Burimuah et al., Afr. J. Infect. Dis. (2016) 10(2): 134 – 145
DOI:10.21010/ajid.v10i2.10

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

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Abstract

Background: Avian influenza (AI) is an important zoonotic disease responsible for significant losses in most sub-Saharan countries. However, the role of poultry other than chicken in the epidemiology of the disease, especially after the first AI outbreak in Ghana, has not been fully elucidated. The objective of this study is to determine whether the AI virus infection that was reported in the area in May 2007 was circulating silently in ducks in nine randomly selected farms in the Sunyani Municipality, Ghana.
Materials and Methods: The sample size was calculated using Epi info version 3.4.1 at 95% confidence level, absolute precision of 5% and assuming 0.5 prevalence of Avian Influenza A virus in ducks. Samples collection was done simultaneously with questionnaire administration to farmers. A total of 526 samples made up of 384 cloacal swabs and 142 feather tissues from ducks from a commercial duck farm, seven backyard holdings and one live birds market in six randomly selected communities in the Sunyani Municipality, Brong Ahafo region of Ghana. The samples were processed and subjected to Influenza Type A Matrix Gene analysis using RRT-PCR.
Results: All the 526 samples subjected to Influenza Type A Matrix Gene analysis using RRT-PCR were negative for Influenza Type A viruses. However, it was observed that bio-security practices which are keys to reintroduction of the virus in the area were not adhered to in 89 % of the sites investigated. Our finding also revealed that only the commercial farm investigated in this study complied with fifteen (78.9%) of the nineteen different farm practices observed.
Conclusion: Though AI was not detected in the ducks sampled, there is the need for continuous surveillance and education of stakeholders on standard bio-security and farm management practices in the area.

Key words: Avian influenza type A viruses, ducks, Sunyani Municipality, RRT-PCR, Surveillance, Re-introduction.

Introduction

Avian Influenza (AI) is an infectious disease of birds caused by influenza A viruses. Migratory waterfowls - most notably wild ducks - are the natural reservoirs of all influenza A viruses (Hinshaw and Webster, 1982; Webster *et al*., 1992; Stallknecht and Brown 2007).
There are 16 main subtypes of influenza A viruses, of which strains within the H5 and H7 subtypes cause Highly Pathogenic Avian Influenza (HPAI), which is highly contagious and rapidly fatal resulting in nearly 100% mortality in infected domestic flocks (Center for Infectious Diseases Research and Policy, 2007).

Recently, avian influenza has acquired world-wide attention because the H5N1 virus had traversed interclass barriers (Perkins and Swayne, 2003) and had been transmitted from birds to mammals (cats, swine, and humans). Substantial number of documented cases in humans are associated with severe disease and several fatalities (Klempner and Shapiro, 2004; Webster, 2006). There are several further lines of evidence suggesting that the H5N1 virus has acquired increased pathogenic potency for several mammalian species. Chickens and turkeys are particularly susceptible to epidemics; direct or indirect contact of domestic flocks with wild waterfowl has been implicated as a frequent cause (Easterday, *et al* 1997; Stalknecht and Brown, 2007). Birds that survive infection often excrete virus for up to 10 days, orally and in faeces, thus facilitating further spread (Olsen *et al*., 2005; Swayne and Beck, 2005). Suspicions that birds may be carrying highly pathogenic virus along their migratory routes were underscored following the detection of outbreaks in wild and domestic birds in the Russian Federation and adjacent parts of Kazakhstan, so also Turkey, Romania, and Croatia (OIE, 2005, WHO, Global Alert and Response).

Most avian influenza viruses affecting humans have caused mild respiratory symptoms or conjunctivitis, with one important exception: the H5N1 strain. The H5N1 strain has caused severe disease throughout the world with high fatality rates starting from 1997, till date. Studies comparing virus samples overtime show that H5N1 has become progressively more pathogenic for mammals, and is now hardier than in the past, surviving several days longer in the environment (Olsen *et al*., 2005; Beck, 2005; EC, 1992 amended 2004). In 2004, H5N1 caused fatal disease in naturally infected large felines (tigers and leopards) and experimentally infected domestic cats -

DOI:10.21010/ajid.v10i2.10

species not previously considered susceptible to disease caused by any influenza A virus. Several mutations in the virus have been detected during 2005, but the significance of these mutations in terms of virulence and transmissibility in humans is not fully understood.

In April 2007, despite a ban on importation of poultry and poultry products from HPAI H5N1 affected countries (mostly Southeast Asian countries) and several biosecurity measures enforced by the Government of Ghana, the first outbreak of H5N1 was reported in Ghana by VSD at a small – scale poultry farm at Kakasunanka, near Michel Camp in the Tema Metropolis (April 24, 2007). Subsequently, outbreaks of AI were reported in Sunyani in the Brong Ahafo region (May 15, 2007) and Aflao in the Volta region in the same year (June 13, 2007). To date, the possible reasons for the emergence and spread of the disease in the country have not been determined.

The disease is associated with enormous economic loss and the cost of veterinary interventions and public education on prevention and control of the disease was estimated at 2 million US dollars of which came from donor partners including USAID and FAO (VSD Annual Report, 2009).

