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EFFECT OF ANTIPYRETIC USAGE ON THE ACCURACY OF THE WORLD HEALTH ORGANISATION CASE DEFINITION FOR INFLUENZA-LIKE ILLNESSES

BY

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DECLARATION

I, Joseph Asamoah Frimpong, declare that except for references to other people's work which have been duly acknowledged, this work is the result of my own original research and that this thesis, either in whole or in part has not been presented elsewhere for another degree.

SIGNATURE…… DATE…….

JOSEPH ASAMOAH FRIMPONG (STUDENT)

SIGNATURE…… DATE……..

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DEDICATION

I dedicate this project work to the Almighty God for his knowledge, wisdom, understanding and uncommon favour. I also dedicate this work to my parents, Mr. and Mrs. Frimpong, for their support and blessings.

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LIST OF ABBREVIATIONS

ARIs	Acute Respiratory Infection
CDC	Centres for Disease Control and Prevention
CHPS	Community based Health Planning Services
cRNA	Complementary Ribonucleic Acid
Ct	Cycle threshold
dsDNA	double stranded Deoxyribonucleic Acid
GHS	Ghana Health Service
HA	Hemagglutinin
HDSS	Health Demographic Surveillance System
HSC	Human Specimen Control
IL	Inter Leukin
ILI	Influenza Like illness
M1	Matrix 1 protein
M2	Matrix 2 protein
mRNA	Messenger Ribonucleic Acid
NA	Neuraminidase
NIC	National Influenza Centre

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NMIMR	Noguchi Memorial Institute for Medical Research
NP	Nucleic Protein
NP/OP	Nasopharyngeal/Oropharyngeal
NS1	Non Structural protein 1
NS2	Non Structural Protein 2
NS2	Non Structural protein 2
NTC	Negative Template Control
OHC	Old Ningo Health Centre
OPD	Outpatient Department
OSU	Osudoku Health Centre
PA	Acid Polymerase protein
PB1	Basic Polymerase protein 1
PB2	Basic Polymerase Protein
PB2	Basic Polymerase protein 2
PCR	Polymerase Chain Reaction
PDA	Personal Digital Assistant
PGE	Postaglandin
PHC	Prampram Health Centre
POAH	Anterior hypothalamus

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PTC	Positive Template Control
RNA	Ribonucleic Acid
RNP	Ribonucleic Protein
SD Card	Secured Digital Memory Card
SOD	Shai-Osudoku District Hospital
SONPD	Shai-Osudoku/Ningo Prampram District
SQL	Structured Query Language
TNF	Tumor Necrosis Factor
US-NAMRU-3	United States Naval Medical Research Unit-3
vRNA	Viral Ribonucleic Acid
VTM	Viral Transport Medium
WHO	World Health Organisation

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ABSTRACT

Introduction

Influenza is a major cause of morbidity and mortality globally especially during pandemics. For this reason countries have set up surveillance systems to continually monitor Influenza like illnesses (ILI) for early detection of outbreaks and rapid response. However, in sub-Saharan Africa, specifically Ghana where medication can be purchased over the counter, it is more likely that people might have taken some antipyretics before reporting to the health facility. This may result in patients not having a measured axillary temperature ≥37.5°C by the time they present to the health facility. These cases are more likely not to meet the WHO case definition and will be missed by the surveillance system. This study was conducted to assess the effect of antipyretics on ILI case detection.

Method

A cross sectional study was conducted in 4 health facilities in the Shai-Osudoku and Ningo-Prampram districts of Ghana from September 2013 to May 2014. Nasopharyngeal and oropharyngeal specimens were collected from 321 patients seeking ambulatory care at the health facilities who met the case definition of "History of fever or measure fever ≥37.5°C (Axillary) with cough" and consented to be part of the study. Swabs were tested for influenza virus using molecular assays. Data collection tool used was a structured questionnaire. Univariate and bivariate analysis were carried out using statistical package for the social sciences (SPSS version 17; IBM Corporation, Armonk, NY).

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Results

Out of 321 participants, 236 (73.52%) had a history of fever and 85 (26.48%) had measured fever ≥ 37.5^{o}C. A total of 95(29.60%) participants took antipyretics before visiting the facility. Out of these 95 participants, 62(65.26%) had a history of fever. Participants who took antipyretics before reporting to the facility were more likely to have a history of fever (OR: 1.78, CI: 1.05 – 3.00, p-value: 0.03). A total of 39 (56.52%) of the influenza cases that had a history of fever were missed by the WHO case definition for ILI. However, patients with measured fever ≥ 37.5^{o}C were 3 times more likely to have had an influenza infection compared to patients with history of fever. Sensitivity of influenza case detection was found to be higher in patients with history of fever and cough (56.52%, CI: 43.38 – 66.23)

Conclusion

A larger proportion of patients with ILI present to the health facility with a history of fever compared to those with measured fever ≥ 37.5^{o}C. Among those with history of fever, a greater proportion takes in antipyretics prior to seeking care at the health facility which results in a measured body temperature < 37.5^{o}C which results in influenza cases been missed. An assessment of the use of antipyretics and history of fever show a positive effect on the sensitivity of Influenza case detection.

Keywords: Influenza, fever, antipyretics

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Influenza-like-illness (ILI) is a term used to describe a group of respiratory diseases presenting with a common set of non-specific symptoms suggestive of influenza or other respiratory viruses. The symptoms commonly include sudden onset of fever, shivering, chills, malaise, and dry cough, loss of appetite, body aches, sore throat and nausea, typically in connection with a sudden onset of illness (Aguilera et al., 2002).

Examples of infections that may manifest ILI symptoms include bronchitis, pneumonia, appendicitis, common cold, meningitis, tuberculosis, urinary tract infection, sexually transmitted diseases, septic arthritis, influenza etc. (McBratney, 2011)

Influenza is an important contributor to Acute respiratory tract infections (ARIs) and it is estimated that 20% of children and 5% of adults suffer influenza infections each year(World Health Organization, 2004). Acute respiratory tract infections (ARIs) are among the most common causes of morbidity and mortality worldwide. Studies have shown that mortality associated with influenza varies by age group, chronic disease status, and influenza virus type and subtype. The World Health Organisation (WHO) seasonal influenza fact sheet No.211 indicated that seasonal influenza affects 5-10% of the world's population resulting in 250,000 to 500,000 deaths annually(World Health Organization, 2014b).

The influenza virus is known to undergo continuous antigenic drift and this makes the effectiveness of flu vaccine dependent on the continuous monitoring of circulating

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influenza strains globally(Ryan, Zoellner, Gradl, Palache, & Medema, 2006). Antigenic shift also occurs occasionally leading to pandemics resulting in the emergence of novel strains which claim the lives of millions of people (Neumann, Noda, & Kawaoka, 2009; World Health Organization, 2004). In 2009, a novel influenza virus emerged in the human population causing the first pandemic of the 21st century (Neumann & Kawaoka, 2011).

A review of seasonal influenza epidemiology in sub-Saharan Africa, found that 10% of outpatient ARIs and 7% of children admitted in the hospital for ARIs tested positive for influenza (Gessner, Shindo, & Briand, 2011).

The earliest reports of influenza in Ghana date back to the pre-independence era when the country was severely affected by the influenza pandemic of 1918–1919. The disease was introduced by shipping along the southern coast and spread overland throughout the territory known then as the Gold Coast. Mortality rates varied regionally and to some extent by occupation. The influenza epidemic killed 100,000 or more people in less than six months and is described as the worst short-term demographic disaster in the history of Ghana (Patterson, 2009). Virological and serological investigations of an influenza-like epidemic in Ghana in 1973 confirmed influenza A2/Hong Kong/1/68 as the causative agent and cases were more frequent in the minor rainy season from October to December. The estimated attack rate was between 2.5% and 5%.

