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Research Article

Is There Cross-Species Transmission to Pigs of Avian Influenza Virus Within Poultry Farms with Previous Outbreak of Avian Influenza?

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ABSTRACT

Interspecies interaction between pigs and poultry has not been well elucidated in Ghana. Although avian influenza circulated in some small commercial holdings with mixed poultry-pig system in the Greater Accra Region of Ghana, the investigation on possible cross transmission from poultry to pigs has not been elucidated. To this end, 350 blood and nasal swab respectively from pigs were screened for influenza A by Real Time RT-PCR and enzyme-linked immunosorbent assay (ELISA). There was no evidence of avian influenza in pigs within the poultry farms. However, biosecurity compliance among the farmers was suboptimal, suggesting possible cross transmission in the future as pig population density continues to increase. Therefore, active surveillance in those farms should be strengthened for unpredictable future pandemic.

Keywords: *Avian influenza, Biosecurity, cross transmission, Ghana, influenza A, interspecies, pig, poultry*

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INTRODUCTION

Livestock production plays a vital role in the lives of people, especially the rural household. This is due to the enormous benefits associated with livestock rearing such as food, income draught power, manure and social status (Banson *et al*. 2015). As a result, livestock production has been captured as one of the sectors for achieving sustainable development goal (poverty reduction), especially in developing countries like Ghana (UNDP, 2018). Piggery production has also played a significant role in that direction as a result of increase in pork consumption over the years (Banson and Josephine, 2014). However, local production of pork has not been able to meet the demand for pork in Ghana. This is partly attributable to the dominance of small-scale commercial holdings (20-100), constituting 95% of the total pork production, and backyard production (1-20) system (Banson and Josephine 2014). This has further been exacerbated by diseases, such as ASF (African swine fever), viral diarrhea in piglet and other bacterial infections (Segales *et al*. 2005). In Ghana, due to the high prevalence of African swine fever, pig production has been affected dramatically, leading to drastic reduction in pig population and diversification of source of livelihood by some pig farmers. Therefore, full scale commercial pig production is gradually shifting to commercial smallholdings characterized by the combination of pigs and poultry (chicken and ducks, turkey and guinea fowl) either in the same vicinity or less than 10 meters apart. This was attributed to the fear of African swine fever and the quest to diversify livelihood as a way of averting risk. Although this system could make economic sense; it dire consequence in respect of interspecies transmission of influenza A, leading to unpredictable future pandemic cannot be discounted. There are several lines of evidence of inter-species transmission of influenza A virus among several animal species coupled with reassortment of IAV genes suggested to occur in areas where humans and livestock get in close contact (Webster *et al*. 1992). Pigs challenged with influenza A avian origin has also demonstrated the ability of the virus to replicate in pigs respiratory tract without a clinical signs, and this is dependent on high infection pressure, route of inoculation and close contact between species.

This research was therefore motivated by a previous report on prevalence of influenza A in poultry in three production systems (live bird, backyard and commercial) in some part of the Greater Accra region where commercial poultry had 1.3% of the total prevalence of influenza A from chicken (Shaban *et al*. 2021). Evidently, all influenza positive samples from commercial system arose from commercial smallholdings

where pigs and poultry were kept within the same compound (Shaban *et al*. 2021). However, evidence of interspecies interaction between pigs and poultry has not been well elucidated in Ghana. It is therefore critical to evaluate if there interspecies transmission in pigs within the poultry farms with previous outbreak of avian influenza in Greater Accra Region of Ghana. It also examined the biosecurity consciousness and hygiene practices on the farm, since human- avian-swine interaction comes with a high risk of future pandemic.

MATERIALS AND METHODS

Farm characteristics: Sixteen farms (16) small commercial holdings were identified to be keeping pig and chicken (local and exotic) on the same farm (figure 1A). The swine pens were separated according to pig type- growers, sows, weaners and boar. However, swine pens were built closed to poultry cages as shown in figure 1B. Farm structures were built with block and wood with metal sheet roof. Pigs were sometimes allowed to roam within the farm premises feeding on leftovers as shown in fig 1C, some with pigs and goats in Fig 1D. The pig population ranged between 20 and 70 per farm. Only three out of the sixteen farms visited were fully fenced with gates.