Unlike chickens, some domestic ducks are known to be resistant to the viruses and can be asymptomatic carriers of the viruses, thus acting as a "silent reservoir" that perpetuates transmission (Swayne and Beck, 2005 and European Commission (EC), 1992 amended 2004).

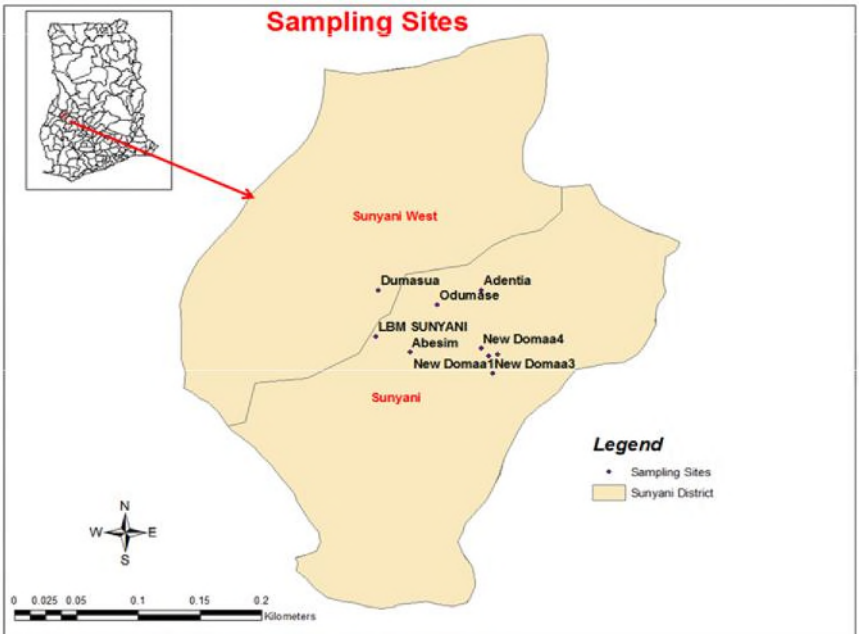
The experience of a second outbreak of HPAI in Togo in 2008 after the 2007 outbreak has shown the ability of H5N1 virus to persist discreetly among traditional farms (scavenging poultry) where chicken mortality is common and usually go unreported. Although the role of ducks and related species in the viral circulation of avian influenza was reported in many countries including Nigeria in the 2008 (Alice *et al.,* 2008), the status of avian influenza virus infections in domestic ducks in Ghana especially that associated with the Sunyani Municipality which shares border with La Cote d'Ivoire, a country which reported AI outbreaks in 2006 has not been determined, hence this study.

Method

Study Area

The study was conducted in the Sunyani Municipality, one of the twenty-two districts of the region which is the capital of the Brong Ahajo region. It lies between latitude 7^0 20'N and 7^0 05'N and longitude 2^0 30'W and 2^0 10'W and shares boundaries with Sunyani West District to the north, Dormaa District to the west, and Asutifi District to the South and Tano North District to the East. The Municipality has a total land area of 829.3 square kilometers (320.1 square miles).

Sunyani Municipality (Fig 1) had an estimated ducks population of 25,036 (according to the Statistics, Research and Information Directorate data, MOFA, 2005) before the 2007 H5N1 outbreak. Many of these ducks were destroyed during the 2007 HPAI outbreaks. Presently, fourteen ducks farmers in the Municipality who abandoned the trade because of the outbreaks in 2007 have become operational again. The population of ducks keepers in the Municipality is estimated at 107. Duck population in the area was estimated at 5,000 after collecting census data from the various active ducks rearing sites in the Municipality.



Source: Epidemiology Unit, VSD, Accra Ghana, 2011.
Figure 1: Map showing Sampling Sites in the Sunyani Municipality, July 2009- October, 2010.

Study Design

A descriptive cross-sectional study using active surveillance approach was carried out as indicated in the scheme below. It entailed simultaneous collection of cloacal swabs and feather tissues from three hundred and eighty-four domestic ducks, and the administration of a structured questionnaire to 17 ducks owners/workers on issues of husbandry practices and bio-security at their farms or premises (households). The study was carried out from July 2009 to August 2010.

Data Collection Technique

Data was collected with structured questionnaires (Appendix II). The questionnaire was developed and pre-tested in Berekum, an adjacent district before it was administered to 17 persons who were either owners or workers of the ducks sampled as well as live birds vendors at the Sunyani live birds market whose birds were sampled. Questionnaire administration was done simultaneously with sample collection.

Sample Size Determination

The sample size was calculated by the Epi info version 3.4.1 at 95% confidence level, absolute precision of 5% and assuming 0.5 prevalence of Avian Influenza among domestic ducks in Sunyani:

$$N=z^2\,p\,(1-p)/d^2 = \frac{(1.96)^2(0.5)(0.5)}{(0.05)^2} = \frac{0.9604}{0.0025} = 384.16$$ **(Jones *et al.*, 2003)**

- where N= sample size,
- z= risk of Type 1 error=1.96 at 95% confidence level
- p= prevalence of AI = 0.5 (arbitrary proxy)
- d= absolute precision = 5% = 0.05
- Total number of domestic ducks sampled =**384**
- Sample size = 384 cloacal swabs plus 142 feather tissues = **526**

Sampling Method

Thirteen (13) epidemiological units (epi units) or communities in the Sunyani Municipality were identified for the study. Simple random sampling method was used to select a community at a time for ducks to be identified in households and farms within that community for sample collection. This procedure was repeated till the desired sample size for the study was attained from nine (9) sites in six communities.