Another report two decades later in 1996, described upper respiratory infections as the second most frequent clinical diagnosis after malaria with a peak in the dry harmattan season from November to February. Serological investigation of unusual cases of febrile episodes during the rainy season (June to September) of that year

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found a high prevalence (more than 95% of cases investigated) of high titres (≥ 1:320) to influenza A H3N2, indicating that this virus was likely associated with those febrile episodes (Mingle , Ofori-Adjei ,Ofori-Adjei, 1996; Addy, Mingle, 1973; Nowacki, Addy, 1975).

Influenza symptoms are non-specific and this highlights the need for a diagnostic confirmation of an Influenza infection. To narrow the scope of suspected cases of influenza, a case definition that considers more associated symptoms is required by a surveillance system.

The WHO case definition for ILI is "measured fever ≥37.5°C (Axillary), AND cough, with illness onset in the last ten days" (World Health Organization, 2014). This case definition has been adopted by most Influenza Sentinel Surveillance systems around the globe geared towards early detection and rapid response to outbreaks. However, there seem to be a limitation in the application of this case definition in some parts of sub-Saharan Africa

In sub-Saharan Africa, specifically Ghana, the purchase of medication over the counter is a common practise which increases the likelihood of self-medication. These medication purchased over the counter may have some antipyretic properties (Adu-Sarkodie, 1997). Antipyretics are a group of medications that are known to reduce fever(Aronoff & Neilson, 2001). Fever is a symptom that emanates as a result of an infection or host immune response to non-infectious inflammatory stimulus(Hawksworth et al., 2009; O'Grady et al., 2008).

The use of antipyretics under such circumstance may result in patients not having a fever (measured axillary temperature of ≥37.5°C) by the time they present to the health facility. Some of these patients are more likely to be missed by a surveillance

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system which uses the WHO case definition for ILI which requires a measured axillary temperature of ≥37.5°C. There are other factors aside from the use of antipyretics that may influence the WHO case definition for ILI but the focus of this study will be on the use of antipyretics.

Data from the Ghana Health Service (GHS) indicated that infectious diseases constituted more than 40% of all deaths in the country(Ghana Health Service, 2009). An annual report of the Shai-Osudoku/Ningo Prampram District (SONPD) administration also showed that ARIs were the second most common cause of outpatient department (OPD) attendance (15% of all attendances) after malaria. In the same year, 88% of suspected cases of influenza sent to the National Influenza Centre (NIC) at Noguchi Memorial Institute for Medical Research (NMIMR) were found to be A (H1N1) pdm09 influenza virus(Dangme West District Health Services Directorate, 2010). Influenza therefore may constitute a substantial proportion of overall morbidity and it will be important to understand the nature of the disease.

1.2 Problem statement

Anecdotal reports indicate that in Ghana, some patients' take antipyretics before reporting to the health facility. This has the potential of reducing fever prior to visiting a health facility for care thus resulting in some patients not meeting the WHO case definition for ILI which is "A patient with a measured axillary temperature ≥37.5°C with cough

Due to the flexible nature of buying medications over the counter without any form of prescription from a qualified physician, most influenza-like symptoms including fever and cough are treated by patients before reporting to the health facility. Self-

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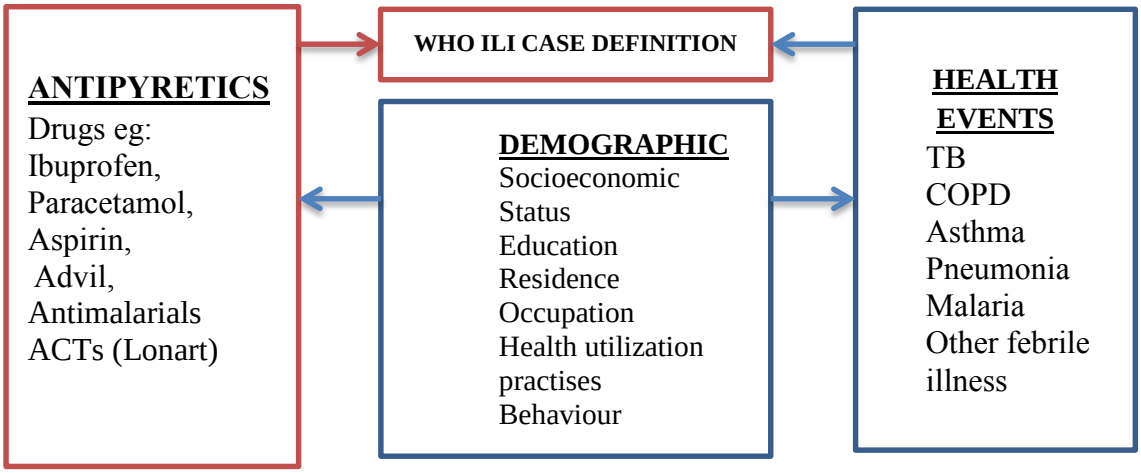
medication might imply that some ILI cases do not present to health facilities and are missed by the surveillance system and those that do present may not meet the case definition due to the use of antipyretics.

These factors may result in the under estimation of the burden of ILI including influenza in Ghana. It would also have an effect on the sensitivity of the surveillance system. There is therefore the need to have case definition that takes into account the use of antipyretics. Studies to ascertain the impact of antipyretic usage on the WHO case definition for ILI has not been done in Ghana.

1.3 Conceptual framework: Factors influencing the sensitivity and specificity of WHO ILI case definition.



The WHO case definition requires a measured body temperature ≥37.5°C and cough. Factors such as the use of antipyretics and health related events can either reduce or increase the sensitivity and specificity of the case definition. Antipyretics such as Ibuprofen, Aspirin and Paracetamol has the tendency of reducing the body temperature to less than 37.5°C resulting in patient not meeting the case definition. Health Events such TB, Asthma and Malaria also present with fever and cough. These

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cases are likely to be captured by the ILI case definition thereby inflating the number of ILI cases. The use of antipyretics and health events can be influenced by demographic characteristics such as socio-economic status, health utilization practises, educational level and occupation. For instance, people who cannot afford hospital bills will initially try to manage the disease at home and probably send the patient to the facility when self-medication fails. For the purpose of this study the focus was on the effect of antipyretic usage on the WHO ILI case definition.

1.4 Justification

For an epidemic prone disease such as Influenza, there is the need to have a sensitive case definition that takes into account factors including the use of antipyretics for early detection of outbreaks and interventions.

Findings from this study may help to ascertain which case definition best fits our setting to help strengthen the influenza surveillance system in Ghana. This may also enable the health sector to early detect and implement influenza outbreak interventions to reduce the morbidity and mortality rate of influenza infection during epidemics. It would help policy makers make evidence-based decisions in terms of allocating resources and prioritizing diseases of public health concern in Ghana.

1.5 Main objective

To determine the effect of antipyretic usage on the WHO case definition for ILI

1.5.1 Specific objectives:

- To determine the proportion of ILI cases with measured fever ≥37.5°C and ILI cases with history of fever.

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- To assess antipyretic usage among ILI cases with measured fever ≥37.5°C and ILI cases with history of fever.
- To determine the effect of antipyretic usage and history of fever on the sensitivity and specificity of ILI case detection.

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CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The Influenza Virus

Influenza virus belong to the family Ortho myxoviridae derived from the Greek words orthos, meaning "standard, correct," and myxa, meaning "mucus" (Cheung & Poon, 2007). Influenza viruses are the etiological agents of influenza(Smith, Andrewes, & Laidlaw, 1933) which is characterized by a segmented minus stranded RNA genome (Röhm, Zhou, Süss, Mackenzie, & Webster, 1996; Shih et al., 2005; Webster, Laver, Air, & Schild, 1982). The family of Orthomyxoviridae has four genera namely; Influenza virus A, Influenza virus B, Influenza virus C, and Thogotovirus (Cheung & Poon, 2007).