Sampling technique: The research employed purposive sampling by targeting commercial smallholdings which tested positive for influenza A for poultry during the previous sampling. A criterion for selection was based on farms with any of the poultry (chicken, ducks, guinea fowl and turkey) and pig. Weaners, growers and finishers were all eligible for selection. However, samples were collected from only chicken and pigs because the commercial smallholdings had only chicken and pigs during the sampling period. All the pigs (350) were sampled due to low pig population.

Farm observations: Direct observation was also employed to assess farm practices and animal interaction. Farmers/caretakers were visited during contact hours with the animals and during feeding, which was done early in the morning and evening as claimed by all the farmers. Activities were then observed. This was also complemented by observations on basic integrated hygiene and biosecurity practices.

Blood and nasal swab collection: Blood and nasal swabs were collected from pigs. Nasal swabs and blood were collected from the same animal. Nasal swabs were collected aseptically with a cotton wool swab. Specimens of both nostrils were obtained with one swab into a vial containing 1.5 ml of viral transport medium. Blood was collected into a 5 ml vacutainer glass serum tube without EDTA. Tubes and cryovials collected were transported on ice to the Veterinary Services laboratory of Ministry of Food and Agriculture, Accra for processing. Samples were stored at 4 oC, and used immediately. Those to be used within 2 weeks were frozen at -20 oC and those to be used in a month or later at -80 oC. Serum aliquots were dispensed into 2 ml cryovials and stored at -20 oC.

Laboratory analysis: Sampled swabs were transported to the Veterinary Services Laboratory, Ghana and pooled into five samples per pool to optimize the reagent. Virus RNA was extracted from pooled swab VTM using the QIAamp Viral RNA Mini kit (Qiagen) according to manufacturer's protocol. The RNA carrier was added to AVL kit-supplied buffer, subsequently 400 ul of this mixed with 100 ul of each sample and left to incubate for 10 minutes at room temperature. 400 ul of absolute ethanol was added to the mixture and applied to QIAamp spin column. The column was with kit-supplied buffer and RNA eluted in 60 ul of elusion buffer. The samples were then screened for influenza A by a one-step rtRT-PCR targeting the matrix gene, a highly conserved gene in all influenza A viruses.

Viral RNA was screened for influenza virus A by RT qPCR using primers for M gene detection (M-5 forward: AGATGAGYCTTCTAACCGAGGTCG; M-5 reverse: TGCAAANACATCYTCAAGTCTCTG; Probe: FAMTCAGGCCCCCTCAAAGCCGA-BHQ1). Thermal cycling conditions were as follows: 50 °C for 15 min, 95 °C for 2 min, then 40 cycles of 95 °C for 10 s and 60 °C for 30 s. A Ct (cycle of threshold) value < 40 was considered as Influenza A virus (M-gene) positive

Detection of influenza A antibody: Sera from pigs were also screened by Enzyme-linked immunosorbent Assay using IDEXX flock chek Avian Influenza MultiS-Screen Antibody Test kit, targeting nucleoprotein for influenza A viruses (Tse *et al*. 2012). Although the kit is species specific by design - chicken ostrich, duck goose turkey- it has been authenticated to be applied to pig sera with an adjusted cutoff of S/N ratio ≤ 0.673 with 72% sensitivity and 99% specificity (Ciacci-Zanella *et al*. 2012). Readings and calculations were done according to manufacturer's instruction

RESULTS

Table 1 depicted evidence of influenza A in chicken in the commercial holdings keeping both poultry and pigs as presented in Shaban *et al*. (2021). Other poultry samples were not collected because those commercial holdings kept only chicken and pigs during sampling. Serum and swab samples were then collected from pigs and screened for influenza A as shown in Table 2.

There was no evidence of influenza A in pigs for rtRTPCR as shown in Table 1. Sera from the same animals from which nasal swabs were collected were screened for antibody against influenza A to determine exposure of the pigs to influenza A. However, there was no exposure of the pigs to influenza A as indicated in Table 2.

