on comb and ruffled feathers. Of the respondents who could identify sick animals by clinical signs, very few (*n* = 29, 22%) could not name any poultry disease. The majority of respondents (*n* = 101, 76.5%) correctly named at least one poultry disease with one or more associated clinical sign(s). In total, respondents named 12 different poultry diseases (Figure 1). NDV, Gumboro disease and Coccidiosis were most frequently named while infectious bronchitis and salmonellosis were the least frequently mentioned (Figure 1).

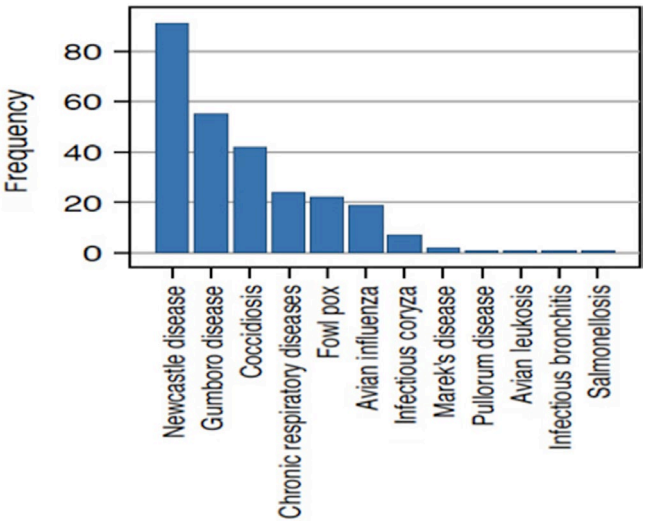

FIGURE 1 Poultry diseases named by respondents (*n* = 266)

3.5 | Awareness and self-protection from zoonotic poultry diseases

In all, 87 (57.2%) respondents were aware that they could become infected with certain poultry diseases. Of those, nearly half (*n* = 39, 44.8%) could name at least one zoonotic poultry disease. Respondents named avian influenza (AI), NDV and salmonellosis as diseases they could contract from their poultry. AI was the most frequently named

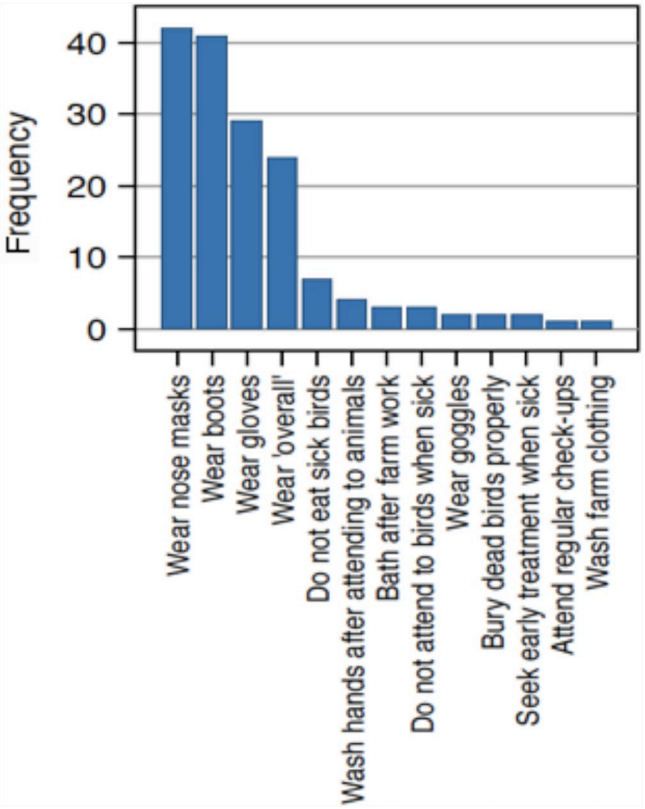

FIGURE 2 Zoonotic preventive measures named (*n* = 161)

(74.5%) while NDV and salmonellosis accounted for 15.7% and 9.8%, respectively. Respondents could name 13 different ways to protect themselves from becoming infected with pathogens from poultry. The

TABLE 1 Demographic characteristics of respondents

Variable	*N* (%)
Female sex	21 (13.8)
Age (years)	
<20	12 (7.9)
20–29	65 (42.8)
30–39	34 (22.4)
40–49	19 (12.5)
50–59	16 (10.5)
60–69	4 (2.6)
>70	2 (1.3)
Education level	
Primary	11 (7.2)
Middle school	57 (37.5)
Senior high school	30 (19.7)
Tertiary	41 (27.0)
None	13 (8.6)
Length of employment at present farm	
<1 yr	29 (19.1)
1–5 yrs	75 (49.3)
>5 yrs	48 (31.6)

TABLE 2 Personal protective equipment usage and hygiene practices among respondents

Parameter	*N* (%)
Change clothes before attending to poultry	148 (97.4)
Type of clothes worn to attend to poultry	
Overall	4 (2.7)
Own clothes	144 (97.3)
Change clothes before exiting farm	148 (100)
Wear protective footwear	74 (48.7)
Change footwear before leaving the farm	151 (99.3)
Wear nose mask	3 (2.0)
Wear gloves	0 (0.0)
Wash hands before leaving farm	148 (97.4)

wearing of nose mask, wellington boots, gloves and overalls while working was the most frequently used zoonotic preventive method. Avoiding the consumption of sick birds, proper disposal of dead birds and regular washing of farm clothing were less frequently mentioned (Figure 2).

The majority of respondents (*n* = 88, 57.9%) had good knowledge about poultry diseases. The age, educational level and experience of respondents influenced their knowledge level score on poultry diseases. Respondents older than 29 years of age were 60% more likely to have good knowledge of poultry diseases than respondents 29 years and below (RR = 1.6, 95% CI = 1.2–2.1). Respondents with a higher education level were about twice as likely to have good knowledge about poultry diseases than respondents with lower education levels (RR = 1.8, 95% CI = 1.4–2.4). Respondents with more than 5 years of employment on the farm were 50% more likely to have good knowledge about poultry diseases than respondents who have spent 5 years

TABLE 3 Factors influencing respondents' knowledge of poultry diseases

		Crude RR		Regression model	
Parameter	High level *N* (%)	RR	95% CI	aRR	95% CI
Sex					
Female	9 (42.9)	Ref.	0.4–1.2		
Male	79 (60.3)	0.7			
Age (years)					
Up to 29	35 (45.5)	Ref.	1.2–2.1		
>29	53 (70.7)	1.6			
Education level					
Low level	34 (42.0)	Ref.	1.4–2.4	1.7	1.3–2.3
High level	54 (76.1)	1.8			
Duration of employment on farm (years)					
Up to 5	52 (50.0)	Ref.	1.2–1.9	1.4	1.1–1.7
>5	36 (75.0)	1.5			

Abbreviations: aRR, adjusted risk ratio; CI, confidence interval; RR, risk ratio.

TABLE 4 Factors influencing respondents' knowledge of zoonotic poultry diseases

Parameter	Good level *N* (%)	Crude RR		Regression model	
		RR	95% CI	aRR	95% CI
Sex					
Female	5 (23.8)	Ref	0.4–2.2		
Male	33 (25.2)	1.0			
Age (years)					
Up to 29	10 (13.0)	Ref	1.5–5.5		
>29	28 (37.3)	2.9			
Education level					
Low level	4 (5.6)	Ref	3.6–26.0	9.6	3.6–25.5
High level	34 (47.9)	9.7			
Duration of employment on farm (years)					
Up to 5	20 (19.2)	Ref	1.1–3.3	1.9	1.2–2.9
>5	18 (37.5)	2.0			

Abbreviations: aRR, Adjusted risk ratio; CI, confidence interval; RR, risk ratio.

or less on the farm (RR = 1.5, 95% CI = 1.2–1.9). In binomial regression, estimates comparable to the crude results were calculated (Table 3).

The knowledge level of respondents on ZPD varied considerably from their knowledge of poultry diseases. A quarter (*n* = 38, 25%) of respondents had good knowledge about ZPD. Respondents with a higher education level were 10 times more likely to have good knowledge of ZPD than respondents with a lower education level (RR = 9.7, 95% CI = 3.6–26.0). Respondents, who worked on the farm for >5 years, were about twice as likely to have good knowledge of ZPD than respondents with up to 5 years of employment experience (RR = 2.0, 95% CI = 1.1–3.3). The binary regression yields comparable results as the crude estimates, highlighting that the chosen variables were unconfounded (Table 4).

4 | DISCUSSION

Proper biosecurity measures (i.e. the implementation of measures that reduce the risk of the introduction and spread of disease agents, FAO/OIE/World Bank, 2008) when adequately practiced on the farm can reduce the risk of introduction and spread of pathogens on farms and further reduce risk of transmission to farmers (Nyaga, 2007). Farmers in Ghana set and operate their own biosecurity standards based largely on their own experience (Aning, Turkson, & Asuming-Brempong, 2009). Our study showed that farms did not comply with all the recommended biosecurity practices and may therefore be at higher risk of outbreaks of infectious diseases on farms and possible ZID spread to humans.

The majority of farmers (81%) have been on the same poultry farm for over 1 year, yet the adoption of on-farm disease mitigating measures like cleaning and disinfection was low. Farmers practiced multi-species (especially chickens and pigs) and multi-age farming (multiple flock of different ages) without the use of footbaths. This does not provide sufficient disinfection of housing and zero fallow period to reduce microbial load in the poultry house. The practice of multi-species provides an enabling environment for generating re-assortment of influenza viruses with zoonotic and pandemic potential from influenza viruses that may be circulating among poultry and pigs in the region (Adeola, Olugasa, & Emikpe, 2015, 2016). Generated waste (including bedding materials of wood shavings together with poultry faeces, feathers, feed and farm dust) is deposited largely on the farm premises which may contaminate the farm environment with potential microbes of economic, environmental and public health importance such as Salmonella sp. and AIV (Andoh et al., 2016; Stephens & Spackman, 2017; Vadari, Mason, & Doerner, 2006; WHO, 2006).

Farms retailed their live birds at the farm gate. This practice brings retailers onto the farm premises regularly exposing them to pathogens circulating on the farm and its environs. The practice also introduces pathogens from carriages of the retailers such as vehicles and cages, onto the farm premises. Aning et al. (2009) observed that public transport is mostly used to move birds in Ghana and that these vehicles are not adequately disinfected before and after being used to transport the birds, posing a public health risk. The unregulated movement of live birds in the country (which is prohibited only during AI outbreaks) further aid the spread of infectious pathogens within and/or between regions in the country and cause exposure risk of the public to airborne zoonotic pathogens such as low pathogenic AI that the birds may be shedding without demonstrating obvious clinical signs.

The wearing of appropriate PPE and adequate farm hygiene practices by farm workers reduces their risk of exposure to occupational health hazards (European Commission, 2012). The majority of respondents have worked on their poultry farms for over 1 year and may have been exposed to infected poultry and the contaminated environment of the farm. Respondents washed their hands regularly but did not utilise PPE for farm activities such as vaccinations and treatment of sick birds. Although washing of hands is a good hygiene practice, it is the use of detergents such as

soap in hand washing that is effective in reducing risk of infection significantly. We did not explore the use of detergents by respondents and can therefore not determine the reduced risk level of hand washing. Respondents did not wear nose masks and remain at high risk of airborne transmitted ZIDs such as AI if circulating in the farms (Harder, Buda, Hengel, Beer, & Mettenleiter, 2016). Gloves were not worn and farm clothing was predominantly a separate set of clothes (often a T-shirt and a pair of shorts or trousers) that the farmer kept on the farm. The use of these separate clothing does not provide the same level of protection as would be provided by overalls (Odo et al., 2015). Interestingly, the majority of respondents had formal education and largely recognised that wearing of PPE is an important preventive measure for ZIDs yet did not implement their use. According to the FAO (2014), poultry farmers in Ghana received extensive training on biosecurity and biosafety practices after the original AI outbreak. However, this study reveals poor adoption and implementation among respondents, implying that education and awareness alone may not be enough to bring about behavioural change among farmers. Behavioural change among respondents may require a multidisciplinary approach including communication and economic analysis. The poor adoption of PPE we observe here is similar to that reported in Bangladesh and Thailand (Odo et al., 2015; Sarker, Sumon, Khan, & Islam, 2016).

Infectious diseases are of major concern to the global poultry industry as frequent outbreaks reduce net profit margins. Our respondents had good knowledge about poultry diseases, particularly those that are endemic and have major economic importance in the country (FAO, 2014). In the multivariate analysis, this good knowledge of poultry diseases was predicted by long years of employment on the farm and having a higher level of education. This good veterinary knowledge and associated predictive factors did not influence respondents' preventive practices on the farm showing knowledge gaps of farm husbandry practices and disease mitigation. This observation is contrary to that reported in China where good veterinary knowledge coupled with longer farming experience of respondents correlated with higher adoption and implementation of disease preventive practices (Huang, Zeng, & Wang, 2016).