2.2. Taxonomy of Influenza Viruses

Influenza can be classified as A, B and C viruses. The distinction between these viruses is based on the antigenic differences between their nucleoproteins (NP) and matrix (M) proteins (Lamb & Krug, 2001). Influenza A and B viruses has 8 RNA genomic segments, as compared to influenza C virus which contains only 7 RNA genomic segments (Desselberger, Racaniello, Zazra, & Palese, 1980). All the types of influenza viruses are known to naturally infect humans; however, only influenza A virus is known to cause influenza pandemics (Potter, 1998). The influenza virus type A can further be subtyped based on the antigenic variation of the hemagglutinin (HA) and neuraminidase (NA) surface glycoproteins (Cheung & Poon, 2007). There are 16 known subtypes of HA(Fouchier et al., 2005) and 9 known subtypes of NA (Laver,

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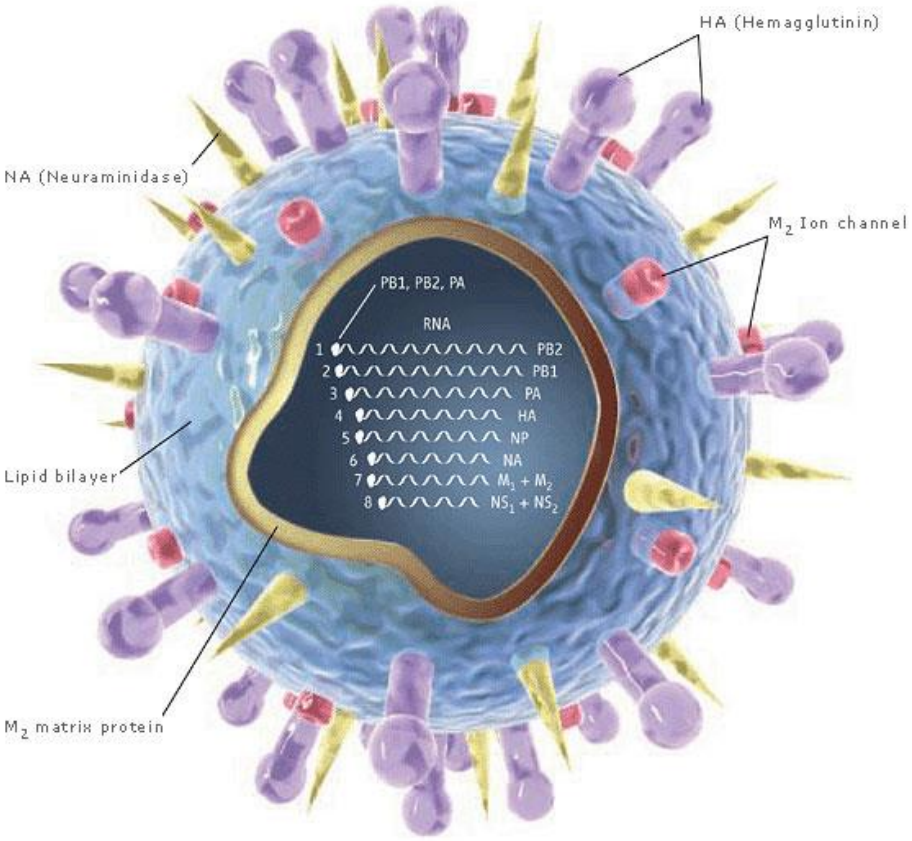
Colman, Webster, Hinshaw, & Air, 1984). The resultant subtype is derived based on the specific HA and NA of the surface glycoprotein. Examples include A(H1N1), A(H3N2) and A(H9N2).

2.3 Structure of the Influenza virus

Figure 1: schematic diagrams showing the Structure of Influenza A virus.



Source: Science Vol. 312, p 380.

The morphology of the influenza virus particle is dependent on several viral proteins. The influenza virus is enveloped with pleomorphic virions of shapes ranging from small spherical to long filamentous forms. The HA, NA, M1 and M2 are known to have effects on the morphology of the influenza virus particles (Burleigh, Calder,

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Skehel, & Steinhauer, 2005; Elleman & Barclay, 2004; Enami & Enami, 1996; Mitnaul, Castrucci, Murti, & Kawaoka, 1996; Roberts, Lamb, & Compans, 1998).

The Influenza A viral particle has a lipid envelope; derived from the cell membrane of the host during the viral budding process. Three viral proteins, HA, NA and M2 are embedded in the lipid envelope. The HA and NA are spike glycoproteins attached to the lipid bilayer by short sequences of hydrophobic amino acids (Lamb & Krug, 2001). The M2 protein is an integral membrane protein that functions as an ion channel for the acidification of the viral particle during viral infection (Pinto, Holsinger, & Lamb, 1992; Wang, Lamb, & Pinto, 1994).

Underneath the viral lipid envelope is a matrix 1 (M1) protein layer (Ruigrok, Calder, & Wharton, 1989). Inside the virion, are eight viral ribonucleic acid (vRNA) segments which are bound to the nucleoprotein (NP) and to the influenza virus RNA polymerases forming a ribonucleoprotein (RNP) complex (Lamb & Choppin, 1983). Each NP monomer interacts with approximately 20 nucleotides of the vRNA(Lamb & Krug, 2001). The RNA polymerase complex is made up of three polymerase subunits: basic polymerase protein 2 (PB2) basic polymerase protein 1 (PB1) and acidic polymerase protein (PA) (Jennings, Finch, Winter, & Robertson, 1983). The non-structural protein 2 (NS2) which appears to function as a nuclear export protein is also present in low amounts in the viral particle (O'Neill, Talon, & Palese, 1998; Richardson & Akkina, 1991).

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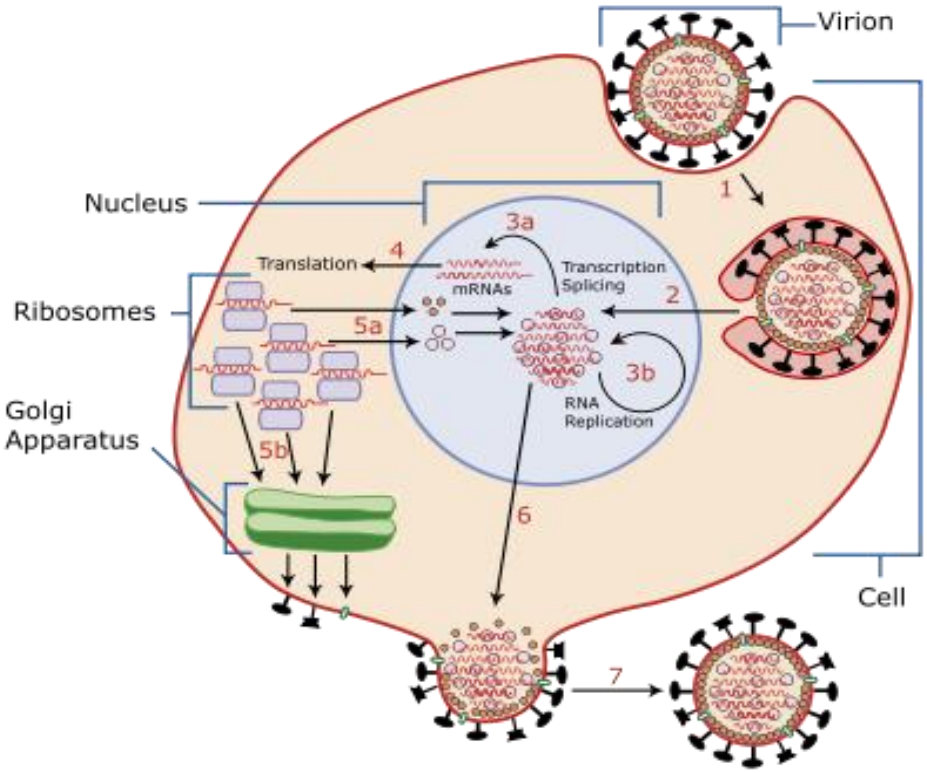
2.4 Replication Cycle of Influenza Virus

The influenza virus genome has negative-sense RNA as shown in Figure 2. However, unlike other negative-sense single stranded RNA viruses, the transcription and translation site for the influenza virus is in the nucleus of an infected cell (Herz, Stavnezer, Krug, & Gurney, 1981; Jackson, Caton, McCready, & Cook, 1982; Palese, 1977). All eight RNA segments of the virus have the first 12-13 nucleotides at their 3' and 5' ends conserved. Each segment also contains 2 to 3 segment specific nucleotides near each end. These RNA sequences are partially complimentary and can form a pan handle structure (Robertson, 1979; Skehel & Hay, 1978).