Ethical Considerations and Consent

Approval for the study was duly obtained from the Scientific Technical Committee of Noguchi Memorial Institute for Medical Research (NMIMR). A written informed consent was obtained from the Brong Ahafo Regional Veterinary Officer, Commercial and Backyard ducks owners, and vendors at the Live Birds Market in the selected communities to carry out the study.

Sample Collection and Processing

384 Cloacal swabs and 142 feather tissues (calamuses) were obtained from backyard ducks, a commercial farm and a live birds market. All field samples collected were pooled separately by type (cloacal swabs separate from feather tissues). Two cloacal swabs from two (2) different birds from the same site were placed into a 2.0 ml tube while ten (10) feather calamuses with similar characteristics were pooled into a 25 ml vacutainer tube with both tubes containing 2 ml and 5 ml of viral transport medium (VTM) which contained 2.5% Veal Infusion Broth (SIGMA), 0.5% Bovine Serum Albumin (SIGMA), 100 µg/ml Gentamycin sulphate (SIGMA) and 2 µg/ml Fungizone (Amphotericin B) solution. The vacutainer tubes containing these samples were properly labelled with information that described a unique identification number and dates of collection. They were placed on ice in “cold boxes” from the field and transported to the Sunyani Veterinary office where they were stored at -70 ºC. RNA extraction and RRT-PCR was carried out in the Virology Department of NMIMR in Accra.

Preparation for RNA Extraction

The 526 samples (384 cloacal swabs and 142 feather tissues) collected from the field were pooled into 43 eppendoff tubes according to the farm/household where samples were obtained from. Thirty one (31) of these pooled samples were cloacal swabs and 12 were feather specimens. Pooling of the samples was done to ensure optimization of the use of reagents.

RNA Extraction

Single stranded viral RNA was extracted using the QIAamp® Viral RNA Mini Kit commercially available from QIAGEN

DOI:10.21010/ajid.v10i2.10
(Qiagen, Hilden, Germany). The viral RNA mini spin procedure as recommended by the manufacturer was used and the manufacturer's instructions were followed.

Real-Time Reverse Transcription-PCR PLATE SETUP

The RT-PCR plate setup for both protocols; Spackman *et al*, 2002 and CDC protocol (WHO, 2009) was the same and as follows: the Negative and Positive Controls were distantly placed (A1 and H12 wells of the plates respectively) to avoid contamination. The field samples were placed systematically from the A4 well to C9 well of the PCR plate.

Spackman *etal* Protocol

The Qiagen one-step RT-PCR kit was used with a 20µl reaction mixture under the following conditions: 0.8 µl of kit supplied enzyme mixture (including RT and hot-start *Taq* polymerase), 10 pmol of each primer, 0.3 µM probe, 400 µM (each) dNTPs, 3.75mM $MgCl_2$ and 6.5U of RNase inhibitor (Promega, Madison, Wisconsin). Reverse transcription was achieved at 50^{O}C for 30 minutes .Taq polymerase activation was at 90^{O}C for 15 minutes. A two-step PCR cycling protocol was then used for the matrix gene primer and probe set as follows 45 cycles of 94^{O}C for 0 seconds for denaturation and 60^{O}C for 20 seconds annealing. Real-time RT-PCR was performed with Applied Biosystems Incorporated (ABI) 7300 system thermocycler and software.

Confirmation of Results Using Real-Time Reverse Transcription-PCR, CDC Protocol (WHO, 2009)

The negative results obtained using the Spackman protocol were confirmed as described by the Centres for Disease Control and Prevention, Atlanta USA in their protocol CDC Real-time RTPCR (rRT-PCR) Protocol (Table 1) for Detection and Characterization of Influenza (version 2009) using reagents from the Invitrogen One-Step Superscript III RT-PCR kit. Primers designed and supplied by the CDC were used at a concentration of 20 pmol each in a 25 µl reaction mix with 0.5 µl kit supplied enzyme, 0.25 µM probe (designed by the CDC) and 12.5 µl of kit supplied 2X reaction mix, a buffer containing 0.4 mM of each dNTP, 2.4 mM MgSO4. Cycling conditions for all primer sets (Table 2) was 50^{O}C for 30 minutes for the reverse transcriptase step, a *Taq* polymerase activation step of 95^{O}C for 2 minutes and 45cycles of 95^{O}C for 15 seconds and 55^{O}C for 30seconds denoting denaturing and annealing steps (Table 3, 4 and 5). Flourescent data was collected during the annealing step at 55^{O}C. Real-time RT-PCR was performed with the Applied Biosysytems Incorporated 7300 system thermocycler and software.

