EPIDEMIOLOGY OF AVIAN INFLUENZA IN DOMESTIC POULTRY AND WILD BIRDS IN THE TEMA METROPOLIS

FENTENG DANSO EDWARD

SCHOOL OF PUBLIC HEALTH

COLLEGE OF HEALTH SCIENCES

UNIVERSITY OF GHANA, LEGON

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By

FENTENG DANSO EDWARD

THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF MASTERS OF PHILOSOPY IN APPLIED EPIDEMIOLOGY AND DISEASE CONTROL

NOVEMBER, 2010

DECLARATION

I hereby declare that this dissertation except for references to other peoples work which have been duly cited, this work is the result of my own research and that this dissertation has neither in whole nor in part been presented for any other degree.

Candidate

……..

FENTENG DANSO EDWARD

Academic Supervisors

……..

DR. WILLIAM KWABENA AMPOFO

(Virology Dept., Noguchi Memorial Institute for Medical Research)

…….

PROFESSOR BAWA AWUMBILA

(Dept. of Animal Science, University of Ghana, Legon)

DEDICATION

This work is dedicated to my dear wife Kate Fenteng Danso and my lovely children, Kwame Fenteng Danso and Abena Asantewa Fenteng Danso.

ACKNOWLEDGEMENT

My special thanks go to my academic supervisors; Dr. William Kwabena Ampofo and Professor Bawa Awumbila for their guidance throughout the research.

I am grateful to the School of Public Health (SPH), Veterinary Services Directorate (VSD) and Centers for Disease Control and Prevention (CDC), Atlanta, USA for offering me the golden opportunity to pursue the post graduate programme in Applied Field Epidemiology and Disease Control.

My sincere gratitude goes to Professor Edwin Afari, Dr. Agyen Frimpong, Mr. David Mensah, Dr. E. B. Koney, Dr. Michael Aryee, Dr. Samuel Sackey, Dr. Priscilla Awo Nortey, Dr. Richard Suu-ire, Dr. Joseph Awune, Dr. Owusu Darlington, Dr. Andy Kwabena Alhassan, Dr. Nathaniel Yebuah, Dr. Mark Hansen, Dr. Abuh Joseph,

Dr. (Mrs.) Benita Anderson, Miss Cedonia Luuse, Mr. Kofi Bonney, Miss Ivy Asante, Mrs. Beatrice Amanquah, Miss Veronica Mensah, Mr. Joseph Asare Yirenkyi, Tema Metropolis Veterinary staff, staff of Wildlife Division of Forestry Commission at Sakumono Ramsar site, National Influenza Center team members of the Noguchi Memorial Institute for Medical Research and all people who in various ways made it possible for me to go through this work.

My warm thanks to United States Naval Medical Research Unit #3 for all their support.

ABSTRACT

BACKGROUND

Avian influenza (AI) is an infectious disease of birds caused by influenza type A viruses. Migratory waterfowl - most notably wild water fowls are the natural reservoir of all influenza A viruses. There are 16 subtypes of influenza A viruses, of which H5 and H7 subtypes are the most pathogenic

In April 2007, the first outbreak of HPAI was reported in Ghana in a small scale poultry farm at Kakasunanka, near Michel Camp in the Tema Metropolis. There were subsequent outbreaks of the AI virus at Adjei Kojo in Tema Metropolis, Sunyani Municipality in the Brong Ahafo and Aflao in Ketu South District in the Volta regions in the same year. All infected poultry farms were stamped out.

This study sought to determine the current profile of Avian Influenza viruses in domestic commercial poultry, backyard poultry, live bird markets and wild birds in the Tema Metropolis over a one year period and covering the dry and wet seasons.

METHODS

From May 2009 to March 2010, we administered a semi-structured questionnaire to poultry farmers and conducted a cross sectional study on 1282 field samples involving fresh faeces, tracheal and cloacal swabs from domestic poultry, live bird markets and wild birds from 16 communities in the Tema Metropolis. These samples were then

subjected to real-time Reverse Transcriptase- Polymerase chain reaction analysis for Influenza A virus.

RESULTS

All the 1282 avian samples tested, were negative for Influenza A viruses. However, Newcastle disease virus was detected in 8% (5/63) of the farms where birds sampled showed respiratory and nervious signs. Commercial farms accounted for 5%, backyard for 2% and live birds market 1%. Also, adherence by farmers to good poultry management practices and proper bio-security measures was found to be low.

CONCLUSIONS

There was no evidence of circulation of AI H5N1 among domestic poultry and wild birds in the study population, between May 2009 and March 2010. This negative result for AI virus in the study shows that measures taken by poultry farmers and other stakeholders were probably effective. However, VSD should conduct further education of farmers on good poultry practices and bio-security.

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LIST OF ABBREVIATIONS

AI	Avian Influenza
AIV	Avian Influenza virus
^{O}C	Degree Celsius
CDC	Centers for Disease Control and Prevention
Ct	Cycle Threshold
DEFRA	Department for Environment, food and Rural Affairs
DIVA	Differentiating Vaccinated from Infected Animals
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleoside triphosphate
ELISA	Enzyme Linked Immunosorbent Assay
EU	European Union
FAO	United Nations Food and Agriculture Organization
HA	Haemagglutinin
HI	Haemagglutination Inhibition
HPAI	Highly Pathogenic Avian Influenza
HPAIV	Highly Pathogenic Avian Influenza Virus
LPAIV	Low Pathogenic Avian Influenza Virus
µl	micro liter
ml	milliliters

NA	Neuraminidase
NMIMR	Noguchi Memorial Institute for Medical Research
OIE	International Organization for Epizootic
RNA	Ribonucleic Acid
RRT-PCR	Real Time Reverse Transcriptase Polymerase Chain Reaction
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
SARS	Severe Acute Respiratory Syndrome
USAID	United States Agency for International Development
VSD	Veterinary Services Directorate
WHO	World Health Organization

CHAPTER ONE

1.1 INTRODUCTION

Fowl plague which is now known as highly pathogenic avian influenza was first recognized as an infectious disease of birds in Italy, in 1878 (Perroncito, 1878). In 1901, Centanni and Savonuzzi, identified a filtrable agent as responsible for causing the disease, and in 1955, Schäfer characterized these agents as influenza type A viruses.

In 1997, there was an outbreak of AI in Southern China (Hong Kong) that affected 1.4 million chickens and eighteen people of whom six persons died (Kaye and Pringle 2005). With the assumption that the human infection came from poultry, all birds were culled. Five years after the 1997 China outbreak, the disease resurfaced again in China and by 2005 most Asian countries had experienced outbreaks although they were successfully managed. In spite of these successes in control, the AI virus appears to have become endemic in most of the outbreak countries. The Asian experienced is similar in African countries that have experienced AI outbreaks including Egypt, South Africa, Nigeria, and Benin.

In April 2007, the first outbreak of AI was reported in Ghana at a small – scale poultry farm at Kakasunanka, near Michel Camp in the Tema Metropolis. This was despite several biosecurity measures such as a ban on importation of poultry and poultry products from Southeast Asia which affected European and African countries, disinfection of farm workers and provision of equipments and vehicles to curtail the entrance of the disease

into Ghana. AI however, appeared in Sunyani in the Brong Ahafo region and Aflao in the Volta region.

The economic losses due to AI can be described through the following: Compensation to poultry farmers whose birds are affected, high cost of equipments and disinfectants. Also, is the cost of human resources needed to control the outbreak and losses in international trade in terms of ban on poultry and poultry products.

Following the outbreak of AI in Ghana, the Government of Ghana paid compensation to poultry farmers for the birds that were culled. The compensation ranged from 70% to 90% of the market prices for day-old chicks, broilers, cockerels and layers, while table and fertile eggs were paid at rates of 50% and 60% of market prices respectively. In all, a total of 13,391 birds were affected and 36,376 birds were destroyed. The Government paid an amount of 160,000 US dollars as compensation to affected farmers (VSD. Annual reports 2007, 2009). The cost of Veterinary interventions and public education on prevention and control of the disease was estimated at 2 million US dollars. Much of this financial support came from donor partners including USAID and FAO (VSD Annual report 2009).

The purpose of this study therefore was to determine the current profile of avian influenza viruses in domestic poultry and wild birds in the Tema Metropolis during the period May 2009 to March 2010.

1.2 PROBLEM STATEMENT

The highly pathogenic avian influenza A (H5N1) outbreak in Asia, Europe, Near East and Africa is not expected to disappear in the short term. It is possible that H5NI virus infections have become endemic among domestic birds in certain areas and that sporadic human infections resulting from direct contact with infected poultry or wild birds will continue to occur.

Migratory water fowls, most notably wild ducks, are natural reservoirs of avian influenza and are most resistant to Al infection. Live bird markets, movement of poultry and poultry products, and movement of poultry farm workers have probably all played an important role in the spread of the disease in Africa.

The H5N1 virus was detected for the first time in Ghana, on a small –scale poultry farm at Kakasunanka, near Michel camp in the Tema Metropolis in April 2007. The second outbreak also occurred in the Sunyani Municipality in May 2007 and the third outbreak occurred in the month of June 2007 at Aflao. Subsequently, these areas were declared free of H5N1 in September, 2007. However, the current profile of Avian Influenza viruses in domestic commercial poultry, backyard, live bird markets and wild birds in the Tema Metropolis where an outbreak had occured is not known. Also, bio-security practices among backyard and commercial poultry farmers in the Tema Metropolis are yet to be reviewed to determine current risk status for AI infection.

1.3 JUSTIFICATION

Following the arrival of the HPAI disease into Ghana, it is important to determine the profile of avian influenza viruses in domestic poultry and wild birds. The H5N1 virus, which has spread from Asia to Europe and Africa, poses a real public health threat as H5N1 can occasionally infect humans. In Ghana, backyard poultry and wild birds may be acting as a silent reservoir for the H5N1 virus and other low pathogenic avian influenza viruses. The concern is greatest in rural areas, where traditional free-ranging ducks, chickens and wildlife mingle frequently with each other, sharing the same source of water and feed.

The experience of the 2008 outbreak of HPAI in Togo has shown the ability of the H5N1 virus to persist discreetly among traditional farms (scavenging poultry) where chicken mortality is common and usually goes unreported (FAO EMPRES, 2008).

The role of domestic birds and wild birds in the maintenance and spread of H5N1 and other influenza A viruses has not been investigated in Ghana, though other countries such as China and Nigeria have also experienced outbreaks of HPAI have conducted such studies.

In Ghana, outbreaks of H5N1 were recorded in 2007 at Kakasunanka and Adjei Kojo all in Tema Metropolis of the Greater Accra region. The metropolis is notable for its significant poultry production and it is also a major port city in Ghana.

Hence, there is the need to determine the current profile of AI infection in commercial birds, backyard birds, live bird markets and wild birds in Tema Metropolis.

1.4 GENERAL OBJECTIVE

To determine the current profile of Avian Influenza viruses in domestic commercial poultry, backyard poultry, live bird markets and wild birds in Tema Metropolis between May 2009 and March 2010 covering the dry and wet seasons.

1.5 SPECIFIC OBJECTIVES

1. To determine current profile of avian influenza virus infection in domestic poultry (commercial poultry, backyard poultry and live bird markets) and wild birds in the Tema Metropolis.
2. To assess Bio-security procedures and practices on commercial poultry farms, backyard farms and live bird markets in the Tema Metropolis.

1.6 RESEARCH QUESTIONS

1. Is the AIV that was detected in poultry in Tema, in April, 2007 still circulating in domestic poultry, backyard birds and wild birds in the Tema Metropolis?

2. Are the bio-security processes and procedures for HPAI in Tema Metropolis adequate?

CHAPTER TWO

LITERATURE REVIEW

2.1 AVIAN INFLUENZA DISEASE

Avian influenza (AI) is a disease of viral etiology that ranges from a mild or even asymptomatic infection to an acute, fatal disease of chicken, turkey, guinea fowl, and other avian species, especially migratory waterfowl (Alexander 1982, Hinshaw and Webster 1982, Beard 1989, Webster *et al* 1992, Easterday *et al* 1997, Stalknecht and Brown 2007). Wild water birds have been identified as natural reservoir host of avian influenza viruses. Generally, the infection is asymptomatic as influenza A virus strains of low pathogenicity co-exist in almost perfect balance in wild water birds (Webster *et a*l 1992, Alexander 2000).Recently, avian influenza has acquired world-wide attention when a highly pathogenic strain of the subtype H5N1, which probably arose before 1997 in Southern China, gained enzootic status in poultry throughout South East Asia. The H5N1 virus had traversed interclass barriers (Perkins and Swayne, 2003) and had been transmitted from birds to mammals (cats, swine, and humans). Although not an entirely unprecedented event (Koopmans *et al* 2004, Hayden and Croisier 2005), the substantial number of documented cases in humans, associated with severe disease and several fatalities raised serious concerns about a pandemic potential of the H5N1 strain (Klempner and Shapiro 2004; Webster *et al* 2006). There are several further lines of evidence suggesting that the

H5N1 virus has acquired increased pathogenic potency for several mammalian species. Justifiably, this has caused world-wide public health concern (Kaye and Pringle 2005).

2.2 HIGHLY PATHOGENIC AVIAN INFLUENZA H5N1 OF ASIA LINEAGE

According to the International Organization for Epizootics (OIE) 2008, more than sixty countries in Asia, Europe and Africa's HPAI H5N1 infection in domestic poultry, captive and wild birds are linked to the Asian lineage virus. By November 2005, over 200 million domestic birds had died from the disease or been slaughtered in attempts to control its spread in western Eurasia and Africa. Also, by March 2008, the World Health Organisation had confirmed more than 370 human cases of H5N1 with a case fatality rate of 60% (World Health Organisation 2008). Sporadic deaths of wild birds due to H5N1 have been reported since 2002 and the first outbreak involving a large number of wild birds was reported in May 2005, in Qinghai province, China (Chen *et al*. 2005; Liu *et al*. 2005). Between 2002 and 2008, the virus has infected a wide range of wild bird species (Olsen *et al*. 2006; USGS National Wildlife Health Center 2008; Lee 2004), but which species are important in H5N1 HPAI movement and whether the virus will become enzootic in wild bird populations is still unknown (Brown *et al*, 1998).

The H5N1 virus has also infected a limited number of domestic, captive and wild mammals, including captive Tigers (*Panthera tigris*), Leopards (*Panthera pardus*), domestic pigs in southeast Asia, domestic cats and a wild Stone Marten Martes foina in Germany. These cases were the result of 'spillover' infection from birds. There is no known reservoir of HPAI H5N1 virus in mammals and there remains no sound evidence that the virus can be readily transmitted from mammal to mammal (Perkins and Swayne 2003).