Figure 2: Schematic diagram of the replication cycle of the Influenza A virus



Source: Cox and Kawaoka, 1997.

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2.4.1 Adsorption, entry and uncoating of virus particle

The influenza virus binds to the host cell surface by fixing the outer top of the haemagglutinin to the sialic acid of a host cell's glycoproteins and glycolipids (Steinhauer & Wharton, 1998) as shown in step 1 of Figure 2. The sialic acid linkage to the penultimate galactose, alpha 2, 3 or alpha 2, 6 in birds or humans respectively, determines host specificity. Since sialic acid- presenting carbohydrates are present on several cells of the organism, the binding capacity of the HA explains why multiple cell types in the host organism can be infected.

From step 2; the virus particle is then taken up by the cell through a clathrin-coated receptor mediated endocytosis process. When internalized, the clathrin molecules are liberated and the vesicle harbouring the whole virus fuses with endosomes. The contents of the vesicles are digested through a stepwise lowering of the pH within the phagosome (Potter, 1998).

The lowering of the pH is stopped by the action of the M2 protein which induces the partial liberation of the fusion peptide of the HA. This allows the fusion of the HA with the membrane of the vesicle and liberation of the ribonucleoproteins into the cytoplasm. The influx of ions from the endosome to the virus particle leads to disconnection of the different viral proteins; M1-protein aggregation is disrupted and ribonucleoproteins no longer adhere to the M1- protein complex. Uncoating is completed within 20 - 30minutes of virus attachment to the cell (Steinhauer & Wharton, 1998).

2.4.2 Transcription of mRNA

Transcription of mRNA takes place in the nucleus of an infected cell as indicated in step 3 of Figure 2. It is initiated by a capped RNA fragment, which is cleaved from

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host mRNA by a cap snatching mechanism (Braam et al., 1983, Krug et al., 1979, Plotch et al., 1979). The PB2 polymerase subunit binds to the 5' end of the host cell mRNA and cleaves it about 10-15 nucleotides downstream from the cap structure after predominantly an A or G residue (Plotch et al., 1981). This cleavage requires a methylated cap structure on the RNA substrate (Bouloy et al., 1980). The endonuclease activity of PB2 is stimulated by vRNA (Cianci et al., 1995, Hagen et al., 1994, Li et al., 1998). The viral polymerase complex thus uses the host mRNA for viral mRNA synthesis (Shih and Krug, 1996). The short capped oligonucleotide obtained after endonuclease cleavage is used by the viral polymerase as a primer for transcription. Initiation of viral transcription requires the interaction between the 5' and 3' conserved sequences of vRNA (Luo et al., 1991) and it is terminated at a track of 5-7 U residues approximately 17 nucleotides from the 5' end of vRNA. A poly A tail is then added to the mRNA transcript and this polyadenylation is known to be a host – independent process (Poon et al., 1998 and Plotch and Krug, 1977).

2.4.3 Synthesis of cRNA

The mechanism for switching from transcription to replication is poorly understood but it is generally accepted that the first step in replication is the production of positive strand copies cRNA of each segment. cRNA is a full length copy of the vRNA and can be used as a template for genomic RNA synthesis. cRNA is different from products of transcription in that the 5' ends are not capped. Again cRNA is not polyadenylated (Hay et al., 1982).

2.4.4 Influenza virus protein Expression

The replication and transcription of the Influenza viral genome is a highly selective process (Smith and Hay, 1982) as illustrated in steps 5 of figure 2. Immediately after infection, primary transcription occurs and all eight mRNAs are synthesized in equal

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amounts (Hay et al., 1977). This is followed by the second transcription stage which can be further subdivided into early and late stages. First, NS1 and NP vRNA are synthesized and become the predominant viral proteins in infected cells at this stage (Hay et al., 1977). During the late stage, the NS1 protein synthesis is reduced and HA, NA and M1 mRNAs are expressed (Shapiro et al., 1987). Most of the capped and polyadenylated viral mRNAs are transported from the nucleus into the cytoplasm for protein synthesis. Membrane-bounded proteins, such as HA, NA, and M2 use the secretory pathway of the trans-Golgi network for protein maturation. HA and NA proteins are post-translationally modified and transported to the cell surface for integration into the cell membrane (Shapiro et al., 1987).

2.4.5 Influenza virus assembly and release of progeny virus

Generally, the RNPs are transported to the nucleus where the polymerase complex binds to viral RNA, cleaves the viral RNA by its endonuclease activity and simultaneously leads to elongation. The production of vRNA is limited by the NP in favour of mRNA and both are transported to the cytoplasm where viral proteins are generated at the ribosome. Part of the viral mRNA is spliced by cellular enzymes so that finally viral proteins such as M1 and NS2 can be synthesized without any further cleavage. Some of the newly synthesized viral proteins are transported to the nucleus where they bind to viral RNA to form RNPs. Other newly synthesized viral proteins are processed in the endoplasmic reticulum and the Golgi apparatus where glycosylation occurs. These modified proteins are transported to the cell membrane where they stick in the lipid bilayer. Different viral components are transported to the lipid bilayer differently. The nucleocapsids are assembled in the nucleus and then move to the cell surface. HA and NA are synthesized in the endoplasmic reticulum and transported to the lipid bilayer. Finally when the desired concentration is reached

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at the plasma membrane; RNPs and M1 proteins aggregate and condenses to produce a viral particle. The particle is extruded from the membrane and is liberated by neuraminidase activity (Wright and Webster, 2001). New progeny viruses are produced within 8 - 10hrs as indicated in steps 6 and 7 of Figure 2.

2.4.6 Shedding of Influenza virus

Immuno-histological pictures show that foci of virus-producing cells are clustered in the mucous layer of the respiratory tract, in the gut and even in endothelial layers, myocardium and brain. Within nasal secretions, millions of virus particles per ml are shed, so that a 0.1 µl aerosol particle contains more than 100 virus particles. A single human infectious dose of influenza virus might be between 100 and 1,000 particles. At least during the early course of influenza infection, the virus can be found also in the blood and in other body fluids (Wright and Webster, 2001).

2.4.7 Infectivity of Influenza Virus

Infectivity of influenza virus particles is preserved depending on temperature, pH and salinity of the water, and ultraviolet irradiation. At 4°C, the half-life of infectivity is about 2-3 weeks in water. Due to the conformation of the lipid bilayer, survival under normal environmental conditions should be shorter. Infectivity of the influenza virus particle is easily inactivated by all alcoholic disinfectants, chlorine and aldehydes. As far as it is known, temperatures above 70°C will destroy infectivity in a few seconds (Wright and Webster, 2001).

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2.5 Genetic Mutation

The influenza virus is known to undergo continuous antigenic changes and this makes the effectiveness of flu vaccines dependent on the continuous monitoring of circulating influenza strains globally (Neumann et al., 2009). These antigenic changes are known as antigenic drift and antigenic shift.

2.5.1 Antigenic Drifts

Minor antigenic changes called antigenic drift are due to the low fidelity of the viral polymerase as well as host immune selection leading to the accumulation of point mutations in the HA and NA genes, resulting in amino acid sequence changes in the protein (Shin-Ru, et al., 2005). These unpredictable point mutations that occur results in minor changes on the surface proteins of the virus which results in new strains that may not be recognized by antibodies to earlier influenza strains. This process works as follows: a person infected with a particular influenza virus strain develops antibody against that strain. This emphasizes the need for continuous monitoring of circulating strains to inform annual vaccine formulation.