Table 1: PCR Master Mix Formula (Spackman *et al.*, 2002)

MASTER MIX	VOLUME (µl)	VOLUME/TUBE	TOTAL
Water	3.1	3.1	155
5 x Buffer of Qiagen kit	4	4	200
MgCl (Promega)	1	1	50
dNTPs	0.8	0.8	40
M+64(FAM-TAMRA) probe	0.3	0.3	15
M+25 Primer	0.5	0.5	25
M+124 Primer	0.5	0.5	25
ROX dye working dilution (1:100)	1	1	50
Qiagen one-step Enzyme Mix	0.8	0.8	40
		12	**600**
Template Volume	8	Number of tubes: **50**	
Reaction Volume	**20**		

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Table 2: PCR Primer and Hydrolysis Probe Sequence for AI Virus Detection (Spackman)

Specificity	Primer/probe	Sequence "(5'-3')
Influenza Matrix	A M + 25	AGA TGA GTC TTC TAA CCG AGG TCG
	M - 124	TGC AAA AAC ATC TTC AAG TCT CTG
	M + 64	FAM-TCA GGC CCC CTC AAA GCC GA-TAMRA

FAM, 6-carboxyfluorescein; TAMRA, 6-carboxytetralrhodamine. Source: Spackman *et al* 2002

Table 3: Amplification Cycling (Spackman)

RT Reaction	Starting	Denaturation	Annealing
50°C	94°C	94°C	60°C
30 min	15 min	0 sec	29 sec
		45 cycles	

Table 4: PCR Master Mix Formula (CDC Protocol)

MASTER MIX	VOLUME (µl)	VOLUME/TUBE		TOTAL
Nuclease Free Water	5.0	5.0		230
2 x Reaction Mix	12.5	12.5		575
Probe (FAM-TAMRA)	0.5	0.5		23
Forward Primer	0.5	0.5		23
Reverse Primer	0.5	0.5		23
25 x RT-PCR Enzyme	1.0	1.0		46
Total		**20**		**920**
Template Volume	**5.0**	5.0	Number of tubes: 46	
Reaction Volume	**25**			

DOI:10.21010/ajid.v10i2.10

Table 5: Amplification cycling (CDC Protocol)

RT Reaction	Starting Denaturation	Denaturing	Annealing
50°C	95°C	95°C	55°C
30 min	2 min	15 sec	30 sec
		45 cycles	

Data Analysis/Processing

Both quantitative and qualitative data obtained from the questionnaires and data from the RRT-PCR testing was double entered into Epidata Software (2007 version) and coded accordingly. This was then exported to SPSS Software version 17.0 for analysis. Data was analyzed into percentages and tables based on adherence or non-adherence of each farm premises to the 19 independent variables or biosecurity practices (Tables 11a-11c) investigated in this study. Test of association (chi-square) was also performed on certain variables (age and sex) to determine the significance.

Results

Descriptive Characteristics of Birds Sampled

Apart from being raised for meat and eggs, some ducks in the area were also kept as pets or for ornamental value. It was observed that the ducks in the area were mostly hybrids from the *Anas platyrhynchos domesticus* family, particularly the Mallards. Eighty four percent (323/384) of the ducks sampled were hybrids; the remaining 16% (61/384) were made up of thorough Mallard breeds (Aylesbury, peking and pennine).

Out of the 526 samples collected (Table 6), 58.6% (308/526) were from backyard holdings, 34% (179/526) from a commercial farm and 7.4% (39/526) from a live birds market (LBM).

Seventy six percent (292/384) of the ducks were females (ducks) and 24% (92/384) were males (drakes) (Table 7). Adult ducks (> 1 year old) represented 79.9% (307/384) of the sampled population while growers or ducklings (< 1 year old) formed only 20.1% (77/384) of the same population (Table 8). The number of ducks of age greater than 1 year compared to those less than 1 year was found to be statistically significant at $p<0.0001$. The same was for male and female ducks sampled (Tables 9 and 10).

The male to female ratio in breeding pens was found to be 5-8 females (ducks) to a male (drake).

Table 6: Details of samples collected from ducks in the Sunyani Municipality (July 2009 - October 2010).

Farm Number	Farm Size	Quantity of Samples Collected		Location of Farm
		Cloacal swabs	Feather samples	
1*	1,113	119	60	Dumasua
2	25	24	7	New Dormaa
3	12	12	7	New Dormaa
4	23	21	7	New Dormaa
5	241	57	20	Abesim
6**	38	30	10	New Dormaa
7*	44	32	7	Sunyani LBM
8	78	46	17	Odomase
9	75	43	7	Adantia
Total	1,649	384	142	

DOI:10.21010/ajid.v10i2.10
* Farm numbers 1 and 7 are; a Commercial Farm and a Live Birds Market (LBM) respectively. The remaining seven are backyard holdings. ** The only duck farm sampled that experienced outbreak in 2007.

Table 7: Sex Distribution of Ducks by Farm/Household in Sunyani Municipality (July 2009 – August 2010).

Farm Number	Male		Female		Total	
	Number	%	Number	%	Number	%
1	25	20	100	80	125	100
2	5	27.8	13	72.2	18	100
3	3	25	9	75	12	100
4	6	28.6	15	71.4	21	100
5	16	28.1	41	71.9	57	100
6	8	26.7	22	73.3	30	100
7	11	34.4	21	65.6	32	100
8	10	21.7	36	78.3	46	100
9	8	18.6	35	81.4	43	100
Total	92	24	292	76	384	100

The above Table shows the number of males and female ducks in each of the nine sites studied. The calculated *Pearson* Chi-square was 5.195 with a $p < 0.0001$. The difference was found to be statistically significant at 99.9% CL in all the nine sites.