2.3 EMERGENCE OF HPAI H5N1 IN POULTRY IN SOUTHEAST ASIA

Highly Pathogenic Avian Influenza H5N1 first received widespread recognition following the 1997 outbreak in poultry in Hong Kong with subsequent spread of the virus to humans. During that outbreak, 18 human cases were reported and six patients died. The outbreak was effectively controlled through slaughtering of all domestic chickens in infected farms as well as those held by wholesale facilities and vendors in Hong Kong. A precursor to the 1997 H5N1 strain was identified in Guangdong, China, where it caused deaths in domestic geese in 1996 (Webster et al. 2006).

Between 1997 and 2002, different reassortments (known as genotypes) of the virus emerged, in domestic goose and duck populations, which contained the same H5 HA gene but had different internal genes (Guan *et al*. 2002; Webster *et al*. 2006).

In 2002, a single genotype emerged in Hong Kong and killed captive and wild water birds in nature parks. This genotype spread to humans in Hong Kong in February 2002 (infecting two, killing one).

Between 2003 and 2005, the HPAI H5N1 spread in an unprecedented fashion across southeast Asia, affecting domestic poultry in Vietnam, Thailand, Indonesia, Cambodia, Laos, Korea, Japan, China and Malaysia. In April 2005, the first major outbreak in wild birds was reported. Some 6345 wild birds were reported dead at Qinghai Lake in central China. Species affected included Great Black-headed Gull (*Larus ichthyaetus*), Bar-headed Goose (*Anser indicus*), Brown-headed Gull (*Larus brunnicephalus*), Great

Cormorant (*Phalacrocorax carbo*) and Ruddy Shelduck (*Tadorna ferruginea*) (Chen *et al.* 2005; Liu *et al.* 2005).

2.4 ECONOMIC CONSEQUENCES OF HPAI

Outbreaks of highly pathogenic avian influenza can be catastrophic for single farmers and for the poultry industry of an affected region as a whole. Economical losses are usually only partly due to direct deaths of poultry from HPAI infection. Measures established to prevent further spread of the disease levy a heavy toll. Nutritional consequences can be equally devastating in developing countries where poultry is an important source of animal protein. Once outbreaks have become widespread, control is difficult to achieve and may take several years (WHO 2004).

This is illustrated by the Southeast Asian outbreaks which resulted in high avian mortalities, resulting in the destruction of over one hundred and fifty million (150,000,000) birds accounting for losses in revenue estimated at over ten billion US dollars (Diouf 2005). This was a serious setback for the agricultural development. There was associated poverty for many rural farmers who depended solely on small scale backyard poultry farming for household income.

2.5 DESCRIPTION OF AVIAN INFLUENZA VIRUS

Influenza viruses are spherically or longitudinally shaped enveloped particles with an eight-fold segmented, single-stranded RNA genome of negative polarity. Influenza viruses belong to the *Orthomyxoviridae* family and are classified into types A, B or C based on antigenic differences of their nucleo- and matrix proteins. Avian influenza viruses (AIV) belong to type A (Sidorenko and Reichl 2004, Center for Infectious Diseases Research and Policy 2007).

The main antigenic determinants of influenza A and B viruses are the haemagglutinin (HA) and the neuraminidase (NA) transmembrane glycoproteins, capable of eliciting subtype-specific and immune responses which are fully protective within, but only partially protective across, different subtypes. On the basis of the antigenicity of these glycoproteins, influenza A viruses currently cluster into sixteen HA (H1 - H16) and nine NA (N1 - N9) subtypes. These clusters are substantiated by phylogenetic analyses and

deduced amino acid sequences of the HA and NA genes, respectively (Fouchier *et al* 2005).

The conventional nomenclature for influenza virus isolates requires connotation of the influenza virus type, the host species, the geographical site, serial number, and year of isolation. For influenza virus type A, the haemagglutinin and neuraminidase subtypes are added in brackets. One of the parental avian strains of the current outbreaks of H5N1 of Asian lineage was isolated from a goose in the Chinese province, Guangdong. Accordingly, it is designated A/goose/Guangdong/1/96 (H5N1) (Class *et al* 1998) while the isolate originating from the first-documented human case of Asian lineage H5N1 infection from Hong Kong (Xu *et al* 1999) is referred to as A/HK/156/97 (H5N1).

The Haemagglutinin, a glycosylated and acylated protein consisting of 562 - 566 amino acids, is incorporated in the viral envelope. The globular head of its membrane-distal, knob-like external domain is associated with binding to cellular receptors composed of oligosaccharides which terminally carry derivates of neuraminic acid (Watowich *et al* 1994). The exodomain of the second transmembrane glycoprotein, the neuraminidase (NA), exerts sialolytic enzymatic activity and liberates virus progeny captured at the

surface of infected cells during egress. This function prevents viral aggregation during egress, and possibly also facilitates the drifting of the virus through the mucus layers of the targeted epithelial tissues leading to viral attachment (Matrosovich *et al* 2004).

This renders the neuraminidase an interesting target of antiviral agents (Garman and Laver 2004). Mutually attuned and co-ordinated actions of the antagonistic glycoprotein species

HA and NA of a viral strain are pivotal for effective attachment and release processes of the virions (Wagner *et al* 2002).

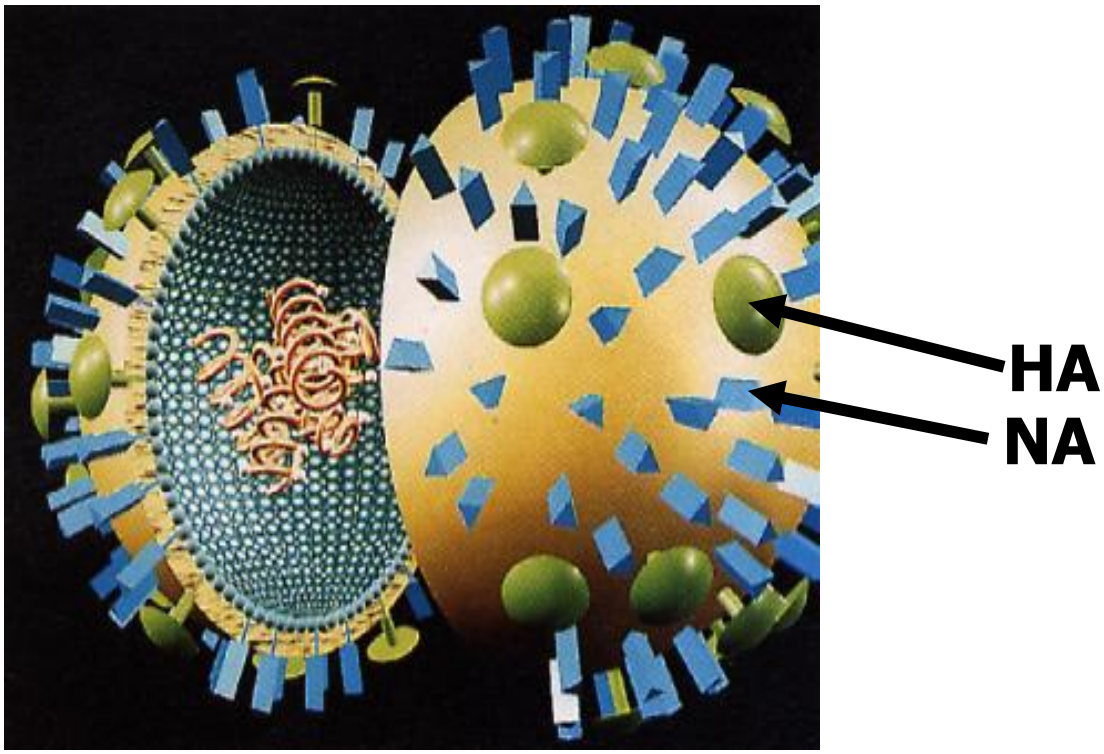


Figure1. Avian Influenza Viral features. Source: Hoffmann 2006

2.6 EPIDEMIOLOGY OF AVIAN INFLUENZA

Wild aquatic birds, notably members of the orders *Anseriformes* (ducks and geese) and *Charadriiformes* (gulls and shorebirds), are carriers of the full variety of influenza virus A subtypes, and thus, most probably constitute the natural reservoir of all influenza A viruses (Webster 1992, Fouchier *et al* 2003, Krauss *et al* 2004, Widjaja *et al* 2004). While all avian species are thought to be susceptible, some domestic poultry species - chicken, turkey, guinea fowl, quail and pheasants - are known to be especially vulnerable to the sequelae of infection.

Avian influenza A viruses generally do not cause disease in their natural hosts. Instead, the viruses remain in an evolutionary stasis, as molecularly signalled by low *N/S* (non-synonymous vs. synonymous) mutation ratios indicating purifying evolution (Gorman *et al* 1992, Taubenberger *et al* 2005). Host and virus seem to exist in a state of a meticulously balanced mutual tolerance, clinically demonstrated by absence of disease and efficient viral replication. Large quantities of virus of up to $10^{8.7}$ x 50% egg-infective dose (EID_{50}) per gram faeces can be excreted (Webster *et al* 1978). When transmitted to highly vulnerable poultry species, usually mild, if any, symptoms ensue. Viruses of this phenotype are referred to as low pathogenic (LPAIV) and, in general, only cause a slight and transient decline in egg production in layers or some reduction in weight gain in fattening poultry (Capua and Mutinelli 2001). However, strains of the subtypes H5 and H7 carry the potential to mutate to a highly pathogenic form after transmission and adaptation to the new poultry hosts. The highly pathogenic forms of H5 and H7 or of other subtypes had not been previously observed in wild birds (Webster 1998). Therefore,

one may conclude that highly pathogenic forms are artificial, made possible as a result of man-made interference with a naturally balanced system.

Once Highly Pathogenic Avian Influenza Virus (HPAIV) become established in domestic poultry, a highly contagious disease results and wild birds are no longer an essential factor for the spread (Swayne and Suarez 2000). This might have changed fundamentally since early 2005, when a large outbreak of the Asian lineage H5N1-related HPAI was observed among thousands of wild aquatic birds in a nature reservation at Lake Qinghai in the North West of China (Chen *et al* 2005, Liu *et al* 2005). As a result of this, further spread of this virus towards Europe during 2005 may have been initiated (OIE 2005).

Infected birds excrete virus in high concentration in their faeces and also in nasal and ocular discharges. Once introduced into a flock, the virus is spread from flock to flock through direct contact or by usual methods involving the movement of infected live birds or illegal trade or their unprocessed products, contaminated equipment, egg flats feed trucks. Unintended mechanical passing-on of virus through human movements probably has been the main factor in the spread of HPAIV (Stegaman et al 2003).

Airborne transmission may occur if birds are in close proximity and with appropriate air movement. Birds are readily infected via instillation of virus into the conjunctival sac, or the tracheal. Preliminary field and laboratory evidence shows that Avian Influenza virus can be recovered from the yolk and albumen of eggs laid by hens at the peak of the disease. The possibility of vertical transmission is unresolved, however, it is unlikely that infected embryos could survive and hatch. Attempts to hatch eggs in disease cabinets from a broiler breeder flock at the height of disease failed to result in any Avian

Influenza-infected chickens. However, broken contaminated eggs could be the source of virus infection to chicks after they hatch in the same incubator (Hoffmann *et al* 2006).

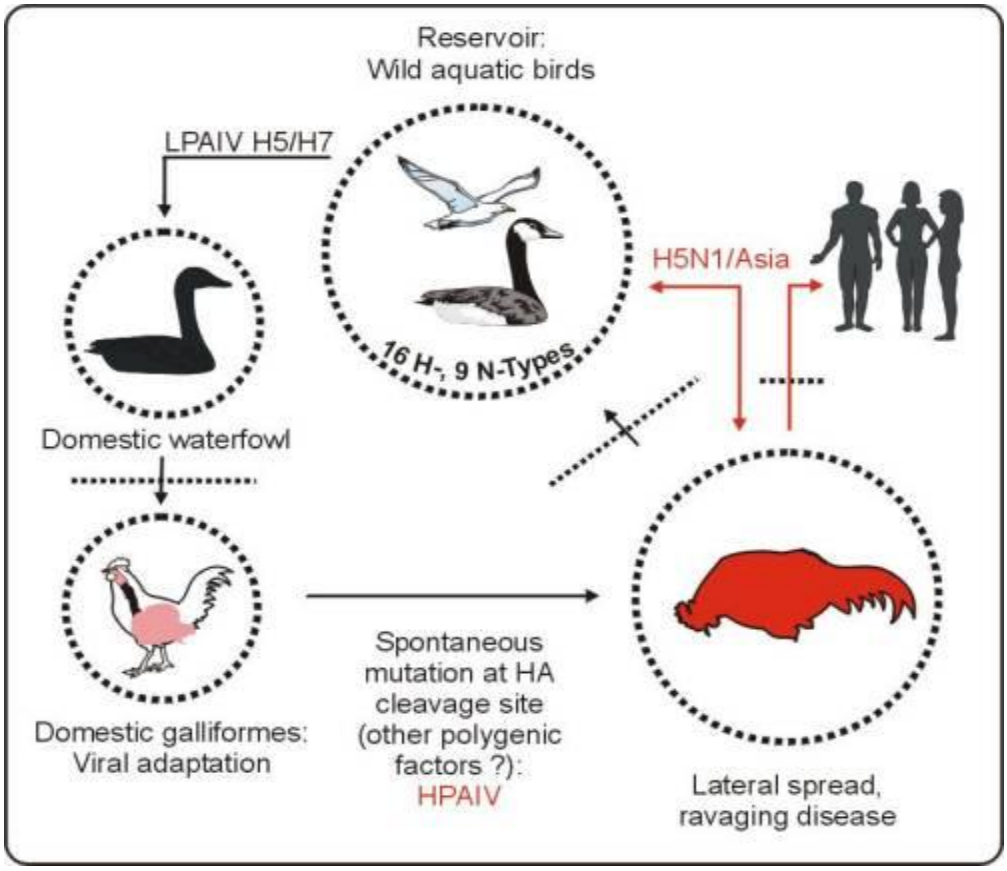


Figure 2. Avian Influenza Transmission Cycle. Source: Hoffmann 2006

2.7 GEOGRAPHICAL SPREAD OF HPAI H5N1 OUT OF SOUTHEAST ASIA

In July 2005, Russia reported its first outbreaks of H5N1 in domestic flocks in six regions of western Siberia and dead wild birds were reported in the vicinities of some of these outbreaks. Kazakhstan reported its first outbreak in August 2005 in domestic birds. In the same month, 89 wild birds described as migratory species were reported infected at two lakes in Mongolia (OIE 2005).