As part of the study, farm observation revealed that researchers and other visitors entered all the 16 farms under study without restrictions, and footbaths were absent or not functional except in only one farm. None of the farmers/caretakers used nose mask and one farmer used gloves during our visit. Changing farm specific clothing and shoes was not practiced in any of the farms. Hand washing practice was not at its best as only18.8% of the farms had their workers use water and soap to wash their hands after farm work. Birds were seen perching on the swine pens since the swine pens were not fully housed as shown in fig 1B. Pigs were also seen moving around the poultry pens as shown in Fig 1C and D in all the 16 farms visited.

Table 1:
Distribution of Influenza A prevalence by rt RTPCR in poultry

	Production levels								
	Live-bird market			Backyard			Commercial		
	No. of samples Tested	No. of positives (%)	Binomial Exact 95% CI*	No. of sample tested	No. of positives (%)	Binomial Exact 95% CI*	No. of samples tested	No. of positives (%)	Binomial Exact 95% CI*
Chickens	450	120 (26.7)	22.6, 31.0	350	7(2.0)	0.8, 4.1	420*	10(2.4)*	1.2, 4.3
Ducks	230	2 (0.9)	0.1, 3.1	140	1(0.7)	0.0, 3.9	-	-	-
Guinea fowls	150	12 (8.0)	4.2, 13.6	-	-	-	-	-	-
Pigeons	100	5 (5.0)	1.6 11.3	-	-	-	-	-	-
Turkeys	100	0	3.6	100	0(0)	3.6	-	-	-
Total	**1030**	**139 (13.5)**	**11.5, 15.7**	**590**	**8(1.4)**	**0.6, 2.7**	**420**	**10(2.4)**	**1.2, 4.3**

**10 out of 420 positives from commercial production system were recorded by commercial small holdings keeping both chicken and pigs (Shaban et al., 2021)*

Table 2:
Influenza A prevalence in pigs by rtRTPCR and enzyme-linked immunosorbent assay in commercial smallholdings in part of the Greater Accra region of Ghana

Sample type	Number	Positives	Prevalence %
Nasal swab	350	0	0
Sera	350	0	0

Table 3:
Biosecurity practices in the pig-poultry smallholdings in parts of the Greater Accra region

Variables	Respondents (N= 16) % (n)
Checking visitors before entry	Yes /observed 0% (0) No/observed 100%(16)
Functional footbath at the entrance	Yes /observed 6.3%(1) No/ observed 93.8%(15)
Changing farm-specific clothing and footwear	Yes/ observed 0%(0) No/ observed 100%(16)
Footwear	Bare foot 0%(0) Flip-flops(easy wear) 68.8%(11) Functional boot 31.3% (5)
Face mask	Yes /observed 0% (0) No /observed 100% (16)
Hand wear	Bare hand 93.8% (15) Gloves 6.2% (1)
Hand washing practice	No washing 25% (4) Water only 53.3% (9) Water and soap 18.8% (3)

DISCUSSION

Influenza A continues to impact livestock production, especially in the low middle income countries where there is low investment (FAO 2010). Therefore, fewer animals of different species are kept on the same compound to diversify source of livelihood as a way of averting risk. African swine fever in Ghana has recently affected pig production (Sánchez-Vizcaíno *et al*. 2012), causing commercial pig producers to cut down scale of production and complementing it with poultry production to serve as a buffer to keep them in business. This was revealed during field interaction with the farmers. However, the consequences of interspecies transmission of pathogen coupled with pigs serving as mixing vessel for the emergence of novel genotype cannot be underestimated (Alexander and Brown 2000, Dawood *et al*. 2012). This is more critical as some samples from chicken from the sixteen (16) farms under study tested positive for influenza A (Shaban *et al*. 2021). Therefore, influenza A spillover to pigs on those farms could be anticipated. A wide range of research worldwide has demonstrated seroprevalence of the subtypes of the influenza A virus known as H1N1, H1N2, H3N2 circulating in pigs (Nelson and Vincent 2015). However, current result of the study showed no evidence of influenza A in pigs on those farms. The result mirrored a systematic active surveillance study of influenza A in pigs in Côte d'Ivoire, Benin, and Togo with no evidence of influenza A in pigs reared with poultry (Couacy-Hymann *et al*. 2012).

