ENSIGN COLLEGE OF PUBLIC HEALTH, KPONG, EASTERN REGION, GHANA

SURVEILLANCE FOR AVIAN INFLUENZA VIRUS IN MAJOR LIVE BIRD MARKETS IN ACCRA IN THE GREATER ACCRA REGION

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A Thesis submitted to the Department of Community Health in the Faculty of Public Health in partial fulfilment of the requirements for the award of Master of Public Health degree.

APRIL, 2017.

DECLARATION

I hereby certify that except for reference to other people's work, which I have duly cited, this Project submitted to the Department of Community Health, Ensign College of Public Health, Kpong, is the result of my own investigation, and that it has not been presented for any other degree elsewhere.

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DEDICATION

This work is dedicated to my adorable daughter; Awurabena Korantemaa Ofori-Koranteng, and my parents for their focussed commitment to my education.

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DEFINITION OF TERMS

Antigen: A protein on the surface of an influenza (flu) that can stimulate an immune response.

Avian Influenza: The disease caused by infection with avian (bird) influenza (flu) Type A viruses.

Biosecurity: A set of preventative practices aimed at reducing the potential for the introduction, delivery, and transmission of disease causing organisms onto and between sites, animals and humans.

High Pathogenic Avian Influenza: An extremely contagious, multi-organ systemic disease of poultry leading to high mortality.

Live Bird Market: A food market that offers both poultry meat and live birds either for sale or for slaughter.

Low Pathogenic Avian Influenza: Any genetic subtype of avian influenza A that causes mild, nonfatal disease in infected birds.

Targeted Surveillance: The tailoring of a surveillance program based on increased likelihood of infection in a particular species or area rather than doing a completely random sampling.

ABBREVIATIONS

AFP ……...:……………………………………………………………… .Avian Flu Pandemic

AI: ………………...……………………………………………………Avian Influenza

AIV: ……………………………………………………………...Avian Influenza Virus

AVL: ……………………………………………………...Accra Veterinary Laboratory

BSA: ………………………………………………….................Bird Sellers Association

CDC: ………………………………………………………Centers for Disease Control

CFR: ……………………………………………………...………..Case Fatality Rate

ELISA ………………………………………. …..Enzyme-Linked Immunosorbent Assay

FAO: ………………………………………………Food and Agricultural Organization

GAR: ……………………………………………..………………Greater Accra Region

GDP: …………………………………………….……………Gross Domestic Product

HA: …………………………………………………….……………Haemaglutinin Antigen

HPAI: ……………………………………………………..High Pathogenic Avian Influenza

IMF: ……………………………………………………………International Monetary Fund

KAP: ………………………………………………….....Knowledge, Attitudes and Practices

LBM: ………………………………………………………………………Live Bird Market

LPAI: …………………………………………………....Low Pathogenic Avian Influenza

LPT: ………………………………………………………………..Live Poultry Trader

NA: ……………………………………………………………….Neuraminidase Antigen

OIE: …………………………………………………...Office International des Epizootics

RADTK: ……………………………………………………..Rapid Antigen Detection Test Kit

rRT-PCR: ………………………...Real-Time Reverse Transcriptase Polymerase Chain Reaction

SPSS: ………………………………………………...Statistical Package for Social Sciences

VSD: …………………………………………………...Veterinary Services Directorate

WHO: ……………………………………………………World Health Organization

ABSTRACT

Between January and February, 2017, a total of 438 samples were collected from 154 apparently healthy birds in five major live bird markets (Dome, Agbogbloshie, Kantamanto, Mallam-Atta, and Pokuase) all within Accra in the Greater Accra Region of Ghana. Real-time Reverse Transcriptase Polymerase Chain Reaction (rRT-PCR), and indirect Enzyme-Linked Immunosorbent Assay (ELISA) were carried out on all samples. A questionnaire was used to gain insight into live poultry traders' knowledge, attitudes and practices towards avian influenza biosecurity in these major markets. The overall positive detection of influenza type A virus in the samples was 6.62%. The results of all AI positive detections in both the molecular and serological assays were negative when subtyped for HPAI/H5N1, the implicated influenza A strain reported and confirmed in all episodes of AI in the country thus far. Further, all five (100%) LBMs surveyed in this study tested positive for influenza A. This finding suggests the need for prompt and further consecutive laboratory investigation for complete molecular sequencing and allow for phylogenetic analysis to determine the subtype in circulation. Survey results indicated poor biosecurity practices. Majority of birds in all five surveyed markets were obtained from multiple sources without records. These results accurately reflect the status of the disease in the study areas. Intensified surveillance is therefore needed to keep track of AI situation in the country since type A viruses are capable of constant mutation. Biosecurity measures must be enforced in full in all live bird markets in the country.

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CHAPTER ONE

INTRODUCTION

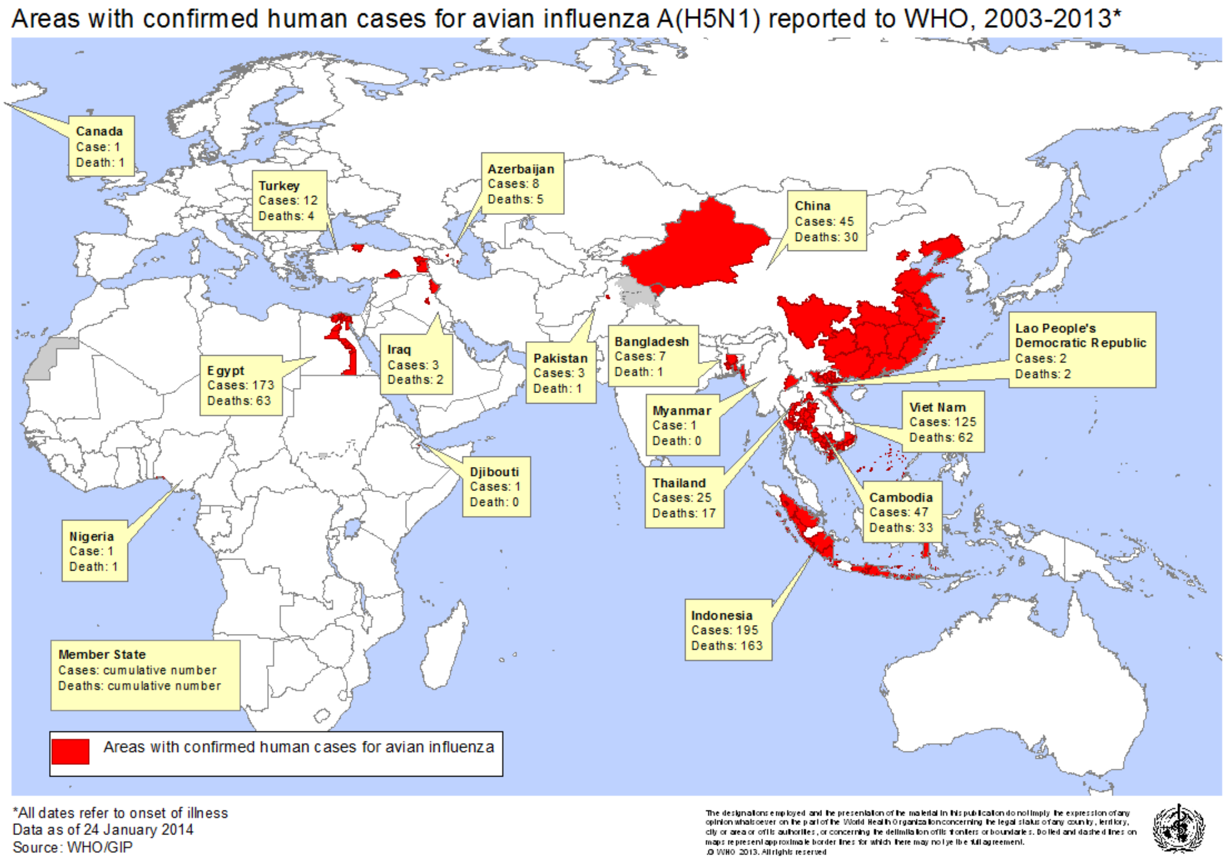
1.1 Background to the Study

Avian influenza viruses (AIVs) belong to the family Orthomyxoviridae, and are of the influenza type A genus which are classified into subtypes on the basis of 18 different haemagglutinin subtypes (H1-H18) and 11 different neuraminidase subtypes (N1-N11) (WHO, 2015). AIVs infecting poultry birds are further sub divided into two distinct groups as highly pathogenic avian influenza (HPAI) and low pathogenic avian influenza (LPAI). HPAI viruses usually cause severe disease in chicken and up to 100 percent mortality, while LPAI viruses cause subclinical infections and little or no mortality in all avian types (CDC, 2014).

Avian influenza can infect a wide variety of birds, including chickens, turkeys, ducks and geese. Wild waterfowl (ducks, geese, shorebirds) tend to be more resistant and are, in fact, reservoirs of the disease (Swayne and Suarez, 2000). According to Sturm-Ramirez *et al.*, (2004), most of the avian influenza viruses replicate preferentially in the gastrointestinal tract (GIT) of wild ducks. The viruses are excreted at high levels in faeces and transmitted through the faecal-oral route.

Epizootic outbreaks of HPAI H5N1 (also known as avian influenza or bird flu) have serious implications from veterinary, medical and public health standpoints (Taisuke and Yoshihiro, 2001; Yoshiyuki, 2006; Suarez *et al*., 2003). Avian influenza (AI) viruses are species-specific and seldom cross the species barrier. Notwithstanding, subtypes H5, H7 and H9 have been implicated in both avian and mammalian infections, including humans. These subtypes are capable of mutating to cause interspecies transmission (Kuiken *et al*., 2006; Olsen *et al*., 2000; Suarez *et al*., 2003 and Choi *et al*., 2004). According to the FAO (2016), the transboundary transmission of

HPAI virus due to the H5N1 subtype from Southeast Asia to over 60 countries, has caused high direct mortalities in over 550 million affected poultry flocks worldwide, with additional losses due to culling. Both farmers and traders have suffered loss of income as a result of market disruption caused by control activities and also market shock due to consumer concerns for human health.



Map 1: Areas with confirmed human cases for avian influenza A(H5N1) reported to WHO, 2003-2013.

For the FAO, OIE and others related in securing the livelihoods of developing country communities, this is ample justification in itself to merit a major campaign to prevent further spread of the disease within infected areas and progressively work toward its eradication. Although transmission from bird to humans is infrequent, there has been no evidence of sustained human-to-human transmission. However, from 2003-2015, 844 laboratory-confirmed human cases of AI H5N1 virus infections, including 449 deaths, were reported to WHO from 16 countries. Overall, the most affected countries are Egypt and Indonesia. In 2014, and 2015, Egypt reported most cases and has become the most affected country with the highest number of human cases (WHO, 2015).

AIV was first officially reported in Africa in 2005 (Adene and Oguntande, 2006). Awuni *et al*., (2010) posit that within three months of the outbreak in Nigeria, where it recorded a high case fatality in birds, the virus rapidly spread to a number of other African countries, including Egypt, Niger, Cameroon, South Africa and other west African countries such as Benin, which is close to Ghana. On the assumption that the Nigerian experience left in its trail a human casualty because of contact with infected birds, there was widespread fear and panic among industry players and the general populace in Africa. However, in April 2007, Ghana recorded its first case of HPAI H5N1 virus infection in a commercial poultry farm at Kakasunanka (close to a water body that is visited by migratory wild birds and waterfowls annually) within the Tema metropolis in the Greater Accra Region. This outbreak caused considerable socioeconomic losses in the poultry industry. By June the same year, seven other outbreaks were confirmed at different locations in the country (Awuni *et al*., 2010). Despite successful containment of the situation through active case search, quarantines, movement controls, temporary ban on importation of poultry and poultry products, education, among other enhanced biosecurity measures, the country again recorded another major outbreak in 2015. Some sporadic cases are still being reported (OIE, 2017).

Almost all human infections have been related to close contact with infected or sick birds or their faeces, secretions or contaminated fomites and products in domestic settings, for example live bird or wet markets. Several studies have implicated live bird markets (LBMs) as a thriving hub for the zoonotic and pathogenic dissemination of AIV and other infections as they provide increased contact and bonding between humans and live animals in a high density setting (Indriani *et al*., 2010; Wang *et al*., 2006; Cardona *et al*., 2009; Mullaney, 2003; Joseph *et al*., 2014; Yu *et al*., 2013). Thus, unprotected exposure to birds infected with H7N9 at LBMs in China has been found to be the major risk factor for human infection. Live poultry traders (LPTs) are mostly small scale traders who sell, and sometimes slaughter and process live birds in these wet markets. The nature of their business makes them have an interface with both consumers and farmers who buy from these wet markets to restock their farms. They thus, can act as key informants to officials involved in the tracing sources of poultry diseases (Chinyere, 2010). They are to a large extent influential and, their knowledge and practice of AI biosecurity in LBMs is crucial and must thus be captured in any attempt to holistically prevent and/ or stamp out AIV/H5N1 from Ghana. Surveillance for influenza viruses from LBMs, thus, provides information that is relevant for both human and veterinary public health. Quite a number of studies use live birds in LBMs in detecting AIVs (Wang *et al*., 2006; Cardona *et al*., 2009; Garber *et al*., 2007). A study by Sheta *et al*., (2014) in backyard poultry in Egypt established that AIV could be isolated at a much higher rate from faecal samples of birds. Another study in New York that tested some environmental aspects of LBMs for AIV identified that the virus could be isolated from samples obtained from the poultry areas of LBMs. The study also established that the level of environmental contamination decreased with proper adherence to strict hygienic practices (Trock *et al*., 2008).