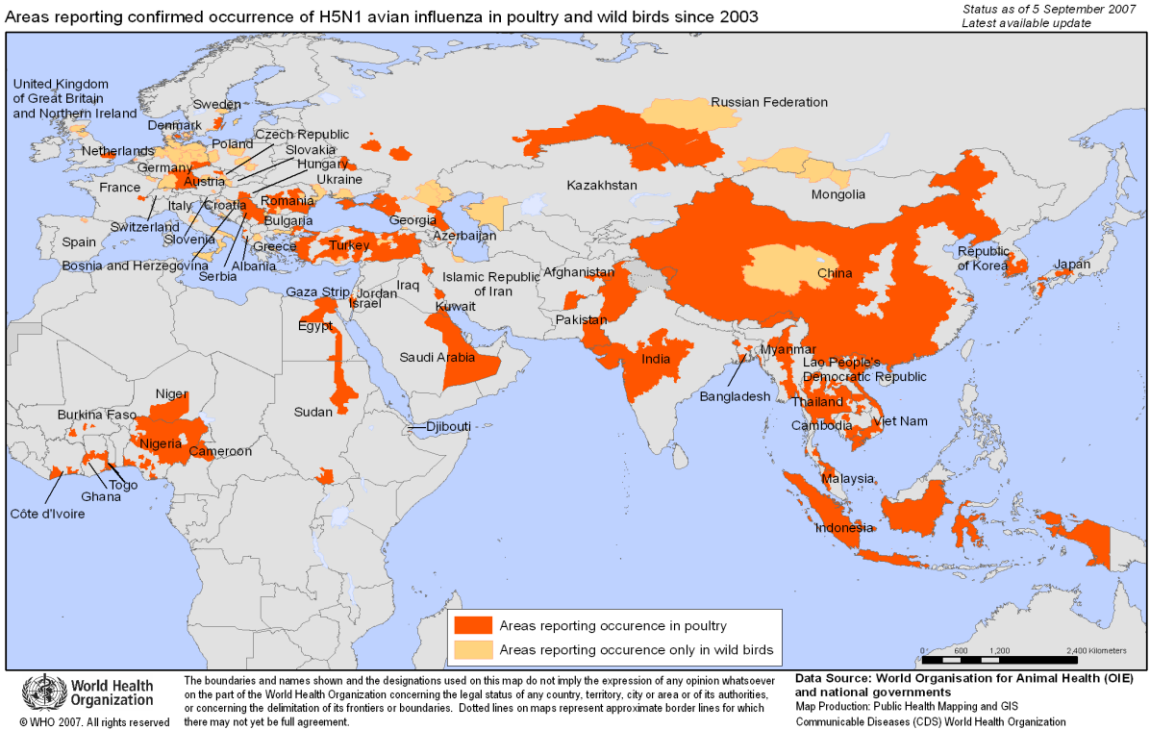
Europe reported its first outbreaks in October 2005 when H5N1 infection was detected in domestic birds in Romania and Turkey. In the same month, Romania reported sporadic cases in wild birds as did Croatia and European parts of Russia. In November, the virus spread to domestic birds in the Ukraine, and the Middle East reported its first case: a flamingo kept as a captive bird in Kuwait. During December 2005, two outbreaks were reported in European Russia in wild swans (species unreported) in regions near the Caspian Sea.

In the first half of 2006, the spread of HPAI H5N1 continued across Europe (Sabirovic et al. 2007; Hesterberg et al. 2009) and the Middle East and into Africa. Between January and May, infection was reported in 24 European countries with the majority of cases occurring in February and March in wild birds. During the same period, outbreaks were reported across central Asia and the Middle East, affecting domestic birds in Azerbaijan, India, Bangladesh, Pakistan, Iran and Iraq, with Azerbaijan also reporting infected wild birds. The first reported outbreak in Africa occurred in January 2006 in poultry in Nigeria, and by the end of April 2007, eight other African nations had reported outbreaks:Burkina Faso, Cameroon, Djibouti, Egypt, Ghana, Cote d'Ivoire, Niger and

Sudan (OIE 2008).

By May 2006, reports of outbreaks in Europe, the Middle East and Africa had for the most part decreased in frequency. Small numbers of cases of infection were reported in Hungary, Spain and the Ukraine in June; Pakistan and Russia in July; and one case was identified in a captive swan in Germany in August. Egypt was exceptional, continuously reporting outbreaks throughout 2008. It is also considered likely that outbreaks continued in poultry in Nigeria (UN System Influenza Coordinator & World Bank 2008). Throughout the time HPAI H5N1 was spreading across central Asia, Europe, the Middle East and Africa; it maintained a stronghold in poultry in Southeast Asia. In 2006, outbreaks were reported in Cambodia, China, Hong Kong, Indonesia, Korea, Laos, Malaysia, Myanmar, Thailand and Vietnam (Figure 3: H5N1 outbreaks).

Figure3:H5N1outbreaks



2.8 OUTBREAKS OF HPAI H5N1 SINCE 2006 AND THE CURRENT SITUATION

2.8.1 GLOBAL HPAI H5N1 SITUATION

Compared with 54 countries reporting 1,470 outbreaks to the OIE in 2006, thirty countries reported 638 outbreaks in 2007 (OIE 2008). In 2007, six European countries (Poland, Hungary, Germany, the United Kingdom, Romania and the Czech Republic) reported sporadic and relatively isolated outbreaks in poultry that were quickly controlled. Outbreaks in domestic birds were also reported in European parts of Russia and in Turkey.

Infected wild birds were reported in Germany, France, the United Kingdom and the Czech Republic; and birds at a rehabilitation centre were affected in Poland. In the Middle East and central Asia, poultry outbreaks occurred throughout 2007. Some 350 outbreaks were reported from Egypt and Bangladesh alone. Poultry (and in some cases captive birds) were also affected in India, Kuwait, Saudi Arabia, Pakistan, Afghanistan and Israel with most outbreaks occurring between February and April, and again between October and December. Again, as in 2006, poultry outbreaks continued across Southeast Asia. Sporadic cases in wild birds were reported in Japan and Hong Kong SAR. In January and February 2008, a small number of wild bird cases were detected in the United Kingdom; large numbers of poultry outbreaks occurred in India and parts of Southeast Asia; and the virus was considered to be enzootic in poultry in Egypt, Indonesia and Nigeria; and possibly enzootic in Bangladesh and China (UN System Influenza Coordinator & World Bank 2008). Globally, 63 countries have reported avian influenza outbreaks since 2006 to March, 2010.

2.8.2 AFRICA HPAI H5N1 SITUATION

In Africa, HPAI H5N1 was reported in domestic birds in Nigeria, Egypt, Togo, Ghana and Benin; and is considered to have become endemic in Egypt (OIE 2008; UN System Influenza Coordinator & World Bank 2008).

Also, in the African Union/Interafrican Bureau for Animal Resources progress report from May 2007 to February, 2009, it was reported that eleven African countries namely; Benin, Burkina Faso, Cameroon, Cote d'Ivoire, Ghana, Niger, Nigeria, Togo, Egypt, Djibouti and Sudan had been infected since the emergence of AI virus in domestic Africa. So far, there have been 52 reported H5N1 human cases with 23 fatalities in Africa. From March, 2009 to March, 2010, only Egypt reported outbreaks in domestic poultry.

2.8.3 GHANA HPAI H5N1 SITUATION

On April 24th, 2007, Ghana reported her first outbreak of HPAI H5N1 virus infection on a small-scale poultry farm situated at Kakasunanka near Michelle camp in the Tema Metropolitan area of the Greater Accra region. This virus was detected by the using of the rapid test kit for influenza type A viruses. The Emergency Preparedness Team of VSD immediately moved to the outbreak area and stamped out all in-contact birds of the infected poultry farm. Further tests conducted at Noguchi Memorial Institute for Medical Research (NMIMR) and international reference laboratory for HPAI at the Instituto Zooprofilattico Spermantalle delle Venezie, Padova, Italy and the United States NAMRU-3 in Cairo, Egypt, also confirmed the presence of H5N1 virus. An active search for HPAI virus was conducted in the Tema Metropolis and as a result, three positive

cases were detected on three farms at Adjei Kojo. Ban on movement and sale of poultry and poultry products in the outbreak area was placed.

A total of 13,391 birds were affected and died naturally. Further more, 36,376 apparently healthy birds were destroyed on the infected farms. On May 15, 2007, a second outbreak of HPAI H5N1 virus was detected on a backyard farm at New Dormaa in the Sunyani Metropolis of the Brong Ahafo region. The third outbreak was reported at Aflao in the Ketu South district of the Volta region on the June 13, 2007.

The source of introduction of HPAI H5N1 virus into Ghana has not been traced. However, according to Mabbett (2007), the virus strain in Ghana was between 98.8% and 99.6% similar to other isolates from Burkina Faso, Cote d'Ivoire, Nigeria and Sudan.

2.9 MAJOR OUTBREAKS OF HPAI H5N1 IN WILD BIRDS

Prior to HPAI H5N1, reports of HPAI in wild birds were very rare. Species of wild birds, especially water birds, are susceptible to infection by the HPAI H5N1 virus. Close contact between poultry and wild birds can lead to cross-infection, from poultry to wild birds and from wild birds to poultry. Additionally, species that live in and around poultry farms and human habitations may serve as "bridge species" that could potentially transmit the virus between poultry and wild birds either by direct contact between wild birds and poultry kept outside or by indirect contact with contaminated materials. While there is no sound evidence that wild birds have carried the virus long distances on migration (Feare and Yasué 2006), analysis of genetic sequences and other largely indirect evidence suggests that wild birds are likely to have contributed to spread (Chen *et al*. 2005).The following table (Table 1) summarises the known major outbreaks of HPAI H5N1 in wild birds.

Year	Month(s)	Location(s)	Description of affected birds
2005	April	Qinghai Lake in central China	6345 waterbirds, the majority of which were Great Black-headed Gulls *Larus ichthyaetus*, Bar-headed Geese *Anser indicus* and Brown-headed Gulls *Larus brunnicephalus*
	August	Lake Erhel & Lake Khunt in Mongolia	89 waterbirds including ducks, geese and swans
	October – November	Romania & Croatia	Over 180 waterbirds, mainly swans
2006	**January**	Coastal area in the vicinity of Baku, Azerbaijan	Unspecified number of birds reported to the OIE as "various migratory birds"
	January – May	23 countries in Europe including Turkey and European Russia	Most cases occurred in ducks, geese and swans but a wide variety of species was infected including other waterbirds and raptors
	February	Rasht, Iran	153 wild swans
	May	Multiple locations in Qinghai province, China	Over 900, mainly waterbirds, and mostly Bar-headed Geese *Anser indicus*
	May	Naqu, Tibet	Over 2,300 birds – species composition unclear but 300 infected Bar-headed Geese *Anser indicus* were reported
	June	Lake Hunt in Bulgan, Mongolia	Twelve waterbirds including swans, geese and gulls
2007	June	Germany, France and the Czech	Over 290, mainly waterbirds, found mostly in Germany

Table 1. Major known outbreaks of highly pathogenic avian influenza H5N1 in wild birds

Source; OIE Disease Information Reports and German Friedrich-Leoffler Epid. bulletins

2.10 AVAIN INFLUENZA AND WETLANDS

Given the ecology of the natural hosts of LPAI viruses, it is unsurprising that wetlands play a major role in the natural epidemiology of avian influenza. As with many other viruses, avian influenza virions survive longer in colder water (Liu et al. 2003; Stallknecht et al. 1990), and the virus is strongly suggested to survive over winter in frozen lakes in Arctic and sub-Arctic breeding areas. Thus, as well as the waterbird hosts, these wetlands are probably permanent reservoirs of LPAI virus (Rogers et al. 2004; Smith et al. 2004) (re-)infecting water birds arriving from southerly areas to breed (shown in Siberia by Okazaki et al. 2000 and Alaska by Ito et al. 1995). Indeed, in some wetlands used as staging grounds by large numbers of migratory ducks, avian influenza viral particles can be readily isolated from lake water (Hinshaw et al. 1980).

An agricultural practice that provides ideal conditions for cross-infection and thus genetic change is used on some fish-farms in Asia: battery cages of poultry are placed directly over troughs in pig-pens, which in turn are positioned over fish farms. The poultry waste feeds the pigs, the pig waste is eaten by the fish or acts as a fertiliser for aquatic fish food, and the pond water is sometimes recycled as drinking water for the pigs and poultry (Greger 2007). These kinds of agricultural practices afford avian influenza viruses, which are spread via the faecal-oral route, a perfect opportunity to cycle through a mammalian species, accumulating the mutations necessary to adapt to mammalian hosts. Thus, as the use of such practices increases, so does the likelihood that new influenza strains infectious to and transmissible between humans will emerge (Culliton 1990; Greger 2007).

As well as providing conditions for virus mutation and generation, agricultural practices,

particularly those used on wetlands, can enhance the ability of a virus to spread. The role of Asian domestic ducks in the epidemiology of HPAI H5N1 has been closely researched and found to be central not only to the genesis of the virus (Hulse-Post *et al*. 2005; Sims 2007), but also to its spread and the maintenance of infection in several Asian countries (Shortridge *et al* 1998). Typically this has involved flocks of domestic ducks used for 'cleaning' rice paddies of waste grain and various pests, during which they can potentially have contact with wild ducks using the same wetlands. Detailed research (Gilbert *et al*. 2006; Songserm *et al*. 2006) in Thailand has demonstrated a strong

association between the HPAI H5N1 virus and abundance of free-grazing ducks.(Gilbert *et al*. 2006) concluded that in Thailand "wetlands used for double-crop rice production, where free-grazing duck feed year round in rice paddies, appear to be a critical factor in HPAI persistence and spread".