2.5.2 Antigenic Shifts

Antigenic shift is a severe form of genetic variation which evolves as a result of major changes in the sequence of the viral surface antigens resulting in the sudden development of a completely new subtype of the virus (Webster, et al., 1982). These extreme changes are thought to be the result of genetic re-assortment between human and animal influenza viruses. Antigenic shift can occur either through direct animal (poultry)-to-human transmission or through mixing of human influenza A and animal influenza A virus genes to create a new human influenza A subtype virus. A global influenza pandemic (worldwide spread) may occur if three conditions are met: A new

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subtype of influenza A virus is introduced into the human population, the virus causes serious illness in humans and the virus can spread easily from person to person in a sustained manner. Influenza types B and C are not known to undergo such extreme changes. (Wright and Webster, 2001).

Antigenic shifts occur occasionally leading to pandemics resulting in the emergence of novel strains which claim the lives of millions of people (World Health Organization, 2014a, Hannoun C, 1994). During epidemics in temperate regions, Influenza affects 30% to 40% of children (Adams and Marano, 1995, Glezen et. al., 1997). Studies in the temperate regions have generated substantial data on disease burden in terms of morbidity, mortality and socioeconomic costs which has supported the development and expansion of influenza control programs, primarily by vaccinating groups at highest risk for serious complications from influenza infection (Ryan et al.,2006, O' Brien et al.,2004).

2.6 Clinical signs and symptoms of Influenza Infection

Influenza virus can be transmitted through large respiratory particle droplets which maybe as a result of sneezing coughing or blowing of the nose near a susceptible host. Due to the large nature of the particles, they stay in suspension over a short period and can travel within a short distance. Transmission is mainly from person-person contact or contact with a contaminated surface. (Brankston, 2007).

Influenza symptoms are non-specific and pose a challenge in differentiating it from other diseases. The common symptoms include fever, shivering, chills, malaise, dry cough, loss of appetite, body aches, sore throat and nausea, typically in connection with a sudden onset of illness. (Aguilera et al., 2002). Examples of infections that may manifest ILI symptoms include Bronchitis, Pneumonia, Appendicitis, Common cold,

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Meningitis, Tuberculosis, Urinary tract infection, sexually transmitted diseases, Septic arthritis, etc.

2.7 Laboratory diagnosis of Influenza by Real - Time Reverse Transcriptase Polymerase Chain Reaction (rRT-PCR)

Polymerase chain reaction (PCR) is a molecular technique which utilizes a pair of synthetic oligonucleotides or primers, each hybridizing to one strand of a dsDNA target, with the pair spanning a region of interest which will be reproduced exponentially. The hybridized primer acts as a substrate for a DNA polymerase which creates a complimentary strand through addition of deoxynucleotides.

There are two main types of Polymerase chain reaction (PCR), these are conventional and real time PCR. For the purpose of this study, real time PCR was used. The Polymerase Chain Reaction (Freymuth et al., 1995) is now used as the new gold standard for detecting a wide variety of templates across a range of scientific specialties including virology. The process of PCR can be put in 3 steps: dsDNA separation at temperatures >90°C, primer annealing at 50 – 70°C and extension at 72 – 78°C. The challenge associated with conventional PCR is assessing the amplicons generated (Guatelli, Gingeras, & Richman, 1989).

Unlike the conventional PCR, the amplicons of real time PCR assay can be visualized as the amplification takes place. The labeling of the primers, probes or amplicons with flurogenic molecules makes the monitoring of accumulating amplicons possible. During the detection, an amplification curve which is a typical sigmoid curve is obtained from a plot of fluorescence activity against the cycle number. Early amplification cannot be viewed because the detection signal is indistinguishable from the background. The point at which the fluorescence passes from insignificant levels

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to clearly distinguishable levels is called the threshold cycle (C_T). The C_T value is proportional to the number of target copies present in the sample. For this study a C_T value < 37 was considered as positive for the target template (RNA).

2.8 Epidemiology of Influenza

Influenza is a major cause of morbidity and mortality globally especially during pandemics which spread in most parts of the world (World Health Organization, 2014). Pandemic influenza is known to cause more deaths compared to seasonal epidemics. During the early circulation of the A(H1N1)pdm09, an estimate of 105 700–395 600 people died of associated respiratory illness (Dawood, Iuliano, & Reed, 2012).

2.8.1 Global burden of Influenza

Influenza is known to occur worldwide with an annual attack rate of 5% - 10% in adults and 20% - 30% in children. The populations at risk are pregnant women, healthcare workers, young children, the elderly and individuals with underlying medical conditions (World Health Organization, 2014).

Severe cases of these illnesses can result in hospitalization among these vulnerable groups. It is estimated that 3 – 5 million of these severe cases occur each year resulting in about 250,000 to 500,000 deaths (World Health Organization, 2014).

A study conducted by Neuzil, K. et al (Neuzil, Reed, Mitchel, Simonsen, & Griffin, 1998)indicated that pregnant women without preexisting medical conditions had 21.7 events per 10, 000 women months during the influenza season. However pregnant women with preexisting medical conditions showed a higher risk of influenza infection.

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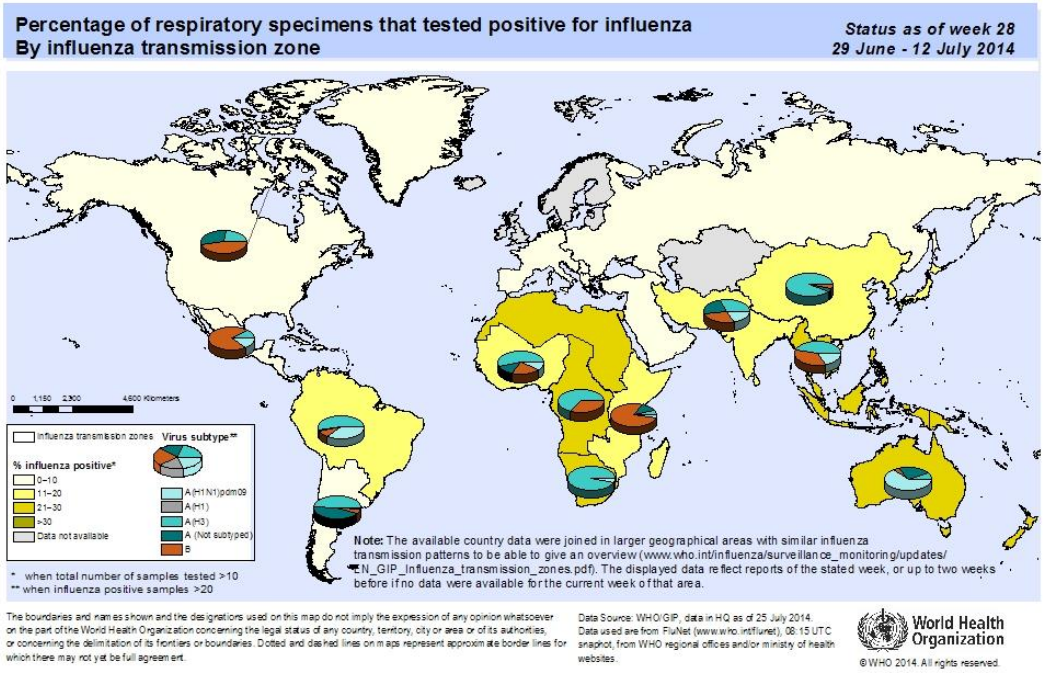
Hartert et al., 2003 indicated that pregnant women with Asthma as a preexisting medical condition were 10 times more like to be hospitalized. Globally, health care workers are also at increased risk of transmitting as well as acquiring an influenza infection. Healthcare workers are more likely to report to duty even when they are not well which may result in nosocomial transmission to patients. On the contrary, they are more likely to get into contact with patients with severe medical conditions.

The globally disease burden of influenza is substantially in children under five years of age. Nair et al., 2011, showed that out of 20.5 million cases of influenza associated acute lower respiratory infection 13% was in children under five.