As respondents stay longer on the farm, they gain experience in raising the animals and are better able to recognise and treat diseases. This may influence their choice of health care assistance when needed. A study by Turkson (2009) shows that farmers in Ghana rely on their own experience and that of their colleague farmers to buy and dispense drugs to their animals rather than to seek professional assistance. However, we observed skewness towards veterinarians for both disease treatment and vaccination services despite respondents' good knowledge of poultry diseases. This observation agrees with recent report on the use of antibiotics in the poultry industry in the same region (Boamah, Agyare, Odoi, & Dalsgaard, 2016).

The majority (n = 114, 75%) of respondents did not have good knowledge about ZPD according to our score. For the few who had, their good knowledge score was predicted by higher education level and long employment on the farm, similar to that reported in China and Italy on AIV (Abbate, Di Giuseppe, Marinelli, & Angelillo, 2006; Chen et al., 2015). Our respondents were predominantly aware of the zoonotic potential of AIV and to a lesser extent, NCDV and the foodborne pathogen Salmonella. However, they were unaware to the zoonotic potentials of Avian chlamydiosis that cause flu-like symptoms among others, and other foodborne pathogens such as Campylobacter (Andoh et al., 2017; European Commission, 2002; Fraser, Williams, Powell, & Cook, 2010; Osei-Tutu & Anto, 2016).

The limited knowledge of farmers about ZPD may account for their relatively poor attitude towards the wearing of PPE. Farmers were unaware of the zoonotic risk of certain diseases from the animals they keep and the health implications thereof. Our study did not directly assess the knowledge of the transmission route of ZPD among the farmers. However, we identified an implementation gap in which respondents are aware of preventive methods against ZPD but do not put them into practice.

Poultry farmers in the Ashanti region of Ghana have a good knowledge of poultry diseases, which may cause them to treat their birds when sick rather than seek professional help. However, their understanding of becoming infected with specific pathogens from their poultry is low. Farmers' husbandry practices and attitude are not enough to prevent infections and or reduce spread on the farm, thereby increasing their risk of becoming infected with ZPD. The reason(s) for the poor adoption and implementation of biosecurity and biosafety measures among the farmers despite their awareness of these measures should be explored and appropriate interventions instituted.

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CONFLICT OF INTEREST

Authors and funders declare no conflict of interest.

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REFERENCES

Abbate, R., Di Giuseppe, G., Marinelli, P., & Angelillo, I. F. (2006). Knowledge, attitudes, and practices of avian influenza, poultry workers, Italy. *Emerging Infectious Diseases*, *12*(11), 1762–1765. https://doi.org/10.3201/eid1211.060671

Adeola, O. A., Olugasa, B. O., & Emikpe, B. O. (2015). Detection of pandemic strain of influenza virus (A/H1N1/pdm09) in pigs, West Africa: Implications and considerations for prevention of future influenza pandemics at the source. *Infection Ecology & Epidemiology*, *5*, 30227. https://doi.org/10.3402/IEE.V5.30227

Adeola, O. A., Olugasa, B. O., & Emikpe, B. O. (2016). Antigenic detection of human strain of influenza virus A (H3N2) in Swine populations at three locations in Nigeria and Ghana during the dry early months

of 2014. *Zoonoses and Public Health*, *63*(2), 106–111. https://doi.org/10.1111/zph.12210

Andoh, L. A., Ahmed, S., Olsen, J. E., Obiri-Danso, K., Newman, M. J., Opintan, J. A., … Dalsgaard, A. (2017). Prevalence and characterization of Salmonella among humans in Ghana. *Tropical Medicine and Health*, *45*, 3. https://doi.org/10.1186/s41182-017-0043-z

Andoh, L. A., Dalsgaard, A., Obiri-Danso, K., Newman, M. J., Barco, L., & Olsen, J. E. (2016). Prevalence and antimicrobial resistance of Salmonella serovars isolated from poultry in Ghana. *Epidemiology and Infection*, *144*(15), 3288–3299. https://doi.org/10.1017/S0950268816001126

Aning, K. G., Turkson, P. K., & Asuming-Brempong, S. (2009). *Pro-Poor HPAI risk reduction strategies in Ghana —Background paper*. Retrieved from https://assets.publishing.service.gov.uk/media/57a08bcded915d622c000ed7/wp02_IFPRI.pdf

Asante, I. A., Bertram, S., Awuni, J., Commey, A. N. O., Aniwa, B., Ampofo, W. K., & Gabriel, G. (2016). Highly pathogenic avian influenza A(H5N1) virus among poultry, Ghana, 2015. *Emerging Infectious Diseases*, *22*(12), 2209–2211. https://doi.org/10.3201/eid2212.160639

Ayim-Akonor, M., Obiri-Danso, K., Toah-Akonor, P., & Sellers, H. S. (2018). Widespread exposure to infectious bronchitis virus and *Mycoplasma gallisepticum* in chickens in the Ga-East district of Accra, Ghana PUBLIC INTEREST STATEMENT. *Cogent Food & Agriculture*, *32*, 1-11. https://doi.org/10.1080/23311932.2018.1439260

Boamah, V. E., Agyare, C., Odoi, H., & Dalsgaard, A. (2016). Antibiotic practices and factors influencing the use of antibiotics in selected poultry farms in Ghana. *Journal of Antimicrobial Agents*, *2*(2), 1–8. https://doi.org/10.4172/2472-1212.1000120

Chen, S., Li, Z., Hu, M., Guo, S., Wu, J., Wang, B., … Chen, H. (2015). Knowledge, attitudes, and practices (KAP) relating to avian influenza (H10N8) among Farmers' markets workers in Nanchang, China. *PLoS ONE*, *10*(5), e0127120. https://doi.org/10.1371/journal.pone.0127120

European Commission. (2002). *Avian Chlamydiosis as a zoonotic disease and risk reduction strategies report of the scientific committee on animal health and animal welfare*. Retrieved from https://ec.europa.eu/food/sites/food/files/safety/docs/sci-com_scah_out73_en.pdf

European Commission. (2012). *Protecting health and safety of workers in agriculture, livestock farming, horticulture and forestry Protecting Social Europe*. Luxembourg: Publications office of the European Union. Retrieved from http://ec.europa.eu/progress

FAO/OIE/World Bank (2008). *Biosecurity for highly avian influenza. Issues and options* (p. 73). Rome, Italy.

FAO (2009). *FAO and traditional knowledge: The linkages with sustainability, food security and climate change impacts*. Rome, Italy: FAO. Retrieved from http://www.fao.org/3/a-i0841e.pdf

FAO (2014). *Poultry Sector Ghana. FAO animal production and health livestock country reviews*. Rome, Italy: FAO. Retrieved from http://www.fao.org/3/a-i3663e.pdf

Fraser, R. W., Williams, N. T., Powell, L. F., & Cook, A. J. C. (2010). Reducing Campylobacter and Salmonella infection: Two studies of the economic cost and attitude to adoption of on-farm biosecurity measures. *Zoonoses and Public Health*, *57*(7–8), e109–e115. https://doi.org/10.1111/j.1863-2378.2009.01295.x

Ghana Ministry of Food and Agriculture (2015). *Facts and figures*. Retrieved from http://www.agrofood-westafrica.com/fileadmin/user_upload/messen/agrofood-Westafrica/Brochure/AGRICULTURE-IN-GHANA-Facts-and-Figures-2015.pdf%0Ahttp://cairngorms.co.uk/discover-explore/facts-figures/

Halliday, J. E. B., Allan, K. J., Ekwem, D., Cleaveland, S., Kazwala, R. R., & Crump, J. A. (2015). Endemic zoonoses in the tropics: A public health problem hiding in plain sight. *The Veterinary Record*, *176*(9), 220–225. https://doi.org/10.1136/vr.h798

Harder, T. C., Buda, S., Hengel, H., Beer, M., & Mettenleiter, T. C. (2016). Poultry food products—A source of avian influenza virus transmission to humans? *Clinical Microbiology and Infection*, *22*(2), 141–146. https://doi.org/10.1016/J.CMI.2015.11.015

Huang, Z., Zeng, D., & Wang, J. (2016). Factors affecting Chinese broiler farmers' main preventive practices in response to highly pathogenic avian influenza. *Preventive Veterinary Medicine*, *134*, 153–159. https://doi.org/10.1016/j.prevetmed.2016.10.013

Mangesho, P. E., Neselle, M. O., Karimuribo, E. D., Mlangwa, J. E., Queenan, K., Mboera, L. E. G.,… Rweyemamu, M.. (2017). Exploring local knowledge and perceptions on zoonoses among pastoralists in northern and eastern Tanzania. *PLOS Neglected Tropical Diseases*, *11*(2), e0005345. https://doi.org/10.1371/journal.pntd.0005345

McDermott, J. J., & Arimi, S. (2002). Brucellosis in sub-Saharan Africa: Epidemiology, control and impact. *Veterinary Microbiology*, *90*(1–4), 111–134. https://doi.org/10.1016/S0378-1135(02)00249-3

Nyaga, P. (2007). *Food and agriculture organization of the United Nations Strategies for the prevention and control of infectious diseases (including Highly Pathogenic Avian Influenza) in Eastern Africa GOOD BIOSECURITY PRACTICES IN SMALL SCALE COMMERCIAL AND SCAVENGING PR*. Retrieved from http://www.fao.org/3/a-al838e.pdf

Nyanteng, V. K., Takyi, P., Lawford, P., Acheamfuor, B., Nii, E., & Tawiah, O. (2013). *2010 population and Housing census. Regional analytical report. Ashanti region*. Retrieved from http://www.statsghana.gov.gh/docfiles/2010phc/2010_PHC_Regional_Analytical_Reports_Ashanti_Region.pdf

Odo, N. U., Raynor, P. C., Beaudoin, A., Somrongthong, R., Scheftel, J. M., Donahue, J. G., & Bender, J. B. (2015). Personal protective equipment use and handwashing among animal farmers: A multi-site assessment. *Journal of Occupational and Environmental Hygiene*, *12*(6), 363–368. https://doi.org/10.1080/15459624.2015.1006635

OIE. (2019). *OIE (World Organization for Animal Health), ' Situation report for highly pathogenic avian influenza' 2019*. Retrieved from http://www.oie.int/en/animal-health-in-the-world/update-on-avian-influenza/2019/.

Osei-Tutu, B., & Anto, F. (2016). Trends of reported foodborne diseases at the Ridge Hospital, Accra, Ghana: A retrospective review of routine data from 2009–2013. *BMC Infectious Diseases*, *16*, 139. https://doi.org/10.1186/s12879-016-1472-8

Sarker, S. K., Sumon, S. M. M. R., Khan, M. A. H. N. A., & Islam, M. T. (2016). Knowledge, attitude and practices survey on avian influenza in three districts of Bangladesh. *Bangladesh Journal of Veterinary Medicine*, *14*(1), 27. https://doi.org/10.3329/bjvm.v14i1.28819

Stephens, C. B., & Spackman, E. (2017). Thermal Inactivation of avian influenza virus in poultry litter as a method to decontaminate poultry houses. *Preventive Veterinary Medicine*, *145*, 73–77. https://doi.org/10.1016/j.prevetmed.2017.06.012

Thornton, P. K. (2010). Livestock production: Recent trends, future prospects. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *365*(1554), 2853–2867. https://doi.org/10.1098/rstb.2010.0134

Thornton, P. K., Kruska, R. L., Henninger, N., Kristjanson, P. M., Reid, R. S., Atieno, F., … Ndegwa, T. (2002). *Mapping poverty and livestock in the developing world*. Health San Francisco, 1, 126. Retrieved from http://www.ilri.cgiar.org/Infoserv/Webpub/fulldocs/Mappoverty/media/PDF_chapters/B2_Front.pdf

Turkson, P. (2009). Use of drugs and antibiotics in poultry production in Ghana. *Ghana Journal of Agricultural Science*, *41*(1), https://doi.org/10.4314/gjas.v41i1.46142

Vadari, Y., Mason, B. P., & Doerner, K. C. (2006). Isolation from poultry litter and characterization in high phosphate conditions of *Staphylococcus* spp. capable of growth. *Letters in Applied Microbiology*, *43*, 64–70. https://doi.org/10.1111/j.1472-765X.2006.01901.x

WHO (2006). The control of neglected zoonotic diseases A route to poverty alleviation. *World Health*, *1*, 1-41. Retrieved from www.who.int/zoonoses/Report_Sept06.pdf

WHO (2011). *WHO|Neglected zoonotic diseases*. Retrieved from http://www.who.int/neglected_diseases/diseases/zoonoses/en/

WHO (2019). *Cumulative number of confirmed human cases for avian influenza A(H5N1) reported to WHO, 2003–2019*. Retrieved from https://www.who.int/influenza/human_animal_interface/2019_02_12_tableH5N1.pdf?ua=1

Zinsstag, J., Schelling, E., Roth, F., Bonfoh, B., de Savigny, D., & Tanner, M. (2007). Human benefits of animal interventions for zoonosis control. *Emerging Infectious Diseases*, *13*(4), 527–531. https://doi.org/10.3201/eid1304.060381

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

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ORIGINAL ARTICLE

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

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 Nzussouo 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 subclinical 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 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.

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.