1.2 Problem Statement

The tendency for LPAI viruses to mutate into HPAI strains connotes that there should be continuous and intensified surveillance to detect the circulating AIV subtype and to keep real time track of the dynamics of the virus. This is of high public health significance, especially in designing appropriate intervention measures to interrupt the spread of the virus. There are a lot of published works describing AIV endemicity in LBMs in several Asian countries (Indriana *et al*., 2010; Kung *et al*., 2002; Martin *et al*., 2011), Europe (Loth *et al*., 2007; Weiner and Harder, 2006), the United States (APHIS, 2015; Trock *et al*., 2008, Bulaga *et al*., 2001) and some places in Africa, such as Nigeria (Oluwayelu *et al*., 2015; Aika-Raji *et al*., 2015; Joseph *et al*., 2014), Uganda (Kirunda *et al*., 2014) and Egypt (Sheta *et al*., 2014). Conversely, not much is known about the LBMs, and by extension the practices employed by LPTs in Ghana as far as AIV/H5N1 is concerned. The poultry industry in Ghana is still at its infant stage, hence the need to track any health related situation that may cripple it. As earlier established, AI poses a real public health threat as HPAI can occasionally infect humans. Although some studies were carried out following the outbreak of H5N1 in Ghana in 2007, (Awuni *et al*., 2010), not much is known about any targeted surveillance of live bird markets in Ghana. Persons who take care of poultry in LBMs have close contact with these birds. This situation puts such persons at high risk should the disease strike their birds. It is therefore imperative to have sufficient knowledge on outbreaks of AI in our LBMs. Aside from this, there is ample proof of the paucity of knowledge of some Ghanaian poultry farmers, and especially live bird tradesmen on biosecurity measures in major LBMs and its value in the identification of AIV. This study, therefore, seeks to do a focussed surveillance on the major live bird markets in Accra by obtaining baseline information regarding the trade activities of LPTs operating at the major

LBMs in Accra on AI biosecurity and to use tracheal, blood, and cloacal samples in detecting the presence of, as well as the circulating subtype(s) of AIV in these LBMs if any.

1.3 Rationale of the Study

The report from this study can help orient policy for developing and improving current approaches to address behaviour change among poultry traders on biosecurity in LBMs. Again, this study is intended to be a part of the on-going surveillance for AI/H5N1 in poultry in Ghana. Additionally, it is hoped that it will contribute to knowledge and resources on the general practices of LPTs in LBMs. Further, it is expected that the outcome of this study will help update the Epidemiology Unit of the Veterinary Services Directorate, Accra about the current trends of avian influenza, especially in live bird markets.

1.4 Hypothesis/Conceptual Framework

Based on the study objectives, three hypotheses were set to guide the study.

Hypothesis 1

H_0: Avian influenza is not currently circulating among birds in major live bird markets in Accra.

H_1: Avian influenza is currently circulating among birds in major live bird markets in Accra.

Hypothesis 2

H_0: No new AIV is currently circulating in live bird markets in Accra.

H_1: New AIV is currently circulating in live bird markets in Accra.

Hypothesis 3

H_0: There is no significant relationship between live poultry traders' knowledge of avian influenza biosecurity and their current practices.

H_1: There is a significant relationship between live poultry traders' knowledge of avian influenza biosecurity and their current practices.

Conceptual framework: Active avian influenza surveillance approach.

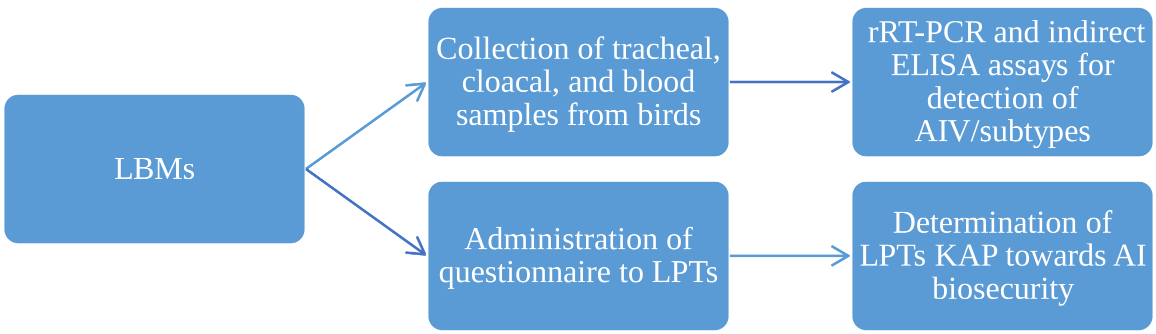


Figure 1.1: Conceptual framework ***Source: Researcher's construct***

1.5 Research Questions

a) To what extent is AIV currently circulating among birds in LBMs in Accra?

b) What is/are the subtype(s) of the circulating AIV in these markets?

c) What is LPTs knowledge, attitude and practises towards AI biosecurity in these markets?

d) What is the possible association between LPTs knowledge of AI biosecurity and their current practices?

1.6 General Objective of the Study

The general objective of this study was to use the active avian influenza surveillance approach to detect the presence of AIVs, as well as determine the circulating strain(s) from samples (tracheal, cloacal and blood) obtained from seemingly healthy birds in the major live bird markets in Accra and to obtain baseline information regarding the trade activities of LPTs operating at these markets.

1.7 Specific Objectives of the Study

Specifically, this study sought:

a) To determine the current profile of AIV from tracheal, cloacal, and blood samples of seemingly healthy birds in the major LBMs in Accra through an active case search.

b) To identify the circulating subtype(s) of AIV in these markets.

c) To establish LPTs knowledge, attitudes and practices (KAP) towards AI biosecurity in these major LBMs.

d) To determine the possible association between LPTs knowledge of AI biosecurity and their current practices.

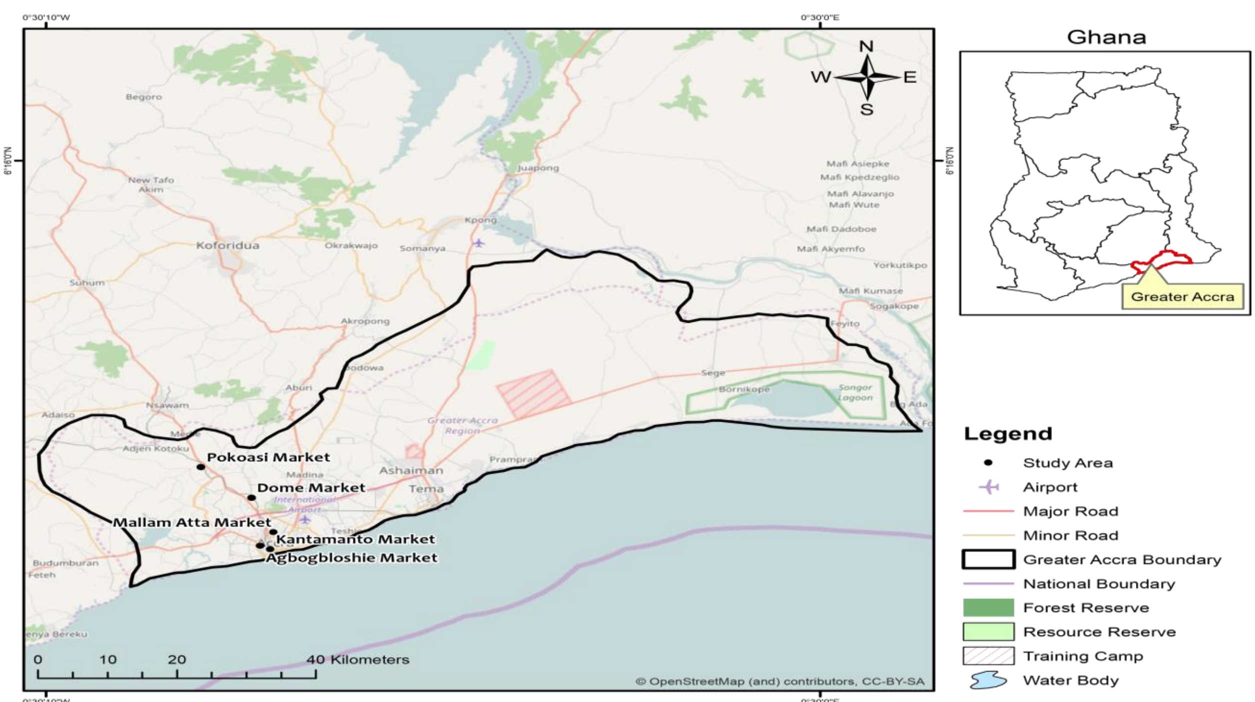
1.8 Profile of the Study Area

The Government of Ghana website (ghana.gov.gh) describes the study area, Greater Accra Region, as the smallest of the 10 administrative regions in terms of area, occupying a total land surface of 3245 square kilometres or 1.4% of the total land area of Ghana. In terms of population, however, it is the second most populated region, with a population of 4,010,054 in 2010, accounting for 15.4% of Ghana's total population (Ghana Statistical Service, 2010). The region is divided into sixteen administrative areas, including, but not limited to Accra Metropolitan Area (AMA), Ga-East Municipal and Ga-West Municipal. It has a coastline of approximately 225 km stretching from Kokrobite in the West to Ada in the East. The soils have low organic contents with shallow top soils which limit the capacity for crop production. The vegetation is mainly coastal savannah shrubs interspersed with thickets.

The region is relatively dry since it falls within the dry coastal equatorial climatic zone with temperatures ranging between 20ºC and 30ºC and annual rainfall from 635 mm along the coast to

1140 mm. The main rivers that flow through the region are the Volta and Densu. Because the region is bordered on the South by the Gulf of Guinea, there are ecologically very important but highly polluted lagoons and wetlands in AMA, Tema and Dangme East which are seasonally visited by migratory birds.

The region may be regarded as the hub of avian influenza in Ghana by virtue of the fact that it was the site for the first outbreak of AI in the country, and continues to be implicated as the region hardest hit in terms of losses due to AI in the country. The influx into and mix of people, and livestock in the region offers an ideal environment for AI viruses to flourish and re-assort themselves. Despite being the capital, Accra is characterised in parts by inadequate and/ or poor infrastructure, high population density, low sanitation and a high burden of infectious diseases. The headquarters of the Ministry of Food and Agriculture, as well as the national veterinary laboratory headquarters are both located in this region. These factors make the study ideal in this area.



Map 2 .1: Map showing the study area and study sites ***Source: Epi Unit, VSD, Accra.***

1.9 Scope of the Study

The study is a targeted surveillance of only five LBMs, even though there are other LBMs and commercial poultry farms in Accra. The choice of these markets was necessitated by the fact that they constitute the most vibrant of all LBMs in the capital and for that matter the Greater Accra region. Again, only clinically healthy birds were sampled for the study. This was to determine whether the virus is circulating silently in seemingly healthy birds or not.

1.10 Organisation of the Study

To facilitate reading and comprehension, the study is organised under six distinct chapters as follows:

Chapter one is a transformation of the research proposal and features the background to the study, statement of the problem, rationale of the study, hypothesis/conceptual framework, research questions, general objectives, specific objectives, profile of the study area, scope of the study and organization of the study. Chapter two is the literature review where works done by other researchers that relate to this study are brought into focus to situate this work in context for comparison.

Chapter three gives details of the research methodology, ethical consideration and limitations of the study. Chapter four presents the findings of the study. The first part of the chapter presents results from the laboratory-based analysis and the second part presents results of the key study variables of the survey questionnaires. Chapter five discusses the findings of the study in relation to the research questions, objectives of the study, key variables and literature review. Chapter six summarizes the key findings of the study, draws conclusion and makes appropriate recommendations.

CHAPTER TWO

LITERATURE REVIEW

2.1 Avian influenza virus

Avian influenza (AI), commonly called 'bird flu' is a highly contagious viral disease affecting several species of food producing birds (chickens, turkeys, guinea fowl, quails etc.), as well as pet birds and wild birds. It is an infection caused by influenza viruses that occur naturally in birds, and less commonly in pigs and humans (OIE, 2016).

2.2 Etiology

The Center for Food Security and Public Health (2015 cited in CDC, 2014; OIE, 2015; Olsen *et al.*,2002; Swayne, 2008 and Tong *et al.*, 2012, 2013) states that avian influenza results from infection by viruses belonging to the species influenza A virus, genus influenza virus A and family Orthomyxoviridae. These viruses are also called type A influenza virus. Influenza A viruses are classified into subtypes based on two surface proteins, the haemagglutinin (HA) and neuraminidase (NA). A virus that has a type 1 HA and type 2 NA, for example, would have the subtype H1N2. At least 16 haemagglutinins (H1 to H16), and 9 neuraminidases (N1 to N9) have been found in viruses from birds, while two additional HA and NA types have been identified, to date, only in bats. Fouchier & Munster (2009) established that some haemagglutinins, such as H14 and H15, seem to be uncommon, or perhaps are maintained in wild bird species or locations that are not usually sampled.

2.3 General Overview of Avian Influenza

Avian Influenza Virus (AIV) prior to the 1990s was primarily a disease of wild birds. These wild birds were the natural reservoir of the virus and infections were intermittently spilled over to house-trained poultry. Only few circumstances of self-limiting illness in human were reported (Wong & Yeun, 2006). According to Xu *et al.* (1999), in 1997, an influenza virus derived from pure avian-origin which belonged to the highly pathogenic AIV of H5N1 subtype (A/H5N1) was able to cross avian-human species boundaries without pre-adaptation in "a mixing vessel" host, namely pigs as hypothesized for years. Six (6) out of eighteen (18) infected persons were killed by the virus in Hong Kong and distressed the poultry industry in the region (Xu *et al.*, 1999).

Also, Causey & Edwards (2008) assert that, avian influenza A virus (an orthomyxovirus) is a zoonotic pathogen with a natural reservoir entirely in birds. The influenza virus genome is an 8-segment single-stranded RNA with high potential for in situ recombination. Two segments code for the haemagglutinin (H) and neuraminidase (N) antigens used for host-cell entry. At present, 18 H and 11 N subtypes are known, for a total of 198 possible different influenza subtypes, each with potentially different host susceptibility. With 110,000 species of birds found in nearly every terrestrial and aquatic habitat, there are few places on earth where birds cannot be found. The avian immune system differs from that of humans in several important features, including asynchronous B and T lymphocyte systems and a polymorphic multigene immune complex, but little is known about the immunogenetics of pathogenic response. Postbreeding dispersal and migration and a naturally high degree of environmental vagility mean that wild birds have the potential to be vectors that transmit highly pathogenic variants great distances from the original sources of infection.

According to the World Health Organisation (WHO), while H5N1 has not yet proven the ability to spread well from person to person, the high case deadliness related with reported infection, ongoing spread of the virus in bird populations, and the probability for influenza viruses to transform and adapt to other hosts mean H5N1 remains an unending public health concern. As of August 10, 2012, H5N1 infection had been detected in 608 individuals in 15 countries globally (WHO, 2012).