Paradoxically, other African countries including Ghana, Kenya, Nigeria and have reported influenza A prevalence in pigs, predominantly human pandemic H1N1 and H3N2 (Adeola *et al*. 2015, Matilda *et al*. 2020, Munyua *et al*. 2018). A plausible explanation to this difference could probably be ascribed to a relatively high population of pigs in those areas which could have facilitated human-pig transmission. The African scenario is in sharp contrast with that of the European countries where influenza A in pig is endemic with > 50% seroprevalence (Harington 2005). This was partly attributed to large pig population coupled with low biosecurity practices as cursors to promote influenza transmission between humans and pigs (Meseko *et al*. 2014). Wain-Hobson (2014) also opined that high density and the complex interactions between pigs, poultry and human have been implicated as main concern during influenza surveillance, especially in South-East Asia.

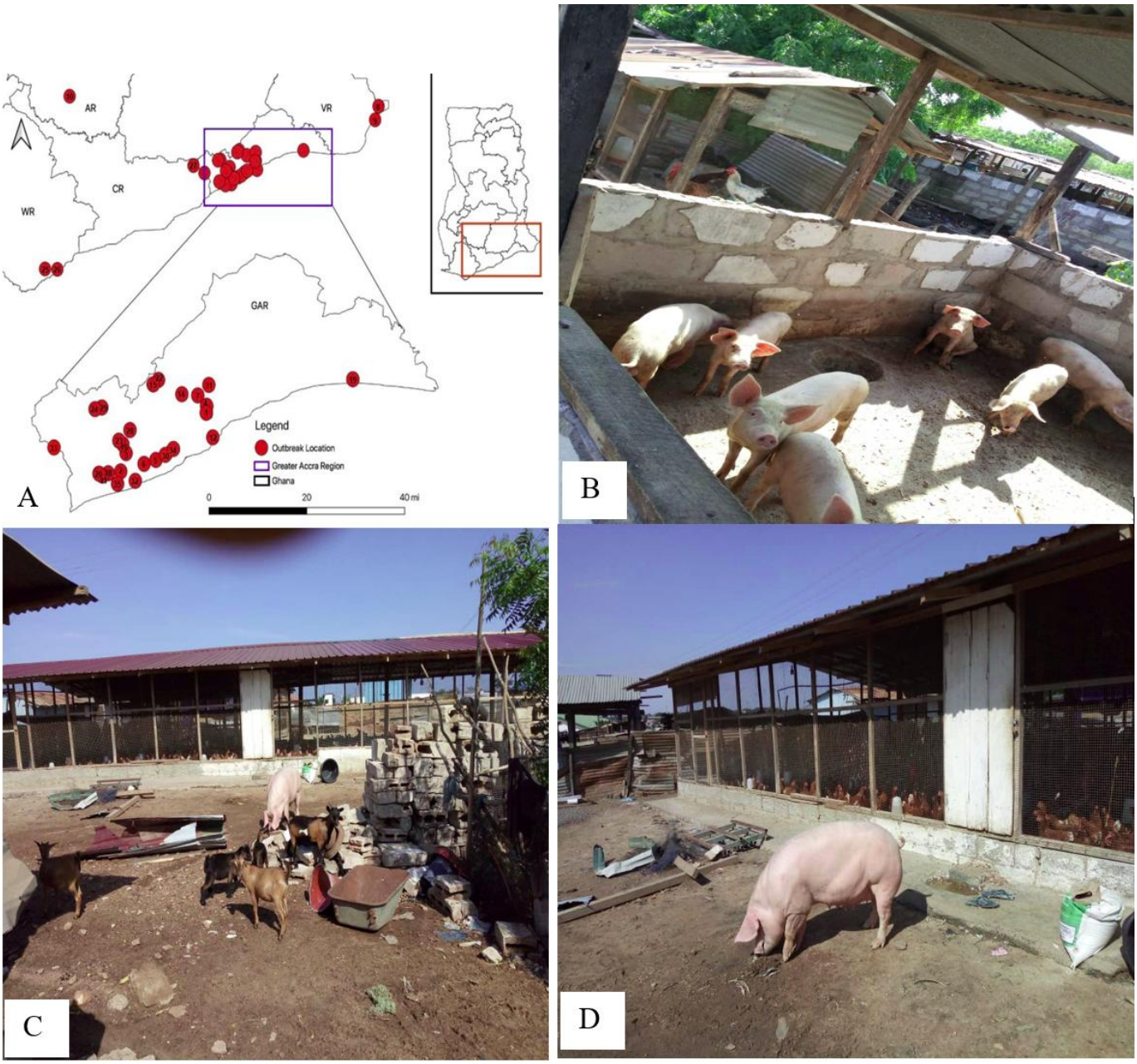

Plate 1

1A. The sites showing the outbreak of pathogenic Avian Influenza A in the Greater Accra region of Ghana. Adapted from (Tasiame et al., 2020) 1B: Semi-fenced swine pen closed to a poultry pen 1C: Pig-goat interaction on the farm. 1D: A pig moving around the poultry pen

Absence of influenza A (avian influenza) in pigs within poultry farm as reported by this study does not rule out the possibility of influenza A to circulate in pig population within poultry farms. That notwithstanding, studies have demonstrated the ability of avian type of influenza A to replicate in the lower lungs of pigs inducing no clinical sign hence minimizing transmission between pigs and other species (Choi *et al*. 2005). This scenario might have arisen during sampling. Again, virus subtype, infection dose and route of inoculation have been implicated to facilitate avian influenza transmission to pigs (De Vleeschauwer *et al*. 2009). Therefore, lack of evidence of the virus in pigs could have been underpinned by low infection pressure and contact restriction. Therefore, outbreak of highly pathogenic avian influenza (H5N1) in those integrated poultry-pig farms may induce infection pressure leading to a spillover.

Observation of farmers' activities during our visit raised a serious issue of biosecurity. Co-mingling of chicken and pigs was observed in all the farmers visited. This depicted a threat to biosecurity, increasing the risk of interspecies transmission of the virus (Webster *et al*. 1992).