DOI:10.21010/ajid.v10i2.10

Table 8: Age Grouping of Ducks Sampled in the Sunyani Municipality, (July 2009 – August 2010).

Farm Number	Ducks greater than one year old		Ducks less than 1 year old		Total	
	Number	%	Number	%	Number	%
1	94	75.2	31	24.8	125	100.0
2	5	27.8	13	72.2	18	100.0
3	12	100.0	0	0.0	12	100.0
4	15	71.4	6	28.6	21	100.0
5	54	94.7	3	5.3	57	100.0
6	30	100.0	0	0.0	30	100.0
7	26	81.3	6	18.8	32	100.0
8	40	87.0	6	13.0	46	100.0
9	31	72.1	12	27.9	43	100.0
Total	307	79.9	77	20.1	384	100.0

The above Table shows the number of adult ducks (age > 1 year) and that of ducklings (age < 1 year) in each of the nine sites studied. The calculated *Pearson* Chi-square was 54.501 with a $p < 0.0001$. This was found to be statistically significant at 99.9% CL in all nine cases.

Sources of Parent Stock

Sources of ducks in all the sites were identified. The commercial farm acquired parent stock from a farm in Dormaa Ahenkro in 2005 (Anonymous). Our findings also indicated that 71.4% (5/7) of the backyard holdings acquired their birds from live birds markets near and far. We also found that various species of birds in the live birds market investigated came from sources within and outside the municipality. Birds were purchased by customers for different purposes such as consumption (household, and restaurant), offering in ceremonies/gifts and religious festivals, and for replacement stock for farmers.

The study further showed that transportation and management of birds by market vendors involved poor biosecurity practices. Collectors (intermediary men) and vendors did not separate birds according to species and sources of birds. Also, birds were mixed in cages during transportation and at the market place.

Molecular Investigation for Avian Influenza

Two different RT-PCR protocols (Spackman *et al.,* 2002 and the CDC Real Time PCR protocol) were applied on 526 ducks samples (384 cloacal swabs and 142 feather tissues) to determine the presence of AI virus in ducks. All the samples were negative for AI virus (Table 9, Fig 2, 3). This included ducks sampled from the only duck farm in the Sunyani Municipality which was affected by the

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2007 HPAI H5N1 outbreaks. This farm was closed down as a result of the outbreak in May 2007 and became operational three years ago with current population of 38 ducks (Table 8).

Table 9: Results of RRT-PCR Tests for AI conducted at the NMIMR Virology Department using both Spackman *et al.*, 2002 and CDC Protocols (WHO, 2009), between October 2010 and April 2011 respectively

Sample	Target Gene	Cycle Threshold (C_t)
Positive Control	Flu-A Matrix Gene (Qiagen)	27.50/20.50
Negative Control	,,	Undetected
Commercial Ducks Samples	,,	Undetected
Backyard Ducks Samples	,,	Undetected
Live Birds Market Samples	,,	Undetected

"Undetected"= Negative results. The 27.50 cycle threshold value recorded for the "Positive Control" is a Positive result. The cut-off point for this test was ≤ 35 c_t value.

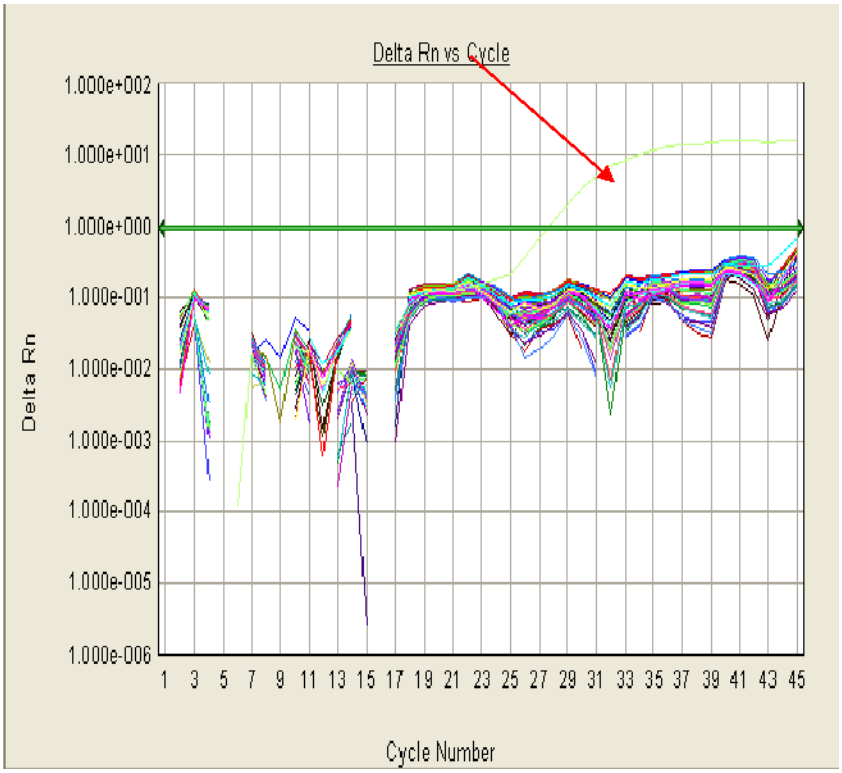

Figure 2: Real-Time PCR (Spackman *et al* 2002) Amplification Plot for AI Virus Detection in the 43 Pooled samples, April 12, 2011

DOI:10.21010/ajid.v10i2.10

Figure 2 shows RRT-PCR amplification plot for Flu A Matrix Gene analysis for the 43 pooled samples analyzed using the Spackman *et al*. (2002) Protocol. The Positive Control **(light green line above baseline indicated with an arrow)** increased exponentially with a cycle threshold (C_T) value of **27.50**. The Negative Control and the 43 pooled samples were undetected by the system's software. The cut off point for the test was a threshold < 35 C_T value. The RRT-PCR lasted for less than three hours with 45 cycles.