With regards to the number of reported cases, Van Kerkhove (2013) reported that, Indonesia, Vietnam, and Egypt have so far reported the largest numbers of human cases reported, with each having reported more than 100 cases. Van Kerkhove (2013) further made it known that, in Western Europe and the Americas, no human cases have yet been reported. Although the apparent case fatality rate (CFR) of H5N1 is high (approximately 59%), this may be an overestimate of the true CFR because any milder cases may never be identified under current surveillance systems in countries affected by H5N1.

AIVs have also been found to have immense effects on economies. Capua and Marangon (2006) state that, from December 1999 through April 2003, more than 50 million birds died or were depopulated after HPAI infection in the European Union, causing severe economic losses to the private and public sectors. Similar steps were taken in other countries and continents where the virus was found. Such losses point to the importance of improving upon the policies and control measures in place to help combat the disease.

2.4 The Global Impact of Avian Influenza

The International Monetary Fund (IMF) in 2006 expressed the growing concern about the possibility of an avian flu pandemic (AFP) and its consequences for humans and the worldwide economic and financial system. The IMF further stated that, while such pandemics are not new (with the last one occurring in 1968), health professionals are mainly apprehensive about the current strain of the virus (H5N1). This strain has spread swiftly in bird populations, caused high death among poultry, and intermittently infected humans, with about half of the cases proving fatal. Human infections however remain infrequent as the strain has not been spreading easily from birds to humans, nor has it been spreading from person to person.

The World Health Organisation has also expressed concern on the difficulty in predicting the evolutions in influenza viruses, thereby making it difficult to know if or when a virus such as H5N1 might become easily transmittable among humans. This has made it impossible to tell when another pandemic will arise, whether it will involve H5N1, or another strain, or whether it will be mild or severe. However, the WHO expresses its awareness of the possibility of a pandemic occurring once a virus mutates into a form that allows for efficient human-to-human transmission. In such an instance, infections could spread quickly, and, if the virus has a high mortality rate, could threaten millions of lives around the world because of the ease of movement and interconnection of countries. The pandemic might emerge in repetitive waves with varying intensity, including durations beyond 6 weeks, as was the case with past pandemics. The outbreak of a pandemic as a result of avian influenza will also cause a decline in the consumption of poultry products as a result of grave concerns over food safety. This can have significant implications for the meat and livestock industry globally. For instance, the 2003 outbreak of another highly pathogenic avian flu (H7N7 virus) in the Netherlands led to the destruction of around 30 million birds and direct economic costs estimated at more than €150 million. Also, in some South-East Asian countries, avian influenza has dealt a significant blow to their economies in which the disease was endemic. Following the 2003-2004 outbreaks of highly pathogenic H5N1 bird flu, the total losses in GDP as a result of damage to the poultry sector in Asia amounted to almost €8 billion.

2.5 Wild Birds and Avian Influenza

According to the FAO (2017), the highly pathogenic avian influenza H5N1 strain has spread from domestic poultry to a large number of species of free-ranging wild birds, including non-migratory birds and migratory birds that can travel thousands of kilometres each year. The regular contact and interaction between poultry and wild birds has increased the urgency of understanding wild bird diseases and the transmission mechanisms that exist between the poultry and wild bird sectors, with a particular emphasis on avian influenza. In general, avian influenza viruses in wild birds can be transmitted to and from poultry, and potentially to and from some other domestic animals and people. Wild birds have been shown to be a reservoir for low pathogenic virus strains, with low prevalence though.

As of October 2007, more than 190,000 wild birds in the U.S. had been tested for influenza viruses under the Wild Bird Surveillance Plan. For wild birds, a total of 98 HPAI positives were detected between December 2014 and June 2015 from a total of 7,084 samples. The majority of these were hunter-harvested waterfowls collected in the Pacific Flyway, but 16 HPAI positives were associated with three wild bird mortality events involving snow geese (Chen caerulescens) and ringed-necked ducks (Aythya collaris) in the Mississippi Flyway. Seven captive raptors were also reported to have died from these viruses after being fed meat from infected wild waterfowl. Although experts agree that wild birds and other wildlife can carry and spread avian influenza viruses in the environment, little is known about the role wildlife plays in spreading the disease to domestic poultry. Also, phylogenetic investigations of the current highly pathogenic H5N8 AI virus strains (within clade 2.3.4.4) and those strains circulating from 2014 to 2015 indicate that the virus evolved and returned. However, virological and serological evidence, as well as results of field surveys in Central Eurasia between 2014 and 2016, indicate that sustained transmission and independent maintenance of the virus in wild bird populations during this period is unlikely. Thus, monitoring techniques, surveillance, habitat use and migration patterns are all important aspects of wildlife and disease ecology that need to be better understood to gain insights into disease transmission between these sectors.

2.6 Live Poultry Traders Knowledge, Attitude and Practice towards Avian Influenza Biosecurity in Live Bird Markets.

Avian influenza is a viral disease of domestic and wild birds. It has a complex ecological distribution with almost unpredictable epidemiological features thus, placing it topmost in the World Organization for Animal Health list A poultry diseases. This has led to the virus being considered as a threat to global public health. The recent pandemics caused by highly pathogenic AIV (H5N1) in domestic poultry is currently rated phase 3 by the World Health Organization on the pandemic alert scale. For this reason, prevention and control is highly dependent on awareness and protective behaviours of the general population as well as high risk-groups.

Most people purchase live poultry from LBMs around the world. Live bird markets are needed for marketing poultry. These markets, however, have been linked to many outbreaks of avian AI and its spread. They are considered high risk areas as a result of the bulk of large numbers of mixed poultry species of unknown disease status. This has placed traders at LBM at increased risk of acquiring AI infection through contact with sick poultry or their products. Issues of great concern arise where a potential AI transmission could lead to a pandemic especially from resource-constrained countries with weak health systems and veterinary services. Therefore, understanding the knowledge, attitude, and practices of live poultry traders and their perceptions of HPAI and biosecurity is critical to reducing transmission risk and controlling the disease. Some researchers (Turkson & Okike, 2016; Kurscheid *et al*., 2015; Kumar *et al.*, 2013; Kirunda *et al*., 2014; Neupane *et al*., 2012) in an attempt to gain insight into this problem, have carried out some studies to help make information available on how to reduce the spread of the virus in LBMs. This was mainly to make known to poultry traders the risks involved and some practices that can be adopted to minimize its effects.

In assessing the knowledge, attitude, and practices of live poultry traders, Kurscheid *et al.* (2015) conducted a survey at 17 live bird markets on the islands of Bali and Lombok in 2008 and 2009. A total of 413 live poultry traders were interviewed. More than half (58%) of live poultry traders interviewed knew that infected birds can transmit HPAI viruses but were generally unaware that viruses can be introduced to markets by fomites. Cleaning cages and disposing of sick and dead birds were recognized as the most important steps to prevent the spread of disease by respondents. Two thirds (n = 277) of respondents were unwilling to report sudden or suspicious bird deaths to authorities. Bali vendors perceived biosecurity to be of higher importance than Lombok vendors and were more willing to improve biosecurity within markets than traders in Lombok. Collectors and traders selling large numbers (>214) of poultry, or selling both chickens and ducks, had a better knowledge of HPAI transmission and prevention than vendors or traders selling smaller quantities or only one species of poultry. Also, education was strongly associated with better knowledge but did not influence positive reporting behavior. It was concluded at the end of the study that, most live poultry traders have limited knowledge of HPAI transmission and prevention and are generally reluctant to report bird deaths. Greater effort is therefore needed to engage local government, market managers and traders in education and awareness programs, regulatory measures and incentive mechanisms. Similarly, understanding and evaluating the social responses to such an integrated approach could lead to more effective HPAI prevention and control.

In the study of Kumar *et al.* (2013), a pretested and semi-structured survey instrument was administered to both live bird market and poultry farm workers in two most populous cities in Karnataka in South India to collect data on demographics, knowledge, attitude, and practices among them. Findings revealed that, there was a higher level of biosecurity practices adopted in poultry farms compared with those adopted in live bird markets. Knowledge regarding AI was acceptable but poorly correlated with actual biosecurity practices. Live bird market and poultry farm workers were also identified as the weakest link in the prevention and control of the spread of AI in the two most populous cities studied in Karnataka. Risk reduction models of behavior change targeting these groups was recommended as being important toward the control and prevention of AI spread.

Sutanto (2013) also proposed that, the level of knowledge and perception of safe poultry handling practices regarding the risk of highly pathogenic avian influenza among workers in LBMs can be assessed using a knowledge, attitudes, and practices study. The results of the study illustrated that, despite being given information, LBM workers had no detailed understanding of avian influenza, had a less perceived risk of experiencing avian influenza, and had a low compliance with precautionary behaviors. As a result, biosecurity in the LBMs was highly inadequate, increasing the threat of another serious outbreak of HPAI in poultry and perhaps in humans as well. Encouragingly, workers' interest in learning more about avian influenza was high in this survey.

Therefore, designing and implementing avian influenza educational programs and measuring their effectiveness should be priorities to encourage the population to take a more active role.

Using structured questionnaire, Musa *et al*. (2013) survey of poultry farmer's knowledge, attitudes, and practices in two Nigerian states revealed the presence of risk farming practices that may enable avian influenza high chance of introduction/reintroduction. There existed significant statistical association between farmer's educational levels and AI awareness and zoonotic awareness. Poultry rearing of multiage and species (81%), multiple sources of stock (62%), inadequate dead-bird disposal (71%), and access to LBMs. (62%) constituted major biosecurity threats in these poultry farming communities. Haemagglutination inhibition (HI) test detected antibodies against H5 avian influenza (AI) in 8 of the 400 sera samples; rapid antigen detection test kit (RADTK) was negative for all the 400 cloaca and tracheal swabs. These results and other poultry diseases similar to AI observed in this study could invariably affect avian influenza early detection, reporting, and control. A policy initiative was recommended towards attitudinal change and increasing efforts on awareness of the implications of future HPAI outbreaks in Nigeria.

Another survey was carried out by Abdullahi *et al*. (2009) among 140 Nigerian poultry traders in traditional live poultry markets enquiring on their knowledge, attitudes and infection control preventive practices regarding AI. Per the results, knowledge was inadequate and the respondents perceived the infection to be a low occupational hazard. Wearing protective equipment and hand washing were also not routine practices. In a logistic regression model, high educational level and risk perception were independent predictors of knowledge of AI with [Odds Ratio (95% Confidence Intervals)] 2.16 (1.03-4.54) and 5.36 (1.70-16.91)] respectively. Belief that AI is a preventable and serious disease independently predicted behavior modification practices 4.05 (1.28-12.81) and 3.24 (1.29-8.14) respectively. The authors recommended that, knowledge of transmission and preventive measures should be improved. Also, more effective information should be provided to this high risk group as well as improvements in infrastructure and working conditions to enable a change in their behavior.

In Uganda, it has been observed that demographic characteristics of poultry traders/handlers influence activities and decision-making in LBMs. Hence, Kirunda *et al*. (2014) investigated the influence of socio-demographic characteristics of poultry handlers: age, sex, religion, educational background, level of income, location of residence and region of operation on 20 potential risk factors for introduction and spread of AI in LBMs. Study sites included 39 LBMs in the four regions of Uganda. Data was collected using a semi-structured questionnaire administered to 424 poultry handlers. In the study, it was observed that educational background was a predictor for slaughter and processing of poultry in open sites. Location of residence was associated with slaughter of poultry from open sites and selling of other livestock species. Region influenced stacking of cages, inadequate cleaning of cages, feeders and drinkers, and provision of dirty feed and water. Specifically, bird handlers with secondary level of education (OR = 12.9, 95% CI: 2.88–57.4, $P < 0.01$) were more likely to be involved in open site slaughter of poultry than their counterparts without formal education. Comparatively, urbanite bird handlers were less likely to share poultry equipment (OR = 0.4, 95% CI: 0.22–0.63, $P < 0.01$) than rural resident handlers. Poultry handlers in the Northern part were 3.5 times more likely to practice insufficient cleaning of cages (OR = 3.5, 95% CI: 1.52–8.09) compared to those in Central region. It can therefore be concluded that, some socio-demographic characteristics of poultry handlers are predictors to risky practices for introduction and spread of AI viruses in LBMs in Uganda.

Finally, in the Ghanaian context, the specific objective of Turkson and Okike (2016) was to assess the practices, capacities and incentives of actors involved in HPAI control to provide information for prevention and control in Ghana. Questionnaires were designed based on specific practices, incentives and capacities associated with each mitigation measure that was being assessed. Two peacetime preventive mitigation measures (biosecurity and reporting) and two outbreak containment measures (culling with compensation and movement control) were selected for evaluation. Supply chain actors were characterised based on baseline information. The study found statistically significant differences between certain actors for practices (biosecurity, reporting, culling and compensation and movement controls), incentives (reporting and movement control) and capacities (reporting and movement control). The findings served as a means to help improve education and messages on HPAI and to assist in providing technical assistance directed at particular actors to prevent and control future HPAI H5N1 outbreaks in Ghana.

From the studies presented, it can be concluded that, traders at LBM are at a high risk of contracting avian influenza. Also, LBMs are high risk areas where AIV can easily spread. Thus, to improve upon the knowledge, attitude and practices of live poultry traders to reduce the incidence of the spread of AIV, there is the need to educate them on the necessity of adhering to safety measures. Some of these measures include hand washing, using cleaning and disinfecting procedures, using protective clothing, not over-stocking cages, among others. The various means through which information can be made available to traders are radio, TV, social media, and newspapers. Governments must also provide proactive control measures which is highly acceptable to poultry workers.

2.7 Possible Associations between Live Poultry Traders Knowledge of Avian Influenza Biosecurity and their Current Practices.

Influenza A viruses that cause HPAI also infect humans. In many developing countries such as Ghana, poultry and humans live in close proximity, thus increasing risk for the spread of HPAI from birds to humans. Live bird markets in particular have been seen as a vital connection in the pathways that lead to the advent and reintroduction of AI infection. These markets enable the congregation of large populations of birds from various sources in moderately small spaces (Senne, 2007). This points to the need for live poultry traders to have adequate knowledge, positive attitudes, and acceptable practices to reduce the spread.