Research elsewhere reported lower seroprevalence (prevalence ratio 0.75, or 25% lower) in swine farms fenced with a bird proof relative to farm without bird proof netting (Amass *et al*. 1999). Uncontrolled access to farms as observed in the farm has been implicated to perpetuate herd infection (Poljak *et al*. 2008, Simon-Grife *et al*. 2011). It has been

reported also that mechanical transmission of influenza virus is possible through birds and other wild species (Allerson *et al*., 2013). Basic hygiene, such as washing hands with soap and water was not a common practice on the farms despite the fact soap and detergents are known to kill influenza viruses (Aielo *et al*. 2010). The use of protective clothing was extremely low on the farms given the benefit associated with the use of protective clothing as a barrier for the transmission of influenza A virus via pathogenic animal secretions and feces. Proper use of protective clothing has been implicated to reduce the risk of interspecies transmission (Kumarsean *et al*. 2009). Since these measures are sparingly used on the farms, there could be a high tendency of interspecies transmission.

In conclusion, There was no evidence of influenza A in pigs in the commercial smallholdings under study. That notwithstanding, influenza A circulates in the commercial smallholdings in poultry (Shaban *et al*, 2021). This is suggestive of possible future spillover to other species, such as pig and a sustained interspecies transmission as a result of low biosecurity compliance observed. In this study, the possible reason for no evidence of cross species transmission may be the small pig population, low infection pressure, virus subtype (H9) and contact restriction which could have hampered spillover to pigs.

Current livelihood diversification among some pig farmers as a result of African swine fever and urbanization envisages increased mixed animal production. Basic biosecurity practices in the farms were substantially low. As a result of this, emphasis should be placed on biosecurity compliance and continued surveillance to avert interspecies transmission which may occur as animal density increases over time, leading to unpredictable future pandemic. It is therefore recommended to intensify biosecurity compliance and surveillance among commercial smallholdings for strategic control of the virus in Ghana.

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