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 km^2. 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 μl 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 μl 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 μl of In-type internal control (IC) RNA (Qiagen) was added to each sample prior to isolation. RNA was eluted in 30 μl 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 μl 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 ≥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 ≥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

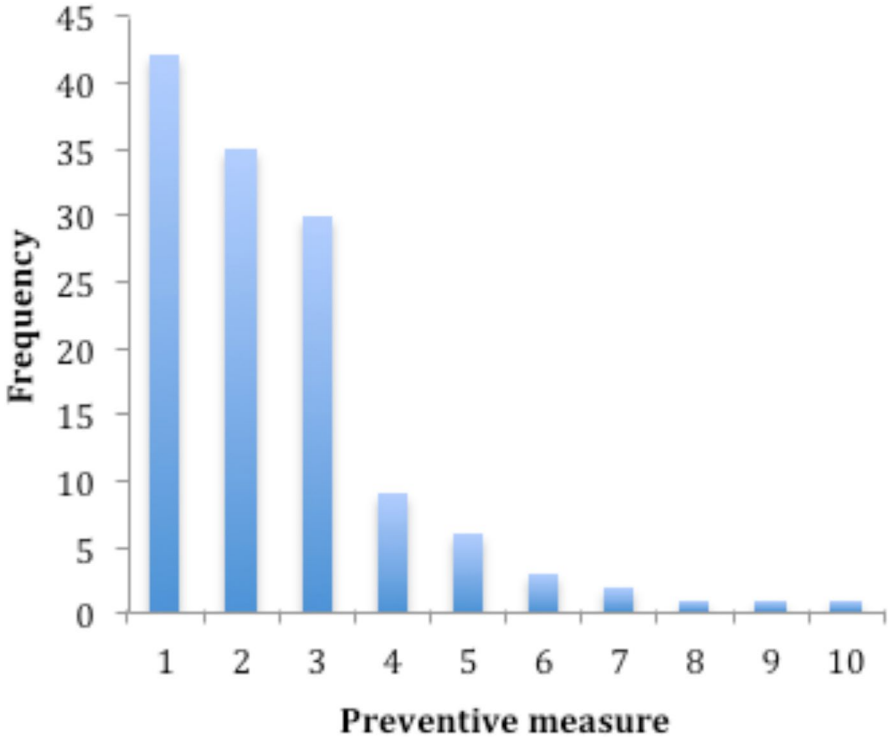

FIGURE 1 Preventive measures against swine zoonoses as mentioned by farmers. Key: 1 = Wear surgical mask, 2 = Wear protective footwear, 3 = Wear gloves, 4 = Wear dedicated farm clothing when working, 5 = Stay away from swine when sick, 6 = Bath after farm work 7 = Wear goggles, 8 = Wash farm clothing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

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 sub-groups holding

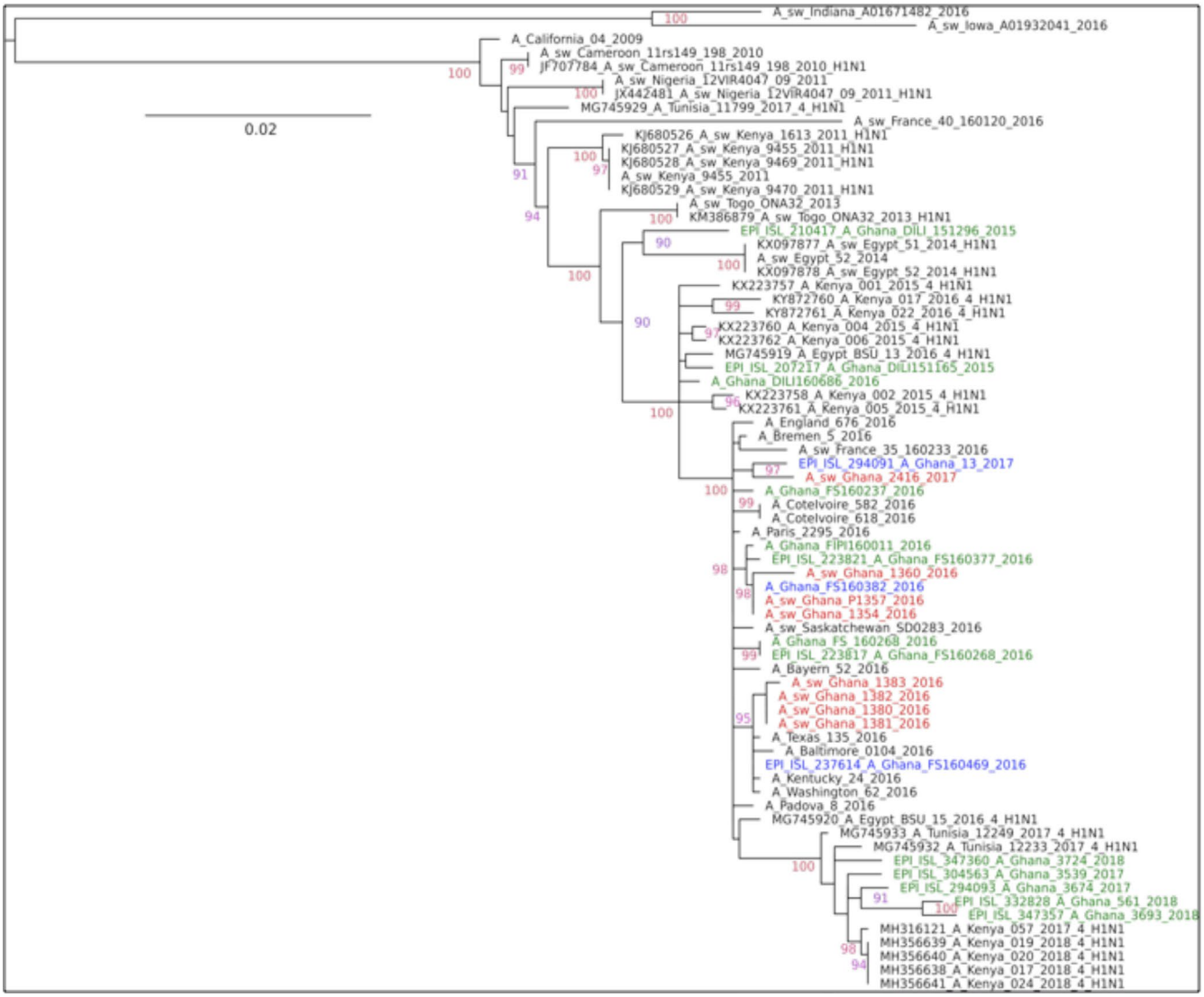

FIGURE 2 Phylogenetic relationship between HAs of H1N1pdm09 viruses identified in swine population in Ghana in our study (red), H1N1 from humans in Ghana (blue and green) and H1N1 from humans and swine from Africa (other than Ghana), Europe and North America (black)

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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REFERENCES

Adeola, O. A., Olugasa, B. O., & Emikpe, B. O. (2015). Detection of pandemic strain of influenza virus (A/H1N1/pdm09) in pigs, West Africa: Implications and considerations for prevention of future influenza pandemics at the source. *Infection Ecology & Epidemiology*, *5*, 30227. https://doi.org/10.3402/IEE.V5.30227

Adeola, O. A., Olugasa, B. O., & Emikpe, B. O. (2016). Antigenic detection of Human Strain of Influenza Virus A (H3N2) in Swine populations at three locations in Nigeria and Ghana during the dry early months of 2014. *Zoonoses and Public Health*, *63*(2), 106–111. https://doi.org/10.1111/zph.12210

Adeola, O. A., Olugasa, B. O., Emikpe, B. O., & Folitse, R. D. (2019). Syndromic survey and molecular analysis of influenza viruses at the human–swine interface in two West African cosmopolitan cities suggest the possibility of bidirectional interspecies transmission. *Zoonoses and Public Health*, *66*(2), 232–247. https://doi.org/10.1111/zph.12559

Annan, A., Owusu, M., Marfo, K. S., Larbi, R., Sarpong, F. N., Adu-Sarkodie, Y., … Eckerle, I. (2015). High prevalence of common respiratory viruses and no evidence of Middle East Respiratory Syndrome Coronavirus in Hajj pilgrims returning to Ghana, 2013. *Tropical Medicine and International Health*, *20*(6), 807–812. https://doi.org/10.1111/tmi.12482

Awuni, J. A., Bianco, A., Dogbey, O. J., Fusaro, A., Yingar, D. T., Salviato, A., … Monne, I. (2019). Avian influenza H9N2 subtype in Ghana: Virus characterization and evidence of co-infection. *Avian Pathology*, *48*(5), 470–476. https://doi.org/10.1080/03079457.2019.1624687

Ayim-Akonor, M., May, J., Ralf, K., Harder, T., & Mertens, E. (2019). Molecular and serological prevalence of influenza A viruses in poultry and poultry farmers in the Ashnati region, Ghana. *Infection Ecology & Epidemiology*, *9*(1), 1698904. https://doi.org/10.1080/20008686.2019.1698904

Corzo, C. A., Culhane, M., Juleen, K., Stigger-Rosser, E., Ducatez, M. F., Webby, R. J., & Lowe, J. F. (2013). Active surveillance for influenza A virus among swine, midwestern United States, 2009–2011. *Emerging Infectious Diseases*, *19*(6), 954–960. https://doi.org/10.3201/eid1906.121637

Couacy-Hymann, E., Kouakou, V. A., Aplogan, G. L., Awoume, F., Kouakou, C. K., Kakpo, L., … Ducatez, M. F. (2012). Surveillance for influenza viruses in poultry and swine, West Africa, 2006–2008. *Emerging Infectious Diseases*, *18*(9), 1446–1452. https://doi.org/10.3201/eid1809.111296

Deng, Y.-M., Iannello, P., Smith, I., Watson, J., Barr, I. G., Daniels, P., … Wong, F. Y. K. (2012). Transmission of influenza A(H1N1) 2009 pandemic

viruses in Australian swine. *Influenza and Other Respiratory Viruses*, *6*(3), e42–e47. https://doi.org/10.1111/j.1750-2659.2012.00337.x

Ducatez, M. F., Awoume, F., & Webby, R. J. (2015). Influenza A(H1N1) pdm09 virus in pigs, Togo, 2013. *Veterinary Microbiology*, *177*(1–2), 201–205. https://doi.org/10.1016/j.vetmic.2015.02.028

Eisfeld, A. J., Neumann, G., & Kawaoka, Y. (2014). Influenza A virus isolation, culture and identification. *Nature Protocols*, *9*(11), 2663–2681. https://doi.org/10.1038/nprot.2014.180

Epperson, S., Jhung, M., Richards, S., Quinlisk, P., Ball, L., Moll, M., … Influenza A (H3N2)v Virus Investigation Team (2013). Human Infections With Influenza A(H3N2) variant virus in the United States, 2011–2012. *Clinical Infectious Diseases*, *57*(Suppl. 1), S4–S11. https://doi.org/10.1093/cid/cit272

FAOSTAT. (2008). Retrieved from http://www.fao.org/faostat/en/#data/QA/visualize

Fobian, K., Fabrizio, T. P., Yoon, S.-W., Hansen, M. S., Webby, R. J., & Larsen, L. E. (2015). New reassortant and enzootic European swine influenza viruses transmit efficiently through direct contact in the ferret model. *Journal of General Virology*, *96*, 1603–1612. https://doi.org/10.1099/vir.0.000094

Forberg, H., Hauge, A. G., Gjerset, B., Hungnes, O., & Kilander, A. (2013). Swine influenza in Norway: A distinct lineage of influenza A(H1N1) pdm09 virus. *Influenza and Other Respiratory Viruses*, *7*(Suppl. 4), 21–26. https://doi.org/10.1111/irv.12194

Ghana Statistical Service (2013). *2010 Population and housing census: National analytical report*. Retrieved from http://www.statsghana.gov.gh/docfiles/publications/2010_PHC_National_Analytical_Report.pdf

Harder, T. C., Grosse Beilage, E., Lange, E., Meiners, C., Dohring, S., Pesch, S. … Starick, E. (2013). Expanded cocirculation of stable subtypes, emerging lineages, and new sporadic reassortants of porcine influenza viruses in swine populations in northwest Germany. *Journal of Virology*, *87*(19), 10460–10476. https://doi.org/10.1128/JVI.00381-13

Henritzi, D., Zhao, N., Starick, E., Simon, G., Krog, J. S., Larsen, L. E., … Harder, T. C. (2016). Rapid detection and subtyping of European swine influenza viruses in porcine clinical samples by haemagglutinin- and neuraminidase-specific tetra- and triplex real-time RT-PCRs. *Influenza and Other Respiratory Viruses*, *10*(6), 504–517. https://doi.org/10.1111/irv.12407

Hoffmann, B., Hoffmann, D., Henritzi, D., Beer, M., & Harder, T. C. (2016). Riems influenza a typing array (RITA): An RT-qPCR-based low density array for subtyping avian and mammalian influenza a viruses. *Scientific Reports*, *6*, 27211. https://doi.org/10.1038/srep27211

Hoffmann, E., Stech, J., Guan, Y., Webster, R. G., & Perez, D. R. (2001). Universal primer set for the full-length amplification of all influenza A viruses. *Archives of Virology*, *146*(12), 2275–2289. https://doi.org/10.1007/s007050170002