Figure 3: Distribution of influenza cases in Influenza transmission zone, 2014



Source: (World Health Organization, 2014a), global influenza surveillance and response system

Figure 3 shows the global distribution of influenza cases that have been reported as Epi-week 28 of 2014. Out of 25 675 respiratory specimen tested, 3184 were positive for influenza viruses, of which 2844 (89.3%) were typed as influenza A and 340

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(10.7%) as influenza B. Of the sub-typed influenza A viruses, 416 (17.6%) were influenza A(H1N1)pdm09 and 1948 (82.4%) were influenza A(H3N2). Of the characterized B viruses, 89 (97.8%) belong to the B-Yamagata lineage and 2 (2.2%) to the B-Victoria lineage. Influenza activity was seen to increase in the southern hemisphere (World Health Organization, 2014a)

2.9 Influenza in West-Africa

The prevalence and incidence of Influenza in Africa is not well established in most parts of the tropics, especially Africa. In temperate regions most of the influenza cases are reported between December and March while in tropical and subtropical regions it is highly variable but the following three patterns have been observed: first, infections occur all year round with peaks related to the rainy seasons, as seen in India and Senegal, secondly infections occur all year round with biannual peaks associated to rainy season and winter months; and finally infections occur year round without a clear seasonality (Chow et al., 2006, Bechett et al., 2004, Doraisingham et al., 1988, Dosseh et al., 2000, Nguyen et al., 2007, Rao and Banerjee, 1993, Tsai et al., 2001) . Until recently, the burden of influenza in Africa as a whole and West Africa in particular was believed to be negligible. However, reports from the Gambia, Senegal, Ivory Coast, and Gabon, have indicated that influenza is circulating in West Africa and may be causing epidemics regularly(Yazdanbakhsh & Kremsner, 2009). Again, the setting up of influenza sentinel surveillance systems in tropical and subtropical countries in response to the worrying situation created by avian influenza and the recent influenza A(H1N1)pdm09 has contributed to an increase in the number of studies about influenza in such countries.

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Contrary to what happens in temperate climates, much less is known about the epidemiology and seasonality of influenza in tropical countries. Gesnner *et al*., 2011, in a recent review, identified the fact that little is known about influenza epidemiology in sub Saharan Africa. Although in recent years, there has been increasing data on the potential magnitude of influenza burden in sub-tropical and tropical areas, these were sporadic outbreak reports or hospital-based studies from wealthier tropical countries (Viboud et al., 2004, Alonso et al. 2007)

2.10 Influenza in Ghana

In 1973, a total of 23,858 cases of influenza A/Hong Kong/1/68 were recorded from selected health centres in the Greater Accra region between the periods of 1st October 1973 to 30th December 1973 (Addy et al., 1976). In 1996, Mingle et al. reported that there was an outbreak of Influenza A (H3N2) in Accra. (Mingle et al., 1996)

Previous studies conducted in Ghana in 1976 proved that Ghana was significantly affected during the Hong Kong influenza epidemic where of the 23,858 cases reported, approximately 51% were children less than 5 years. (Rao and Banerjee, 1993).

For early detection and response to these outbreaks, Ghana identified the need to put in place a surveillance system to monitor the trends and possible predictors of Influenza activity in Ghana.

In 2007, Ghana established an Influenza surveillance system with the aim of understanding the epidemiology and seasonality of the strains of influenza virus that may be circulating in the country.

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The Ghana Health Services in September 2007 with support from the United States Naval Medical Research Unit (US-NAMRU 3), Cairo, the World Health Organization, the Centers for Disease Control and Prevention (CDC), United States and the Noguchi Memorial Institute for Medical Research (NMIMR), University of Ghana, Legon, initiated an influenza sentinel surveillance programme in Ghana. Currently, Ghana has 24 sentinel sites covering all 10 regions in the country.

2.11 Antipyretics

Antipyretics are a group of medications that are known to reduce fever("Merriam-Webster Online Dictionary," 2014). Fever is a symptom that emanates as a result of an infection or host immune response to non-infectious inflammatory stimulus(Hawksworth et al., 2009; O'Grady et al., 2008) and it is one of the top managed symptom by paediatricians(Sullivan & Farrar, 2011). Although fever is considered as a beneficial nonspecific immune response to invasion by microorganisms, it's also known to provide discomfort resulting in the intake of antipyretics to suppress the elevated body temperature (Aronoff & Neilson, 2001). Many parents still administer antipyretics even when there is minimal or no fever. Studies have shown that about one half of parents consider a body temperature less than 38^{o}C as fever. The use of antipyretics will therefore remain as a common practise to relief fever to provide some sort of security(Sullivan & Farrar, 2011). In Ghana, self-medication is a common practice which may increase the use the antipyretics such as paracetamol, prior to visiting a healthcare provider. (Adu-Sarkodie, 1997).

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2.11.1 Mechanism of fever generation

The process of fever generation is initiated when microbial tissues are invaded by microorganisms. This results in inflammatory response causing the activation of local vascular endothelial cells and leukocytes. The activated leukocytes release pyrogenic cytokines interleukin-1b (IL-1b), tumor necrosis factor (TNF), and interleukin-6 (IL-6). Hematogenous dissemination will then allow these endogenous pyrogens to stimulate vascular endothelial cell to produce prostaglandin E_2 (PGE_2) within the central nervous system. Concurrently, peripheral inflammatory signals may be sent along the neural connections (such as the vagus nerve) to trigger central nervous system PGE_2 production (Romanovsky et al., 1998). Neurons within the preoptic area of the anterior hypothalamus (POAH) bearing specific E-prostanoid receptors orchestrate the febrile response after the PGE_2 signal.

The PGE_2 changes the firing rate of these neurons which results in an elevated thermoregulatory set point to a temperature usually equal to or greater than 37.5^{o}C as illustrated in the Figure 4. (Aronoff & Neilson, 2001).

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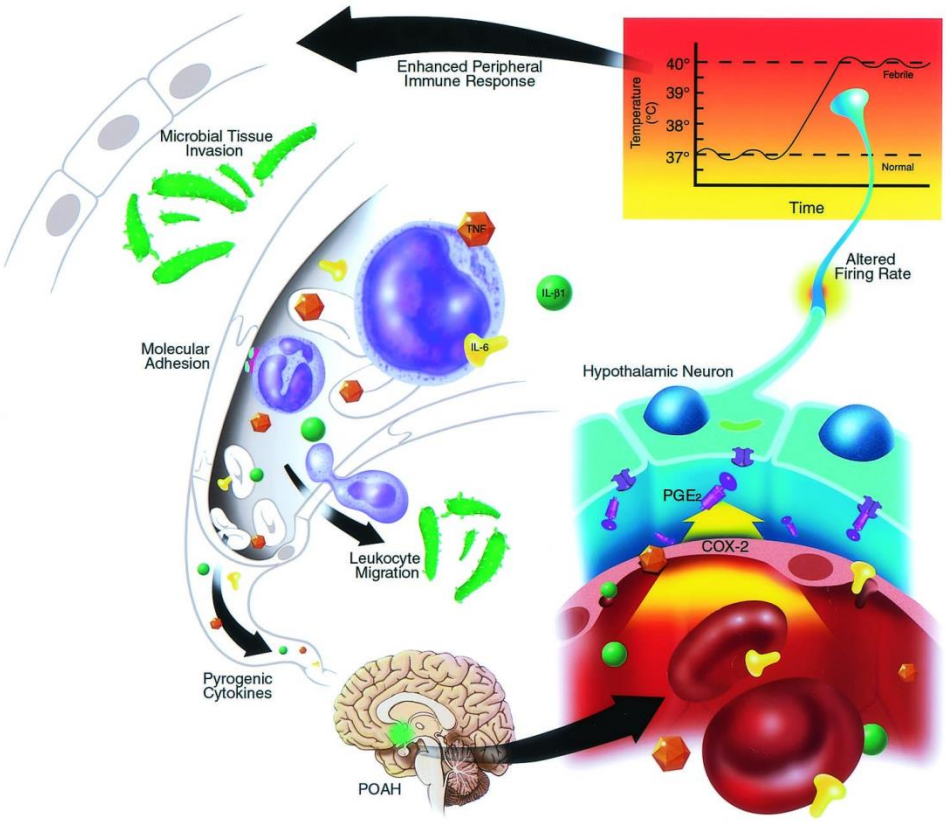
Figure 4: Mechanism of fever generation after an infection



Source: (Aronoff & Neilson, 2001)

2.11.2 Mode of Action of Antipyretics

The mode of action of most antipyretics is by reducing the level of prostaglandin E_2 in the central nervous system. This can be achieved by inhibiting the enzyme cyclooxygenase which plays a major role in increasing the level of prostaglandin E_2 in the hypothalamus. Other mechanisms of action of some antipyretics have been described in recent times. These include reduction in proinflammatory mediators, enhancement of anti-inflammatory signals at sites of injury or boost of antipyretic messages within the brain.(Aronoff & Neilson, 2001)

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CHAPTER THREE

3.0 METHODS

3.1 Study Design

This was a cross-sectional study conducted in 4 health facilities in the Shai-Osudoku and Ningo–Prampram Districts (SONPD) in the Greater Accra region between the periods of September 2013 and May 2014.