According to Akinola *et al*. (2008), approximately 1.5 million birds have died or been depopulated as a result of avian influenza infection among poultry in Nigeria. They further made it known that, one fatal human case had been reported in the country. This prompted them to assess the knowledge of, attitudes to, and compliance with preventive practices for avian influenza infection among poultry workers in a district in Nigeria. A cross-sectional epidemiological study design was conducted using a semi-structured questionnaire administered to obtain information. The results of the study were as follows: nearly all respondents (92.9%) had heard about avian influenza, and their main source of information was the mass media. Only 61.4% correctly defined the infection as a viral infection that occurs in all species of birds. Knowledge of transmission and preventive practices for the infection was varied and incomplete among respondents. The majority (78.6%) agreed that avian influenza is a serious and preventable disease. The perceived risk of infection was however moderate. No vaccination of poultry against avian influenza was reported by 98.6% of respondents, and wearing of personal protective equipment was not a routine practice. There is therefore the need to provide effective and coordinated information to poultry workers about avian influenza and the precautions necessary to avoid spreading the virus among poultry and to humans.

Also, with highly pathogenic avian influenza being an issue of concern as a major potential global threat, Souris *et al*. (2014) in their study evaluated and discussed the level of vulnerability of medium and small-scale commercial poultry production systems in Thailand related to avian influenza virus re-emergence. They developed a survey on 173 farms in Nakhon Pathom province to identify the global level of vulnerability of farms, and to determine which type of farms appears to be more vulnerable. They used official regulations (the Good Agricultural Practices and Livestock Farm Standards regulations) as a reference to check whether these regulations are respected. The results show that numerous vulnerability factors subsist and could represent, in case of HPAI re-emergence, a significant risk for a large spread of the disease. Bio-security, farm management and agro-commercial practices are particularly significant on that matter.

Further, due to its wide spread and the important role live bird markets play in HPAI disease dynamics, ElMasry *et al.* (2015) evaluated the H5N1 HPAI prevalence in representative LBMs in Egypt from 2009 to 2014. They assessed the effects of other variables and evaluated past outbreaks and human cases. It was found that ducks and geese were high-risk species. The end of a calendar year (June to December) was also identified as a high-risk period for positive samples, and the risk in urban LBMs was twice the risk in rural LBMs. This implies that, the knowledge and biosecurity practices in the rural areas was more encouraging than that of their counterparts in the urban areas.

In Ghana, Odoom *et al*. (2012) collected cloacal and tracheal samples from 680 domestic and domesticated wild birds and analysed for influenza A using molecular methods for virus detection. Even though no evidence of the presence of avian influenza infection was found in the 680 domestic and wild birds sampled, biosecurity in the households surveyed was very poor. This shows that, though residents demonstrated good knowledge of pandemic avian influenza, biosecurity practices were minimal. As a result, it was recommended that, sustained educational programmes were required to promote avian influenza surveillance and prevention in the country.

It can be concluded that, generally, biosecurity practices in most live bird markets are poor, despite majority of the traders being knowledgeable of avian influenza infection. To buttress the above, a report by the Food and Agriculture Organisation (2008) on the assessment of the Nigerian poultry market chain to improve biosecurity revealed that, since December 2007, there has been a country-wide programme for disinfecting LBMs, sustained by the Federal Ministry of Agriculture and Water Resources (FMA&WR) and assisted by the World Bank. The public veterinary structure was responsible for routine disinfection of all LBMs. Unfortunately, the equipment (particularly sprayers) and the personnel needed to effectively carry out the process were not always sufficient for the task. Some Local Government Areas have taken the initiative of supplying equipment while elsewhere, marketers' organizations support the veterinary services.

Nevertheless, in most cases, disinfection was not always regular and even if it is, it is carried out on a two-three-week basis. Rules on the use of alternative disinfectants are not regularly respected and the choice of disinfectant depends more on price. This further increases the spread of the virus in the markets. It also shows the poor attitude and biosecurity practices among the traders and key stakeholders.

2.8 Biosecurity/Prevention of Avian Influenza

AI is a highly contagious disease caused by either of the subtypes of influenza Type A virus. Although LBMs have been associated with outbreaks of AI, there are some LBM systems where AI outbreaks are extremely rare events. For instance, the California LBMs have not identified any AIVs since December 2005. Trock *et al*. (2008) further affirmed the above by asserting that, the long-term elimination of H7-strains from the American LBM-system demonstrates that eradication of AIVs from market environments is possible through regular interruptions of the constant bird flow and disinfection, although the role of additional measures such as all-year-round vehicle washing was also evident. This shows that, preventive measures when put in place and adhered to judiciously can help reduce or even eradicate the spread of avian influenza viruses in live bird markets around the world.

Thus, to make prevention and eradication possible, some measures have been proposed by researchers. According to them, adhering to and effectively implementing these measures can help prevent the possibility of a pandemic. For example, Cardona *et al*. (2009) found that, after the outbreak in the three (3) AIV-positive markets in California, traders voluntarily depopulated their inventories, as did the one AIV-positive supplier. The markets and suppliers entered into an agreement immediately before the detection of AIV in which they agreed to respectively purchase and supply to certified participants in the control plan. This has resulted in an LBM system that has remained AIV free since December 2005. This shows that, the implementation of disease control systems that combine good communication relations for all contributing parties, solutions that have good approval, and are rewarding for those who conform would help minimize the risk of LBM acting as sources of AIV to both poultry and humans.

Also, Kurscheid *et al.* (2015) found that, cleaning and disinfecting cages was recognized as the most important step in preventing HPAI in poultry at markets. Other proposed methods include disposing of sick and dead birds which according to the respondents of their survey minimizes the risk of virus transmission and also vaccination. The results of Martin *et al.* (2006) further suggest that the practice of selling live birds directly to consumers in food markets should be discouraged in areas currently experiencing influenza outbreaks among birds, especially in large modern cities where there may be a threat to the casual market visitor. To ElMasry *et al.* (2015), LBMs must be reorganized to make it possible to trace the sources of AIVs in poultry.

In the views of Kathleen *et al.* (2008), AI viruses are best controlled through international collaborative animal and public health campaigns to prevent, detect, respond to, and control the disease in wildlife, production animals, and humans, including workers. They state the education of employers and training poultry workers as critical components of worker protection. Fang *et al.* (2016) agreed with the importance of the education as a preventive measure by proposing that, the supervision and information distribution by local public health facilities should be strengthened to prevent the unofficial trading of live poultry during live poultry markets closure periods, which is often neglected in routine live poultry markets management measures. Other recommendations made to prevent the spread of AI among poultry workers were the use of good hygiene and work practices, personal protective clothing and equipment, vaccination for seasonal influenza viruses, antiviral medication, and medical surveillance.

Data collected by Offeddu *et al.* (2016) also show that, AIV-circulation can also be significantly reduced in the LPM-environment and among market-birds through temporary live poultry market closure, periodic rest days, market depopulation overnight, and improved hygiene and disinfection. Overall, the findings indicate that the length of stay of poultry in the market is a critical control point to interrupt the AIV-replication cycle within LPMs. In addition, temporary closure of bird markets was associated with a significant reduction of the incidence of zoonotic influenza.

Additionally, in ensuring biosecurity in relation to the spread of AI, some recommendations made by Kayali *et al.* (2016) were: revamping veterinary and public health surveillance and conducting joint human–animal interface surveillance and risk-assessment exercises; encouraging poultry owners to report outbreaks and providing them appropriate compensation; intervening, when poultry outbreaks are reported, by culling infected poultry and setting monitoring zones around each focus point; encouraging the use of disinfectants in backyards where poultry are raised; increasing awareness about the effects of avian influenza; and ensuring vaccine efficacy not only in a laboratory setting but also in the field.

A report by the Humane Society of United States also revealed that, due to the awareness of both the seriousness of the public health threat and the inconvenience of outright biosecurity in China, the government has set the precedence of trying to ban all live bird markets in Shanghai, its largest city, as well as the capital city of Beijing. Hong Kong has also decided to phase them out. It was also reported that, the Chinese government has allegedly advised all large cities to slowly call off the killing and sales of live fowls in the market. In the same way, bans have purportedly been recommended in Singapore, Japan, and Vietnam's Hanoi, Hai Phong, Vinh and Ho Chi Minh City. In Taiwan, since 2008, those found publicly slaughtering birds in LBMs may face a 500,000 NT fine (approximately $15,000 USD). Though there has never been a recorded outbreak in Taiwan, the chairman of its National Science Council explained that, every nation in the world is obligated to take part in the prevention of the epidemic, though an outbreak of bird flu cannot be predicted in the country.

However, Lau *et al.* (2007) cautioned that, the prospective benefits of public health procedures need to be prudently balanced against their possibly important societal and economic costs. In their view, whether the short-term closure of LBMs should be continued and expanded to reduce the transmission and protect public health needs to be decided by the local and national authorities. Such a decision, they averred, should consider the possible destructive impact on those working in the poultry trade, pricing of the poultry and the potential for unplanned consequences such as the spread of infected animals through the movement of the poultry and the dislocation of poultry trading to other areas.

2.9 Detection of Outbreak of New Subtypes of Avian Influenza in Live Bird Markets.

In live bird markets around the world, the observation for influenza viruses has been recognized as an effective means for discovering circulating influenza subtypes in poultry population (Webster, 2004). This markets have been known to serve as ideal locations for virus mixing and transmission because of their nature of gathering birds from various farms together with the practices of mixing newly arrived birds with those that have been in the market for extended periods. According to Nguyen *et al.* (2001), influenza viruses since the 1970s have been isolated from birds in LBMs in multiple countries. For example, from 7% to 30% of faecal swabs from ducks were positive for circulating H3, H4, H5, H6, H7, and H9 influenza virus subtypes in LBMs in Taiwan, Vietnam, and Hong Kong in the 1980s before the onset of the H5N1, H7N2, and H9N2 poultry epidemics in Southeast Asia. In early 2013, a new re-assortant influenza A (H7N9) virus also emerged in eastern China. Exposure to H7N9 infected poultry at live bird markets (LBM) was implicated as the main risk factor for human infection (Zhou *et al.* 2015).

Lee *et al*. (2006) likewise analyzed the evolution of H9 influenza viruses isolated from Korean chicken farms from 2002 to 2004. Phylogenetic analysis of the 12 viruses studied revealed three genotypes of H9N2 viruses and showed that re-assortment had occurred. One isolate, Ck/Kor/164/04, belonged to the H9N8 subtype. Its HA and *PB1* genes were similar to those of the H9N2 viruses, but its other genes were closely related to H3N8 viruses. In the study by Luan et al. (2016), the application of the pan-IAV RT FRET-PCR to oral-pharyngeal and cloacal swab specimens collected from healthy poultry in 34 live bird markets in 24 provinces of China revealed that 9.2% of the animals (169/1,839) or 6.3% of their oral-pharyngeal or cloacal swabs (233/3,678) were positive for IAV, and 56.8% of IAV-positive samples were of the H9N2 subtype.

A systematic surveillance program for avian influenza viruses has also been in place since 2009 in Egypt. Commercial and semi-commercial farms, abattoirs, backyard flocks, and live bird markets located in different governorates are sampled on a monthly basis regardless of the presence of disease symptoms in poultry. The rate of avian influenza infection during August 2009–July 2010 was 5%, was exclusively attributable to H5N1 infection, and was more concentrated in the commercial production sector. In 2011, H9N2 viruses emerged and were detected by this program and other surveillance activities. H9N2 viruses was frequently detected as well (Kayali *et al.* 2016).

Another surveillance project in Kenya by Munyua *et al*. (2013) aimed at identifying influenza A virus in poultry traded in five LBMs in Kenya. Each market was visited monthly where oropharyngeal and cloacal specimens were collected from poultry and environmental specimens for virological testing for influenza A by real time RT-PCR. On each visit, information was collected on the number and types of birds in each market, health status of the birds, and market practices. During March 24, 2009–February 28, 2011, 5221 cloacal and oropharyngeal swabs were collected. Of the 5199 (99·6%) specimens tested, influenza A virus was detected in 42 (0·8%), including 35/4166 (0·8%) specimens from chickens, 3/381 (0·8%) from turkeys, and 4/335 (1·2%) from geese.

Live bird markets have been known to play a major role in enabling emergence or reemergence of influenza viruses. For this reason, monitoring of live bird and food markets should be implemented in countries where such markets are still common. This is because continued monitoring of influenza viruses in poultry in LBMs would help in discovering new introductions of AIVs in the poultry population that would be of public health and socioeconomic impact to the poultry industry in every country. Early detection of new potentially dangerous influenza viruses could lead to early application of control measures that could minimize the public health impact of outbreaks of HPAI viruses and decrease the impact on the livelihoods along the poultry value chain. Increased public awareness about the risks for influenza virus in association with live bird and food markets must also be intensified to help prevent and control infection in humans. In addition, raw bird meats should be handled with care, and eating of raw bird meats should be totally avoided if possible.

Since LBMs will continue to play an important role in the spread of poultry diseases, there is a need to restructure live bird markets in relation to biosecurity. Doing this will make the markets more hygienic and help reduce the spread of avian influenza and other poultry infections. The formulation of policies, their design, and enforcement must also be user-friendly by considering its socio-cultural and economic impacts.

CHAPTER THREE

METHODOLOGY

3.1 Study Design

The study was a cross-sectional, quantitative laboratory-based analysis of samples taken from various domesticated avian species using active avian influenza epidemiological surveillance approach and a social survey (questionnaire) to assess LPTs knowledge, attitude and practices towards AI biosecurity in the major LBMs in Accra.

3.2 Laboratory-based Approach

3.2.1 Survey Animals and Study Sites

Tracheal, cloacal, and blood samples were taken from apparently healthy domesticated birds; turkey *(Meleagris gallopavo)*, ducks (*Anas platyrhynchos domesticus*), guinea fowls (*Numida meleagris*), doves/pigeon (*Family columbidae*) and chicken (*Gallus gallus domesticus*) from Dome, Agbogbloshie, Kantamanto, Mallam-Atta, and Pokuase LBMs between January and February, 2017. These sites fall under Ga East and Ga West municipalities, and the Accra Metropolitan area, all within the Greater Accra Region (GAR) of Ghana.