Hogan, B., Ammer, L., Zimmermann, M., Binger, T., Krumkamp, R., Sarpong, N., … Eibach, D. (2017). Burden of influenza among hospitalized febrile children in Ghana. *Influenza and Other Respiratory Viruses*, *11*(6), 497–501. https://doi.org/10.1111/irv.12507

Ito, T., Couceiro, J. N., Kelm, S., Baum, L. G., Krauss, S., Castrucci, M. R., … Kawaoka, Y. (1998). Molecular basis for the generation in pigs of influenza A viruses with pandemic potential. *Journal of Virology*, *72*(9), 7367–7373. https://doi.org/10.1128/JVI.72.9.7367-7373.1998

Jones, A. H., Ampofo, W., Akuffo, R., Doman, B., Duplessis, C., Amankwa, J. A., … Dueger, E. L. (2016). Sentinel surveillance for influenza among severe acute respiratory infection and acute febrile illness inpatients at three hospitals in Ghana. *Influenza and Other Respiratory Viruses*, *10*(5), 367–374. https://doi.org/10.1111/irv.12397

Kirunda, H., Erima, B., Tumushabe, A., Kiconco, J., Tugume, T., Mulei, S., … Wabwire-Mangen, F. (2014). Prevalence of influenza A viruses in livestock and free-living waterfowl in Uganda. *BMC Veterinary Research*, *10*, 50. https://doi.org/10.1186/1746-6148-10-50

Kong, W., Wang, F., Dong, B., Ou, C., Meng, D., Liu, J., & Fan, Z.-C. (2015). Novel reassortant influenza viruses between pandemic (H1N1) 2009 and other influenza viruses pose a risk to public health. *Microbial Pathogenesis*, *89*, 62–72. https://doi.org/10.1016/J.MICPATH.2015.09.002

Larison, B., Njabo, K. Y., Chasar, A., Fuller, T., Harrigan, R. J., & Smith, T. B. (2014). Spillover of pH1N1 to swine in Cameroon: An investigation of risk factors. *BMC Veterinary Research*, *10*, 55. https://doi.org/10.1186/1746-6148-10-55

Li, O. T. W., Barr, I., Leung, C. Y. H., Chen, H., Guan, Y., Peiris, J. S. M., & Poon, L. L. M. (2007). Reliable universal RT-PCR assays for studying influenza polymerase subunit gene sequences from all 16 haemagglutinin subtypes. *Journal of Virological Methods*, *142*(1–2), 218–222. https://doi.org/10.1016/j.jviromet.2007.01.015

Meseko, C., Globig, A., Ijomanta, J., Joannis, T., Nwosuh, C., Shamaki, D., … Starick, E. (2018). Evidence of exposure of domestic pigs to Highly Pathogenic Avian Influenza H5N1 in Nigeria. *Scientific Reports*, *8*(1), 5900. https://doi.org/10.1038/s41598-018-24371-6

Meseko, C. A., Heidari, A., Odaibo, G., & Olaleye, D. (2019). Complete genome sequencing of H1N1pdm09 swine influenza isolates from Nigeria reveals likely reverse zoonotic transmission at the human-animal interface in intensive piggery. *Infection Ecology & Epidemiology*, *9*(1), 1696632. https://doi.org/10.1080/20008686.2019.1696632

Meseko, C. A., Odaibo, G. N., & Olaleye, D. O. (2014). Detection and isolation of 2009 pandemic influenza A/H1N1 virus in commercial piggery, Lagos Nigeria. *Veterinary Microbiology*, *168*(1), 197–201. https://doi.org/10.1016/j.vetmic.2013.11.003

Meseko, C., Olaleye, D., Capua, I., & Cattoli, G. (2014). Swine influenza in sub-Saharan Africa – Current knowledge and emerging insights. *Zoonoses and Public Health*, *61*(4), 229–237. https://doi.org/10.1111/zph.12068

Munyua, P., Onyango, C., Mwasi, L., Waiboci, L. W., Arunga, G., Fields, B., … Njenga, M. K. (2018). Identification and characterization of influenza A viruses in selected domestic animals in Kenya, 2010–2012. *PLoS One*, *13*(2), e0192721. https://doi.org/10.1371/journal.pone.0192721

Njabo, K. Y., Fuller, T. L., Chasar, A., Pollinger, J. P., Cattoli, G., Terregino, C., … Smith, T. B. (2012). Pandemic A/H1N1/2009 influenza virus in swine, Cameroon, 2010. *Veterinary Microbiology*, *156*(1–2), 189–192. https://doi.org/10.1016/j.vetmic.2011.09.003

Nyanteng, V. K., Takyi, P., Lawford, P., Acheamfuor, B., Nii, E., & Tawiah, O. (2013). *2010 population and Housing census. Regional analytical report. Ashanti region*. Retrieved from http://www.statsghana.gov.gh/docfiles/2010phc/2010_PHC_Regional_Analytical_Reports_Ashanti_Region.pdf

Osoro, E. M., Lidechi, S., Nyaundi, J., Marwanga, D., Mwatondo, A., Muturi, M., … Njenga, K. (2019). Detection of pandemic influenza A/H1N1/pdm09 virus among pigs but not in humans in slaughterhouses in Kenya, 2013–2014. *BMC Research Notes*, *12*(1), 628. https://doi.org/10.1186/s13104-019-4667-4

Shinde, V., Bridges, C. B., Uyeki, T. M., Shu, B., Balish, A., Xu, X., … Finelli, L. (2009). Triple-reassortant Swine Influenza A (H1) in humans in the United States, 2005–2009. *New England Journal of Medicine*, *360*(25), 2616–2625. https://doi.org/10.1056/NEJMoa0903812

Simon, G., Larsen, L. E., Dürrwald, R., Foni, E., Harder, T., Van Reeth, K., … Loeffen, W. (2014). European surveillance network for influenza in pigs: Surveillance programs, diagnostic tools and Swine influenza virus subtypes identified in 14 European countries from 2010 to 2013. *PLoS One*, *9*(12), e115815. https://doi.org/10.1371/journal.pone.0115815

Talla Nzussouo, N., Duque, J., Adedeji, A. A., Coulibaly, D., Sow, S., Tarnagda, Z., … McMorrow, M. (2017). Epidemiology of influenza in West Africa after the 2009 influenza A(H1N1) pandemic, 2010–2012. *BMC Infectious Diseases*, *17*(1), 745. https://doi.org/10.1186/s12879-017-2839-1

Taubenberger, J. K., & Kash, J. C. (2010). Influenza virus evolution, host adaptation, and pandemic formation. *Cell Host & Microbe*, *7*(6), 440–451. https://doi.org/10.1016/j.chom.2010.05.009

Tse, M., Kim, M., Chan, C. H., Ho, P. L., Ma, S. K., Guan, Y., & Peiris, J. S. (2012). Evaluation of three commercially available influenza A type-specific blocking enzyme-linked immunosorbent assays for seroepidemiological studies of influenza A virus infection in pigs. *Clinical and Vaccine Immunology*, *19*(3), 334–337. https://doi.org/10.1128/CVI.05358-11

Yang, H., Chen, Y., Qiao, C., He, X., Zhou, H., Sun, Y., … Chen, H. (2016). Prevalence, genetics, and transmissibility in ferrets of Eurasian avian-like H1N1 swine influenza viruses. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(2), 392–397. https://doi.org/10.1073/pnas.1522643113

Zhang, Y., Aevermann, B. D., Burke, D. F., Dauphin, G., Gu, Z., & Li, X. (2011). Influenza Research Database: An integrated bioinformatics resource for influenza virus research. *Nucleic acids research*, *45*, D466–D474.

Zhu, H., Webby, R., Lam, T. T. Y., Smith, D. K., Peiris, J. S. M., & Guan, Y. (2011). History of swine influenza viruses in Asia. *Current Topics in Microbiology and Immunology*, *370*, 57–68. https://doi.org/10.1007/82_2011_179

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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Supplemental Table 1. HI titers of human-like IAVs and European swIAVs in swine sera (2016-2017)

Sample ID	HAI titer pH1N1	HAI titer H3	HAI titer H1avN1av	HAI titer H3swN2
P1139	20	320	<10	<10
P1209	20	320	<10	<10
P1260	80	640	<10	<10
P1261	160	640	80	20
P1262	160	640	<10	<10
P1263	320	≥1280	80	40
P1264	160	640	<10	<10
P1265	320	320	40	<10
P1266	320	320	20	<10
P1267	320	320	10	<10
P1268	160	320	<10	<10
P1269	160	640	20	<10
P1270	160	640	20	<10
P1271	640	≥1280	80	40
P1272	160	640	160	<10
P1273	320	640	160	<10
P1274	≥1280	≥1280	640	<10
P1432	80	≥1280	20	80
P1475	160	320	20	<10
P1477	320	640	160	20
P1479	80	320	160	20
P1488	320	640	160	10
P1489	160	≥1280	40	10
P1490	160	≥1280	80	40
P1510	80	640	20	10
P1511	80	640	80	10
P1517	320	640	160	10
P1518	80	640	40	10
P1520	160	640	20	10
P1521	320	≥1280	80	<10
P1527	80	640	20	10
P1539	80	640	<10	10
P1541	80	320	<10	40
P2489	160	≥1280	80	160
P2490	160	≥1280	80	80
P2498	640	≥1280	160	80
P2503	320	640	80	40
P2506	640	1280	80	160

HI titers ≥ 40 are considered positive.

Supplemental Table 2. Amino acid substitutions and phenotypes identified in H1N1pdm09 from swine in Ghana (2016-2017) compared with A/swine/Alberta/25/2009(H1N1)

Protein	Substitution present	Phenotype	Reference
PB2	627E	Decreased virulence and replication efficiency in mice	(Bogs et al., 2011)
PB2	701D	Decreased virulence	(Li et al., 2005)
PB2	89V, 309D, 339K, 477G, 495V, 627E, 676T	Increased polymerase activity	(Li et al., 2009)
PA	149S	Decreased virulence in mammals	Leung et al., 2010)
NA	275H, 278E, 295N	Sensitive to Oseltamivir	(Earhart et al., 2009; Hurt et al., 2009a)
NA	223I, 247S, 119E, 136Q	Sensitive to Oseltamivir, Zanamivir and Peramivir	(Hurt et al., 2009a; Hurt et al., 2009b; Okomo-Adhiambo et al., 2010; Pizzorno et al., 2012)

References:

Bogs, J., Kalthoff, D., Veits, J., Pavlova, S., Schwemmle, M., Mänz, B., ⋯ Stech, J. (2011). Reversion of PB2 - 627E to - 627K during replication of an H5N1 Clade 2.2 virus in mammalian hosts depends on the origin of the nucleoprotein. Journal of Virology, 85(20), 10691–10698. https://doi.org/10.1128/JVI.00786-11

Earhart, K. C., Elsayed, N. M., Saad, M. D., Gubareva, L. V., Nayel, A., Deyde, V. M., … Tjaden, J. A. (2009). Oseltamivir resistance mutation N294S in human influenza A(H5N1) virus in Egypt. Journal of Infection and Public Health, 2(2), 74–80. https://doi.org/10.1016/j.jiph.2009.04.004

Hurt, A. C., Holien, J. K., Parker, M., Kelso, A., & Barr, I. G. (2009). Zanamivir - resistant influenza viruses with a novel neuraminidase mutation. Journal of Virology, 83(20), 10366-10373. https://doi.org/10.1128/JVI.01200-09

Leung, B. W., Chen, H., & Brownlee, G. G. (2010). Correlation between polymerase activity and pathogenicity in two duck H5N1 influenza viruses suggests that the polymerase contributes to pathogenicity. Virology, 401(1), 96–106. https://doi.org/10.1016/j.virol.2010.01.036

Li, J., Ishaq, M., Prudence, M., Xi, X., Hu, T., Liu, Q., & Guo, D. (2009). Single mutation at the amino acid position 627 of PB2 that leads to increased virulence of an H5N1 avian influenza virus during adaptation in mice can be compensated by multiple mutations at other sites of PB2. Virus Research, 144(1–2), 123–129. https://doi.org/10.1016/j.virusres.2009.04.008

Li, Z., Chen, H., Jiao, P., Deng, G., Tian, G., Li, Y., … Yu, K. (2005). Molecular basis of replication of duck H5N1 influenza viruses in a mammalian mouse model. Journal of Virology, 79(18), 12058–12064. https://doi.org/10.1128/JVI.79.18.12058-12064.2005

Okomo‐Adhiambo, M., Nguyen, H. T., Sleeman, K., Sheu, T. G., Deyde, V. M., Garten, R. J., … Gubareva, L. V. (2010). Host cell selection of influenza neuraminidase variants: Implications for drug resistance monitoring in A(H1N1) viruses. Antiviral Research, 85(2), 381–388. https://doi.org/10.1016/j.antiviral.2009.11.005

Pizzorno, A., Abed, Y., Bouhy, X., Beaulieu, E., Mallett, C., Russell, R., & Boivin, G. (2012). Impact of mutations at residue I223 of the neuraminidase protein on the resistance profile, replication level, and virulence of the 2009 pandemic influenza virus. Antimicrobial Agents and Chemotherapy, 56(3), 1208–1214. https://doi.org/10.1128/AAC.05994-11

8.0 General Discussion

Infectious pathogens of animal origins occasionally cross the host species barrier to cause disease and sometimes death in humans, and persons in direct contact with these animals are particularly at high risk. An effective control method of zoonoses requires a multidisciplinary approach of 'One Health' where both animal- and human- health practitioners work together simultaneously to institute useful and effective control strategies in their respective population with animal health practitioners making the additional effort to interupt transmission from animals to humans.