2.11 WILDLIFE CONSERVATION IMPLICATIONS

Prior to the outbreak of HPAI H5N1, reports of HPAI in wild birds were very rare. The broad geographical scale and extent of the disease in wild birds is both extraordinary and unprecedented, and the conservation impacts of HPAI H5N1 have been significant. It is estimated that between 5-10% of the world population of Bar-headed Goose Anser indicus died at Lake Qinghai, China in spring 2005 (Chen et al. 2005; Liu et al. 2005). At least two globally threatened species have been affected: Black-necked Crane, Grus nigricollis in China and Red-breasted Goose, Branta ruficollis in Greece. Approximately 90% of the world population of Red-breasted Goose is confined to just five roost sites in Romania and Bulgaria, countries that have both reported outbreaks, as also have Russia

and Ukraine where these birds also over-winter (Bird Life International 2007). However, the total number of wild birds known to have been affected has been small in contrast to the number of domestic birds affected, and many more wild birds die of commoner avian diseases each year. Perhaps a greater threat than direct mortality has been the development of public fear about waterbirds resulting in misguided attempts to control the disease by disturbing or destroying wild birds and their habitats. Such responses are often encouraged by exaggerated or misleading messages in the media. Currently, wildlife health problems are being created or exacerbated by unsustainable activities such as habitat loss or degradation, which facilitates closer contact between domestic and wild animals. Many advocate that to reduce risk of avian influenza and other bird diseases, there is a need to move to markedly more sustainable systems of agriculture with significantly lower intensity systems of poultry production. These need to be more biosecure, separated from wild waterbirds and their natural wetland habitats resulting in far fewer opportunities for viral cross-infection and thus pathogenetic amplification (Greger 2007). There are major animal and human health consequences (in terms of the impact on economies, food security and potential implications of a human influenza pandemic) of not strategically addressing these issues. However, to deliver such an objective in a world with an ever-growing human population and with issues of food-security in many developing countries, will be a major policy challenge (Sonaiya 2007, Roland-Holst *et al*., 2008).

2.12 CLINICAL PRESENTATION OF AI IN POULTRY

Following an incubation period of usually 3 to 5 days but rarely up to 21 days, depending upon the characteristics of the virulence of the infecting virus, species affected, sex and

age of the bird, the clinical presentation of avian influenza in birds is variable and symptoms are fairly unspecific (Elbers *et al* 2005). Therefore, a diagnosis solely based on the clinical presentation is impossible.

The symptoms following infection with low pathogenic AIV may be as discrete as ruffled feathers, transient reductions in egg production or weight loss combined with a slight respiratory disease (Capua and Mutinelli 2001). Some low pathogens (LP) strains such as certain Asian H9N2 lineages, adapted to efficient replication in poultry, may cause more prominent signs and also significant mortality (Bano *et al* 2003, Li 2005).

In its highly pathogenic form, the illness in chickens and turkeys is characterised by a sudden onset of severe symptoms such as depression, ruffled feathers, cyanotic comb and wattles, haemorrhages on the shanks and mortality can approach 100 % within 48 hours (Swayne and Suarez 2000). Spread within an affected flock depends on the form of rearing: in herds which are litter-reared and where direct contact and mixing of animals is possible, spread of the infection is faster than in caged holdings (Capua *et al* 2000). Also, many birds die without premonitory signs so that sometimes poisoning is suspected in the beginning (Nakatami *et al* 2005). It is worth noting, that a particular HPAI virus isolate may provoke severe disease in one avian species but not in another.

In industrialised poultry holdings, a sharp rise followed by a progressive decline in water and food consumption can signal the presence of a systemic disease in a flock. In laying flocks, a cessation of egg production is apparent. Individual birds affected by HPAI often reveal little more than severe apathy and immobility (Kwon *et al* 2005). Oedema, visible at feather-free parts of the head, cyanosis

of comb, wattles and legs, greenish diarrhoea and laboured breathing may be inconsistently present. In layers, soft-shelled eggs are seen initially, but any laying activities cease rapidly with progression of the disease (Elbers *et al* 2005). Nervous symptoms including tremor, unusual postures (torticollis), and problems with co-ordination (ataxia) dominate the picture in less vulnerable species such as ducks, geese, and ratites (Kwon *et al* 2005). During an outbreak of HPAI in Saxonia, Germany, in 1979, geese compulsively swimming in narrow circles on a pond were among the first conspicuous signs leading to a preliminary suspicion of HPAI.

2.13 PATHOLOGY OF AI

Birds that die of peracute disease may show minimal gross lesions, consisting of dehydration and congestion of viscera and muscles In birds that die after a prolonged clinical course, petechial and ecchymotic haemorrhages occur throughout the body, particularly in the larynx, trachea, proventriculus and epicardial fat, and on serosal surfaces adjacent to the sternum. There is extensive subcutaneous oedema, particularly around the head and hocks. The carcass may be dehydrated. Yellow or grey necrotic foci may be present in the spleen, liver, kidneys and lungs. The air sac may contain an exudate. The spleen may be enlarged and haemorrhagic (Perkins and Swayne 2003)

Avian influenza is characterised histologically by vascular disturbances leading to oedema, haemorrhages and perivascular cuffing, especially in the myocardium, spleen, lungs, brain and wattles. Necrotic foci are present in the lungs, liver and kidneys. Gliosis, vascular proliferation and neuronal degeneration may be present in the brain (Perkins and Swanyne 2003, Kwon *et al* 2005, Brojer *et al* 2009).

2.14 DIFFERENTIAL DIAGNOSIS OF AI FROM OTHER DIEASES

clinically, the less severe forms of AI may be confused with many other respiratory or enteric diseases in poultry (Elbers et al 2005). However, in the laboratory, AI can only be differentiated from other acute poultry respiratory diseases such as Newcastle, infectious laryngotracheitis, duck plague, acute fowl cholera and other septicemia diseases, by serological tests (Agar gel-diffusion test) or virus isolation and molecular detection by the RT-PCR (Animal health advisory leaflet 8; 1996). Avian Influenza should be suspected in any disease outbreak in poultry that persists despite the application of preventive and therapeutic measures for other diseases (Elbers et al 2005).Newcastle disease which has very similar signs and lesions as AI is characterized by the sudden onset of watery discharge from the nostrils, labored breathing, facial swelling, paralysis, trembling and twisting of the neck. Mortality ranges from 10 to 80% depending on host immunity. It causes also, drastic reduction in egg-laying and production of soft-shelled eggs. Lesions of Newcastle disease include hemorrhages of peyers patches in the intestines, hemorrhages in the ovaries, proventiculus, intestine lining and caecal tonsils.

2.15 LABORATORY DIAGNOSTIC PROCEDURES OF AVIAN INFLUENZA

2.15.1 COLLECTION OF SPECIMENS

Specimens should be collected from several fresh carcasses and from diseased birds of a flock. Ideally, adequate sampling is statistically backed up and diagnosis is made on a flock basis. When sampling birds suspected of HPAI, safety standards must be observed to avoid exposure of the sample collectors to potentially zooanthroponotic HPAIV

(Bridges *et al* 2002). Guidelines have been established by the CDC (CDC 2005).For virological assays, swabs obtained from the cloacal and the oropharynx generally allow for a sound laboratory investigation. The material collected on the swabs should be mixed into 2-3 ml aliquots of a sterile isotonic transport medium containing antibiotic supplements and a protein source (e.g. 0.5 % [w/v] bovine serum albumin, up to ten percent of bovine serum or a brain-heart infusion).At autopsy, carried out under safe conditions and avoiding spread of disease, unpreserved specimens of brain, trachea/lung, spleen and intestinal contents are collected for isolation of the virus.For serological purposes, native blood samples are taken. The number of samples collected should surffice detection with a 95 % confidence interval for a parameter with a prevalence of 30 % (CDC, 2005).

2.15.2 TRANSPORTATION OF SPECIMENS TO THE LABORATORY

Swabs, tissues and blood should be transported chilled but not be allowed to freeze. If delays of greater than 48 hours are expected in transit, these specimens should be frozen and transported on dry ice. In all cases, transport safety regulations should be punctiliously observed to avoid spread of the disease and accidental exposure of personnel during transport. It is highly advisable to contact the assigned diagnostic laboratory before sending the samples and, ideally, even before collecting them.

2.15.3 DIRECT DETECTION OF AVIAN INFLUENZA VIRUS INFECTION

Basically, there are two (parallel) lines of diagnostic measures that attempt to (a) isolate and subtype the virus by classical methods (OIE Manual 2005) and (b) molecularly detect and characterize the viral genome (Ng et al, 2005).

Conventionally, AI virus is isolated by inoculation of swab fluids or tissue homogenates into 9- to 11-day-old embryonated chicken eggs, usually by the chorioallantoic sac route (Woolcock *et al* 2001). Depending on the pathotype, the embryos may or may not die within a five-day observation period and usually there are no characteristic lesions to be seen in either the embryo or the allantois membrane (Mutinelli *et al* 2003b). Eggs inoculated with HPAIV-containing material usually die within 48 hours. The presence of a haemagglutinating agent can be detected in harvested allantoic fluid.

Haemagglutination (HA) is an insensitive technique requiring at least $10^{6.0}$ particles per ml. If only a low virus concentration is present in the inoculum, up to two further passages in embryonated eggs may be neccessary for some LPAIV strains, in order to produce enough virus to be detected by HA. In the case of HPAIV, a second passage using diluted inoculum may be advantageous for the optimal production of haemagglutination (Woolcock *et al* 2001).

Haemagglutinating isolates are antigenically characterised by haemagglutination inhibition (HI) tests using (mono-) specific antisera against the 16 H subtypes and, for control, against the different types of avian paramyxoviruses which also display haemagglutinating activities. The NA subtype can be subsequently determined by neuraminidase inhibition assays, again requiring subtype-specific sera (Aymard *et al* 2003). In case isolates of the H5 or H7 lineages are encountered, their intravenous

pathogenicity index (IVPI) needs to be determined to distinguish between LP and HP biotypes (Allan *et al* 1977). This is achieved by inoculation of ten 6-week old chickens with the egg-grown virus isolate (0.1 ml of a 1 in 10 dilution of allantoic fluid containing an HA titre greater than 1 in 16). The chickens are observed over a period of ten days for clinical symptoms. Results are integrated into an index which indicates a HPAI virus when values greater than 1.2 are obtained.

Alternatively, a HPAI isolate is encountered when at least seven out of ten (75 %) inoculated chickens die within the observation period (Hoffmann *et al* 2006).

The classical procedures can lead to a diagnosis of HPAI within five days but may demand more than a fortnight to rule out the presence of AIV. In addition, high quality diagnostic tools (SPF eggs, HA- and NA-subtype specific antisera) and skilled personnel are a prerequisite. Currently, cell culture applications for the isolation of AIV that can achieve the sensitivity of embryonated hen eggs require the use of Biosafety Level 3 laboratory facilities.

A more rapid approach, especially when exclusion of infection is demanded, employs molecular techniques, which could also follow a cascade style: the presence of influenza A specific RNA is detected through the reverse transcription-polymerase chain reaction (RT-PCR assay) which targets fragments of the M gene, the most highly conserved genome segment of influenza viruses (Fouchier *et al* 2000, Spackman *et al* 2002), or the nucleocapsid gene (Dybkaer *et al* 2004). When a positive result is obtained, RT-PCRs amplifying fragments of the haemagglutinin genes of subtypes H5 and H7 are then

conducted to detect the presence of notifiable AIVs (Dybkaer *et al* 2004, Spackman *et al* 2002, Oraveerakul *et al* 2004). Then, a further molecular diagnosis of the pathotype (LP versus HP) is feasible after sequencing a fragment of the HA gene spanning the endoproteolytic cleavage site. Isolates presenting with multiple basic amino acids are classified as HPAI.

Various techniques have been developed for the detection of Asian lineage H5N1 strains (Collins *et al* 2002, Payungporn *et al* 2004). Non-H5/H7 subtypes can be identified by a canonical RT-PCR and subsequent sequence analysis of the HA-2 subunit (Phipps *et al* 2004). There are also specific primers for each NA subtype. A full characterisation might be achievable within three days, especially when real time PCR techniques are used (Perdue 2003, Lee *et al* 2004). However, DNA chips could also be used for the typing of AI viruses (Li 2001, Kessler *et al* 2004). An exclusion diagnosis is possible within a single working day.

The disadvantages of molecular diagnostics is the cost of equipment, reagents and consumables, although, if available, many samples can be analysed by less personnel in shorter times in comparison to virus isolation in eggs. However, it is a well established fact that each PCR or hybridisation reaction, in contrast to virus isolation in eggs, harbours an intrinsic uncertainty related to the presence of specific mutations in a given virus at the binding sites of primers and/or probes, which might produce a false negative results. Thus, a combination of molecular (e.g. for screening purposes) and classical methods (e.g. for final characterisation of isolates and confirmation of diagnosis of an index case) may help to counterbalance the disadvantages of the two principles.

Rapid assays have been designed for the detection of viral antigen in tissue impression smears and cryostat sections by use of immunofluorescence, or by antigen-capture enzyme-linked immunosorbent assay (ELISA) and dip-stick lateral flow systems in swab fluids. So far, these techniques have been less sensitive than either virus isolation or PCR, and therefore might be difficult to approve for a legally binding diagnosis, especially of an index case (Davison *et al* 1998, Selleck 2003, Cattoli *et al* 2004).

2.15.4 INDIRECT DETECTION OF AVIAN INFECTION VIRUS INFECTION

Serology on a herd basis may be useful for screening purposes (Beck *et al* 2003). For the detection of AIV-specific antibodies in serum samples from birds, or in egg yolk in the case of laying flocks, the haemagglutination inhibition (HI) assay using reference subtype antigens still represents the gold standard test. Group-specific antibodies (influenza virus type A) against the nucleocapsid protein can also be detected by agar gel immunoprecipitation and by enzyme-linked immunosorbent assays (ELISA) (Meulemans *et al* 1987, Snyder *et al* 1985, Jin *et al* 2004). Competitive ELISA formats allow the examination of sera of all bird species, independent of the availability of species-specific conjugates (Shafer 1998, Zhou *et al* 1998). An ELISA format for the detection of H7-specific antibodies has been reported (Sala *et al* 2003), but there is no commercial assay presently available for the detection of H5-specific antibodies in avian sera.Subtype-specific antibody kinetics depend on the viral strain characteristics and, primarily, on the host species. In gallinaceous birds, AIV-specific antibodies reliably become detectable during the second week following exposure; antibodies in egg yolk are detectable after a

delay of a few days (Beck *et al* 2003). The production and detection of antibodies in *Anatidae* species are much more variable (Swayne and Suarez 2003).

2.16 CONTROL MEASURES AGAINST HIGHLY PATHOGENIC AVIAN INFLUENZA (HPAI)

Due to its potentially devastating economic impact, HPAI is subject world-wide to vigilant supervision and strict legislation (OIE Terrestrial Animal Health Code 2005). Measures to be taken against HPAI depend on the epidemiological situation of the region affected. In the European Union (EU) where HPAIV is not endemic, prophylactic vaccination against avian influenza is generally forbidden. Thus, outbreaks of HPAI in poultry are expected to be conspicuous due to the clinically devastating course of the disease. Consequently, when facing such an outbreak, aggressive control measures, e.g. stamping out affected and contact holdings, are put in place, aiming at the immediate eradication of HPAI viruses and containing the outbreak at the index holding (OIE 2008).