The GAR was purposively selected for this study as a result of previous outbreaks of AI in poultry in 2007, 2015, and 2016 in the region. It has been the hardest hit region with AIV thus far in Ghana (Asantewaa, 2015). Second, Accra was chosen because of the number of LBMs available for the study. A quick scan through available literature revealed that scholarship has not paid any particular attention to LBMs within the region although a few studies have targeted commercial poultry farms and wild birds in the region (Awuni *et al*., 2010; Fenteng, 2010). A list of the wet markets in Accra was provided by the Epidemiology Unit of the VSD, Accra. The afore-mentioned markets were selected because they are always functional and serve as the focal points of live poultry business in Accra as could be determined by the density and volume of trade. Based on variations in LBM infrastructure, LBMs used in this study had their own unique characteristics and thus samples were obtained, and respondents enrolled as were available in each LBM.

3.2.2 Sampling Procedure

The multi-stage sampling method was used and this involved three stages. The first stage comprised the selection of the LBMs. The second stage involved the selection of vendors while the third stage involved the selection of birds for sample collection.

The purposive sampling method was used for the selection of the five LBMs in terms of the density, volume of trade and number of stalls, and vendors available at these markets. Also, the other markets in Accra were not functionally active. The participatory surveillance sampling was used to enrol vendors following the eligibility criteria set for the study. According to Mariner *et al*., (2014), for diseases with significant impact, participatory methods explicitly seek to improve understanding of livestock systems in order to enhance interpretation of information and for setting intervention strategies that lead to more constructive action by all stakeholders. Further, participatory surveillance programmes help strengthen epidemiological intelligence to inform decision making and action. Finally, the convenient sampling method was used to select seemingly healthy birds for sample collection among the various mixed species.

3.2.3 Eligibility Criteria

Only sellers registered with the respective Fowl Sellers' Association (FSA) in each LBM were enrolled for this study as freelance sellers could change their location at will. Again, only vendors actively involved in the bird trade business, and have experience of five years or more were selected. Lastly, samples were collected from only seemingly healthy birds in each market where farmers consented.

3.2.4 Sample Size

According to the U. S. Interagency Strategic Plan, to detect AI at or below 1.5% prevalence with a 95% level of confidence, 200 individual birds from the population of interest should be sampled. Thus, the required sample size was calculated as; n = log (1-c) / log (1-p) = log (1-0.95) / log (1-0.015) = 199, where (n) is the sample size; (c) is the desired level of confidence, 95%; and (p) is the estimated prevalence, 1.5%. Some sellers considered the mode of sample collection to be too invasive and did not allow samples to be taken from their birds and so limited the number of birds to 154 in this study.

3.3 Preparation for Lab work

3.3.1 Preparation of Virus Transport Medium (VTM)

2.0 ml of transport medium prepared from benzylpenicillin (2×10^{6}1U/litre), streptomycin (200mg/litre) and gentamycin (250 mg/litre) was dispensed into sterile plastic screw-capped cryovials and stored at room temperature for a day prior to use.

3.3.2 Sample Collection, Transportation and Storage

This was done in conformity with guidelines for the collection and preservation of samples of avian influenza H5N1 determination (WHO, 2006). A total of 438; comprising 154 each of tracheal and cloacal swabs, and 130 blood (differential figure because some vendors considered that to be too invasive) samples were taken from apparently healthy adult and grower birds using sterile cotton swab sticks and sterile needles and syringes.

For tracheal swabs, birds were gently restrained and their beaks held open. Swabs were then carefully introduced down the trachea and gently rubbed up and down along the trachea. For cloacal swab, birds were held securely and gently swab sticks were introduced into the cloaca through the vent to ensure contact with the mucous membranes based on the size of each bird. Stained swabs were gently removed and immediately placed into the transport media tubes. Birds were watched for clinical signs of stress before blood collection. All blood samples were taken from the brachial vein. To allow for serum separation, immediately after collecting the required volume of blood, tubes were placed on their sides.

All samples obtained were uniquely labelled to match with information recorded on the field data collection sheet. Samples were then conveyed in coolers with dry ice packs to the Accra Veterinary Laboratory for storage and analyses. Samples were stored in an Ultra-low Sanyo® freezer at -70ºC until processed and tested.

3.4 Laboratory Procedures

3.4.1 AI Virus Detection Methods

All tracheal and cloacal swabs were tested with real-time reverse polymerase chain reaction (rRT-PCR), while sera samples were screened with indirect enzyme-linked immunosorbent assay (ELISA).

3.4.2 Sample Preparation

Samples were first thawed and vortexed to obtain a homogenized mixture of the sample on the swabs and VTM.

3.4.3 RNA Extraction/Molecular Detection

Extraction of RNA was done at the RNA, cDNA Extraction lab with QIAamp® Viral RNA Mini Kit (50), QIAGEN GmbH, Hilden, Germany. 50 µl of prepared Buffer AVL containing carrier RNA was pipetted into a 1.5 ml microcentrifuge tube. Then, 140 µl of the prepared samples was added to the Buffer AVL-carrier RNA in the microcentrifuge and mixed by pulse vortexing for 15 seconds until a uniform mixture was obtained. This was followed by incubation at room temperature 20ºC for 10 minutes. Briefly, the tube was centrifuged to remove drops from the inside of the lid. 560 µl of absolute ethanol (99.7-100) was then added to the sample and mixed by pulse-vortexing for 15 seconds. Tubes were then briefly centrifuged again to remove drops from inside of the lid. 630 µl of the resultant solution (Buffer AVL containing carrier RNA, sample and absolute ethanol) was then applied to the QIAamp Mini column (in a 2 ml collection tube) without wetting the rim. This was spun at 8000 rpm for 1 minute, and the QIAamp Mini column was placed in a clean 2 ml collection tube and the tube containing the filtrate discarded.

Step was repeated until all the lysate had been loaded onto the spin column. The QIAamp Mini column was carefully opened and 500 µl of Buffer AW1 was added and centrifuged at 8000 rpm for 1 minute. The QIAamp Mini column was then placed in a new a clean 2 ml tube and the filtrate thereof discarded. Again, 500 µl of Buffer AW2 was added to the QIAamp Mini column and centrifuged at full speed of 14000 rpm for 3 minutes and the supernatant was discarded. Finally, the QIAamp Mini column was placed in a clean 1.5 ml microcentrifuge tube while the filtrate was discarded. The QIAamp Mini column was carefully opened and 60 µl of Buffer AVE equilibrated to room temperature for 1 minute. This was then centrifuged at 8000 rpm for 1 minute and stored in a 1.5 ml Eppendorf tubes at -70ºC in an ultra-low freezer at the main corridor of the Accra Veterinary Laboratory.

3.4.4 Master Mix Preparation and rRT-PCR

The MM-gene preparation was performed at the P3 Lab in a SterileGARD® Biosafety cabinet. The QIAGEN® one step cycling protocol was used for the real-time PCR amplification. The master mix prepared for general influenza A for each of the six sets of 26 reactions before the addition of 5 µl of extracted RNA sample involved Taq universal probes master mix, i-script advanced reverse transcriptase, (5µM) M+25, (5µM) M-124, (1µM) M+64 probe and RNase free water.

Table 3.1: PCR primers and probes sequence for general Influenza A detection

Specificity	Primer/Probe	Sequence
AIV	M+64	5'-TCA GGC CCC CTC AAA GCC GA-3'-TAMRA
	M+25	5'-AGA TGA GTC TTC TAA CCG AGG TCG-3'
	M-124	5'-TGC AAA AAC ATC TTC AAG TCT CTG-3'

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

3.4.5 Amplification Cycles and Virus Detection

Thermo scientific PIKO REAL 24 (Real-Time PCR System, Finland) was used to run the test under the following working conditions; Reverse transcription (50ºC, 10 minutes); Activation of Taq (95ºC, 45 seconds); Annealing (60ºC, 45 seconds) and Holding (4ºC, infinite time). Results were obtained by the device in-built autoanalysis software programme with a positive control cycle threshold (cq) based on the respective cq values of both the extraction positive and negative controls as well as the master-mix positive and negative controls used.

3.5 Indirect ELISA Procedure/Serological Analysis

The QIAGEN flocktype® AIV Ab (2), Leipzig GmbH, Germany protocol was used to screen all 130 sera samples for AIV (Qiagen flocktype AIV Ab Handbook, 2013). The kit used was already quoted with antigen plate according to the manufacturer. 100 µl of test controls were pipetted in duplicates and 1:500 samples into the Test Plate wells. This was incubated for 30 minutes at 25ºC. Solution was then removed from the wells by aspiration. Then, each well was rinsed 3x with 300 µl of prepared Wash Buffer. Buffer was removed after each rinse. This was followed by pipetting 100 µl of Conjugate to each well and incubated at 25ºC for 30 minutes. Substrate TMB Solution was then added after rinsing each well 3x with Wash Buffer. This was then incubated for 10 minutes at 25ºC in the dark and timed. Reaction was then stopped by adding 100 µl Stop Solution per each well. Optical Density (OD) values were measured in the Spectrophotometer machine at 450 nm within 20 minutes after stopping the reaction. The ratio (S/P) of sample OD to mean OD of the Positive Control according to the kit protocol as: S/P= OD_{sample}-MV OD_{nc}/MV OD_{pc}-MV $OD_{nc,,}$ where OD –optical density, MV-mean value, nc-negative control, pc-positive control.

3.6 ONE STEP real-time RT-PCR Avian Influenza H5

The One Step rRT-PCR for the detection of AI H5 (H5N1 Duplex Master Mix) was performed according to the protocol by Slomka *et al* (2007). Samples that tested positive for influenza A were isolated and screened for H5N1 using H5N1 specific probes, primers and reagents. Primers used for this cycle are listed in Table 3.2.

Table 3.2: Primers used for H5 Detection

Specificity	Primer/Probe	Sequence
H5	Primer forward: H5-Kha 1	5'-CCT CCA GAR TAT GCM TAY AAA ATT GTC-3'
	Primer reverse: H5-Kha 3	5'-TAC CAA CCG TCT ACC ATK CCYTG-3'

Source: Slomka et al., (2007).

3.6.1 Amplification Cycle for ONE STEP RT-PCR AI H5 Detection

Reaction conditions used were as follows: 50ºC for 30 minutes and 94ºC for 15 minutes with Annealing temperature of 94ºC for 30 seconds, 58ºC for 1 minute and 68ºC for 2 minutes on a 40 cycle run with a final extension of 68ºC for 7 minutes and at 4ºC ∞.

3.7 Questionnaire Survey

3.7.1 Study Design and Instrument

The questionnaire design was based on published literature on the subject and best practice methodology. The questionnaire (Appendice C) was adapted and modified from a study previously done by Chinyere (2010) to suit the objectives of this study. These questionnaires were used to obtain relevant information from all fifty (50) respondents who consented (Appendice B) to be part of this study and allowed samples to be taken from their birds. Questionnaires were used to validate findings obtained through the quantitative laboratory based approach. Further, questionnaires were used because of their time and cost effectiveness. They also covered a wider scope of the information needed for the study. The structured questionnaire comprised sections on respondent's socio-demographic data; knowledge of signs and symptoms of avian influenza infection; knowledge of avian influenza human health risks and preventive measures; live poultry traders' attitude towards avian influenza biosecurity; and current practices of live poultry traders relative to avian influenza biosecurity/preventive measures.

3.7.2 Validity and Reliability

The data collectors were adequately trained and the data collection tool was pre-tested with five LPTs at the Madina LBM, which has similar characteristics to the sites surveyed. Questionnaire validation was done to ensure a high face validity. All LBMs were also inspected to make sure questionnaire addressed objectives of the study.

3.7.3 Questionnaire Administration

The face-to-face method of administering questionnaire was used with all its inherent disadvantage of time consumption. This was to establish a strong rapport and collaboration with the vendors. It also ensured a high response rate. (Kumekpor, 2002).

All questionnaires were administered by a study team, consisting of data collectors working with the VSD, Accra prior to sample collection.

3.7.4 Data Issues

Microsoft Excel (Microsoft, Redmond, WA, USA) and Statistical Package for Social Sciences (SPSS) computer programme, version 20 were used for the data entry, descriptive and statistical analyses. Descriptive statistical tables were generated for the representation of the data analyzed to aid in easy discussion.

3.8 Study Approval and Ethics

Scientific review for this study protocol was obtained from the Ethical Review Board (ERB) of Ensign College of Public Health, Kpong. Written permission was also sought from the Veterinary Services Directorate (headquarters, Accra) for use of laboratory for sample storage, processing and analyses. Informed oral consent was sought to gain access to the LBMs for sample collection and administration of questionnaire from the market managers. Permission was also sought from the managers of the Bird Sellers' Association (BSA) in the designated markets for the collection and use of samples. Right and dignity of the individual were highly considered and respected. Purpose and objectives of the study were explained and verbal consent was read out to sellers who willingly offered to participate in the study. LPTs were informed that information from the study would be shared with the VSD of the Ministry of Food & Agriculture to enable them take relevant actions related to the control and prevention of AI infections in the markets.

3.9 Limitations of the Study

Lack of prevalence data on AI in Ghana affected the sample size used in this study. There was also the issue of non-compliance on the part of some LPTs who initially agreed for samples to be taken from their birds for the study. Frequent power outages also affected the progress of work at the laboratory. Financial constraint and the late arrival of some reagents were also a challenge. Again, the short duration of the study truncated presentation of further findings from ongoing laboratory analysis of the positive samples detected.

3.10 Assumptions

The study assumed that every LPT has some knowledge, attitude, practices and strategies he or she uses in preventing AI and that the understanding of these perceptions and practices could help form some strong basis for effective intervention in AI prevention and control.

CHAPTER FOUR

RESULTS OF THE STUDY

4.1 Laboratory Findings

A total of 438 (comprising 154 each of tracheal and cloacal swabs; and 130 sera) samples were collected from 154 various domesticated avian species (chicken; duck; turkey; pigeons; and guinea fowl) sold in the five LBMs surveyed in this study. The overall positive detection of AIV in the samples was 6.62%. Of the total samples examined, 15 (3.42%) tested positive for AI with rRT-PCR (Table 4); while 14 (3.19%) screened with indirect ELISA, also tested positive for AI (Table 5). No positive isolates were found in the cloacal swabs of sampled birds. The results of all AI positive detections in both the molecular (rRT-PCR) and serological (indirect ELISA) assays were negative when subtyped for H5N1. Further, all five (100%) LBMs surveyed in this study tested positive for influenza A (Table 4.1).