IAVs have a wide host range and are associated with seasonal epidemics and rare pandemics in humans. In poultry and swine, the virus causes serious economic losses to these animal producers. In addition, the virus can cross the host species barrier to infect humans with increased incidence reported among individuals in direct contact with animals and or contaminated environment. Therefore, circulating viruses in animals are of both veterinary and public health concern. However, limited information exists on influenza viruses circulating in farmed animals in Ghana. Nearly no information exists for virus circulation in farmers. This study was thus undertaken to provide baseline information on influenza transmissions at the human-animal interface in Ghana.

8.1 Research objectives 1 and 2

- Determine the prevalence of IAVs circulating in poultry and poultry-farmers in Ghana
- Evaluate the husbandry practices and zoonotic knowledge of poultry farmers in Ghana

Despite the intense poultry activity in the Ashanti region, no data on AIV infections in poultry at farm levels has been published from the region, leaving a knowledge gap. In our surveillance across 76 poultry farms in the region, AIV was detected in apparently healthy commercial chickens raised in the region but at a low prevalence (Manuscript 1). AIV antibodies were not detected in any of the farms visited. Unlike other countries such as Egypt and Bangladesh, HPAIV H5N1 has not become endemic in the Ghanaian poultry population after the initial introduction in 2007 and re-occurrence in 2015. Thus, the AIV detected in a few of the commercial poultry farms in the region was likely to have been recently introduced into the poultry population shortly before or during our sampling period,

highlighting the importance of regular surveillance as a tool to early pathogen detection. The circulating virus could not be directly subtyped because the initial viral load was low and attempts to isolate the virus via embryonating chicken eggs was not successful. However because the affected chickens exhibited no clinical symptoms, the virus was likely to be an LPAIV rather than an HPAIV. There is no national policy for eradicating LPAIV in the country and the pathogen may therefore circulate continuously in the flock without direct control as would be the case if it was an HPAIV. Biosecurity and biosafety practices were poor on the majority of farms (Manuscript 2). Most farms had multiple age flocks while other farms had other animals mostly pigs. Yet the majority of farms did not have appropriate footbaths. In addition, farm waste including bedding materials was disposed on the premises of most farms (Manuscript 2). These sub-optimal practices may contribute to viral spread within and between flocks on the farm. The continuous circulation of LPAIV in poultry may cause a potential immunosuppressive effect, predisposing the flock to other pathogens. Such co-infections may lead to an increase in disease severity and infection outcomes and thereby reduce animal productivity and profit of the farmers.

Poultry farmers in the region were actively infected with seasonal influenza virus of subtype H1N1 but not with AIV. In addition, antibodies to avian H5 and H7 (the only subtypes analysed in the study) were not detected, implying that poultry farmers in the region have not been exposed to these avian pathogens (Manuscript 1). These subtypes were prioritised for analysis because so far, the highest human infections and deaths due to influenza of avian origin have been H5 and H7 (section 3.1) and also because infections of poultry with these subtypes are reportable to the OIE.

Although active AIV and/or antibodies to selected AIV subtypes were not detected in poultry farmers, the risk of farmers to possible infections with AIVs remains high. This is due to the poor biosafety practices of farmers (Manuscript 2) as reported elsewhere in the country (Agbenohevi et al., 2015; Burimuah et al., 2016; Odoom et al., 2012). A substantial number of farmers were aware of the zoonotic potentials of AIVs. In addition, farmers had good knowledge of several preventive measures that could minimize their risk of zoonotic infections. However, farmers rarely applied these measures when working on the farm. After the maiden outbreak of HPAIV H5N1, several trainings, workshops and education were organized for poultry farmers across the country irrespective of whether the region recorded an outbreak or not. The present study was conducted almost a decade after the first outbreak in the country and during the period when the second outbreak in the country was occurring. The majority of poultry farmers in the study region had worked on their present farm from 1-

5 years. It is interesting to speculate that most of these farmers may not have been involved in poultry farming during the maiden outbreak and may therefore not have attended or be interested in the AIV workshops, training and other educational programmes that were previously organized for the farmers. However for farmers that were in poultry farming during the first AIV outbreak (more than 5 years on the farm), they may have been interested in AIV and possibly attended AIV training workshops and seminars organized by stakeholders. This may be reflected in the high knowledge of poultry zoonoses among individuals who are older than 29 years. However, these farmers did not put their acquired knowledge into practice (Manuscript 2). The region did not record any AIV in the first outbreak and no human infection with AIV has so far been recorded in Ghana. These factors may perhaps contribute to the poor adoption of biosafety practices of farmers in the region.

8.2 Research objective 3

- Identify and genetically characterise IAVs circulating in swine and swine-farmers in Ghana

IAV RNA was detected in swine kept intensively in the Ashanti region. The identified virus phylogenetically clustered closely with IAVs that circulated in humans in the country and other parts of the world in 2016-2017, but distant from similar viruses detected from swine in other African countries, Europe and America (Manuscript 3). The identified viruses do not seem to have become adapted to swine but rather sporadically transmitted directly from humans to swine. Unlike Europe and America where multiple IAVs circulate in swine, only viral antigens of influenza subtype H1N1pdm09 (Corzo et al., 2013; Harder et al., 2013; Simon et al., 2014) was detected in the study region but antibodies to human H3 was also detected indicating previous exposure of swine to this human-like viruses (Manuscript 3). From SSA countries such as Kenya, Cameroon and Togo, similar surveillance studies have often detected influenza H1N1pdm09 in swine (Table 3) demonstrating that the virus circulates actively in the human population with high transmission rate to swine. Using antigen capture ELISA, human-like H3N2 was reported in swine in Nigeria and Ghana at a prevalence of 4% in each country (Adeola et al., 2016). The present study used haemagglutination inhibition assay, which is the gold standard for influenza serology, and demonstrated high titres of human-like H3 antibodies in swine (Manuscript 3).

Farmers were not infected with endemic swine IAVs but seasonal influenza H1N1pdm09 and H3N2. The anthroponosis observed here reflects on the level of biosafety practices carried

out by farmers on their farms. Swine farmers were aware of the zoonotic potential of swine influenza viruses but wore no surgical mask and gloves to reduce their risk of infection and subsequently minimise anthroponoses. The practice of farmers to attend to animals despite experiencing influenza-like illness may be a transmission pathway for swine infections with human influenza viruses.

9.0 Conclusion.

Avian influenza virus, most likely of low pathogenicity was detected in chickens in the region but at low prevalence. AIV antibodies were not detected. The virus was not endemic in poultry but likely to have been sporadically transmitted to the poultry population shortly before or during the sampling period. Poultry farmers were infected and exposed to IAVs associated with seasonal epidemics in humans but not to AIVs.

Swine in the Ashanti region were exposed to and infected with human seasonal IAVs. Endemic swIAVs and possible reassortants thereof were not found in swine in the region during the study period. Swine farmers were however exposed to and infected with influenza viruses that cause seasonal epidemics in humans. The husbandry practices of farmers were poor and farmers may be a source of infection to the swine.

Thus, at the human-swine interface, anthroponoses rather than zoonoses were detected and at the human-poultry interface neither zoonoses nor anthroponoses were detected during the study period.

Most poultry and swine farmers were aware of the possibility of becoming infected with diseases from the animals they keep and were also aware of several basic preventive measures that could reduce their zoonotic risk and possibly anthroponoses, but an implementation gap was observed. The risk of farmers in the region with zoonotic infections remains high.

10.0 Recommendations

1. Influenza surveillance at the human-animal interface should be undertaken regularly in the region and other regions with high animal and human population density to provide continuous information on influenza transmission at the human-animal interface in the country for early pandemic preparedness. Such surveillance efforts should be extended to other live animal-handlers along the value chain such as poultry retailers at live poultry markets and abattoir workers

2. Influenza viruses from surveillance efforts at the human-animal interface should be characterised for early detection of viral reassortants with zoonotic and pandemic propensities
3. Animal-handlers particularly swine farmers should be vaccinated against seasonal influenza virus to minimise anthroponoses and possible generation of reassortants
4. Stakeholders should explore the poor adoption of basic biosecurity and biosafety practices among farmers in the region despite having the knowledge of on-farm disease mitigation strategies. This would help in developing a tailored control programme to minimise the risk of farmers for zoonoses and the risk of anthroponoses to swine

11.0 List of References

Abolnik, C., Gerdes, G. H., Sinclair, M., Ganzevoort, B. W., Kitching, J. P., Burger, C. E., … Olivier, A. J. (2010). Phylogenetic Analysis of Influenza A Viruses (H6N8, H1N8, H4N2, H9N2, H10N7) Isolated from Wild Birds, Ducks, and Ostriches in South Africa from 2007 to 2009. *Avian Diseases Digest*, *5*(s1), e48–e49. https://doi.org/10.1637/9094-878109-digest.1

Abolnik, C., Olivier, A., Reynolds, C., Henry, D., Cumming, G., Rauff, D., … G, C. F. (2016). Susceptibility and Status of Avian Influenza in Ostriches Susceptibility and Status of Avian Influenza in Ostriches. *Avian Diseases*, *60*(1s), 286–295. https://doi.org/http://dx.doi.org/10.1637/11110-042815-Reg

Abolnik, C., Strydom, C., Rauff, D. L., Wandrag, D. B. R., & Petty, D. (2019). Continuing evolution of H6N2 influenza a virus in South African chickens and the implications for diagnosis and control. *BMC Veterinary Research*, *15*(1), 1–20. https://doi.org/10.1186/s12917-019-2210-4

Adeola, O. A., Adeniji, J. A., & Olugasa, B. O. (2010). Detection of Haemagglutination-Inhibiting Antibodies against Human H1 and H3 Strains of Influenza A Viruses in Pigs in Ibadan, Nigeria. *Zoonoses and Public Health*, *57*(7–8), e89–e94. https://doi.org/10.1111/j.1863-2378.2009.01268.x

Adeola, O. A., Olugasa, B. O., & Emikpe, B. O. (2016). Antigenic Detection of Human Strain of Influenza Virus A (H3N2) in Swine Populations at Three Locations in Nigeria and Ghana during the Dry Early Months of 2014. *Zoonoses and Public Health*, *63*(2), 106–111. https://doi.org/10.1111/zph.12210

Adeola, O. A., Olugasa, B. O., & Emikpe, B. O. (2017). Molecular detection of influenza A(H1N1)pdm09 viruses with M genes from human pandemic strains among Nigerian pigs, 2013-2015: Implications and associated risk factors. *Epidemiology and Infection*, *145*(16), 3345–3360. https://doi.org/10.1017/S0950268817002503

Adeola, Oluwagbenga A., Olugasa, B. O., Emikpe, B. O., & Folitse, R. D. (2019). Syndromic survey and molecular analysis of influenza viruses at the human–swine interface in two West African cosmopolitan cities suggest the possibility of bidirectional interspecies transmission. *Zoonoses and Public Health*, *66*(2), 232–247. https://doi.org/10.1111/zph.12559

Adeola, Oluwagbenga A, Adeniji, J. A., & Olugasa, B. O. (2009). Isolation of influenza A viruses from pigs in Ibadan, Nigeria. *Veterinaria Italiana*, *45*(3), 383–390. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/20391401

Adeola, Oluwagbenga A, Olugasa, B. O., & Emikpe, B. O. (2015). Detection of pandemic strain of influenza virus (A/H1N1/pdm09) in pigs, West Africa: implications and considerations for prevention of future influenza pandemics at the source. *Infection Ecology & Epidemiology*, *5*(1), 30227. https://doi.org/10.3402/iee.v5.30227

Agbenohevi, P. G., Odoom, J. K., Bel-Nono, S., Nyarko, E. O., Alhassan, M., Rodgers, D., … Ampofo, W. K. (2015). Biosecurity measures to reduce influenza infections in military barracks in Ghana. *BMC Research Notes*, *8*(1), 1–8. https://doi.org/10.1186/s13104-014-0956-0

Aiki-Raji, C. O., Oyadele, I. O, Ayoade, G.O., Fagbohun, O. A, O. T. A. (2004). Aiki-Raji et al., 2004.pdf. *African Journal of Clinical and Experimental Microbiology*, *5*(3), 278–279.