For these purposes, control and surveillance zones are erected around the index case with diameters varying from nation to nation (3 and 10 kilometres, respectively, in the

European Union). The quarantining of infected and contact farms, rapid culling of all infected or exposed birds, and proper disposal of carcasses, are standard control measures to prevent lateral spread to other farms (OIE – Terrestrial Animal Health Code 2005). It is pivotal that movements of live poultry and also, possibly, poultry products, both within and between countries, are restricted during outbreaks.

In addition, control of H5 and H7 subtypes of LPAI in poultry, by testing and culling of acutely infected holdings, may be advisable in non-endemic areas in order to reduce the risk of a de novo development of HPAIV from such holdings.

Specific problems of this eradication concept may arise in areas with a high density of poultry populations (Marangon *et al* 2004, Stegemann *et al* 2004, Mannelli *et a* 2005) and where small backyard holdings of free roaming poultry prevail (Witt and Malone 2005). Due to the close proximity of poultry holdings and intertwining structures of the industry, spread of the disease is faster than the eradication measures. Therefore, during the Italian outbreak of 1999/2000 not only infected or contact holdings were destroyed, but also flocks with a risk of infection within a radius of one kilometre from the infected farm were pre-emptively killed. Nevertheless, eradication required four months and demanded the death of 13 millions birds (Capua *et al* 2003).

The creation of buffer zones of one to several kilometres around infected farms completely devoid of any poultry was also behind the successful eradication of HPAIV in the Netherlands in 2003 and in Canada in 2004. So, not only the disease itself, but also the pre-emptive culling of animals led to losses of 30 and 19 million birds, respectively. In 1997, the Hong Kong authorities culled the entire poultry population within three days (on the 29th, 30th, and 31st December; 1.5 million birds). The application of such measures, aimed at the immediate eradication of HPAIV at the cost of culling also non-infected animals, may be feasible on commercial farms and in urban settings. However, this will afflict the poultry industry significantly and also prompts ethical concern from

the public against the culling of millions of healthy and uninfected animals in the buffer zones.

Such measures are most difficult to implement in rural areas with traditional forms of poultry holdings where chickens and ducks roam freely and mingle with wild birds or share water sources with them. Moreover, domestic ducks attract wild ducks and provide a significant link in the chain of transmission between wild birds and domestic flocks (WHO 2005). These circumstances may provide the grounds for HPAI viruses to gain an endemic status.

Endemicity of HPAI in a certain region imposes a constant pressure on poultry holdings. As the above mentioned restrictions can not be upheld over prolonged periods without vital damage to a country's poultry industry or, in the developing world, leading to a serious shortage of protein supply for the population, other measures must be considered.

Vaccination has been widely used in these circumstances and may also be a supplementary tool in the eradication process of outbreaks in non-endemic areas.

2.17 VACCINATION

In the veterinary medicine, vaccination pursues four goals: (i) protection from clinical disease, (ii) protection from infection with virulent virus, (iii) protection from virus excretion, and (iv) serological differentiation of infected from vaccinated animals (DIVA principle).

In the field of influenza vaccination, neither commercially available nor experimentally tested vaccines have been shown so far to fulfill all of these requirements (Lee *et al*

2005). The first aim, which is the protection from clinical disease induced by HPAIV, is achieved by most vaccines. The risk of infection of vaccines with and excretion of, virulent field virus is usually reduced but not fully prevented.

This may cause a significant epidemiological problem in endemic areas where exhaustive vaccination is carried out: vaccinated birds which appear healthy may well be infected and excrete the field virus 'under cover' of the vaccine.The effectiveness of reduction of virus excretion is important for the main goal of control measures, that is, the eradication of virulent field virus. The effectiveness can be quantified by the replication factor r0. Assuming a vaccinated and infected flock passes on the infection on average to less than one other flock ($r0 < 1$), the virulent virus is, on mathematical grounds, prone to be extinguished (van der Goot *et al* 2005). When dealing with vaccination against the potentially zoonotic H5N1 virus, reduction of virus excretion also reduces the risks of transmission to humans, since a significant dose of virus seems to be required to penetrate the species barrier between birds and humans. Also, a DIVA technique allows the tracing of field virus infections by serological means in vaccinated birds.

Various vaccine concepts have been developed. Most are still based on inactivated, adjuvant whole virus vaccines which need to be applied by needle and syringe to each animal separately.

Inactivated homologous vaccines, based on the actual HPAI strain, induce proper protection but do not allow a distinction between vaccinated and infected birds serologically.Since the vaccine is made from the current HPAI virus, there is an inherent delay before such vaccines can be used in the field.

Inactivated heterologous vaccines, in contrast, can be used as marker vaccines when the vaccine virus expresses the same HA- but a different NA-subtype compared to the field virus (e.g. H5N9 vaccine vs. H5N2 HPAI). By detection of NA subtype-specific antibodies, vaccinated and infected birds can be distinguished (Cattoli *et al* 2003). However, these methods can be laborious and may lack sensitivity. Nevertheless, such vaccines can be kept in vaccine banks comprising several H5- and H7-subtypes with discordant NA subtypes. Reverse genetics will greatly aid in producing vaccines both for veterinary and medical use with the desired HxNy combinations in a favourable genetic background (Liu *et al* 2003, Neumann *et al* 2003, Lee *et al* 2004, Chen *et al* 2005, Stech *et al* 2005). Currently, inactivated heterologous vaccines have been used the H5N1 hot spots of South East Asia as well as in Mexico, Pakistan and Northern Italy (Garcia 1998, Swayne *et al* 2001). As an alternative DIVA system for use with inactivated vaccines, the detection of NS-1 specific antibodies has been proposed (Tumpey *et al* 2005). These antibodies are generated at high titres by naturally infected birds, but at considerably lower titres when inactivated vaccines are used.

Recombinant live vector-engineered vaccines express an H5 or H7 HA gene from the backbone of viruses or bacteria capable of infecting poultry species (e.g. fowl pox virus [Beard 1991, Swayne *et al* 1997+2000c], laryngotracheitis virus [Lueschow *et al* 2001, Veits *et al* 2003] or Newcastle Disease virus [Swayne 2003] among others). Being live vaccines, mass application via water or sprays is often feasible. While allowing for a clear-cut DIVA distinction, a pre-existing immunity towards the vector virus, however, will grossly interfere with vaccination success. Some field experience with fowl pox recombinants has been collected in Mexico and the U.S.

Finally, successful use of recombinantly expressed HA proteins and of DNA vaccination using HA-expressing plasmids has been experimentally proven (Crawford *et al* 1999, Kodihalli *et al* 1997). Vaccination has been used on a nationwide scale in several countries in South East Asia (Normile 2005).

2.18 BIO-SECURITY MEASURES

The aim of a bio-security programme is to reduce sources and causes of contamination to enable the supply of a healthy, safe and reliable product (Artois *et a*l 2009). According to the Department for Environment, Food and Rural Affairs (Defra 2007), guidelines, Institute de Selection Animale (ISA) of Netherlands outlines the following, bio-security programme for effective control of poultry diseases. These include a buffer zone and clean area.

Persons entering clean area should at least change shoes and clothes. Also, equipment entering the clean area should be cleaned and disinfected. Again, all materials entering this zone should be stored for at least two days in a clean, dry and rodents freed room.

Visitors entering poultry premises should fully understand proper hygienic procedures and records must be kept of all visitors. Farm workers must wash their hands thoroughly with soap after any farm activity. Also, care should be taken at all times to protect the health and safety of farm workers and visitors (OIE Guidelines).

Poultry houses must be cleaned and disinfected after emptying them. Footbaths should be replenished with additional disinfectant daily to maintain sufficient depth and the whole contents of the bath renewed once soiled or at least twice a week (Defra 2007).

CHAPTER THREE

METHOD

3.1 STUDY AREA

The study took place in the Tema Metropolis, located along the eastern coast of Ghana covering an area of 396 square kilometers. It lies within the coastal savannah zone and has a vegetation of grassland and shrub land. Tema metropolis is flat, rising from the coast to 35 meters above sea level. There are a few i*nselbergs* that do not rise more than 65 meters above sea level. Average rainfall is 700 millimeters. It is boarded on the north by Ashaiman, to the south by the Atlantic Ocean, to the East and West by Dangbe West District and Accra Metropolitan Area respectively.

Tema has an estimated total population of 511,459 people according to the 2000 census and less than 10% of the population lives in rural communities (Ghana Statistical Services 2000). Also, it is the major industrial zone of Ghana. It has several communal natural resources such as the Chemu and Sakumono lagoons.

Poultry production in Tema Metropolis could be classified into three categories according to installed capacity, marketing system and level of integration of its operations. These are commercial farms, semi-commercial farms and backyard/village poultry producers. It has an estimated total poultry population of 696,694 according to 2009 VSD annual report.

These are made up of 534,852 layer chicken, 100,586 broiler chicken, 40,492 local chicken and 11,930 cockerels. The rest are 4,434 ducks, 2,920 turkeys and 1,480 guinea fowls. The metropolis also, has two poultry hatcheries and four commercial feed

processing plants namely; Ghana Agro Food Company, Afariwaa Farms, Central Feed Mills and Glamour Farms.

On animal health services delivery, the study area has one government veterinary hospital and two private veterinary hospitals that provide regulatory, field and clinical services.

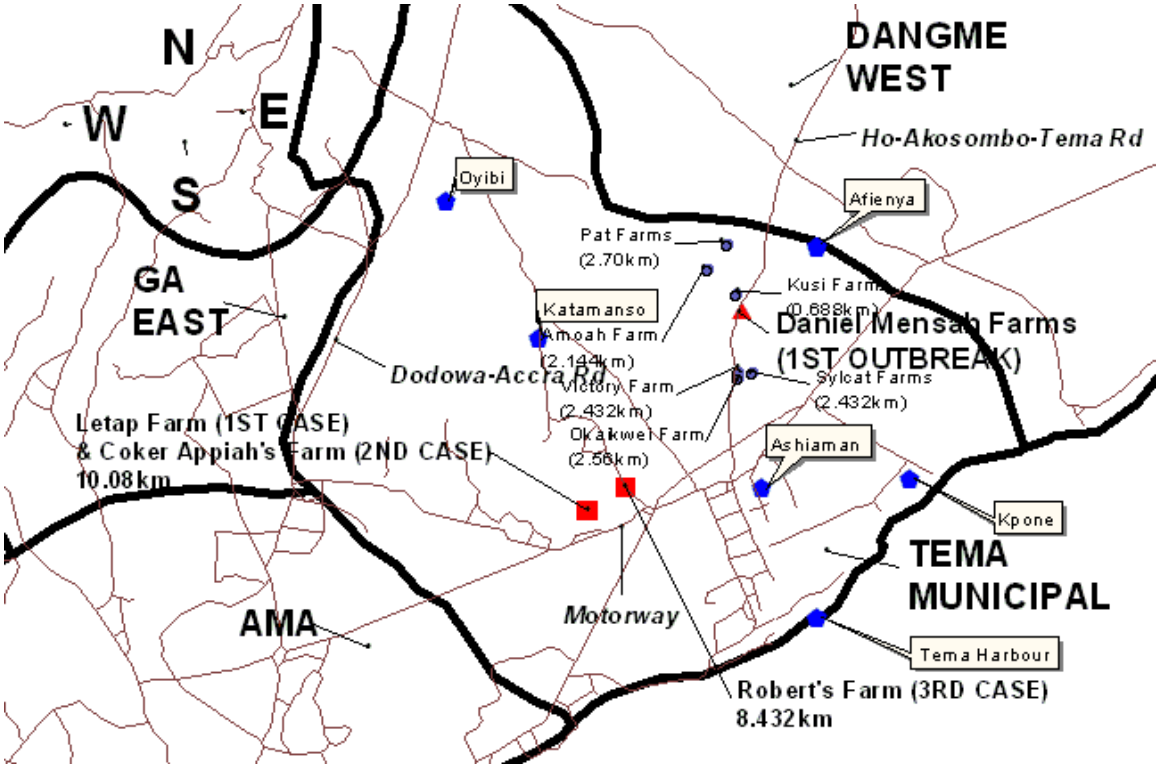


Figure 4. Map of first outbreak areas for HPAI in Ghana (source VSD, 2007 reports)

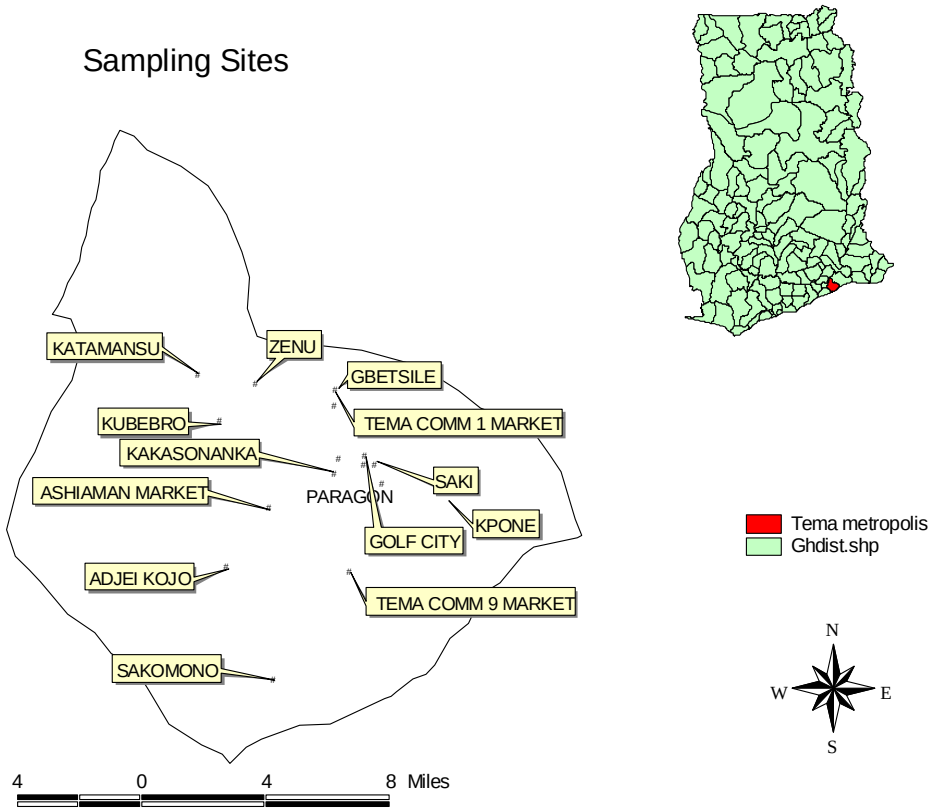


Figure 5. Sampling sites in Tema Metropolis (Source, VSD, 2009)

Commercial poultry and backyard poultry were sampled from Kakasunanka, Bethlehem, Golf City, Serbrepor, Gbetsile, Adjei Kojo, Michelle camp, Saki, Kpone, Kubekro, Zenu and Katamansu. Live bird markets in this study were located at Tema community one, Tema community nine and Ashiaman. Again, all wild birds sampled came from Sakumono Ramsar site.