3.2 Study area

The study area was previously known as Dangme–West district and was divided into two separate districts as Shai-Osudoku and Ningo–Prampram Districts (SONPD) in 2013. Figure 5 shows the map of the study area with the four study sites.

The SONPD are located in the south-eastern part of Ghana and lies between latitude 5˚ 45' South and 6˚ 05' North and longitude 0˚ 05' East and 0˚ 20' West. It is about 40.8 kilometres away from the national capital, Accra. It is the district with the largest land surface area (about 1,700 square kilometres) in the Greater Accra region, covering about 40.5% of the total land size within the Region. The land is flat and at sea level with isolated hills.

Most of the district inhabitants are subsistence farmers or fishermen. Other occupations are petty trading. There are a handful of trained artisans, craftsmen and a few civil servants, mainly migrant employees of government ministries, departments and agencies. The vegetation is mainly coastal savannah.

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Based on the 2010 population census the projected district population for the year 2012 was 130,570. It is a fairly rural coastal district with mostly scattered communities. Communities with the largest populations are only 5-6,000 people and the people are poor and deprived.

There are 24 health facilities in the district. These comprise: 1 District hospital, 4 Health Centers, 13 Community-based Health Planning and Services (CHPS) compounds and 6 private facilities

The district has a Health and Demographic Surveillance System (HDSS) housed within the Dodowa Health Research Centre located in the Dodowa sub-district. The HDSS provides a framework for monitoring demographic dynamics in the district. The HDSS enumerates the district's population, recording every individual, their relationships, ages as well as other attributes. The population is prospectively followed over time to record births, deaths, in and out migrations and selected additional (i.e., geographic, socio-economic) information (Dangme West District Health Services Directorate, 2010).

The Dodowa HDSS provides the infrastructure and opportunity for a comprehensive study by integration of the population data with an effective documentation system at the health facilities. Irrespective of the division of the districts, the HDSS continues to cover the population of both Shai-Osudoku and Ningo-Prampram Districts. For the purpose of this study, Shai-Osudoku district hospital, Prampram health centre, Old Ningo health centre and Osudoku health centre were the selected study sites based on the fact that NMIMR in collaboration with the Centres for Disease Control and Prevention (CDC) and GHS are currently conducting a population based study in the district at the aforementioned facilities with the aim of determining the burden of

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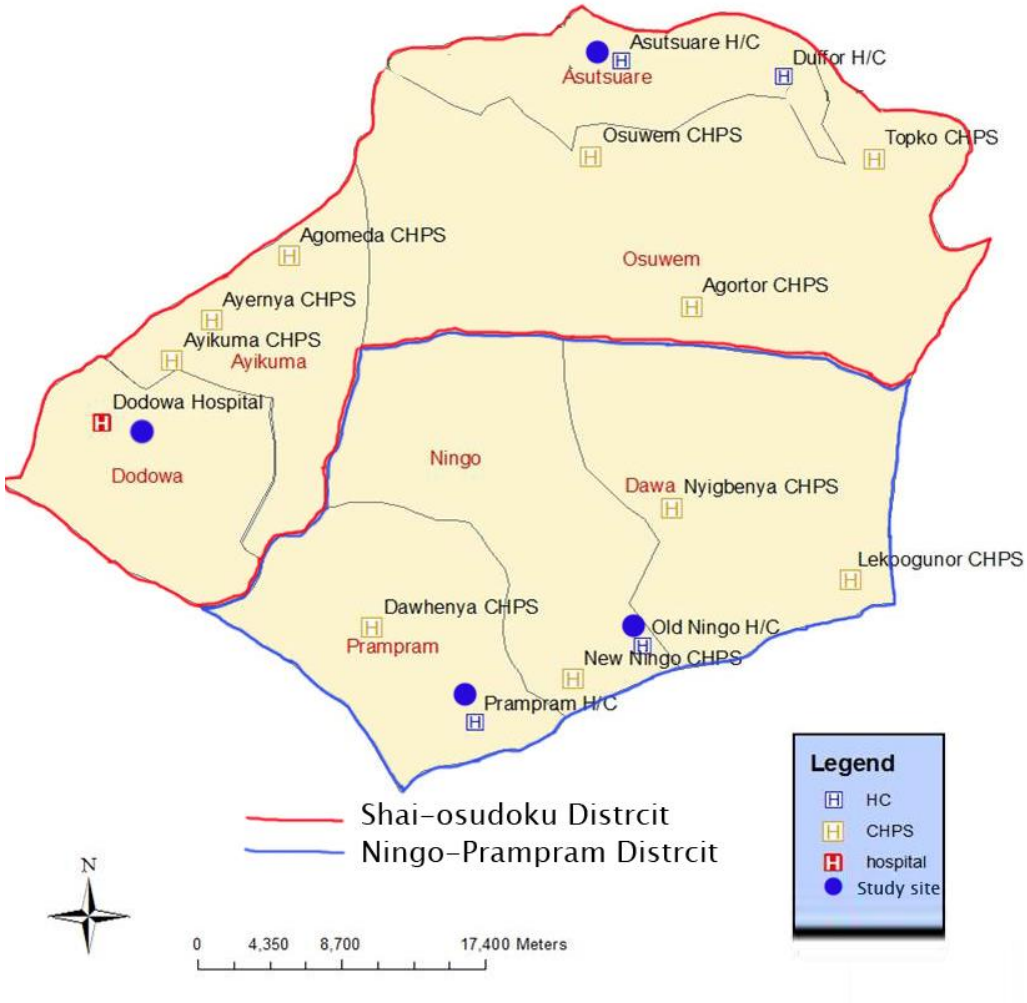
Influenza in the district. This background knowledge made the SONPD a suitable site for this study.

Figure 5: District Map: Shai-Osudoku and Ningo-Prampram District



Source: Dodowa Health Research Centre

3.3 Sample size

The minimum sample size was determined by the formula;

$$N = \frac{Z^2 (P) (1-P)}{(M)^2}$$

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Where N = sample size,

P = estimated prevalence of Influenza in Shai-Osudoku/ Ningo-Prampram District (SONPD) of Ghana. An Integrated Hospital Based Influenza Disease Surveillance (IHBIDS) conducted in the district in 2012 showed an influenza prevalence of approximately 15% among patients who met the WHO case definition for ILI.

Z = confidence level at 95% (standard value of 1.96)

M = margin of error at 4% (standard value of 0.04)

Using the above parameters, the minimum sample size was calculated as;

$$\frac{1.96(0.15)\ (1-0.15)}{(0.04)2} = 306 \text{ samples}$$

Adjusting for non-response rate of 5% where there could be 3% occasional withdrawal of consent and 2% improper handling/storage, we had (0.05 x 306) + 306 = 321. The final sample size was 321.

3.4 Sampling Method

Clinical and demographic data were collected from patients who sort ambulatory care at the study sites and met the inclusion criteria stated in the next paragraph. These patients were identified by trained healthcare providers (physician, nurse, midwife or research assistant) and enrolled into the study until the desired sample size was achieved.