Table 4.1: Percentage distribution of positive samples by status and site

PERCENTAGE DISTRIBUTION OF POSITIVE SAMPLES BY STATUS AND SITE					
BIRD TYPE	NO. OF SAMPLES	STATUS		SITE	
	COLLECTED	TYPE A	H5		
Chicken	95(61.69%)	18(18.95%)	0	A,D,K,M,P	
Guinea fowl	25(16.23%)	5(20%)	0	K,P	
Turkey	11(7.14%)	4(36.36%)	0	K,M	
Duck	8(5.19%)	0(0%)	0		
Pigeon	15(9.74%)	1(6.67%)	0	K	
* A = Agbogbloshie, D = Dome, K = Kantamanto, M = Mallam-Atta, P = Pokuase					

Source: Surveillance 2017.

Table 4.2: rRT–PCR results (M-gene)

rRT-PCR RESULTS FOR TRACHEAL SAMPLES			
S/N	**SITE**	**cq-VALUES**	**EXT +VE CNTRL**
1	Dome	24.90	***24.88***
2	Agbogbloshie	18.47	
3	Agbogbloshie	20.76	
4	Kantamanto	20.95	
5	Kantamanto	23.70	
6	Kantamanto	20.23	
7	Kantamanto	20.76	
8	Kantamanto	23.40	
9	Kantamanto	20.77	
10	Kantamanto	24.43	
11	Kantamanto	25.40	
12	Mallam-Attah	23.47	
13	Pokuase	22.52	
14	Pokuase	23.91	
15	Pokuase	23.43	

Source: Surveillance 2017. ***Lab approved Cq-detection range: 15-29***

Table 4.3: Indirect ELISA results for sera samples

Indirect ELISA results: samples with the S/P ratio < 0.3 are negative Samples with the S/P ratio ≥ 0.3 are positive.

S/N	SITE	BIRD TYPE	S/P RATIO VALUES
1	Dome	Chicken	1.2674
2	Dome	Chicken	1.1307
3	Dome	Chicken	1.5219
4	Dome	Chicken	1.0459
5	Mallam-Attah	Turkey	1.0834
6	Kantamanto	Turkey	1.5768
7	Kantamanto	Turkey	1.2218
8	Agbogbloshie	Chicken	1.0017
9	Kantamanto	Chicken	1.4039
10	Kantamanto	Chicken	1.8936
11	Kantamanto	Chicken	1.5809
12	Kantamanto	Chicken	1.2950
13	Kantamanto	Pigeon	1.4938
14	Mallam-Attah	Turkey	1.4633

Source: Surveillance 2017.

4.2 Survey Results

4.2.1: Socio-Demographic Data

Table 4.4: Socio-Demographic Data of Respondents

Item	Responses	Frequency	Percentage
Sex	Female	9	18
	Male	41	82
Age	15-25 yrs	1	2
	26-35 yrs	9	18
	36-45 yrs	26	52
	46+ yrs	14	28
Religion	Traditional	0	0
	Islamic	25	50
	Christianity	25	50
Educational level	Middle school	16	32
	JHS	4	8
	SHS/Voc/Tech.	22	44
	Tertiary	3	6
	No formal education	5	10
Length of years in the poultry business	5-15 yrs	26	52
	16-25 yrs	14	28
	26-35 yrs	9	18
	36+ yrs	1	2
Sell mixed birds	Yes	47	94
	No	3	6
Sources of birds*	Specific farm in Greater Accra	20	40
	Any poultry farm	32	64
	From other regions	22	44
Records sources of birds	Yes	9	18
	No	41	82
Have had birds culled due to infections	Yes	7	14
	No	43	86
Compensation by government	Yes	9	18
	No	41	82
Given samples for research	Yes	6	12
	No	44	88

Source: Field Survey Data, 2017. **** Involved multiple responses***

Of the 50 persons that answered the questionnaire, 82% were males and 18% were females. The distribution by age of respondents appears to be skewed to 36-45 age group. Out of the 50 respondents, 52% were between this age group; while one of the respondents, representing 2% of the total sample was in the age range of 15-25 years. Overall, majority of the respondents were aged above 25 years. The distribution of respondents by religion was evenly spread among poultry traders, with both Christianity and Islam constituting 50% each of the study sample.

On education, about 32% of the respondents had middle school education; 8% had JHS education; about half (44%) had SHS/Voc/Tec education; 6% had tertiary education, and 10% had no formal education, which is fairly low. A little more than half (52%) of the respondents had poultry trade experience of between 5-15 years, while 28% had between 16 and 25 years of poultry trade experience; followed by 18% of respondents who had experience of between 26-35 years with only 1 (2%) respondent having a trade experience of at least 35 years.

Almost all (94) respondents sold mixed birds in the same cage, while the rest sold only one bird type in their cages in the surveyed markets. More than one-third (82%) of the respondents indicated that they did not keep records of the sources of their birds, while the remaining mentioned that they kept records of their stocks. 43 (86%) of the sample mentioned that they have not had any birds of their stocks culled due to any infection for as long as they have been engaged in the live bird trade. On the contrary, only a few (14%) of the respondents indicated that they have had some birds culled at a point in time since they started trading in the live bird markets due to infection of their birds. Majority (82%) of the respondents mentioned that they have not received any compensation from the government for any losses resulting from infection of their birds. The rest, on the other hand, indicated that they have received some forms of compensation for losses due to infection of their birds. More than four-fifth (88%) of the respondents revealed that they have not allowed samples to be taken from their birds for any study or research work, while the rest indicated that they have allowed samples to be taken from their birds for a study or research since they started trading in live birds.

4.2.2: Knowledge of Signs and Symptoms

Table 4.5: Results on the Knowledge and Signs and Symptoms of AI

Ho: Observed frequencies are equal to expected frequencies

Responses	Yes	No	χ^2	Interpretation	Decision
Source of information on AI					
Family members	5 (10%)	45 (90%)	32.00	Significant	Reject H*o*
Radio	7 (14%)	43 (86%)	46.08	Significant	Reject H*o*
Newspaper	3 (6%)	47 (94%)	38.72	Significant	Reject H*o*
TV	7 (14%)	43 (86%)	25.90	Significant	Reject H*o*
Vet/MOFA officials	31 (62%)	19 (38%)	2.88	Significant	Reject H*o*
Other poultry sellers	31 (62%)	19 (38%)	2.88	Significant	Reject H*o*
Mode of virus infections in human					
Contact with infected surfaces	9 (8%)	41 (92%)	20.48	Significant	Reject H*o*
Contact with infected birds	44 (88%)	6 (12%)	28.88	Significant	Reject H*o*
Contaminated poultry feed	8 (16%)	42 (84%)	23.12	Significant	Reject H*o*
Eating raw/uncooked poultry meat	6 (12%)	44 (88%)	28.88	Significant	Reject H*o*
Signs of AI in human					
Cough	21 (42%)	29 (58%)	1.20	Significant	Reject H*o*
Diarrhoea	11 (22%)	39 (78%)	15.68	Significant	Reject H*o*
Headache	16 (32%)	34 (68%)	6.48	Significant	Reject H*o*
Difficulty in breathing	15 (30%)	35 (70%)	8.00	Significant	Reject H*o*
High temperature	21 (42%)	29 (58%)	1.20	Significant	Reject H*o*
Conjunctivitis	10 (20%)	40 (80%)	18.00	Significant	Reject H*o*
Don’t know	28 (56%)	22 (44%)	0.72	Significant	Reject H*o*
Signs of AI in birds					
Sudden death without signs	43 (86%)	7 (14%)	25.92	Significant	Reject H*o*
Discolouration of wattles, combs, and legs	39 (78%)	11 (22%)	15.68	Significant	Reject H*o* Reject H*o*
Coughing	19 (38%)	31 (62%)	2.88	Significant	Reject H*o*
Sneezing	18 (36%)	32 (64%)	3.92	Significant	Reject H*o*
Don’t know	8 (16%)	42 (84%)	23.12	Significant	Reject H*o*
Conditions of birds that calls for report of suspected AI					
Manifesting suspected signs and symptoms	26 (46%)	23 (44%)	0.18	Significant	Reject H*o*
Sudden death	19 (38%)	31 (62%)	2.88	Significant	Reject H*o*
Don’t know	16 (32%)	34 (68%)	6.48	Significant	Reject H*o*

Source: Field Survey Data, 2017. ***Universal P-value = 0.05%***

An equal number of respondents (50%) were expected to respond to each category (disagree, undecided, agree). However, the observed frequencies were significantly different from the expected frequencies. The table above shows responses on knowledge of signs and symptoms of AI. From the table, it is evident that, majority of respondents had information on AI from the Vet/MOFA officials and other poultry sellers χ^2 *(2, N=50 = 2.88, P<0.05)*. This shows that, officials are being proactive in making information on AI available to live poultry sellers. The impact is however low among the traders. Also, the least effective source of information was through the radio χ^2 *(2, N=50) = 46.08, P<0.05)*. This response was highly significant and this is an indication that, much education/awareness is not done using the radio.

4.2.3: Knowledge of Avian Influenza Human Health Risks and Preventive Measures

Table 4.6: Comparison between the awareness of LPTs on the existence of biosecurity measures against AI infection and prescribed biosecurity measures.

Ho: Observed frequencies are equal to expected frequencies

	Awareness on the existence of biosecurity measures against AI infection				
Prescribed biosecurity measures	**Yes**	**No**	χ^2	**Interpretation**	**Decision**
Do not trade birds of unknown origins	32 (71%)	13 (29%)	8.19	Significant	Reject H*o*
Adopt all-in-all-out management	32 (71%)	13 (29%)	8.19	Significant	Reject H*o*
Minimize contact with feathers, blood, offal, and faecal matter	40 (89%)	9 (11%)	19.35	Significant	Reject H*o*
Clean and disinfect cages and slaughter surfaces thoroughly and frequently	40 (89%)	9 (11%)	19.35	Significant	Reject H*o*
Use personal protective equipment when handling with birds	42 (86%)	7 (14%)	26.13	Significant	Reject H*o*
Leave carcass disposal to appropriate authorities	30 (61%)	19 (39%)	6.87	Significant	Reject H*o*
Do not mix different species of birds in the same cage	35 (71%)	14 (29%)	10.88	Significant	Reject H*o*
Don't know	1 (2%)	44 (98%)	25.95	Significant	Reject H*o*

Source: Field Survey Data, 2017. ***Universal P-value = 0.05%***

An equal number of respondents (50%) were expected to respond to each category (disagree, undecided, agree). However, the observed frequencies were significantly different from the expected frequencies. From the table, it can be deduced from the responses that, respondents had ample knowledge of avian influenza and also the human health risks and preventive measures. The commonly stated preventive measure was personal protective equipment when handling birds χ^2 *(2, N=50)= 26.13, P<0.05)*, followed by minimizing contact with feather, blood, offals and faecal matter. Also, disinfecting cages and slaughter surfaces thoroughly and frequently had equal responses ($\chi^{2,}$ *2, N=50)= 19.35, P<0.05).* The least biosecurity measure mentioned by the traders was leaving carcass disposal to appropriate authorities χ^2 *(2, N=50)= 6.87, P<0.05).*

4.2.4.: Live Poultry Traders Attitude towards Avian Influenza Biosecurity

Table 4.7: Associations between the attitudes of LPTs towards AI biosecurity

Questions	**1**	**2**	**3**	**4**	**P-value**
1.Would you be willing to comply with these biosecurity measures	1				-
2. Given the uncertainty surrounding avian influenza, would you continue to trade as a live poultry trader in this market	-	1			-
3. Are you worried about the extent of vectors, rodents and stray dogs in this market	-	-	1		-
4. Are you worried about the sanitation situation in this market?	-	-	-.305	1	**0.31**

Source: Field Survey Data, 2017 ***Universal P-value = 0.05%***

Table 4.7 shows responses received on the effects of policy implementation on staff performance. A correlation analysis was used to determine the relationship. From the responses presented on the table, it can be seen that, no statistics are computed because all respondents were unanimous that they would be willing to comply with biosecurity measures if they are enforced. However, there was a statistically significant negative association between worry about the extent of vectors, rodents and stray dogs in the study markets and worry about the sanitation situation in these markets (r = -0.305, p< 0.31). This means that, as traders get more worried about sanitation issues in the market, their worry over rodents and stray dogs in the markets will decrease and vice versa.

4.2.5: Current Practices of Live Poultry Traders Relative to Avian Influenza Biosecurity/Preventive Measures

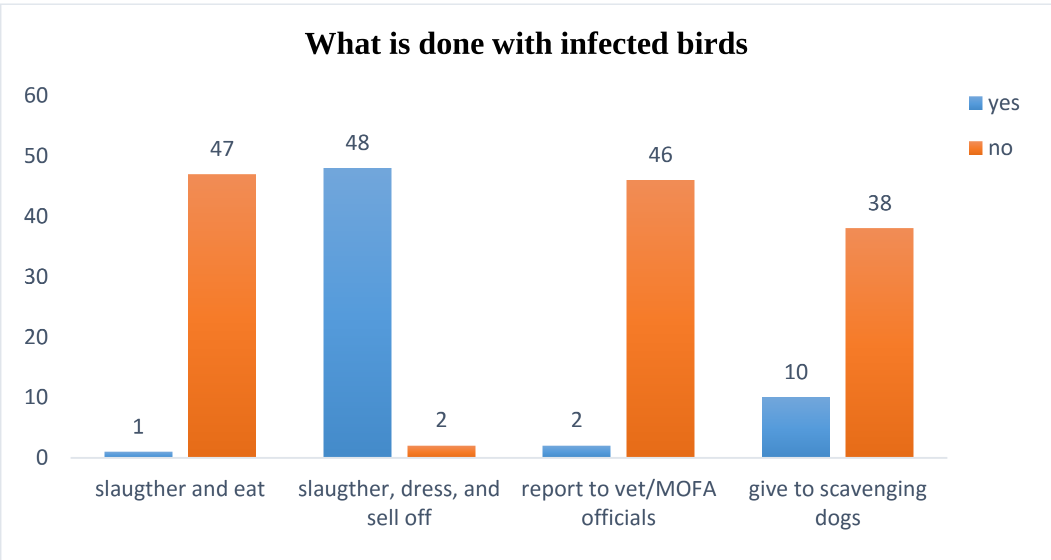
Table 4.8: Associations between current practices of LPTs and avian influenza biosecurity/preventive measures.