The reasons for the choice of Tema Metropolis were that it had experienced an outbreak of Avian Influenza in April 2007 which affected four commercial farms at Kakasunanka

and Adjei Kojo. Thirteen thousand, three hundred and ninety one (13,391) birds were affected and thirty six thousand, three hundred and seventy six (36,376) birds were

destroyed. The outbreak necessitated the institution of further control measures such as quarantine, disinfection and temporal ban of movement and sale of birds and poultry products in and out of the affected areas. The situation was brought under control by September, 2007 when Tema was declared free of the H5N1 virus.

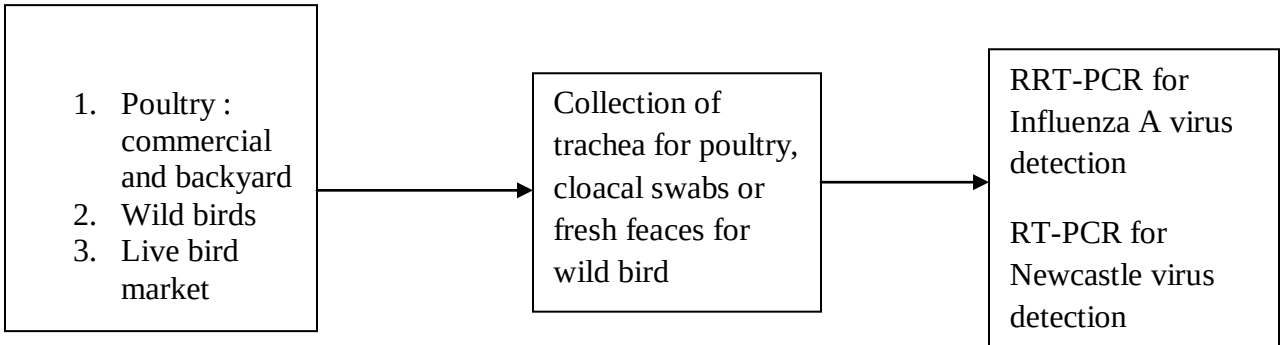
The choice of Tema was also informed by the fact that it is a port city with various commercial activities.

3.2 STUDY DESIGN

The study type was a descriptive and cross sectional using active avian influenza surveillance approach. Thus, the study obtained tracheal specimens from commercial poultry, backyard poultry and live bird markets while cloacal and fresh feaces were obtained from wild birds and ducks for reasons of these being the natural reservoir of avian influenza viruses (Webster 1992, Fouchier *et al* 2003, Krauss *et al* 2004, Widjaja *et al* 2004).

The study also employed both qualitative and quantitative methods of data collection. The qualitative method was used to capture information that was not catered for in the quantitative technique. It was also used to validate findings obtained through quantitative method.

Figure 6: Active Avian Influenza Surveillance



3.3 SAMPLE SIZ

SAMPLE SIZE DETERMINATION

Using the following formula, the sample size was calculated in Epi info version 3.4.1 at 95% confidence level, and prevalence of Avian Influenza among domestic poultry, back yard poultry and wild life birds of 0.5

$$N=\frac{z^2\, p(1-p)}{d^2}$$

Where

N= sample size

z= risk of Type 1 error= 1.96 (at 95% confidence level)

p= prevalence of Avian Influenza= 0.5 (assumed as no previous study to provide reference)

d= precision= 5%

Minimum sample size= 384.

Commercial poultry, backyard poultry and live bird markets accounted for 384 birds each while 121 wild birds were sampled. This was in accordance with the recommendations made by Beal (1983) and Wildlife Health Center and Cooperative Extension, that for wildlife flocks of more than 2000 wild birds at 95% confident level the sample size should be a minimum of 58 birds.

3.4 SAMPLING PROCEDURE

A simple random sampling procedure was used to select communities in the metropolis. Also, using criteria for eligibility based on birds whether apparently healthy or with respiratory signs or gastroenteritis or nervous illness, were conveniently selected for tracheal swabbing, cloacal swabbing, collection of fresh faecal and identification in the case of commercial poultry, backyard poultry and the live bird markets. With regards to the wild birds, mist nets were used to capture wild birds. The captured ones were then marked to avoid recounting upon recapture of the same bird. Cloacal swabbing or collection of fresh faeces and identification were made before release of the birds. In this study, data were collected by reviewing available farm records. Poultry farm owners, poultry farm workers, poultry traders and staff of Wildlife Division of Forestry Commission were interviewed and a semi-structured questionnaire was administered for data collection. All specimens were put in vials containing viral transport medium (VTM) and transported on ice in cool boxes for laboratory analyses at the Virology Department of the Noguchi Memorial Institute for Medical Research.

3.5 MOLECULAR DETECTION

3.5.1 RNA EXTRACTION

Ribonucleic acid was extracted from samples with the QIAamp viral RNA mini kit 250 (Qiagen, GmbH). Briefly, 560 µl of prepared buffer (viral lysis buffer plus RNA carrier) was added to 140 µl of trachea, cloacal and faecal samples obtained from domestic poultry and wild birds in the Tema Metropolis. It was then mixed by pulse vortexing for 15 seconds and then left to stand at room temperature for 10 minutes. Also, it was mixed

by pulse vortexing with 560 µl of absolute ethanol. Then, 630 µl of the mixture was clarified by centrifugation at 8000 xg for 1 minute and repeated. The filtrate tube was discarded and column tube placed in a new collection tube. Then, 500 µl of wash buffer 1 was added and spun at 8000 xg for 1 minute. Again, the filtrate tube was discarded and the spin column placed in a new collection tube. Five hundred µl of wash buffer 2 was added and spun at 14000 xg for 3 minutes. Then, the filtrate was discarded and a new collection centrifuged at 14000 xg for 1 minute. Finally, the RNA was eluted in 40 µl twice with a final product of 80 µl. This was stored in a -70^{0}C ultra low freezer thermo scientific at the P3 laboratory corridor, Noguchi Memorial Institute for Medical Research and 8 µl of RNA was used as template for the real time RT-PCR.

3.5.2 REAL-TIME REVERSE TRANSCRIPTASE-POLYMERASE CHAIN REACTION (RRT-PCR)

The Qiagen one step RT-PCR kit (Protocol: Influenza A Matrix gene AB17300 RT-PCR Spackman *et al.*, 2002) was used with a 20 µl reaction volume under the following amplification cycling conditions; 0.8 µl of Qiagen one-step enzyme (Qiagen GmbH), 10 pmol of each primer, 0.3 µM probe, 0.8 µl of deoxynucleoside triphosphate (dNTPs), 1 µl magnesium chlorite ($MgCl_2$), 4 µl of 5X buffer of Qiagen kit, 1 µl of ROX dye working dilution (1:100) and 6.5 U of RNase inhibitor.

The RT step conditions for forward and reverse primer set were 30 minutes at 50^{0}C and 15 minutes at 94^{0}C. A two-step PCR cycling protocol was used for the matrix gene primer set as follows; 45 cycles of 94^{0}C for 0 seconds and 60^{0}C for 20 seconds. The H5

PCR was as follow; 94^0C for 0 seconds, 57^0C for 20 seconds and 72^0C for 5 seconds for 40 cycles. Primers used for this amplification are listed in table 2.

Table 2. PCR primer and probe sequences for Influenza A virus detection

Specificity	Primer/Probe	Sequence"(5'-3')
Influenza A virus	M + 25	AGA TGA GTC TTC TAA CCG AGG TCG
	M - 124	TGC AAA AAC ATC TTC AAG TCT CTG
	M + 64	FAM-TCA GGC CCC CTC AAA GCC GA-TAMRA
Avian H5	H5+1456	ACG TAT GAC TAT CCA CAA TAC TCA G
	H5-1685	AGA CCA GCT ACC ATG ATT GC
	H5+1637	FAM-TCA ACA GTG GCG AGT TCC CTA GCA-TAMRA
Avian H7	H7+1244	ATT GGA CAC GAG ACG CAA TG
	H7- 1342	TTC TGA GTC CGC AAG AC TAT TG
	H7 + 1281	FAM-TAA TGC TGA GCT GTT GGT GGC A-TAMRA

FAM, 6-carboxyfluorescein; TAMRA, 6-carboxytetralrhodamine.

Source; Spackman et al 2002

RRT-PCR was performed by an advanced pathogen identification device called Applied Biosystems 7300 Real Time PCR System. Results of RRT-PCR were determined by the device autoanalysis software with a positive control cycle threshold (Ct) at 20.45 and a negative control Ct at undetectable (table 10)

3.5.3 CONVENTIONAL RT-PCR FOR NEWCASTLE DISEASE VIRUS (NDV)

Conventional RT-PCR NDV was performed as described in the field and laboratory manual (chapter 11.3). Briefly, the reaction conditions used were as follow; 50^0C for 30 minutes and 94^0C for 15 minutes with annealing temperature of 94^0C for 30 seconds, 55^0C for 1 minute and 68^0C for 1 minute on a 35 cycle run with a final extension of 68^0C for 7 minutes. Fragment size at 270bp (Capua and Alexander. Avian Influenza and Newcastle Disease. A field and laboratory Manual. Chapter 11.3).

Conventional RT-PCR NDV was by Applied Biosystems 2720 Thermal Cycler.

Primers used for this cycle were as follow;

Primer Forward: NOH-For 5' TAC ACC TCA TCC CAG ACA GG 3'

Primer Reverse: NOH-Rev 5' AGT CGG AGG ATG TTG GCA GC 3'

3.6 DATA PROCESSING AND ANALYSIS

Data collected was checked for accuracy, completeness and consistency. The data was coded and fed into SPSS statistic version 17.0 software programme. Also, cleaning of data was ensured by double entry and reconciliation of these entries. Simple frequencies, percentages, graphs and tables were then generated.

3.7 ETHICAL CLEARANCE

Scientific review of the study protocol was sort from the Scientific Technical Committee of the Noguchi Memorial Institute for Medical Research. Contacts were made with Tema Metropolis veterinary officer, Wildlife Division of Forestry Commission; poultry farm owners, local bird owners and poultry traders in the selected communities of the metropolis for their permission and informed consent (Appendix I).

3.8 LIMITATIONS

Financial constraints were the major challenge. Also, late arrival of materials and reagents resulted in the delay in completing the study on scheduled. Again, lack of prevalence data on avian influenza disease in Ghana was a limitation as that could have affected the sample size.

CHARPTER FOUR

RESULTS

4.1 DESCRIPTIVE CHARACTERISTIC OF BIRDS

A total number of one thousand two hundred and eighty two different birds were sampled from 16 different places within 2.5 kilometers to18 kilometers of the Tema Metropolis. Out of these, 384 samples were from Commercial poultry farms, 393 samples were from Backyard farms, 384 samples were from Tema Community One, Tema Community Nine and Ashiaman live birds markets and 121samples were from wild birds at Sakumono Ramsar site (Table 3).

For commercial poultry, tracheal samples were obtained from layer chicken (*Gallus gallus domesticus*). In the backyard and live bird markets, tracheal swabs from local fowls (*Gallus gallus domesticus*), guinea fowls (*Numida meleagris*), pigeons (*Family Columbidea*), turkeys (*Meleagris gallopavo*) and cloacal swabs from ducks (*Anas platyrhynchos domesticus*) were sampled. For the wild birds, cloacal swabs were obtained from white-faced tree duck (*Dendrocygna viduota)*, black-winged stilt (*Himantopus himantopus*), white-backed stilt (*Himantopus himantopus meleaurus*), black tern (*Chlidonias niger*), quails (*Coturnix coturnix*), prantcole (*Glareola nordmanni*), little egret (*Egretta garzetta*), sand plover (*Charadrius mongolus*), spur-winged plover

(*Vanellus spinosus*), ringed plover (*Charadrius hiaticula*), village weaver (*Ploceus cucullatus*), pygmy cormorant (*Phalacrocorax pygmae*us) and goliath heron (A*rdea goliath*) (Table 4).

About, 91% (349/384) of birds sampled from commercial poultry farms were females while 9% (35/384) were males. The sex distribution of the backyard poultry was found out to be at 72% (283/393) for females and 28% (110/393) for males. Again, the study observed 69% (265/384) females and 31% (119/384) males in the case of the poultry birds in live bird markets. However, all wild birds sampled at the Sakumono ramsar lagoon, sex identification was not done. This was due to the fact that there was no expert to determine the sex of the wild birds. We found out that 15% of the farms sampled had grower age range (less than 18 weeks for commercial birds and less than 24 weeks for backyard birds) and 85% had adult birds (more than 18 weeks for commercial birds and more than 24 weeks for backyard birds) at the time of sampling.

Table 3. Descriptive characteristics of Birds

		Count	Percent
Flock Grouping	Commercial	384	30%
	Backyard	393	30.6%
	Live Bird Market	384	30%
	Wild Bird	121	9.4%
	Total	1282	100%
Age Range	Grower	192	15%
	Adults	1090	85%
	Total	1282	100%
		Male	Female
Sex	Commercial	9%(35/384)	91%(349/384)
	Backyard	28%(110/393)	72%(283/393)
	Live Bird Market	31%(119/384)	69%(265/384)
	Wild Bird	Unknown	Unknown

*Wild birds could not be sexed

Table 4. Descriptive characteristic of birds (common/scientific name)

System of Production	Common Name	Scientific Name
Commercial Poultry	Broiler and Layer Chicken	*Gallus gallus domesticus*
Backyard Poultry	Local fowls	*Gallus gallus domesticus*
	Ducks	*Anas platyrhynchos domesticus*
	Guinea fowls	*Numida meleagris*
	Turkey	*Meleagris gallopavo*
Live Bird Market	Local fowls	*Gallus gallus domesticus*
	Ducks	*Anas platyrhynchos domesticus*
	Guinea fowls	*Numida meleagris*
	Turkey	*Meleagris gallopavo*
	Doves/Pigeons	*Family Columbidea*
Wild Birds	White-faced tree duck	*Dendrocygna viduota*
	Black-winged stilt	*Him antopus himantopus*
	White-backed stilt	*Himantopus himantopus melanurus*
	Black tern	*Chlidonias niger*
	Quails	*Coturnix coturnix*
	Pranticole	*Glareola nordmanni*
	Little egret	*Egretta garzetta*
	Sand plover	*Characdrius mongolus*
	Ringed plover	*Charadrius hiaticula*
	Spur-winged plover	*Vanellus spinosus*
	Village weaver	Ploceus cucullatus
	Pygmy cormorant	Phalacrocorax pygmeaus

Note: (1) Live bird markets in this study were located at Tema community one, Tema community nine and Ashiaman.