Akunzule, A. N., Koney, E. B. M., & Tiongco, M. (2009). Economic impact assessment of highly pathogenic avian influenza on the poultry industry in Ghana. *World's Poultry Science Journal*, *65*(3), 517–528. https://doi.org/10.1017/S0043933909000385

Al Khatib, H. A., Al Thani, A. A., Gallouzi, I., & Yassine, H. M. (2019). Epidemiological and genetic characterization of pH1N1 and H3N2 influenza viruses circulated in MENA region during 2009-2017. *BMC Infectious Diseases*, *19*(1), 1–22. https://doi.org/10.1186/s12879-019-3930-6

Almayahi, Z. K., Al Kindi, H., Davies, C. T., Al-Rawahi, B., Al-Jardani, A., Al-Yaqoubi, F., … Al-Abri, S. (2020). First report of human infection with avian influenza A(H9N2) virus in Oman: The need for a One Health approach. *International Journal of Infectious Diseases*, *91*, 169–173. https://doi.org/10.1016/j.ijid.2019.11.020

Arzey, G. G., Kirkland, P. D., Arzey, K. E., Frost, M., Maywood, P., Conaty, S., … Selleck, P. (2012). Influenza virus A (H10N7) in chickens and poultry abattoir workers, Australia. *Emerging Infectious Diseases*, *18*(5), 814–816. https://doi.org/10.3201/eid1805.111852

Asante, I. A., Bertram, S., Awuni, J., Commey, A. N. O., Aniwa, B., Ampofo, W. K., & Gabriel, G. (2016). Highly pathogenic avian influenza A(H5N1) virus among poultry, Ghana, 2015. *Emerging Infectious Diseases*, *22*(12), 2209–2211. https://doi.org/10.3201/eid2212.160639

Awuni, J. A., Bianco, A., Dogbey, O. J., Fusaro, A., Yingar, D. T., Salviato, A., … Monne, I. (2019). Avian influenza H9N2 subtype in Ghana: virus characterization and evidence of co-infection. *Avian Pathology*, *0*(0), 1–7. https://doi.org/10.1080/03079457.2019.1624687

Banson, K., Nguyen, N., Sun, D., Asare, D., Sowah Kodia, S., Afful, I., & Leigh, J. (2018). Strategic Management for Systems Archetypes in the Piggery Industry of Ghana—A Systems Thinking Perspective. *Systems*, *6*(4), 35. https://doi.org/10.3390/systems6040035

Bertran, K., Lee, D.-H., Criado, M. F., Smith, D., Swayne, D. E., & Pantin-Jackwood, M. J. (2018). Pathobiology of Tennessee 2017 H7N9 low and high pathogenicity avian influenza viruses in commercial broiler breeders and specific pathogen free layer chickens. *Veterinary Research*, *49*(1), 82. https://doi.org/10.1186/s13567-018-0576-0

Boschloo, R. (2020). *Analysis poultry sector Ghana*. Retrieved from http://www.rvo.nl/onderwerpen/internationaal-ondernemen/mvo

Böttcher-Friebertshäuser, E., Klenk, H.-D., & Garten, W. (2013). Activation of influenza viruses by proteases from host cells and bacteria in the human airway epithelium. *Pathogens and Disease*, *69*(2), 87–100. https://doi.org/10.1111/2049-632X.12053

Burimuah, V., Ampofo, W. K., Awumbila, B., Yebuah, N., Emikpe, B. O., Tasiame, W., & Folitse, R. D. (2016). The evaluation of domestic ducks as potential reservoir of avian influenza virus in post HPAI H5N1 outbreak area, Sunyani Municipality, Brong Ahafo Region of Ghana. *African Journal of Infectious Diseases*, *10*(2), 134–145. https://doi.org/10.21010/ajid.v10i2.10

Capua, I., Mutinelli, F., Pozza, M. D., Donatelli, I., Puzelli, S., & Cancellotti, F. M. (2002). The 1999-2000 avian influenza (H7N1) epidemic in Italy: Veterinary and human health implications. *Acta Tropica*, *83*(1), 7–11. https://doi.org/10.1016/S0001-706X(02)00057-8

Caron, A., Abolnik, C., Mundava, J., Gaidet, N., Burger, C. E., Mochotlhoane, B., … Cumming, G. S. (2011). Persistence of low pathogenic avian influenza virus in waterfowl in a southern African ecosystem. *EcoHealth*, *8*(1), 109–115. https://doi.org/10.1007/s10393-010-0356-4

CDC. (2018). Highly Pathogenic Asian Avian Influenza A(H5N1) Virus | Avian Influenza (Flu). Retrieved June 19, 2020, from https://www.cdc.gov/flu/avianflu/h5n1-virus.htm

CDC. (2020). Reported infections with variant influenza viruses in the United States since 2005. Retrieved June 20, 2020, from https://www.cdc.gov/flu/swineflu/variant-cases-us.htm#table-infections

Corzo, C. A., Culhane, M., Juleen, K., Stigger-Rosser, E., Ducatez, M. F., Webby, R. J., & Lowe, J. F. (2013). Active surveillance for influenza A virus among swine, midwestern United States, 2009-2011. *Emerging Infectious Diseases*, *19*(6), 954–960. https://doi.org/10.3201/eid1906.121637

Couacy-Hymann, E., Kouakou, V. A., Aplogan, G. L., Awoume, F., Kouakou, C. K., Kakpo, L., … Ducatez, M. F. (2012). Surveillance for influenza viruses in poultry and swine, West Africa, 2006-2008. *Emerging Infectious Diseases*, *18*(9), 1446–1452. https://doi.org/10.3201/eid1809.111296

Dawood, F. S., Iuliano, A. D., Reed, C., Meltzer, M. I., Shay, D. K., Cheng, P.-Y., … Widdowson, M.-A. (2012). Estimated global mortality associated with the first 12 months of 2009 pandemic influenza A H1N1 virus circulation: a modelling study. *The Lancet. Infectious Diseases*, *12*(9), 687–695. https://doi.org/10.1016/S1473-3099(12)70121-4

de Graaf, M., & Fouchier, R. A. M. (2014). Role of receptor binding specificity in influenza A virus transmission and pathogenesis. *The EMBO Journal*, *33*(8), 823–841.

https://doi.org/10.1002/embj.201387442

Deng, Y.-M., Iannello, P., Smith, I., Watson, J., Barr, I. G., Daniels, P., … Wong, F. Y. K. (2012). Transmission of influenza A(H1N1) 2009 pandemic viruses in Australian swine. *Influenza and Other Respiratory Viruses*, *6*(3), e42-7. https://doi.org/10.1111/j.1750-2659.2012.00337.x

Dione, M., Masembe, C., Akol, J., Amia, W., Kungu, J., Lee, H. S., & Wieland, B. (2018). The importance of on-farm biosecurity: Sero-prevalence and risk factors of bacterial and viral pathogens in smallholder pig systems in Uganda. *Acta Tropica*, *187*, 214–221. https://doi.org/10.1016/j.actatropica.2018.06.025

Ducatez, M. F., Awoume, F., & Webby, R. J. (2015). Influenza A(H1N1)pdm09 virus in pigs, Togo, 2013. *Veterinary Microbiology*, *177*(1–2), 201–205. https://doi.org/10.1016/j.vetmic.2015.02.028

Epperson, S., Jhung, M., Richards, S., Quinlisk, P., Ball, L., Moll, M., … Finelli, L. (2013). Human infections with influenza A(H3N2) variant virus in the United States, 2011-2012. *Clinical Infectious Diseases*, *57*(SUPPL.1), 2011–2012. https://doi.org/10.1093/cid/cit272

FAO. (2012). *Worldwide situation : observed trends*. *H5N1 HPAI Global Overview April-June 2012*.

FAO. (2014). *Poultry Sector Ghana. FAO Animal Production and Health Livestock Country Reviews*. Rome. Retrieved from http://www.fao.org/3/a-i3663e.pdf

FAO. (2015). Empres watch countries in West Africa. *Empres Watch*, *32*, *April*, 1–10.

FAO, A.-I. and I. (2017). *Regional strategy for the control of African swine fever in Africa*. Retrieved from www.fao.org/publications

FAO. (2020). Animal Production and Health: Sub-Saharan Africa HPAI situation update. Retrieved from www.fao.org/ag/againfo/programmes/en/empres/HPAI_Africa/situation_update.html

Fasanmi, O. G., Odetokun, I. A., Balogun, F. A., & Fasina, F. O. (2017). Public health concerns of highly pathogenic avian influenza H5N1 endemicity in Africa, *10*. https://doi.org/10.14202/vetworld.2017.1194-1204

Fobian, K., Fabrizio, T. P., Yoon, S.-W., Hansen, M. S., Webby, R. J., & Larsen, L. E. (2015). New reassortant and enzootic European swine influenza viruses transmit efficiently through direct contact in the ferret model. *Journal of General Virology*, *96*, 1603–1612. https://doi.org/10.1099/vir.0.000094

Forberg, H., Hauge, A. G., Gjerset, B., Hungnes, O., & Kilander, A. (2013). Swine influenza in Norway: a distinct lineage of influenza A(H1N1)pdm09 virus. *Influenza and Other Respiratory Viruses*, *7 Suppl 4*(Suppl 4), 21–26. https://doi.org/10.1111/irv.12194

França, M., Stallknecht, D. E., & Howerth, E. W. (2013). Expression and distribution of sialic acid influenza virus receptors in wild birds. *Avian Pathology : Journal of the W.V.P.A*, *42*(1), 60–71. https://doi.org/10.1080/03079457.2012.759176

Freidl, G. S., Meijer, A., de Bruin, E., de Nardi, M., Munoz, O., Capua, I., … FLURISK Consortium, F. (2014). Influenza at the animal–human interface: a review of the literature for virological evidence of human infection with swine or avian influenza viruses other than A(H5N1). *Eurosurveillance*, *19*(18), 20793. https://doi.org/10.2807/1560-7917.ES2014.19.18.20793

Gaidet, N., Dodman, T., Caron, A., Balança, G., Desvaux, S., Goutard, F., … Monicat, F. (2007). Avian influenza viruses in water birds, Africa. *Emerging Infectious Diseases*, *13*(4), 626–629. https://doi.org/10.3201/eid1304.061011

Garten, R. J., Davis, C. T., Russell, C. A., Shu, B., Lindstrom, S., Balish, A., … Cox, N. J. (2009). Antigenic and genetic characteristics of swine-origin 2009 A(H1N1) influenza viruses circulating in humans. *Science*, *325*(5937), 197–201. https://doi.org/10.1126/science.1176225

Gaydos, J. C., Top, F. H., Hodder, R. A., & Russell, P. K. (2006). Swine influenza A outbreak, Fort Dix, New Jersey, 1976. *Emerging Infectious Diseases*, *12*(1), 23–28. https://doi.org/10.3201/eid1201.050965

Ghana Ministry of Food and Agriculture. (2015). Facts and Figures, (2015). Retrieved from http://www.agrofood-westafrica.com/fileadmin/user_upload/messen/agrofood-Westafrica/Brochure/Agriculture-in-Ghana-Facts -and-Figures 2015.pdf%0Ahttp://cairngorms.co.uk/discover-explore/facts-figures/

Ghana Statistical Service. (2013). *2010 Population and Housing Census: National Analytical Report*. Retrieved from http://www.statsghana.gov.gh/docfiles/publications/2010_PHC_National_Analytical_Report.pdf

Gomaa, M. R., Kayed, A. S., Elabd, M. A., Zeid, D. A., Zaki, S. A., El Rifay, A. S., … Kayali, G. (2015). Avian influenza A(H5N1) and A(H9N2) seroprevalence and risk factors for infection among Egyptians: a prospective, controlled seroepidemiological study. *The Journal of Infectious Diseases*, *211*(9), 1399–1407. https://doi.org/10.1093/infdis/jiu529

Hagag, N. M., Erfan, A. M., El-Husseiny, M., Shalaby, A. G., Saif, M. A., Tawakol, M. M., … Naguib, M. M. (2019). Isolation of a Novel Reassortant Highly Pathogenic Avian Influenza (H5N2) Virus in Egypt. *Viruses*, *11*(6). https://doi.org/10.3390/v11060565

Hahn, S., Bauer, S., & Liechti, F. (2009). The natural link between Europe and Africa - 2.1 billion birds on migration. *Oikos*, *118*(4), 624–626. https://doi.org/10.1111/j.1600-0706.2008.17309.x

Harder, T. C., grosse Beilage, E., Lange, E., Meiners, C., Dohring, S., Pesch, S., … Starick,

E. (2013). Expanded Cocirculation of Stable Subtypes, Emerging Lineages, and New Sporadic Reassortants of Porcine Influenza Viruses in Swine Populations in Northwest Germany. *Journal of Virology*. https://doi.org/10.1128/JVI.00381-13

Henritzi, D., Zhao, N., Starick, E., Simon, G., Krog, J. S., Larsen, L. E., … Harder, T. C. (2016). Rapid detection and subtyping of European swine influenza viruses in porcine clinical samples by haemagglutinin- and neuraminidase-specific tetra- and triplex real-time RT-PCRs. *Influenza and Other Respiratory Viruses*, *10*(6), 504–517. https://doi.org/10.1111/irv.12407

Hirve, S., Newman, L. P., Paget, J., Azziz-Baumgartner, E., Fitzner, J., Bhat, N., … Zhang, W. (2016). Influenza Seasonality in the Tropics and Subtropics - When to Vaccinate? *PloS One*, *11*(4), e0153003. https://doi.org/10.1371/journal.pone.0153003

Howden, K. J., Brockhoff, E. J., Caya, F. D., McLeod, L. J., Lavoie, M., Ing, J. D., … Rohonczy, E. B. (2009). An investigation into human pandemic influenza virus (H1N1) 2009 on an Alberta swine farm. *Canadian Veterinary Journal*, *50*(11), 1153–1161. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2764467/