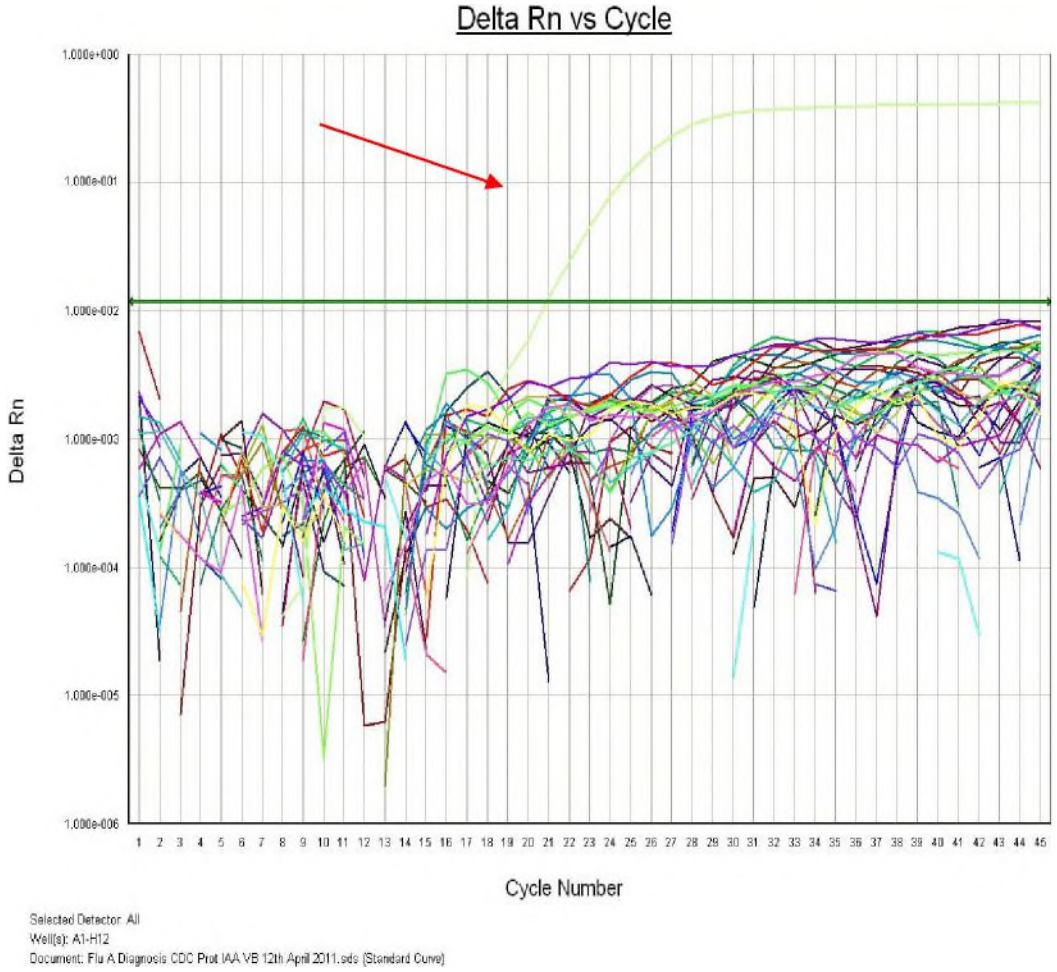

Figure 3: Real-Time PCR (CDC Protocol) Amplification Plot for AI Virus Detection in the 43 Pooled Samples, October 17, 2010

Figure 3: displays the RRT-PCR amplification plot for Flu A Matrix Gene analysis for the 43 pooled samples analyzed using the CDC Protocol. The Positive Control **(light green thin line above baseline indicated with an arrow)** increased exponentially with a C_T value of **20.50**. The Negative Control and the 43 pooled samples were undetected by the system's software. The cut off point was a threshold < 35 C_T value. The test lasted for less than three hours with 45 cycles.