(2) Wild birds were sampled from Sakumono Ramsar site

(3) Commercial poultry and backyard poultry were sampled from Kakasonanka, Bethlehem, Golf city, Serbrepor, Gbetsile, Adjei Kojo, Michelle camp, Saki, Kpone, Kubekro, Zanu and Katamansu

In reviewing the total stock of birds per farm, the study found that in the one hundred and five (105) farms sampled, eight percent (8%) had less than ten birds, while 56.8% farms kept between ten (10) and ninety nine (99) birds. Also, 18.2% of the farms in the study area had a stock populations of between one hundred (100) and nine hundred and ninety nine (999) birds. Farms found to be keeping more than thousand (>1000) birds accounted for seventeen percent (17%) of the total number of farms sampled, (Figure 7).

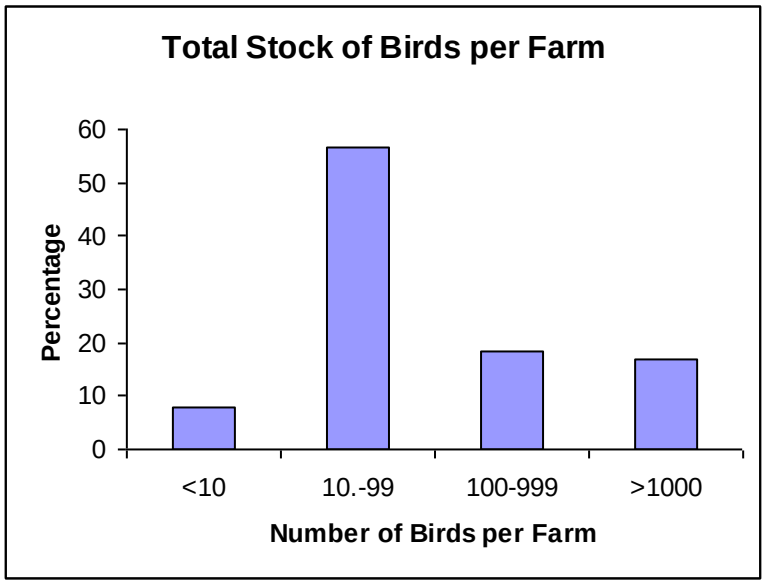


Figure 7: Total stock of birds per farm

The flocks of birds were established from hatcheries in Accra, Dormaa Ahenkro, Kumasi and Tema. Also, day old chicks were imported from Holland and Belgium. Tema accounted for sixty three farms representing 60% of the source of birds introduced into the metropolis, followed by Accra, Holland, Belgium, Kumasi and Dormaa Ahenkrom in that order. Also, wild birds accounted for 16.2% of the number sampled. (Table 5) This profile shows that the source of the birds is from within Tema. This scenario also indicates that major prophylactic measures would probably have to be targeted within Tema.

From Table 5. it was observed that most of the birds were slaughtered for food (50.5%) while a significant number (33%) were sold as live birds in the markets. This informs us that a potential source of spread of Avian Influenza could be from the market since birds bought for home consumption were invariably immediately prepared for consumption.

But this could be a source of infection to humans since handling, slaughter of poultry are linked to H5N1 infectious of infection.

Looking at the trend for recent introduction of birds within the two weeks period (table 5) prior to the day of our sample collection, it was observed that local birds were the most (34.4%) followed by layers (24.8%), then wild birds (16.2%) after which came ducks (11.4%), guinea fowls (5.7%), turkeys (1.9%) and doves (1.0%)..

Sampling took place between May, 2009 and March, 2010. The reasons for these dates were to coincide with wet and dry seasons respectively. The choice of the two climatic

seasons was to find out if Avian Influenza virus infection was more prevalent in one season over the other

Table 5 **Descriptive Characteristics of Birds (source.)**

		Count	Percent
Source of birds	Accra	8	7.6%
	Belgium	5	4.8%
	Dormaa	2	1.9%
	Holland	7	6.7%
	Kumasi	3	2.9%
	Tema	63	60.0%
	wild	17	16.2%
	Total	105	100.0%
Next destination of birds leaving premises	market	35	33.3%
	home consumption	53	50.5%
	game	0	.0%
	no information	17	16.2%
	Total	105	100.0%
	layers	26	24.8%
	broilers	5	4.8%
	Local fowl	36	34.3%
	ducks	12	11.4%
	turkey	2	1.9%
	guinea fowl	6	5.7%
	wild birds	17	16.2%
	dove	1	1.0%
	Total	105	100.0%

4.2: CLINICAL FINDING

The study took into consideration the signs and symptoms associated with Avian Influenza as documented by Capua and Mutinelli (2001) which identified ruffled feathers, marked depression swollen comb, swollen eyes, severe haemorrhages on the shanks, reduction in egg production, weight loss and slight respiratory problems. The signs and symptoms observed were as follows;

Marked Depression: When a bird was not eating, not drinking water, and not reacting to its environment

Respiratory Problems: Bird with difficulty in breathing, sneezing, nasal discharges or opened beak.

Swollen Comb: Puffed up comb with rounded edges

Ruffled Feathers: Bird with raised and rough feathers.

Severe Hemorrhages on Shanks: Bird with reddish patches on the leg.

Swollen Eye: Bird with puffed eye with or without exudates

Reduction in Egg Production: Egg production reduce to 20% or less

In this study, we observed an average mean of 8.4% of the farms sampled had birds with clinical presentations such as marked depression (7.6%), respiratory problems (11.5%), swollen comb (3.7%), ruffled feathers (10.5%) and swollen eyes (8.6%). However, symptoms such as severe hemorrhages on shanks and reduction in egg production were not detected in any of the farms.

Morbidity and Mortality

From the study, it was realized that the highest morbidity rate was 6.7% with p-value of 0.125 at 95% confidence interval and we have sufficient evidence that there was no statistically significant difference so far as morbidity of HPAI H5N1 is concerned. But, it is important as the clinical presentation of Avian Influenza is variable and symptoms are fairly unspecific. (Elbers *et al.* 2005).

With regards to mortalities in the selected farms, we found out that thirteen farms out of the one hundred and five (13/105) farms sampled, reported mortalities ranging from a high of 27.3% to low of 0.1% (Table 6). These mortality rates were observed on commercial and backyard poultry farms at Gbetsile, Kakasunanka, Saki, Zenu, Kpone and Michelle Camp. In the commercial poultry, Saki recorded the highest mortality rate of 1.1% during the study period whiles the rest reported a mortality rate of 0.1%. However, among farms that reported mortalities in backyard poultry, we observed 27.3% mortality rate at Michelle Camp, 14% at Zenu, 4.8% at Gbetsile, 4.3% at Kpone and 2.7% at Saki.

Table 6: Mortality rates (May 2009-March 2010)

Place	**Flock Grouping**	**Mortality Rate**
Gbetsile	Commercial Poultry	0.1%
	Backyard Poultry	4.8%
Kakasunanka	Commercial Poultry	0.1%
Saki	Commercial Poultry	1.1%
	Backyard Poultry	2.7%
Zenu	Commercial Poultry	0.2%
	Backyard Poultry	14%
Kpone	Commercial Poultry	0.1%
	Backyard Poultry	4.3%
Michelle Camp	Backyard Poultry	27.3%

4.3 MANAGEMENT PRACTICES

In commercial holdings, the study revealed that 91.7% used deep littler while 8.3% had their animals caged. The backyard and wild birds had all the animals scavenging. However, the wild birds covered a large area in search of food. The live bird markets exclusively caged all types of different avian species. This shows that the spread of avian influenza virus in an outbreak is likely to be more devastating in the commercial birds (Capus *et al* 2000) and wild birds.

As summarized in Table 7, considering the preparation of feed, we found out that 81.9% of feed was self-prepared whiles about 2% were from commercial source and the remaining sixteen percent had their feed source from scavenging. Seventy-eight point one percent (78.1%) of water delivery was manually administered while 5.7% of water was automatically delivered. Again, we observed that 82.9% of feeders were manual and only 1% of the farms had automatic feeders. This clearly showed that farm hands had direct contact with birds and this could be a potential sources of infection and spread of AI

Table 7. Poultry Management Practices in the Tema Metropolis (May 2009-March 2010)

		Count	Percent
Feed	Self prepared feed	86	81.9%
	Commercial	2	1.9%
	Wild	17	16.2%
	Total	105	100.0%
Water delivery	Automatic	6	5.7%
	Manual	82	78.1%
	wild	17	16.2%
	Total	105	100.0%
Feeders	Automatic	1	1.0%
	Manual	87	82.9%
	wild	17	16.2%
	Total	105	100.0%

4.4 BIO-SECURITY MEASURES

Analysis of the information gathered from poultry farm owners and farm workers as well as direct observations made during the study showed that the mode of disinfection of poultry houses in the study area is as follows:

(a) removal of birds from the houses (slaughtered ,sold alive or dead birds)

(b) removal of feeders and water troughs

(c) removal of litter

(d) removal of dirt from all surfaces

(e) manual washing of floor and other surfaces with detergents

(f) scrubbing or spraying with disinfectants .

(g) reintroduction of birds into house varies between six hours to twenty four hours

From the study, we found out that 33.3% of farm houses were disinfected while 66.7% were not disinfected. This portrayed a high percentage of farm houses that did not follow hygienic principles of disinfection thus making the removal of pathogenic materials impossible.

It was observed that the frequency of disinfection of poultry houses varied from one to four weeks (that is 16.2%) for those who disinfected at one to four weeks while 12.4% disinfected between five to eight weeks intervals.

We found also that 22.9% poultry farms had functional footbaths while 77.1% did not have functional footbaths. This shows that a large proportion of farms have the

potentialities of AI and other diseases being easily introduced into them, if the movements of people in and out of these farm houses are not controlled.

The study showed that only 20% of the commercial farms had the facilities for vehicular dips while 80% did not have. This also stresses the fact of the possibility of the introduction of diseases from vehicles of unknown origins.

The study revealed that 41% of farm workers used protective clothing and 59% did not use protective clothing. This shows that more than fifty percent of farm workers are exposed to possible infections. Furthermore, these farm workers could also be sources of the spread of avian influenza and other infections.

With regards to the disposal of dead birds, it was found out that the methods used included deep burying (45.7%), throwing out of premises (28.6%), burning (4.8%) and use for animal feeding (1.9%). Nineteen percent made up of backyard poultry and wild birds that died were left to decompose wherever they died.

Also, analysis of the biosecurity data gathered indicated that 27.6% of sick birds were quarantined while 72.4% were not quarantined. This high number of unquarantined birds is likely to mitigate against containment measures should an outbreak occur.

During the study we observed that all the three live bird markets namely Community One, Community Nine and Ashiaman in the Tema Metropolis practiced daily sweeping of their premises. It was a common practice to find mixed species of poultry being sold together in the same cages. Furthermore, it was observed that the poultry traders did not separate newly arrived birds from the old stock neither did they separate sick birds from healthy ones.

Table 8: Bio-security Practices by Poultry Farmers in the Tema Metropolis

(May 2009-March 2010)

Activity	Percentage
Farms with Functional Footbaths	22.9%
Farms without Functional Footbaths	77.1%
Farms with Vehicular Dips	20%
Farms without Vehicular Dips	80%
Use of Protective Clothing	41%
Do not use Protective Clothing	59%

Table 9: Disposal of Dead Birds in the Tema Metropolis (May 2009-March 2010)

Activity	Percentage
Deep burying	45.7%
Throwing out of Premises	28.6%
Left to Decompose	19%
Burning	4.8%
Use for Animal feeding	1.9%

4.5 LABORATORY RESULTS

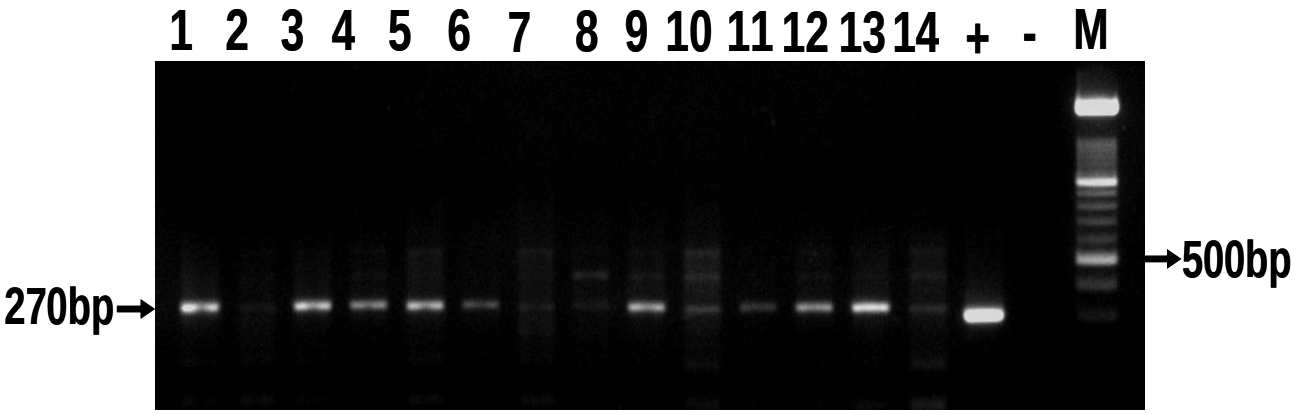
All one thousand two hundred and eighty two birds (commercial poultry, backyard poultry, live bird markets and wild birds) sampled in the Tema Metropolis tested negative for avian influenza virus by the real time RT-PCR assays. Initial Influenza A matrix gene analyses carried out using Spackman *et al*, (2002) protocol for RRT-PCT found that fifty four (54) commercial birds, twenty four (24) backyard birds, eighteen (18) birds sampled in live bird market and two (2) wild birds indicated a possible presence of Influenza A virus.