Hussain, M., Galvin, H. D., Haw, T. Y., Nutsford, A. N., & Husain, M. (2017). Drug resistance in influenza a virus: The epidemiology and management. *Infection and Drug Resistance*, *10*, 121–134. https://doi.org/10.2147/IDR.S105473

Kariithi, H. M., Welch, C. N., Ferreira, H. L., Pusch, E. A., Ateya, L. O., Binepal, Y. S., … Suarez, D. L. (2020). Genetic characterization and pathogenesis of the first H9N2 low pathogenic avian influenza viruses isolated from chickens in Kenyan live bird markets. *Infection, Genetics and Evolution*, *78*(September 2019), 104074. https://doi.org/10.1016/j.meegid.2019.104074

Karlsson Hedestam, G. B., Fouchier, R. A. M., Phogat, S., Burton, D. R., Sodroski, J., & Wyatt, R. T. (2008). The challenges of eliciting neutralizing antibodies to HIV-1 and to influenza virus. *Nature Reviews Microbiology*, *6*(2), 143–155. https://doi.org/10.1038/nrmicro1819

Kim, H., Webster, R. G., & Webby, R. J. (2018). Influenza Virus: Dealing with a Drifting and Shifting Pathogen. *Viral Immunology*, *31*(2), 174–183. https://doi.org/10.1089/vim.2017.0141

Kirunda, H., Erima, B., Tumushabe, A., Kiconco, J., Tugume, T., Mulei, S., … Wabwire-Mangen, F. (2014). Prevalence of influenza A viruses in livestock and free-living waterfowl in Uganda. *BMC Veterinary Research*, *10*, 50. https://doi.org/10.1186/1746-6148-10-50

Koopmans, M., Wilbrink, B., Conyn, M., Natrop, G., van der Nat, H., Vennema, H., … Bosman, A. (2004). Transmission of H7N7 avian influenza A virus to human beings during a large outbreak in commercial poultry farms in the Netherlands. *The Lancet*, *363*(9409), 587–593. https://doi.org/10.1016/S0140-6736(04)15589-X

Krumbholz, A., Lange, J., Dürrwald, R., Hoyer, H., Bengsch, S., Wutzler, P., & Zell, R. (2010). Prevalence of antibodies to swine influenza viruses in humans with occupational exposure to pigs, Thuringia, Germany, 2008-2009. *Journal of Medical Virology*. https://doi.org/10.1002/jmv.21869

Kyriakis, C. S., Brown, I. H., Foni, E., Kuntz-Simon, G., Maldonado, J., Madec, F., … Van Reeth, K. (2011). Virological Surveillance and Preliminary Antigenic Characterization of Influenza Viruses in Pigs in Five European Countries from 2006 to 2008. *Zoonoses and Public Health*, *58*(2), 93–101. https://doi.org/10.1111/j.1863-2378.2009.01301.x

Kyriakis, C. S., Zhang, M., Wolf, S., Jones, L. P., Shim, B. S., Chocallo, A. H., … Tripp, R. A. (2018). Corrigendum to "Molecular epidemiology of swine influenza A viruses in the Southeastern United States, highlights regional differences in circulating strains'' [Vet. Microbiol. 211 (2017) 174–179](S037811351730617X)(10.1016/j.vetmic.2017.10.016). *Veterinary Microbiology*. https://doi.org/10.1016/j.vetmic.2017.11.034

Laisse, C. J. M., Bianchi, M. V., Pereira, P. R., De Lorenzo, C., Pavarini, S. P., & Driemeier, D. (2018). Influenza A virus infection in pigs in southern Mozambique. *Pesquisa Veterinaria Brasileira*, *38*(8), 1484–1490. https://doi.org/10.1590/1678-5150-PVB-5291

Larison, B., Njabo, K. Y., Chasar, A., Fuller, T., Harrigan, R. J., & Smith, T. B. (2014). Spillover of pH1N1 to swine in Cameroon: An investigation of risk factors. *BMC Veterinary Research*, *10*. https://doi.org/10.1186/1746-6148-10-55

Ma, M., Anderson, B. D., Wang, T., Chen, Y., Zhang, D., Gray, G. C., & Lu, J. (2015). Serological evidence and risk factors for swine influenza infections among Chinese swine workers in guangdong province. *PLoS ONE*, *10*(5), 1–13. https://doi.org/10.1371/journal.pone.0128479

Malek Zrelli. (2016). *Immediate notification report*. Retrieved from https://www.oie.int/wahis_2/temp/reports/en_imm_0000021749_20161201_191226.pdf

Mangesho, P. E., Neselle, M. O., Karimuribo, E. D., Mlangwa, J. E., Queenan, K., Mboera, L. E. G., … Rweyemamu, M. (2017). Exploring local knowledge and perceptions on zoonoses among pastoralists in northern and eastern Tanzania. https://doi.org/10.1371/journal.pntd.0005345

McLeod A. & Hinrichs J. (2017). The economics of animal influenza. In D. E. Swayne (Ed.), *Animal Influenza* (2nd ed., pp. 43–73). Ames, Iowa: John Wiley & sons Inc. https://doi.org/10.1007/978-1-4684-5239-6_9

Mena, I., Nelson, M. I., Quezada-Monroy, F., Dutta, J., Cortes-Fernández, R., Lara-Puente, J. H., … García-Sastre, A. (2016). Origins of the 2009 H1N1 influenza pandemic in swine in Mexico. *ELife*, *5*(JUN2016), 1–21. https://doi.org/10.7554/eLife.16777

Mertens, E., Dugan, V. G., Stockwell, T. B., Lindsay, L. A. L., Plancarte, M., & Boyce, W. M. (2013). Evaluation of phenotypic markers in full genome sequences of avian influenza isolates from California. *Comparative Immunology, Microbiology and*

Infectious Diseases, *36*(5), 521–536. https://doi.org/10.1016/j.cimid.2013.06.003

Meseko, C. A., Odaibo, G. N., & Olaleye, D. O. (2014). Detection and isolation of 2009 pandemic influenza A/H1N1 virus in commercial piggery, Lagos Nigeria. *Veterinary Microbiology*, *168*(1), 197–201. https://doi.org/10.1016/j.vetmic.2013.11.003

Meseko, Ca, Heidari, A., Odaibo, G., & Olaleye, D. (2019). Complete genome sequencing of H1N1pdm09 swine influenza isolates from Nigeria reveals likely reverse zoonotic transmission at the human-animal interface in intensive piggery. *Infection Ecology & Epidemiology*, *9:1*(1696632). https://doi.org/10.1080/20008686.2019.1696632

Meseko, Clement, Globig, A., Ijomanta, J., Joannis, T., Nwosuh, C., Shamaki, D., … Starick, E. (2018). Evidence of exposure of domestic pigs to Highly Pathogenic Avian Influenza H5N1 in Nigeria. *Scientific Reports*, *8*(1), 5900. https://doi.org/10.1038/s41598-018-24371-6

Min, J. Y., & Subbarao, K. (2010). Cellular targets for influenza drugs. *Nature Biotechnology*, *28*(3), 239–240. https://doi.org/10.1038/nbt0310-239

Monamele, C. G., Y, P., Karlsson, E. A., Vernet, M.-A., Wade, A., Okomo, M.-C. A., … Dussart, P. (2019). Evidence of exposure and human seroconversion during an outbreak of avian influenza A(H5N1) among poultry in Cameroon. *Emerging Microbes & Infections*, *8*(1), 186–196. https://doi.org/10.1080/22221751.2018.1564631

Monne, I., Meseko, C., Joannis, T., Shittu, I., Ahmed, M., Tassoni, L., … Cattoli, G. (2015). Highly Pathogenic Avian Influenza A(H5N1) Virus in Poultry, Nigeria, 2015. *Emerging Infectious Diseases*, *21*(7), 1275–1277. https://doi.org/10.3201/eid2107.150421

Munyua, P. M., Githinji, J. W., Waiboci, L. W., Njagi, L. M., Arunga, G., Mwasi, L., … Katz, M. A. (2012). Detection of influenza A virus in live bird markets in Kenya, 2009-2011. *Influenza and Other Respiratory Viruses*, *7*(2), 113–119. https://doi.org/10.1111/j.1750-2659.2012.00365.x

Munyua, P., Onyango, C., Mwasi, L., Waiboci, L. W., Arunga, G., Fields, B., … Njenga, M. K. (2018). Identification and characterization of influenza A viruses in selected domestic animals in Kenya, 2010-2012. *PloS One*, *13*(2), e0192721. https://doi.org/10.1371/journal.pone.0192721

Myers, K. P., Olsen, C. W., & Gray, G. C. (2007). Cases of Swine Influenza in Humans: A Review of the Literature. *Clinical Infectious Diseases*, *44*(8), 1084–1088. https://doi.org/10.1086/512813

Myers, K. P, Olsen, C. W., Setterquist, S. F., Capuano, A. W., Donham, K. J., Thacker, E. L., … Gray, G. C. (2006). Are Swine Workers in the United States at increased risk of infection with Zoonotic Influenza Virus ?, *52242*, 14–20.

Naguib, M. M., Kinne, J., Chen, H., Chan, K. H., Joseph, S., Wong, P. C., … Harder, T. C. (2015). Outbreaks of highly pathogenic avian influenza H5N1 clade 2.3.2.1c in hunting

falcons and kept wild birds in Dubai implicate intercontinental virus spread. *Journal of General Virology*, *96*(11), 3212–3222. https://doi.org/10.1099/jgv.0.000274

Naguib, M. M., Verhagen, J. H., Samy, A., Eriksson, P., Fife, M., Lundkvist, Å., … Järhult, J. D. (2019). Avian influenza viruses at the wild-domestic bird interface in Egypt. *Infection Ecology & Epidemiology*, *9*(1), 1575687. https://doi.org/10.1080/20008686.2019.1575687

Neira, V., Allerson, M., Corzo, C., Culhane, M., Rendahl, A., & Torremorell, M. (2018). Detection of influenza A virus in aerosols of vaccinated and non-vaccinated pigs in a warm environment. *PloS One*, *13*(5), e0197600. https://doi.org/10.1371/journal.pone.0197600

Njabo, K. Y., Fuller, T. L., Chasar, A., Pollinger, J. P., Cattoli, G., Terregino, C., … Smith, T. B. (2012). Pandemic A/H1N1/2009 influenza virus in swine, Cameroon, 2010. *Veterinary Microbiology*, *156*(1–2), 189–192. https://doi.org/10.1016/j.vetmic.2011.09.003

Nyanteng, V. K., Takyi, P., Lawford, P., Acheamfuor, B., Nii, E., & Tawiah, O. (2013). 2010 population and Housing census. Regional analytical report. Ashanti region. Retrieved from http://www.statsghana.gov.gh/docfiles/2010phc/2010_PHC_Regional_Analytical_Reports_Ashanti_Region.pdf

Odoom, J. K., Bel-Nono, S., Rodgers, D., Agbenohevi, P. G., Dafeamekpor, C. K., Sowa, R. M. L., … Ampofo, W. K. (2012). Troop education and avian influenza surveillance in military barracks in Ghana, 2011. *BMC Public Health*, *12*, 957. https://doi.org/10.1186/1471-2458-12-957

Okoye, J., Eze, D., Krueger, W. S., Heil, G. L., Friary, J. A., & Gray, G. C. (2013). Serologic evidence of avian influenza virus infections among Nigerian agricultural workers. *Journal of Medical Virology*, *85*(4), 670–676. https://doi.org/10.1002/jmv.23520

Olaleye, O. D., Omilabu, S. A., Baba, S. S., & Fagbami, A. H. (1990). Haemagglutination-inhibiting (HI) antibodies against strains of influenza A virus in horse and pig sera in Nigeria. *Journal of Hygiene, Epidemiology, Microbiology, and Immunology*, *34*(4), 365–370. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2100750

Olsen, C. W., Karasin, A. I., Carman, S., Li, Y., Bastien, N., Ojkic, D., … Broukhanski, G. (2006). Triple reassortant H3N2 influenza A viruses, Canada, 2005. *Emerging Infectious Diseases*, *12*(7), 1132–1135. https://doi.org/10.3201/eid1207.060268

Ortiz, J. R., Katz, M. A., Mahmoud, M. N., Ahmed, S., Bawa, S. I., Farnon, E. C., … Wali, S. S. (2007). Lack of Evidence of Avian-to-Human Transmission of Avian Influenza A (H5N1) Virus among Poultry, *196*, 1685–1691. https://doi.org/10.1086/522158

Osoro, E. M., Lidechi, S., Marwanga, D., Nyaundi, J., Mwatondo, A., Muturi, M., … Njenga, K. (2019). Seroprevalence of influenza A virus in pigs and low risk of acute respiratory

illness among pig workers in Kenya. *Environmental Health and Preventive Medicine*, *24*(1), 53. https://doi.org/10.1186/s12199-019-0808-6

Osoro, E. M., Lidechi, S., Nyaundi, J., Marwanga, D., Mwatondo, A., Muturi, M., … Njenga, K. (2019). Detection of pandemic influenza A/H1N1/pdm09 virus among pigs but not in humans in slaughterhouses in Kenya, 2013–2014. *BMC Research Notes*, *12*(1), 628. https://doi.org/10.1186/s13104-019-4667-4

Rahn, J., Hoffmann, D., Harder, T. C., & Beer, M. (2015). Vaccines against influenza A viruses in poultry and swine: Status and future developments. *Vaccine*. https://doi.org/10.1016/j.vaccine.2015.03.052

Ramos S, MacLachlan M, M. A. (2017). Impacts of the 2014–2015 highly pathogenic avian influenza outbreak on the U.S. poultry sector. *Livestock Dairy Poultry Outlook, LDPM-282-0*, 282–284. Retrieved from https://www.ers.usda.gov/publications/pub-details/?pubid=86281

Rimmelzwaan, G. F., De Jong, J. C., Bestebroer, T. M., Van Loon, A. M., Claas, E. C. J., Fouchier, R. A. M., & Osterhaus, A. D. M. E. (2001). Antigenic and genetic characterization of swine influenza A (H1N1) viruses isolated from pneumonia patients in The Netherlands. *Virology*, *282*(2), 301–306. https://doi.org/10.1006/viro.2000.0810

Shaw M. L. & Palese P. (2013). Orthomyxoviridae. In P. M. H. David M. Knipe (Ed.), *Fields Virology* (6th ed., pp. 1151–1185). Philadelphia: Lippincott Willaims & Wilkins, a Wolters Kluwer business.