Discussion

This study was conducted to increase the body of knowledge on the role of waterfowls (domestic ducks) as potential reservoirs of AI viruses and also to ascertain the profile of AI in domesticated ducks in the Sunyani Municipality area after the H5N1 outbreaks in May 2007. Avian influenza virus was isolated from outbreaks in parts of the Municipality particularly the New Dormaa area during this

DOI:10.21010/ajid.v10i2.10

period (May, 2007). Also, because waterfowls (ducks) have the unique characteristic of being asymptomatic reservoir of the AI virus, the need to verify whether or not the virus was still in circulation is expedient. The results obtained from the present study showed no evidence of the presence of AI virus in the five hundred and twenty six (526) AI samples collected from domestic ducks in nine farms in the Sunyani Municipality. These samples were tested using two different RT-PCR protocols; Spackman *et al.*, (2002) and CDC protocol (WHO, 2009). Hence, the fact that two different "tried and tested" RRT-PCR protocols were applied in this study and both tested negative for the virus in all the samples probably confirmed the validity of the results obtained and therefore suggest that there is currently no circulation of the virus in the area. Similar studies conducted in migratory and resident birds in Argentine, Bolivia and Caribbean countries, identified H1, H3, H4, H10 and H13 subtypes (Douglas *et al.*, 2007; Spackman *et al.*, 2007a; Pereda *et al.*, 2008; Ghersi *et al.*, 2009; Alvarez *et al.*, 2010). In Mexico, results of similar research have been incorporated into activities performed by sanitary officials at poultry farms as part of the campaign for early detection and prevention of HPAI outbreaks (Villarreal-Chavez and Rivera-Cruz, 2003).

A similar study carried out in commercial, backyard and live birds market in the Tema Metropolis of Ghana where there have been outbreaks (May, 2007) from May 2009 to September 2010 also yielded negative results (Danso *et al.*, 2010). This could be connected with destruction of the poultry in the only duck farm where there was an outbreak in 2007 and the subsequent containment measures put in place.

However, evidence gathered by the present study pointed to deterioration of farm management practices particularly biosecurity, and if left unattended to and reintroduction of AI virus occurs, the effect may be more disastrous to the poultry industry than the 2007 outbreaks, with possible human incursions.

In this study, we found out that biosecurity practices which had to do with the use of disinfectants (disinfection of premises 11.1%, personnel disinfection 22.2%, use of footbath 0% and disinfection of vehicles 0%) were considered expensive hence were either not practiced or were less practiced compared to those that were not disinfectant dependent. This was also observed by Danso and others (2010) in their findings.

Activity such as delivery of water and feed to birds could act as a source of contamination which could enhance the spread of poultry diseases when being administered manually without proper hygienic practices. According to Adak G.K *et al.* (1995) and Rodriguez *et al*, (2001), contaminated food and water are believed to be major sources of infection in warm-blooded animals including poultry. However, our study revealed an awfully low patronage (11.1%) for cleaning and disinfection of premises. Also, visitors to these premises did not put on protective gears to avoid been infected by sick birds or to ensure they did not introduce pathogens to farm premises. Again, the study showed that none of the sites investigated practised this very important biosecurity measure (donning of PPEs by visitors). Another equally important biosecurity measure was the disinfection of vehicles to and from the farm premises which was never observed in any of the sites. Though there was no available data on the biosecurity situation in the area prior to this study, it was clear that many farms (89%) did not still adhere to strict biosecurity and farm management practices.

At the live birds market, vendors did not adhere to any of the requirements of bio-security except for daily sweeping. In this study, the difference in sex amongst the ducks investigated was found to be statistically significant with a p-value of 0.0001 at 95% confidence level, and the accepted male/female ratio of ducks in breeding according to Koney (1998) is 1 male to 5-8 females. However, the male/female ratio in this study was found to be 1:3 which suggested that there could be in-fighting among males in mating females since there was deviation from the norm (1 male to 5-8 females). This difference could then encourage males (those in free-range) to migrate to other territories in search of females hence favoring the spread of diseases amongst birds.

However, because high mortality rates were recorded in two backyard holdings and the live birds market (Table 10) which were characterised by non-adherence to biosecurity practices, we suspected possible deficiencies in biosecurity to be the cause of the deaths.

Study Limitation/Constraint

An equal number of feather tissue and cloacal swabs could not be collected due to shortage of Viral Transport Medium (VTM). Also, viral isolation method which is a gold standard test for AI diagnosis was not applied.

Conclusions

There was no evidence of AI virus in domestic ducks in the Sunyani Municipality. Hence domestic ducks in the Municipality are not acting as reservoir of AI viruses. However, adherence to strict farm management and biosecurity practices was not observed by 89% of the sites investigated. Generally, ducks farmers in the Municipality had little knowledge about biosecurity practices. Also, deep burying of dead birds and burning were the most common methods of disposal of dead birds practiced though burying was most preferred (77.8% of the sites). Daily sweeping of the premises was the major sanitation practice in the LBM involved in this study.

References

1. Alice, F., Tony, J., Isabella, M., Annalisa, S., Bitrus, Y., Clement, M., Tinuke, O., Sonia, F., Ilaria, C and Giovanni, C. (2008). Introduction into Nigeria of a distint genotype of avian influenza virus (H5N1). Inst. Zooprofilattico Sperimentale delle Venezie, Padova, Italy and National Veterinary Research Inst., Vom, Nigeria.