Questions	1	2	3	4	5	6	7	P-value
Do you disinfect your cage?	1							-
Do you sell off all birds in your cage before you restock?	-.119	1						-
On the average, how long does a bird remain in your cage before it is sold?	-.003	.261	1					-
Do you exchange equipment such as waterers and feeders with your colleagues?	.127	.014	-.051	1				**-**
How do you feed your birds?	.100	.-189	-.070	-.045	**1**			
Where do you keep unsold birds?	.062	.-036	.286*	-.190	.190	**1**		**.044**
Do you slaughter, process and sell dressed birds to some of your buyers?	.036	.084	-.087	.306*	-.028	-.429*	**1**	**.002**

Source: Field Survey Data, 2017 ***Universal P-value = 0.05%***

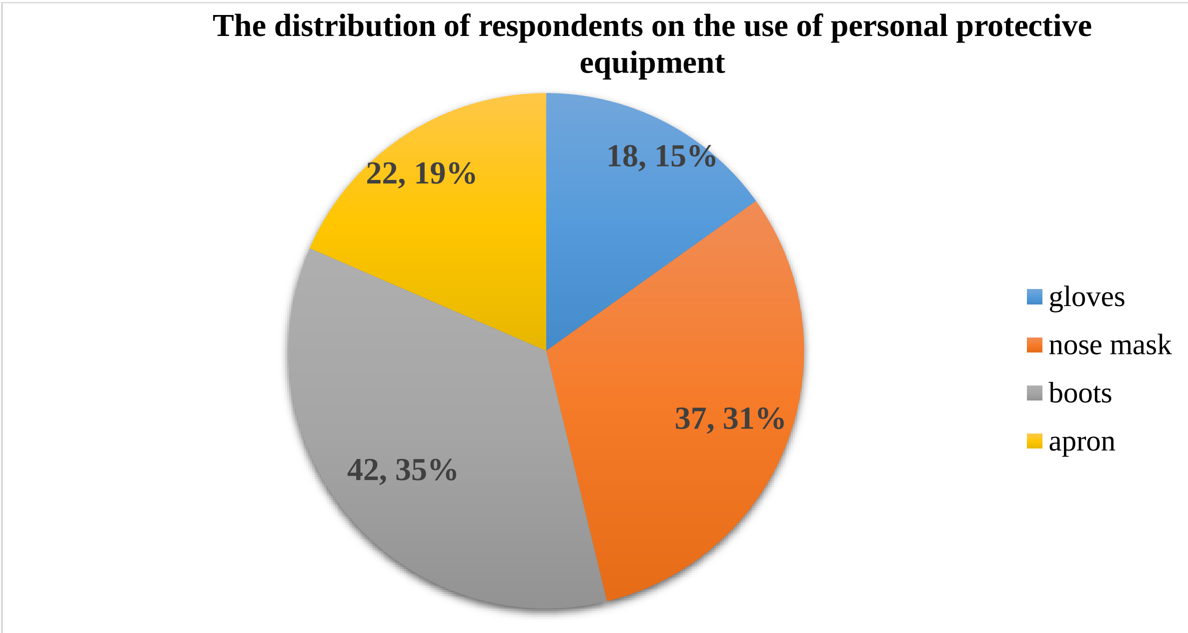
Table 4.8 shows responses received on the effects of policy implementation on staff performance. A correlation analysis was used to determine the relationship. From the responses presented on the table, it can be seen that, a relationship existed between all the statements made under this objective. Not all these relationships were, however, statistically significant. The statistically significant relationships at a significance level of 0.05% are flagged (*). Some of the relationships are positive while others are negative. From the table, a positive statistical association was found between disinfecting cages and where unsold birds are kept (r = 0.286, p< 0.044). The implication is that, the more traders keep birds until they run out of stock, the more they will have to disinfect their cages until they run out of stock. a positive statistical association was found between disinfecting cages and where unsold birds are kept (r = 0.286, p< 0.044). The implication is that, the more traders keep birds until they run out of stock, the more they will have to disinfect their cages until they run out of stock.. The last statistical association which was negative was found between slaughtering, processing and selling birds and where unsold birds are kept (r = -.429, p< 0.002). This implies that, as traders slaughter, process, and sell more birds, the need to keep unsold birds will decrease. This may be as a result of selling majority of their stock to consumers who demanded processed birds instead of live ones, hence storing less or none in some cases.

Figure 4.1: Results on what respondents do with sick birds



Source: Field Survey Data, 2017.

Figure 4.2: Distribution of respondents use of personal protective equipment (PPEs)



Source: Field Survey Data, 2017.

CHAPTER FIVE

DISCUSSION

5.1 Current profile of AIV

The 6.62% detection of positive isolates by both the rRT-PCR and indirect ELISA assays among surveyed birds in this study points to the circulation of AIV in birds sold at the major LBMs in Accra. This finding supports the claim by Webster (2004), and Nguyen *et al.* (2001), that influenza viruses since the 1970s have been isolated from birds in LBMs in several countries. This emphasizes the need for constant epidemiological surveillance of our LBMs in order keep to forestall any threat that might cripple them and endanger public health. This evidence is further highlighted by (Lau et al, 2007).

5.2 Subtype of circulating AIV

The negative results for all AI positive isolates when subtyped for H5 impliedly indicates that possibly a new influenza A subtype might be in circulation in the surveyed markets or perhaps reassortment may have occurred. This is based on the fact that all AI cases in Ghana thus far, reported to the OIE have implicated the AI/H5N1 subtype. This evidence further affirms the assertion that AI viruses are capable of constant mutation (Lee *et al.*, 2006). This mutation or reassortment, if confirmed is of public health concern since some of these changes could lead to a human adapted influenza subtype and underscores the constant risk posed by the virus.

At the time of submitting this thesis, AI specific primers for the detection of other subtypes had also been ordered by the Accra Veterinary laboratory to carry out further consecutive laboratory analysis on the positive isolates as described by Awuni *et al.,* (2010).

5.3 LPTs Knowledge of Signs and Symptoms

Live bird markets have been linked with many outbreaks of avian influenza and its spread; thus, considered as a high risk area due to the large number of mixed poultry birds that are traded there. This has also placed the traders in such markets at a high risk of contracting AI infections through contact with birds that they may not know were infected. The study therefore sought to access the knowledge of LPTs on the signs and symptoms of AI infections among birds and humans.

From the responses received, the respondents indicated they were knowledgeable about the signs and symptoms of AI infections. This agrees with Akinola *et al.* (2008) as stated in the literature, who in their study found that, nearly all respondents (92.9%) had heard about avian influenza. This shows that, there is available information for traders in LBMs on the existence of AI infections.

The traders were aware they could get infected with AI virus through contact with infected surfaces ($\chi^2 = 20.48$) contact with infected birds ($\chi^2 = 28.88$), contaminated poultry feed ($\chi^2 = 23.12$), and eating raw/uncooked poultry meat ($\chi^2 = 288$).

The respondents also showed a high knowledge of the ability to identify the signs and symptoms of AI infections in both human and birds. Some of the signs and symptoms of AI infections that were identified by respondents in man were diarrhoea ($\chi^2 = 15.68$), conjunctivitis ($\chi^2 = 18.00$), and difficulty in breathing ($\chi^2 = 8.00$). However, only few of the respondents ($\chi^2 = 0.72$) could not tell the signs and symptoms of AI infections in man. On the ability to identify the signs and symptoms in birds, respondents identified sudden death without signs ($\chi^2 = 25.92$), and discolouration of wattles, combs, and legs ($\chi^2 = 23.12$) as the signs in birds. The number of respondents who could not identify the signs and symptoms of the infection in birds were however high ($\chi^2 = 23.12$) which is an indication of poor biosecurity practices among LPTs.

This finding is in agreement with that of Sutanto (2013) whose study results illustrated that, despite being given information, LBM workers had no detailed understanding of avian influenza, had a less perceived risk of experiencing avian influenza, and had a low compliance with precautionary behaviours. As a result, biosecurity in the LBMs was highly inadequate, increasing the threat of another serious outbreak of HPAI in poultry and perhaps in humans as well.

5.4 Knowledge of Avian Influenza Human Health Risks and Preventive Measures

In order to help reduce the incidence of AI infections, it is necessary to determine the knowledge of live poultry traders on the human health risks and preventive measures. To achieve this, respondents were asked to indicate their awareness on the existence of biosecurity measures against AI infection and the prescribed biosecurity measures. Their responses showed that, they were aware of the existence of biosecurity measures in helping to curb AI infection. They also identified some prescribed biosecurity measures. These included cleaning and disinfecting cages and slaughter surfaces thoroughly and frequently ($\chi^2 = 19.35$), using personal protective equipment when handling birds ($\chi^2 = 26.13$), leaving carcass disposal to the appropriate authorities ($\chi^2 = 6.87$), not mixing birds of different species in the same cage ($\chi^2 = 10.88$), and adopting all-in-all-out management ($\chi^2 = 8.19$).

Also, a significant number of respondents ($\chi^2 = 25.95$) indicated they did not know the prescribed biosecurity measures though they were aware of its existence. This may serve as a major reason for the poor adoption and use of the prescribed biosecurity measures by the live poultry traders in the markets surveyed. Such instances are not peculiar to these markets alone but have also been found in other parts of the world. To buttress, a report by the Food and Agriculture Organisation (2008) on the assessment of the Nigerian poultry market chain to improve biosecurity according to the literature revealed that, since December 2007, there has been a country-wide programme for disinfecting LBMs, sustained by the Federal Ministry of Agriculture and Water Resources (FMA&WR) and assisted by the World Bank.

Unfortunately, the equipment (particularly sprayers) and the personnel needed to effectively carry out the process were not always sufficient for the task. The disinfection according to the report was also not always regular. The rules on the use of alternative disinfectants were not regularly respected and the choice of disinfectant was highly dependent on its price. This further increased the spread of the virus in the markets and also shows the poor attitude and biosecurity practices among the traders and key stakeholders.

5.5 Live Poultry Traders Attitude towards Avian Influenza Biosecurity

In determining the attitude of LPTs towards AI biosecurity, all respondents agreed they would be willing to comply with these biosecurity measures (r= 1). They also indicated that, given the uncertainty surrounding avian influenza, they would continue to trade as live poultry traders in the markets surveyed for the study (r=1). All respondents were also worried about the extent of vectors, rodents and stray dogs and the sanitation situation in the markets (r= -0.305).

This implies that, respondents will worry less about the presence of vectors, rodents, and stray dogs in the markets if sanitation conditions are not improved. They will, however, be more worried about rodents and stray dogs in the markets if sanitation situations are improved upon in the markets. This is because since sanitation has improved, they will then concentrate on how to get rid of the rodents and stray dogs from spreading the AI infection in the markets.

5.6 Current Practices of Live Poultry Traders Relative to Avian Influenza Biosecurity/Preventive Measures

To help reduce the spread of AI infections in LBMs, it is imperative for live poultry traders to adopt some preventive measures. According to respondents of the survey, some of the practices and preventive measures relative to AI biosecurity to consider includes disinfecting cages, how long birds are kept in their cages before restocking, the exchange of equipment such as waterers and feeders with colleagues, and slaughtering, processing, and selling dressed birds. These practices when effectively implemented will help in the control and prevention of AI virus infections in LBMs during outbreaks.

Other biosecurity/preventive measures have also been identified in the literature. For example, Cardona *et al.* (2009) posit the voluntary depopulation of inventories by traders in LBMs in California has made the market influenza free since 2005 after an outbreak in the market. Also, Kurscheid *et al.* (2015) found that, cleaning and disinfecting cages was recognized as the most important step in preventing HPAI in poultry at markets. Other proposed methods include disposing of sick and dead birds which according to the respondents of their survey minimizes the risk of virus transmission and also vaccination. The results of Martin *et al*. (2006) further suggest that the practice of selling live birds directly to consumers in food markets should be discouraged in areas currently experiencing influenza outbreaks among birds. Data collected by Offeddu *et al.* (2016) also shows that the length of stay of poultry in the market is a critical control point to interrupt the AIV-replication cycle within LPMs.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

The findings of this study not only reveal that influenza A virus currently circulate in the various domesticated avian species sampled from the major bird markets in Accra, but also underscore the need for intensified surveillance in order to keep real time track changes of the virus as an early warning measure to prevent further transmission in Ghana, especially in LBMs. Secondly, this study presents evidence that the subtype of the circulating virus(es) is/are different from the H5N1 strain which has been implicated in all reported and confirmed AI cases and points towards the possibility of the circulation of a novel AIV in the country. It is expected that AI will continue to circulate gradually within LBMs until effective biosecurity measures are enforced. The veterinary service of Ghana has demonstrated their ability to not only contain but eliminate HPAI H5N1 during the first episode of the disease in 2007 and this could be replicated if adequate logistical support and resources are available to implement control measures. In addition to the poor biosecurity recorded in all the surveyed markets, the overall operational practices of LPTs live much to be desired.

6.2 Recommendations

On the basis of the findings of the study and the conclusions reached, the following recommendations are made for the consideration of stakeholders:

6.2.1 MOFA/VSD

Should:

- Conduct regular disease surveillance (both active and passive) of live bird markets as an early warning indicator of AI and to keep track of the dynamics posed by the virus.
- Collaborate with Noguchi Memorial institute of Medical Research and the Ghana Health service to collect samples of bird sellers for routine checks as their operations put them at a high risk of infection with zoonotic diseases such as AI.
- Be resourced by the government with the provision of needed diagnostic materials to aid their operations.
- Frequent LBMs and periodically educate sellers about biosecurity practices.
- Quickly move in and stamp out all birds once a case of AI has been detected in a LBM as a means to prevent further spread. This must be accompanied by the payment of due compensation.
- Subsidize the cost of disinfectants for poultry farmers and LPTs as is done for crop farmers with the provision of subsidy on fertilizers. This will help with farm and LBMs sanitation.
- Ensure that any internal movement of poultry and poultry products must undergo veterinary inspection and be accompanied by a movement permit.
- Provide a national framework that may factor the existing perceptions of the LPTs to standardize their practices.
- Supervise and monitor the activities of LPTs and that of other persons (such as freelance bird sellers) involved in the live bird food chain from farmers to the consumers.