Simon, G., Larsen, L. E., Dürrwald, R., Foni, E., Harder, T., Van Reeth, K., … Loeffen, W. (2014). European surveillance network for influenza in pigs: surveillance programs, diagnostic tools and Swine influenza virus subtypes identified in 14 European countries from 2010 to 2013. *PloS One*, *9*(12), e115815. https://doi.org/10.1371/journal.pone.0115815

Simulundu, E., Ishii, A., Igarashi, M., Mweene, A. S., Suzuki, Y., Hang'ombe, B. M., … Takada, A. (2011). Characterization of influenza A viruses isolated from wild waterfowl in Zambia. *Journal of General Virology*, *92*(6), 1416–1427. https://doi.org/10.1099/vir.0.030403-0

Snoeck, C. J., Abiola, O. J., Sausy, A., Okwen, M. P., Olubayo, A. G., Owoade, A. A., & Muller, C. P. (2015). Serological evidence of pandemic (H1N1) 2009 virus in pigs, West and Central Africa. *Veterinary Microbiology*, *176*(1–2), 165–171. https://doi.org/10.1016/j.vetmic.2014.12.022

Stafford, K., Stafford, Y., Paton, D., & Gamble, P. (1992). Antibodies to some swine diseases in commercial piggeries in Central Zambia. *Revue d'élevage et de Médecine Vétérinaire Des Pays Tropicaux*, *45*(3–4), 229–230.

Starick, E., Lange, E., Grund, C., grosse Beilage, E., Döhring, S., Maas, A., … Harder, T. C. (2012). Reassortants of pandemic influenza A virus H1N1/2009 and endemic porcine

HxN2 viruses emerge in swine populations in Germany. *Journal of General Virology*, *93*(8), 1658–1663. https://doi.org/10.1099/vir.0.042648-0

Steinhauer, D. A. (1999). Role of hemagglutinin cleavage for the pathogenicity of influenza virus. *Virology*, *258*(1), 1–20. https://doi.org/10.1006/viro.1999.9716

Stieneke-Gröber, A., Vey, M., Angliker, H., Shaw, E., Thomas, G., Roberts, C., … Garten, W. (1992). Influenza virus hemagglutinin with multibasic cleavage site is activated by furin, a subtilisin-like endoprotease. *The EMBO Journal*, *11*(7), 2407–2414. https://doi.org/10.1002/j.1460-2075.1992.tb05305.x

Su, S., Fu, X., Li, G., Kerlin, F., & Veit, M. (2017). Novel Influenza D virus: Epidemiology, pathology, evolution and biological characteristics. *Virulence*, *8*(8), 1580–1591. https://doi.org/10.1080/21505594.2017.1365216

Suarez, D. L. (2017). Influenza A virus. In S. D. E (Ed.), *Animal Influenza* (2nd ed., pp. 3–30). Ames, Iowa: John Wiley & sons Inc. https://doi.org/10.1007/978-1-4684-5239-6_9

Sun, H., Xiao, Y., Liu, J., Wang, D., Li, F., Wang, C., … Liu, J. (2020). Prevalent Eurasian avian-like H1N1 swine influenza virus with 2009 pandemic viral genes facilitating human infection. *Proceedings of the National Academy of Sciences*, 201921186. https://doi.org/10.1073/pnas.1921186117

Swayne, David E., Suarez David & Sims, L. D. (2013). Influenza. In D. L. S. and V. N. Editor-in-chief, Swayne, David E; associate editors, John R. Glisson, Larry R. McDougald, Lisa K. Nolan (Ed.), *Diseases of poultry* (13th ed., pp. 181–218). Ames, Iowa: John Wiley & Sons, Inc. https://doi.org/10.1016/B978-1-4557-0297-8.00402-X

Thornton, P. K., Kruska, R. L., Henninger, N., Kristjanson, P. M., Reid, R. S., Atieno, F., … Ndegwa, T. (2002). Mapping Poverty and Livestock in the Developing World. *Health San Francisco*, *1*, 126. Retrieved from http://www.ilri.cgiar.org/Infoserv/Webpub/fulldocs/Mappoverty/media/PDF_chapters/B2_Front.pdf

Tweed, S. A., Skowronski, D. M., David, S. T., Larder, A., Petric, M., Lees, W., … Mak, A. (2004). Human illness from avian influenza H7N3, British Columbia. *Emerging Infectious Diseases*, *10*(12), 2196–2199. https://doi.org/10.3201/eid1012.040961

Van Reeth, K., Brown, I. H., Dürrwald, R., Foni, E., Labarque, G., Lenihan, P., … Koch, G. (2008). Seroprevalence of H1N1, H3N2 and H1N2 influenza viruses in pigs in seven European countries in 2002-2003. *Influenza and Other Respiratory Viruses*, *2*(3), 99–105. https://doi.org/10.1111/j.1750-2659.2008.00043.x

Vijaykrishna, D., Smith, G. J. D., Pybus, O. G., Zhu, H., Bhatt, S., Poon, L. L. M., … Peiris, J. S. M. (2011). Long-term evolution and transmission dynamics of swine influenza A virus. *Nature*, *473*(7348), 519–522. https://doi.org/10.1038/nature10004

Vincent, A.L., Lewis N. & Webby. R. (2017). Global evolution of influenza A virus in swine.

In D. E. Swayne (Ed.), *Animal Influenza* (2nd ed., pp. 459–479). Ames, Iowa: John Wiley & sons Inc. https://doi.org/10.1007/978-1-4684-5239-6_9

Vincent, A., Awada, L., Brown, I., Chen, H., Claes, F., Dauphin, G., … Ciacci-Zanella, J. (2014). Review of Influenza A Virus in Swine Worldwide: A Call for Increased Surveillance and Research. *Zoonoses and Public Health*, *61*(1), 4–17. https://doi.org/10.1111/zph.12049

Vincent, A. L., Ma, W., Lager, K. M., Janke, B. H., & Richt, J. A. (2008). Chapter 3 Swine Influenza Viruses. A North American Perspective. *Advances in Virus Research*, *72*(08), 127–154. https://doi.org/10.1016/S0065-3527(08)00403-X

Wang, D. Y., Qi, S. X., Li, X. Y., Guo, J. F., Tan, M. J., Han, G. Y., … Shu, Y. L. (2013). Human infection with Eurasian avian-like influenza A(H1N1) virus, China. *Emerging Infectious Diseases*, *19*(10), 1709–1711. https://doi.org/10.3201/eid1910.130420

Wei, S.-H., Yang, J.-R., Wu, H.-S., Chang, M.-C., Lin, J.-S., Lin, C.-Y., … Chang, F.-Y. (2013). Human infection with avian influenza A H6N1 virus: an epidemiological analysis. *The Lancet. Respiratory Medicine*, *1*(10), 771–778. https://doi.org/10.1016/S2213-2600(13)70221-2

WHO. (2011). WHO | Neglected zoonotic diseases. Retrieved July 24, 2018, from http://www.who.int/neglected_diseases/diseases/zoonoses/en/

WHO. (2018). Influenza (Seasonal). Retrieved July 13, 2020, from https://www.who.int/news-room/fact-sheets/detail/influenza-(seasonal)

WHO. (2020a). *Avian Influenza Weekly Update Number 744*. Retrieved from http://www.who.int/influenza/human_animal_interface/HAI_Risk_Assessment/en/

WHO. (2020b). Cumulative number of confirmed human cases for avian influenza A(H5N1) reported to WHO, 2003-2020. Retrieved June 19, 2020, from https://www.who.int/influenza/human_animal_interface/2020_MAY_tableH5N1.pdf?ua=1

Zhou, H., Cao, Z., Tan, L., Fu, X., Lu, G., Qi, W., … Zhang, G. (2014). Avian-like a (H1N1) swine influenza virus antibodies among swine farm residents and pigs in Southern China. *Japanese Journal of Infectious Diseases*, *67*(3), 184–190. https://doi.org/10.7883/yoken.67.184

Zinsstag, J., Schelling, E., Roth, F., Bonfoh, B., de Savigny, D., & Tanner, M. (2007). Human Benefits of Animal Interventions for Zoonosis Control. *Emerging Infectious Diseases*, *13*(4), 527–531. https://doi.org/10.3201/eid1304.060381

12.0 Curriculum Vitae

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b. Education and Qualification

- Kwame Nkrumah University of Science and Technology, Ghana
- Master of Philosophy in Clinical Microbiology (Awarded in 2007)

- Kwame Nkrumah University of Science and Technology, Ghana
- Bachelor of Science in Biological Sciences (Awarded in 2004)

c. Work Experience

- 2007 to date: Council for Scientific and Industrial Research (CSIR)-Animal Research Institute, Accra, Ghana
- Position: Research Scientist

- 2003 - 2006: Kumasi Centre for Collaborative Research in Tropical Medicine, Kumasi, Ghana (2003-2006)
- Position: Assistant Research Scientist

e. Publications

1. **Ayim-Akonor M**, Krumkamp R, May J & Mertens E (2020). Understanding attitude, practices and knowledge of zoonotic infectious disease risks among poultry farmers in Ghana. Vet Med Sci. 2020;00:1–8. https://doi.org/10.1002 vms3.257
2. **Ayim-Akonor M**, Mertens E, May J & Harder T (2020). Exposure of domestic swine to influenza A viruses in Ghana suggests unidirectional, reverse zoonotic transmission at the human–animal interface. Zoonoses Public Health. 2020;00:1–11. https://doi.org/10.1111/zph.12751
3. **Ayim-Akonor M**, May J, Krumkamp R, Harder T & Mertens E (2019). Molecular and serological prevalence of influenza A viruses in poultry and poultry farmers in the Ashanti region, Ghana, Infection Ecology & Epidemiology, 9:1, 1698904, DOI:10.1080/20008686.2019.1698904
4. **Ayim-Akonor M**, Obiri-Danso K, Akonor P T & Sellers, H (2018). Widespread exposure to infectious Bronchitis virus and *Mycoplasma gallisepticum* in chickens in the Ga-East District of Accra, Ghana. Cogent Food and Agriculture. Pg 1-11.
5. **Ayim-Akonor M**, Owusu-Ntumy D D, Ohene-Asa, H E, Oduro-Abrokwa, A, Hammond, P, Appenteng, M & Annan D (2018). Serological and molecular surveillance of Infectious Bronchitis Virus in free range chickens and guinea fowls in the Ga-East District of Ghana (2018). Journal of Veterinary medicine. https://doi.org/10.1155/2018/4949580
6. **Ayim-Akonor M** and Akonor PT (2014). Egg consumption patterns, preferences and perceptions among consumers in Accra metropolitan area. International Food Research Journal 21(4): 1457-1463
7. Otsyina HR, Arthur C.T, **Ayim-Akonor** M and Obese FY (2013). Seroprevalence of Peste des Petits Ruminants (PPR) in sheep, goats and cattle in Ghana. Bulletin of Animal Health and Production in Africa 61: 473 – 479
8. **Ayim-Akonor M**, Baryeh K and Asante I (2013). Molecular based survey of pathogens associated with respiratory disease outbreaks in broiler chickens in Accra. Journal of Natural Sciences Research 3(10): 25-31
9. Benno K, Kreuzberg C, Kobbe R, **Ayim-Akonor M**, Appiah Thompson B, Ehemen C, Adjei S, Langefeld I, Adjei O, and May J (2010). Differing effects of HBS and HBC traits on uncomplicated falciparum malaria, anemia and child growth. Blood 115(522): 4551-4558
10. Kobbe R, Kreuzberg C, Adjei S, Thompson B, Langefeld I, Appiah-Thompson P, Abruquah H.H, Benno K, **Ayim M**, Busch W, arks F, Amoah K, Opoku E, Meyer C.G, Adjei O and May J (2007). A randomized controlled trail of extended intermittent preventive antimalarial treatment in infants. Clinical Infectious Diseases. 45: 16-25.