DOI:10.21010/ajid.v10i2.10

2. Alvarez, P. S., Mattiello, P., Rivailler, A., Pereda, C.T., Davis, E.D., Ambrosio, S., Aguirre, C., Espinosa, J. la Torre. Donis, R., and Mattion, N. (2010). First isolation of an H5N1 avian influenza virus from wild Terrestrial non-migratory birds in Argentina. Virology 396, 76-84.
3. Beck JR, Swayne DE, Davison S, Casavant S, Gutierrez C.(2003). Validation of egg yolk antibody testing as a method to determine influenza status in white leghorn hens. Avian Dis 2003; 47: Suppl: 1196-9.
4. Center for Infectious Disease Research and Policy. Avian influenza- a global health care structures. New England Journal of Medicine, 351 (23), 2363-2365.
5. Douglas, K.O., M.C Lavoie, L.M. Kim, and C.L. Suarez, (2007). Isolation and genetic characterization of avian influenza viruses and a Newcastle disease from wild birds in Barbados: 2003-2004. Avian Dis. 51, 781- 787.
6. Easterday, B.C., Hinshaw, V.S and Halvorson, D.A. (1997). Influenza. In Diseases of Poultry, 10th ed., B.W. Calnek, et al, eds., Ames, IA: Iowa State University Press, pp. 583-605.
7. Fenteng D. E (2010). Epidemiology of avian influenza in domestic poultry and wild birds the Tema Metropolis, Gt. Accra reg. Ghana, FELTP, SPH, university of Ghana 2010.
8. Ghersi, B.M., D.L. Blazes, E. Icochea, R.I. Klimov, A.E, Gonzalez, T. Kochel, Y. Tinoco, M.M. Sovero, S. Lindsrom, B. Shu, A. Klimov, A.E. Gonzalez, and J.M. Montgomery, (2007). Avian influenza in wild birds, Central Coast of Peru. Emerg. Infect. Dis. 15, 935-938.
9. Hinshaw, V.S and Webster, R.G. (1982). The natural history of influenza A viruses. A.S Beare (ed). Basic and applied influenza research. CRC Press: Boca Raton, FL, 79-104.
10. Klempner, M.S and Shapiro, D.S. (2004). Crossing the species barrier. one small step to man, one giant leap to mankind. N. Engl. J. Med. 2004; 350: 1171-2.
11. OIE 2005. (World Organisation for Animal Health). Highly pathogenic avian influenza in Mongolia: in migratory birds.
12. Olsen, S.J, Laosiritaworen, Y., Pattanasin, S., Prapasiri, P., and Dowell S.F (2005a). Poultry handling practices during avian influenza outbreak, Thailand. Emerging infect Dis. 11: 1601-1693.
13. Olsen, S.J., Ungchusak, K., Birmingham, M., Bresee, J., Dowell, S.F and Chunsuttiwat, S. Surveillance for avian influenza in human beings in Thailand. Lancet Infect Dis. (2006). Dec; 6 (12):757-8.
14. Pereda, A.J., M. Uhart, A.A Perez M.E Zaccagnini, L. La Sala, J. Decarre, A. Goijman, L. Solari, R. suarez, M.I. Craig, A. Vagnozzi, A. Rimondi, G. Konig, M.V. Terrera, A. Kaloghlian, H. Song, E.M. Sorrell, and D.R. Perez, (2008). Avian influenza virus isolated in wild waterfowls in Argentina; Evidence of potentially unique phylogenetic lineage in South America. Virology 378, 363-370.
15. Perkins, L.E., Swayne, D.E. (2003). Comparative susceptibility of selected avian and mammalian species to a Hong Kong-origin H5N1 high-pathogenicity avian influenza virus. Avian Dis. 2003; 47(3 Suppl):956-67.
16. Rodriguez, L.C., J.M. Cowden, J.G. Wheeler, D. Seth, P.G Wall, P. Cumberland, D.S Tompkins, M.J. Hudson, J.A Roberts, and P.J Roderich. (2001).The study of infectious disease in England: risk factors for cases of infectious intestinal disease with campylobacter jejuni infection. Epidemiol. Infect. 127: 185-193.
17. Spackman, E., K.G., McCracken, Winker, K., and Swayne, D.E. (2007a). An avian influenza virus from waterfowls in South America contains genes from North America avian and equine lineages. Avian Dis. 51, 273-274.
18. Spackman, E., Senne, D.A, Myer,s T.J, Bulaga L.L, Garber L.P, Perdue M.L, Lohman K, Daum L.T, Suarez D.L (2002). Development of a real-time reverse transcriptase PCR assay for type A influenza virus and the avian H5 and H7 hemagglutinin subtypes .J Clin Microbiol; 40: 3256-60.
19. Swayne, D.E and Beck, J.R. (2005). Experimental study to determine if low pathogenicity and high pathogenicity avian influenza viruses can be present in chicken breast and high meat following intranasal virus inoculation. Avian Dis. 49 (1): 81-85.
20. Webster, R. G., Bean, W. J., Gorman, O. T., Chambers, T. M and Kawaoka, Y. (1992). Evolution and ecology of influenza A viruses. *Microbiology Review*; 56: 152-179.
21. Webster, R.G., Peiris, M., Chen, H and Guan, Y. (2006). H5N1 outbreaks and enzootic influenza. Emerging Infect Dis 2006; 12: 3-8.
22. World Health Organization Global Alert and Response. (2005). Geographical spread of H5N1 avian influenza in birds- update 28, 18 August 2005; www.who.inte/entity/csr/2005_08_18/en/index.html.
23. World Health Organization. (2009). Cumulative number of Confirmed Human Cases of Avian Influenza A/ (H5N1) Reported to WHO.