6.2.2 MUNICIPAL AUTHORITIES/MARKET MANAGERS/BSA

Should:

- Invest in constructing modern bird markets within the region.
- Enact bye laws to deal with non-compliant bird sellers towards laid down operational practices.
- Provide a central waste bin in the market for the temporary disposal of dead birds feathers and manure.
- Require poultry sellers to attend short classes on basic biosecurity practices in order to get or renew their license to sell live poultry in the markets.

6.2.3 LIVE BIRD SELLERS

Should:

- Periodically partake in screening programmes to know their health status with regards to influenza A since they have close contacts with birds and therefore are at high risk of infection.
- Register with and become members of the association of bird sellers.
- Report promptly to appropriate authorities any unusual signs and symptoms they may detect among their flock for prompt action.
- Ensure strict compliance to prescribed biosecurity measures.

6.2.4 THE PUBLIC/MEDIA

- The general public should be sensitized to report any unusual deaths in domestic, and wild birds to the nearest veterinary office.
- The mass media should help in creating the needed sensitization and education about AI.

6.3 FURTHER RESEARCH

I recommend that similar studies be conducted in all the other regions of Ghana, and also in all of Ghana’s border towns and villages, especially among free ranging domesticated birds.

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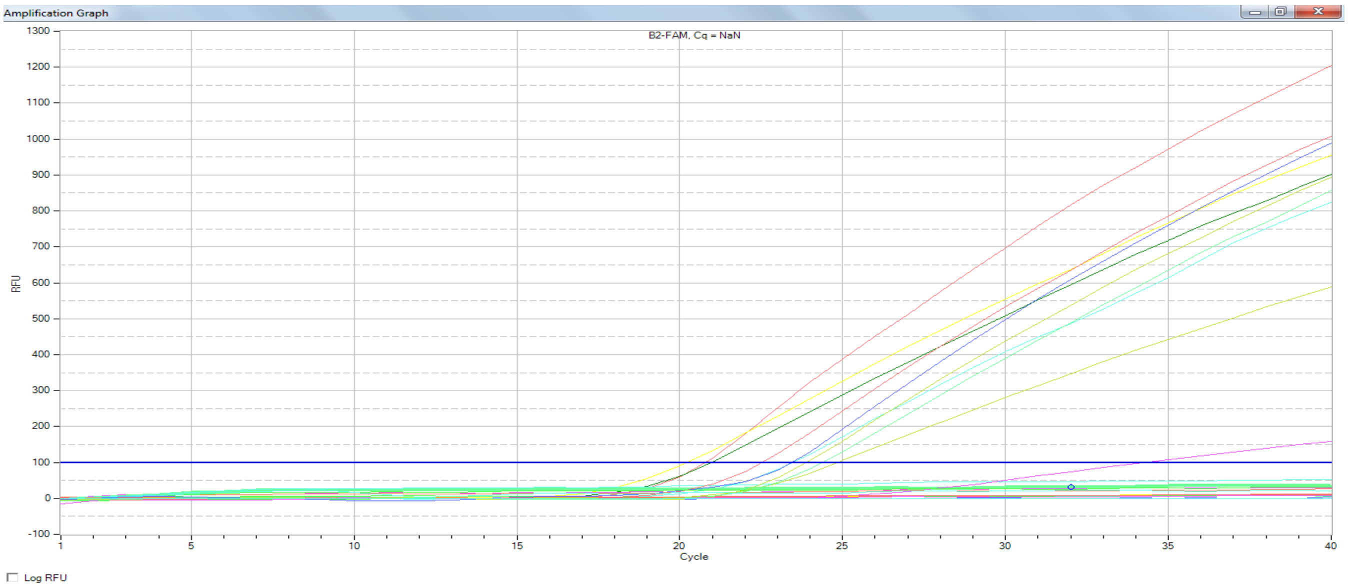
APPENDIX A

PCR OUTPUTS



Graph of M-gene PCR

Results RFU

Well	Replicate	Fluorophore	Sample Type	Sample Name	Target	Cq	Quantity
A01		FAM	Empty	106,107		20.77	0
A02		FAM	Empty	108,109		20.23	0
A03		FAM	Empty	110		20.95	0
A04		FAM	Empty	111,112		NaN	0
A05		FAM	Empty	113,114,115		NaN	0
A06		FAM	Empty	116,117		23.47	0
A07		FAM	Empty	118		NaN	0
A08		FAM	Empty	119		NaN	0
B01		FAM	Empty	120,121		NaN	0
B02		FAM	Empty	122,123		NaN	0
B03		FAM	Empty	124,125		NaN	0

Results of M-Gene PCR showing Cq values of samples.

APPENDIX B

ENSIGN COLLEGE OF PUBLIC HEALTH, KPONG

PARTICIPANT CONSENT FORM

Research Title: Surveillance for Avian Influenza Virus in Major Live Bird Markets in Accra in the Greater Accra Region.

What is this study about? This is a research project being undertaken by Solomon Ofori Koranteng, an MPH student at Ensign College of Public Health, Kpong I am inviting you to participate in this research because you work as a live poultry trader in the metropolis and registered under the Bird Sellers' Association. The purpose of this research is to ascertain your knowledge of AI health risk and preventative measures.

What will I be asked to do if I agree to participate? You will be asked to provide some information about your socio-demographic background such as your level of education, gender, religion, age, length of time in the trade, your trading experience, type and volume of birds you sell, your knowledge of AI biosecurity measures viz your current practices etc.

Would my participation in this study be kept confidential? Yes! Your participation in this study will be kept strictly confidential. The result of the study will be presented in an aggregate form which will not be traceable to you.

Is there any harm from this research? There is no harm/risk whatsoever associated with your participation in this study.

What are the benefits of this research? The result of this study and recommendations will be disseminated to policy makers geared towards efforts at avian influenza control in Ghana.

Do I have to be in this research and may I stop participating at any time? Your participation in this research is entirely voluntary and you may choose not to participate at all. If you agree to participate, you may withdraw your participation at any time without any reason. There is no penalty or loss of any benefits whatsoever if you decide not to participate, since I do not envisage any negative effects on you.

What if I have questions or need further clarifications? This proposal has been reviewed by the Institutional Review Board of Ensign College of Public Health. Should you have any questions regarding this study and your rights as a participant, or if you wish to report any problems regarding this study, please contact:

The Ethical Review Board,

Ensign College of Public Health

P. O. Box AB 136,

Akosombo, E/R, Ghana.

I believe I have been properly informed and that I understand the nature and goals of the study. I freely and voluntarily agree to participate. My questions about the study have been answered. I understand that my identity will not be disclosed and that I may withdraw from the study without giving a reason at any time and this will not negatively affect me in any way.

Participant's ID……………………

Date………………………………….

APPENDIX C

QUESTIONNAIRE ENSIGN COLLEGE OF PUBLIC HEALTH, KPONG

Surveillance for Avian Influenza Virus in Major Live Bird Markets in Accra in the Greater Accra Region of Ghana

Respondent No……………………..Date:……………….Site:………………………

Hello! My name is Solomon Ofori Koranteng. I'm an MPH student at the Ensign College of Public Health. I'm researching on the topic Surveillance for Avian Influenza Virus in Major Live Bird Markets in Accra. All of your answers will be taken and evaluated anonymously. Your statements are only used for this research and will be kept confidential. There are no right or wrong answers.

Please tick (√) against your preferred answer. Multiple responses can be ticked where applicable.

Part I: Socio-Demographic Data

1. Sex: 1. Female ☐ 2. Male ☐
2. Age: 1. 15-25 years ☐ 2. 26-35 years ☐ 3. 36-45 years ☐ 4. 46+ years ☐
3. Religion: 1. Traditional ☐ 2. Islamic 3. ☐ Christianity ☐
4. Other, specify ……………………
4. Educational level: 1. Middle School 2. ☐ JHS ☐ 3. SHS/Voc/Tec ☐ 4. Tertiary ☐ 5. No formal education ☐
5. How long have you been in this poultry selling business?
   1. 5-15 years ☐ 2. 16- ☐ 25 years 3. 26 – 35 years ☐
   4. 36+ years ☐
6. Do you sell mixed birds including free ranging local birds?
   1. Yes ☐ 2. No ☐
7. Where do you get your birds from for sale?

1. Specific poultry farm within the Greater Accra Region ☐ 2. Any poultry farm ☐ 3. From other regions ☐ 4. Other, specify………

8. Do you keep records of the source of your live birds?

   1. Yes ☐ 2. No ☐

9. Have you had any birds culled from your stocks due to any infection since you started business in this market as a live poultry trader

   1. Yes ☐ 2. No ☐

10. If yes, have you ever been compensated by the government for any losses resulting from infection of your birds

    1. Yes ☐ 2. No ☐

11. Have you ever allowed samples to be taken from your birds for purposes of any study/research?

    1. Yes ☐ 2. No ☐

Part 2: Knowledge of Signs and Symptoms

12. Have you heard of avian influenza?

    1. Yes ☐ 2. No ☐

13. If yes, where did you hear about it?

    1. Family members ☐ 2. Radio ☐ 3. TV 4. ☐ Newspaper ☐ 5. Vet/MOFA officials ☐ 6. Other Poultry sellers ☐

    7. Other, specify……………………………………………..

14. Do you think humans can get infected with influenza?

    1. Yes ☐ 2. No ☐

15. If yes, how? 1. Through contact with infected surfaces ☐ 2. Through contact with infected birds 3. ☐ Through contact with contaminated poultry feed. 4. When one ☐ eats raw or uncooked poultry meat ☐ 5. Other, specify……………………………………………

16. What do you think may be the signs of avian influenza in humans?

1. Cough ☐ 2. Diarrhoea ☐ 3. Headache ☐ 4. ☐ Difficulty in breathing 5. High temperature ☐

6. Conjunctivitis ☐

7. Don't know ☐ 8. Other, specify……………………………

17. What are the signs of avian influenza in birds?

1. Sudden death without signs 2. ☐ Discolouration of the wattles, combs and legs ☐

3. ☐ Coughing ☐ 4. Sneezing 5. Don't know 6. Other, ☐ Specify…………………………………………………..

18. In what condition do you think a bird suspected of avian influenza should be reported? 1. Manifesting suspected signs and symptoms ☐

2. Sudden death ☐ 3. Don't know ☐ 4. Other, specify……..

Part 3: Knowledge of Avian Influenza Human Health Risks and Preventive Measures

19. Are you aware about the existence of any prescribed biosecurity measures against avian infection? 1. Yes ☐ 2. No ☐

20. If yes, what do you think are these bio security measures?

1. Do not trade birds of unknown origin ☐ 2. Adopt all-in-all-out management ☐ 3. Minimize contact with feathers, blood, offal, and faecal matter ☐ 4. Clean and disinfect cages and the slaughter surfaces thoroughly and frequently. ☐ 5. Use personal protective equipment when handling birds 6. ☐ Leave carcass disposal to appropriate authorities ☐

7. Do not mix different species of birds in the same cage ☐ 8. Don't know ☐

9. Other, specify…………………………………………….

21. Who /what determines the biosecurity measures you practice?

1. For protection against avian influenza infection ☐ 2. To prevent the spread of avian influenza infection ☐ 3. Government policy ☐

4. Don't know 5. ☐ Other, specify……………………

Part 4: Live Poultry Traders Attitude towards Avian Influenza Biosecurity

22. Would you be willing to comply with these biosecurity measures if they are enforced?

1. Yes ☐ 2. No ☐

23. Given the uncertainty surrounding avian influenza, would you continue to trade as a live poultry trader in this market?

1. Yes ☐ 2. No ☐

24. If yes, why?...

25. Are you worried about the extent of vectors, rodents and stray dogs in this market?

1. Yes ☐ 2. No ☐

26. Are you worried about the sanitation situation in this market?

1. Yes ☐ 2. No ☐

Part 5: Current Practices of Live Poultry Traders Relative to Avian Influenza Biosecurity/Preventive Measures

27. What do you do with sick birds? 1. Slaughter & eat ☐

2. Slaughter, dress and sell off ☐ 3. Report to Vet/MOFA officials ☐

4. Give to scavenging dogs ☐ 5. Other, specify…………………

28. Do you disinfect your cage? 1. Yes ☐ 2. No ☐

29. If yes, how often? 1. Routinely ☐ 2. Periodically ☐

3. Occasionally ☐ 4. Other, specify…………………………..

30. Do you wash your hands prior to and after handling birds?

1. Yes ☐ 2. No ☐

31. If yes, how often? 1. Always ☐ 2. Periodically ☐ 3. Occasionally ☐

32. Do you sell off all birds in your cage before you restock?

1. Yes ☐ 2. No. ☐

33. On the average, how long does a bird remain in your cage before it is sold?

1. One week ☐ 2. Two weeks ☐ 3. Three weeks ☐

4. One month or more ☐ 5. Cannot tell ☐

34. Do you exchange equipment such as waterers and feeders with your colleague vendors operating stalls? 1. Yes ☐ 2. No ☐

☐

35. How do you feed your birds? 1. Put feed on plate

2. Spread out in flock ☐ 3. Other, Specify……………………..

36. Where do you keep unsold birds? 1. Send them home 2. ☐ Under lock and key in my stall overnight 3. Other, ☐ Specify………………………..

37. Do you use any of the following personal protective equipment (PPEs) and how often?

Gloves 1. Never 2. Occasionally 4. Always ☐ 3. Monthly ☐ ☐ ☐

Nose mask 1. Never 2. ☐ 3. Monthly ☐ ☐ ☐ Occasionally 4. Always

Boots 1. Never 2. Occasionally 3. Monthly ☐ ☐ 4. Always ☐ ☐ ☐ ☐ ☐ ☐

Apron 1. Never 2. Occasionally 3. Monthly 4. Always

38. Do you slaughter, process and sell dressed birds to some of your buyers?

1. Yes ☐ 2. No ☐

39. If yes, how often do you clean your slaughter slabs and tools?

1. Before every slaughter ☐ 2. After every slaughter ☐

3. Before and after every slaughter ☐ 4. Every day at the close of business ☐

4. Other, specify………………..

THANK YOU FOR YOUR TIME