Further tests on these samples were done using Spackman *et al* (2002) protocol for Influenza A H5 and the Centre for Disease Control and Prevention, Atlanta (CDC) protocol for influenza A and H5 also tested negative (Table 10). However, for differential diagnostic purposes, the study also further tested samples which showed clinical signs as described in Clinical findings (4.3) for Newcastle Disease Virus. Testing conducted for Newcastle virus found five out of the sixty-three farms representing 8% of total farms that showed signs as outlined in our clinical findings (Figure 8)

RRT-PCR Results

Sample	Detector	Ct (cycle threshold)
Positive Control	Flu-A H5 Qiagen	20.45
Negative Control	Flu-A H5 Qiagen	undetected
Commercial bird samples	Flu-A H5 Qiagen	undetected
Backyard bird samples	Flu-A H5 Qiagen	undetected
Live bird market sampls	Flu-A H5 Qiagen	undetected
Wild bird samples	Flu-A H5 Qiagen	undetected

Table 10: RT-PCR Results for RRT-PCR for Influenza A virus



Lane 1-14: Field samples; lane +: Positive control; lane -: Negative control; and lane M: Molecular Marker (100bp)

Figure 8: Detection of New Castle virus RNA in samples

Note: Required product length is 270 base pair (pb). Primers used for amplification were derived from I. Capua and D. J. Alexander (A field and laboratory manual. Chapter 11.3)

CHAPTER FIVE

DISCUSSIONS

There was no evidence of the presence of AIV among domestic poultry and wild birds' samples in Tema Metropolis which were tested by RRT-PCR during the study from May 2009 to March 2010. The virus had been found in birds in 2007 in an outbreak in Tema Metropolis, especially at Kakasunanka and Adjei Kojo, this result is reassuring in that no avian influenza virus was detected. The study covered only a limited period of time and continuous surveillance will be necessary for early detection of avian influenza virus with potential for avian and human epidemics. It will also be worthwhile to carry out active surveillance in other areas such as Brong Ahafo and Volta regions where outbreaks occurred. Border towns with other countries which have experienced recent outbreaks need to be surveyed as well to have a comprehensive picture of AI profile in Ghana.

It was however, noted that about 8% of the total number of farms sampled were positive for Newcastle disease. Since the symptoms of Newcastle disease and Avian Influenza mimic each other,(Swanyne and Suarez 2000) there is the possibility of Avian Influenza passing for Newcastle disease and vice versa. This could be a problem for an early warning system based on clinical symptoms along as a basis for diagnosis. There is therefore the need to carry out snap laboratory tests for cases that have symptoms of AI and Newcastle diseases.

The situation of negative results for AI in the study area indicate that measures taken by Veterinary Services Directorate supported by USAID, European Union, Government of Ghana and farmers have yielded positive results (VSD 2009 annual report). However, the husbandry systems practiced by farmers will mitigate efforts to contain the disease should there be an outbreak. Looking at management practices, almost 91% of commercial holdings used deep litter and the rest used battle cages. Studies conducted by Capus in 2000 revealed that birds kept on deep litter are more likely to spread the virus within themselves at a faster rate. The commercial holdings are therefore at a high risk of spreading the disease among birds resulting in high mortality rates and huge economic losses.

Recent introduction of birds two weeks prior to the day of sample collection, it was found out that local birds accounted for 34.4%, 24.8% for commercial layers and wild birds stood at 16.2%.This indicates that local birds, layers and wild birds are potential source of introduction of Avian Influenza. However, the danger posed by wild birds should not be over looked since a percentage of (16.2%) is high due to the fact that wild birds travel over a very long distance as compared to layers and local fowls which are most of the time, confined to a very large extent. The potential threat of layers should not be taken lightly, since farmers are reluctant to dispose of them in cases of outbreaks

Observation of feeding and watering activities revealed that 81% of feed was self-prepared and fed manually to the birds, and 78% of water was manually administered. Analyzing the fact that farm workers are a probable source of introduction of the virus and its eventual spread, these management practices have a high propensity of introducing AI and spreading it within farms. Furthermore, with high demand for manual labour, more farm workers are likely to take part in these activities and therefore, there is a further high risk of introduction and spread from several workers (Capua *et al* 2003), unlike in automated practices where one person does the job of many.

Bio-security measures differ depending on the type of poultry production system. In some small, medium and large scale commercial poultry farms bio-security measures include fencing or walling of farms, provision of footbath and vehicle dips with constant replenishment of disinfectant solution. Other measures include use of protective clothing by farm workers, hand washing facilities, disposal of dead birds, disinfection of premises and quarantine facility. Conversely, the free range poultry production is characterized by no or minimal bio-security measures (Sonaiya 2007).

Critical analysis of the bio-security situation in the present study area reveals an interesting picture. A review of activities such as the use of protective clothing and deep burial showed that about fifty percent of farmers practiced them.

Other activities such as disinfection, footbath, and vehicular dips were practiced by between 20% to 30% farmers. Although there is no baseline for comparing, it was observed that compliance with the use of protective clothing and deep burial were high because these activities cost less and are bought or done once in a while. Other activities which have low compliance rate such as use of footbath, vehicular dips and disinfection of poultry houses required recurrent expenditure especially the frequent use of chemicals which are expensive.

At the live bird markets, sellers did not adhere to any of the requirements of bio-security except for daily sweeping. There is a dangerous practice of mixing different species of birds in the same cage and also mixing sick and healthy birds either together in the same cage or keeping apparently sick birds outside the cage but in close proximity. It was also observed that in the three live bird markets visited, two were within the main markets while one was just a road away on the main market but still within easy reach. One factor to be considered, looking at the scenario, it is possible that buyers will purchase these birds and take them to their homes far and near and could spread infection should there be one especially, if the birds bought are for rearing. The potential for transmission of infection from birds to humans under such circumstances is very high and poses danger to human health. Bio-security practices in the backyard poultry were mainly daily sweeping.

Farmers kept all types of avian species in either poultry pens or in the opened compounds. The risk of infection among birds and humans would also be high could there be an outbreak.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

The negative results for AI in the study area indicate that measures taken by poultry farmers in the Tema Metropolis and her development partners during the outbreak in the year 2007 yielded positive result. It also, informs us that the scare associated with HPAI H5N1 infection among poultry farm workers, poultry traders, and veterinarians in particular and the general public is minimal or non-existence.

However, adherence by farmers to good farm practices and bio-security is low as revealed in the study. Commercial poultry farms in the Tema Metropolis generally used deep litter. The backyard poultry or the rural poultry and the wild birds are free ranged. Also, all types of different avian species in the three live bird markets are caged and are mixed up with both healthy and sick birds.

Manual labour is mainly employed in the poultry business and the use of personal protective equipments (PPE) is low since only about 41% of farm workers are using PPE such as wellington boots and overalls only.

In the study, it can be concluded that deep burying of dead birds and throwing outside the premises are the most common methods of disposal of dead birds practiced. Daily sweeping of the premises is the major sanitation practice in the three live bird markets in the Tema Metropolis and traffic control is poor.

There is a need for more intensive and aggressive awareness creation among farmers and the public. It would be still more beneficial if this study could be replicated in other outbreak areas such as Sunyani in the Brong Ahafo and Aflao in the Volta regions to determine the effects of the various interventions.

It is my conviction that this study has been worth the effort and investment in both time, financial and material resources. The recommendations outlined below if carefully considered and implemented, will go a long way to minimize the reintroduction of avian influenza and facilitate containment should the unfortunate situation of reoccurrence arise.

6.2 RECONMMENDATIONS

The Ministry of Food and Agriculture and Veterinary Services should

(1) Continue with both active and passive Avian Influenza surveillance as well as education on AI and bio-security. However, this should be done concurrently with Newcastle disease as symptoms mimic each other.

(2) Training of farmers and technical staf as well as equipping of local veterinary clinics with AI field diagnostic kits.

(3) Encourage commercial farmers as much as possible to automate their activities in the farms. Although the initial cost of automation is expensive, long term benefits will accrue.

(4) Subsidize the cost of disinfectants for poultry farmers as is being done for fertilizers.

(5) Encourage Field Epidemiology and Training Programme residents to replicate this study in other outbreak areas (Sunyani and Aflao) of the country.

The Tema Metropolitan Assembly should

(1) Make conscious efforts to move the live bird market from the main markets in order to minimize possible spread of disease from animals to humans.

(2) Provide modern live bird markets

(3) Enact bye-laws to sanction recalcitrant farmers

The Poultry farmers should

(1) Report any unusual signs of disease or unexpected death among birds as soon as they see them to the appropriate authorities.

(2) Ensure good bio-security practice as outlined in the Bio-security Manual prepared by Veterinary Services Directorate of Ministry of Food and Agriculture for training of field staff, poultry farmers and other stakeholders, March 2008.

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APPENDIX I

Participants Consent form

Name of Principal investigator: Dr. Edward Fenteng Danso

Name of Organisations: Veterinary Services Division of Ministry of Food and Agriculture/ FELTP-Ghana

Study title: EPIDEMIOLOGY OF AVIAN INFLUENZA IN DOMESTIC POULTRY AND WILD BIRDS IN THE TEMA MUNICIPALITY

Dear Participant,

I am doing a study on epidemiology of Avian Influenza in domestic poultry and wild birds in the Tema municipality. This is a study done in collaboration with the Noguchi Memorial Institute for Scientific Research.

Purpose of the study

The purpose of this study is to provide information on the Avian Influenza (AI) prevalence in domestic poultry and wildlife birds as well as information on circulating AI strains and their seasonal distribution.

Study Procedures

If you agree to participate, you will be asked questions regarding poultry management, health status of birds, movement of farm equipments and the use of personnel protective equipments (PPE) by farm workers.

Risks and discomfort

There are hardly any risks associated with your participation in this study.

Benefit

The benefit of taking part in this study is that if you have any questions concerning the Avian Influenza menace, we will try to answer them. You would also be contributing to knowledge that is useful for the successful control of AI. in Ghana.

Incentives

You will not be given any incentives to take part in the study.

Confidentiality

Any information about you that will be collected during the study will be confidential and will be stored in a file which will have only a number assigned to it and not your name.

Right to refuse or withdraw

Your participation in this study is purely voluntary and you are free to withdraw at any point in the study. You would not suffer any penalty for refusing to participate or for withdrawing from the study at any point

Who to contact

This proposal has been reviewed and approved by the Ethical Medical Research Review Committee of the University of Ghana, Legon, whose responsibility it is to make sure that research participants are protected from harm. If you wish to find more about the Review Committee, please contact the chairman of the committee at the University of Ghana, Legon, Accra, Ghana.

CONSENT FOR STUDY PARTICIPATION

I have read the foregoing information, or it has been read to me. I understand that the purpose of the study is to provide information on the Avian Influenza (A) prevalence in domestic poultry and wildlife birds as well as information on circulating AI strains and their seasonal distribution. With the view to providing knowledge that would be useful for the successful control of AI in Ghana. I have had the opportunity to ask questions. I consent voluntarily to participate in this study and understand that I have the right to withdraw with no consequent penalties.

Participant Name Signature/thumb print

……..…….. …….

…….…….. …….

…… ……

Date Place

For further information on the study please contact;

Dr Edward Fenteng Danso

School of Public Health

Department of Epidemiology

University of Ghana, Legon, Accra.

Tel: 0246577745/ 02772577866

Email: yfenteng@yahoo.com

APPENDIX II : QUESTIONNAIRE

Date:

Investigator:

Samples obtained: Y/N

Farm ID:

Sector:

AVIAN INFLUENZA OUTBREAK INVESTIGATION FORM

Farm Name: Phone #:

Location: GIS :

On-farm contact: Farm owner(s):

Birds kept at other location:

LIVESTOCK DATA

Farm activities: □ Layer □ Broiler □ Parent stock □ Poultry □ Non-poultry species □ Other:

Check all that apply

Commercial type/species	Males (>1yr)	Females (>1yr)	<1yr	Age range	Total	Comments

Total flock:

Non-Commercial type/species	Males (>1yr)	Females (>1yr)	<1yr	Age range	Total	Comments

Game fowl

Psitticines

Waterfowl

Total other:

Contact with wild bird species: □ No □Yes, describe…….

List other animal species located on the premises:

Bird Health

Increased illness in birds previous 3 months? Y/N
Describe:

Number sick	Last week	Last month	Last 3 months	Total
Number dead	Last week	Last month	Last 3 months	Total

Vaccination practices

Vaccine	Use and number of birds vaccinated	Frequency
Medication used	Use and number of birds treated	Dates

Birds introduced to the premises in the past 90 days?

Type of bird Location where birds came from: Total number:

Birds leaving the premises in the past 90 days?

Type of bird: Location where birds went to: Total number:

Of birds that left did any return to the premises? Y/N

Type of bird: Location where birds went to: Total number:

MANAGEMENT AND HUSBANDRY

Type of house(s): □ Open □ Closed □ Other:……..

check all that apply

House materials: □ wood □ Cement □ Metal □ Other:……

check all that apply

Disinfection of houses: □Yes □ No

Describe:……..

Type of

husbandary:

check all that apply □ All In/All Out □ Multi-age □ Modification/Other:……

Distance	□ between poultry houses:……..	□ to residential area:……
	□ to nearest poultry farm:……	□ to nearest live market:…….
Feed	Feed delivered to farm?	□ Yes □ No
	Feed stored on premises?	□ Yes □ No
Workers	Number of workers:……..	Number of workers per house:……
	Work on other farms?	□ Yes □ No

Other questions:

□ Perimeter fence	**Feeders**	□ Sick birds isolated
□ Drainage system	□ Automatic □ Manual	□ Quarantine of new birds
□ Personnel disinfection	**Water delivery**	□ Disinfection of vehicles
□ Use of PPE for workers	□ Automatic □ Manual	
□ " " for visitors	□ Individual □ Group	□ Controlled entry and traffic
□ Poultry house disinfection	□ Cleaning of feeders/waterers Frequency:…….	Disposal of dead birds:
Frequency:…… ……..		
□Use of poultry house rest period	□ Waste management	□ Buried □ Burned □Thrown away □ Other
Duration:…… ……...	If yes, how handled?	

SAMPLE COLLECTION

□ Samples collected previously	sample type (circle all that apply): Blood/oral swab/cloacal swab/feces/organ	Results if known:……..
□ Samples collected this visit	Blood/oral swab/cloacal swab/feces/organ	Attach list of samples by identification number and type