Participants were sampled from Monday to Friday, 8:00 am to 5:00 pm each day. Any patient who met the inclusion criteria for the study was enrolled.

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Variables

The data collection focused on the following variables:

Independent variables:

- Measured fever ≥38°C
- History of fever

Dependant variables:

Demographic characteristics:

- Age
- Sex
- Occupation
- Education status
- Place of residence
- Distance from residence to health facility

Clinical characteristics:

- Pre-existing medical conditions
- Influenza vaccination status
- Illness onset
- Clinical symptoms
- Measured body temperature
- Treatment history

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Inclusion criteria

Patients' aged 1 month and older seeking care at the outpatient department (OPD) of the study facilities and met the ILI case definition of;

A history or measured fever of ≥37.5°C (Axillary) and cough with onset within the last ten days and consented to be part of the study.

Exclusion criteria

Patients less than 1 month or older than a month who did not have history or measured fever of ≥37.5°C (Axillary) and cough and or did not give their consent to be part of the study were excluded.

3.5 Data collection

Field staff were trained on the use of PDA and how to fill in the questionnaires accurately. Field staff included physicians, nurses, midwives and research assistants. Demographic and clinical information were collected using structured questionnaires. The information on the questionnaire was then entered onto the PDA. Data captured on the PDAs were backed up on a Secured Digital Memory Card (SD card) weekly and synchronized with a database at NMIMR. Structured questionnaires were picked up from the sites weekly and information entered into a database at NMIMR.

3.6 Ethical Considerations

Ethical clearance was obtained from the Ethical Review Committee of Noguchi Memorial Institute for Medical Research.

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Consent procedures

The risks and benefits of participating in the study were discussed with the patient prior to enrolment. On agreement, the participants were asked to provide written consent and enrolled into the study. A parent/legal guardian was asked to give consent on behalf of participants <18 years. Children aged 5 to 17 years were asked to provide assent. Only parental/guardian consent was obtained for children less than 5 years of age. On giving the informed consent, the patient was enrolled and appropriate biological samples - Oropharyngeal/Nasopharyngeal (OP/NP) swabs were collected for laboratory diagnosis. A questionnaire was administered to the participants to collect basic demographic and clinical information.

Privacy and confidentiality

All data were kept in a confidential manner at the sites and NMIMR where the samples were analysed. For ethical compliance, logbooks, completed consent forms, and Personal Digital Assistant (PDA) were kept in locked file cabinets at the sites and the electronic database was access protected. All electronic copies of the data were password-protected. No personal identifiers were used in labelling clinical samples.

Risks and benefits

Risks: The risk directly associated with sample collection which was slight discomfort when collecting throat and nasal swabs were described to the participants.

Anticipated benefits: It was explained to the participants that no direct benefits were associated with their participation in the study; however, results of this study are likely to influence policy guidelines and thus benefit a larger group.

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3.7 Sample Collection

Cryovials containing Virus Transport Medium (VTM) were obtained from -20°C freezer and thawed by twirling between palms or obtained directly from a 4°C fridge. VTM with swab were maintained at 4°C before testing.

Gloves and all other materials that were used during sample taking were disposed off as potentially infectious waste. Swabs were removed from its wrapper using sterile technique to avoid contamination of the sample collection kit.

Collection of Oropharyngeal swab

Swab was inserted through the patient's mouth to the back of the throat (posterior pharynx) and rolled to scrape the mucosa to take an oropharyngeal swab. The tip of the applicator swab with the specimen was inserted into the vial containing the VTM and the excess shaft was cut off.

Collection of Nasopharyngeal swab

A nasopharyngeal swab was inserted into one nostril straight back (not upwards) and continued along the floor of the nasal passage for several centimetres until reaching the nasopharynx (resistance will be met on reaching the posterior pharynx). The distance from the nose to the ear gave an estimate of the distance the swab should be inserted. In the event of an obstruction, force was not used; swab was removed and tried in the other nostril.

Swab was rotated gently for 5-10 seconds to loosen the epithelial cells. Swab was removed immediately and placed into the vial containing VTM. The excess shaft was cut off and vial properly closed to avoid leakage. An ID and sample collection date was assigned to the vial. Initials of staff who took the sample, date and time of sample

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collection and type of swabs collected was indicated on the laboratory form. Laboratory forms were placed in a folder and kept in a cabinet under lock and key.

3.8 Sample Transport

Samples were collected from study sites at least twice in a week and transported in screw cap leak proof plastic containers wrapped in adsorbent material and kept in cool box with ice packs.

Samples transported were accompanied with the laboratory forms. All samples transported were documented into a Laboratory logbook located onsite.

3.9 Laboratory analysis of respiratory specimen

Samples were processed in the lab within 2 to 3 hours on arrival at NMIMR. Date and time of sample receipt was recorded. Samples were given laboratory IDs on receipt and recorded on the vials and laboratory form. Details on laboratory form were entered into a Structured Query Language (SQL) 2008 database. Samples were thawed by twirling between palms and vortexed. Ribonucleic acid (RNA) was extracted from samples using QIAamp RNA extraction mini kit and stored temporarily at 4°C for PCR testing. RNA was tested for Influenza A, Influenza B and Ribonucleic Protein (RNP) by RT-PCR analysis using Centres for Disease Control and Prevention Protocol. All samples that tested positive for Influenza A were subtyped for targets, Influenza A(H1N1), A(H3N2) and A(H1N1)pdm09. PCR results and date of analysis were recorded on the Lab form and updated in the SQL

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database. Clinical samples and RNA extracts were stored in an Ultra-low freezer and storage location indicated in a log book.

3.9.1 RNA Extraction

Bio-safety cabinet, work surfaces, centrifuges and pipettes were cleaned and decontaminated with 10% bleach, 70% alcohol and RNA ZAP to minimize risk of nucleic acid contamination and degradation.

Single stranded viral RNA was extracted using the QIAamp® Viral RNA Mini Kit commercially available from QIAGEN (Valencia, California, USA). The viral RNA mini spin procedure as recommended by the manufacturer was used and the manufacturer's instructions were followed. Five hundred and sixty µl of lysis buffer containing carrier RNA was added to 140 µl of nasopharyngeal and oropharyngeal specimen and mixed by pulse vortexing. The mixture was incubated at room temperature for 10 minutes. Five hundred and sixty µl of 96 – 100% molecular grade ethanol was added and again mixed by pulse vortexing. Six hundred and thirty µl of the mixture was transferred to the QIAmp Mini spin column and spun at 8000 revolutions per minute (rpm) for 1 minute. This step was repeated for the remaining 630 µl of the mixture. The RNA now bound to the membrane in the spin column underwent two washing steps with 500µl of washing buffers designated AW1 and AW2 to remove all contaminants. Bound RNA was then eluted in 40µl of a special RNAse – free buffer into DNAse/RNAse free tubes. Extracted RNA was stored at -30^{O}C until use. The volume of RNA used as template for rRT-PCR was 5µl.

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3.9. 2 RRT-PCR for influenza virus detection

To avoid contamination, the following precautionary procedures were followed to avoid false positive amplifications:

- Separate areas for assay set up and nucleic acid handling were maintained
- Clean lab coat and powder free gloves were used in setting up the assays.
- Separate, dedicated equipment (e.g. pipettes and micro-centrifuges) and supplies (e.g. micro-centrifuge tubes and pipette tips) were maintained for assay set up and handling of extracted nucleic acids.
- Reagent and reaction tubes were capped or covered as much as possible to avoid contamination

Bio-safety cabinet, work surfaces, centrifuges and pipettes were cleaned and decontaminated with 10% bleach, 70% alcohol and RNA ZAP to minimize risk of nucleic acid contamination and degradation.

All reagents were kept on ice during assay set up. The enzyme was kept at -20^{o}C until needed. Thawed primers and probes kept at 2 – 8^{o}C in the dark were pulse vortexed and stored on ice.

Each sample RNA extract was first tested for Influenza A, Influenza B and RNaseP using their respective primer and probe set. The RNaseP primer and probe set targets the human Rnase P gene and thus serves as an internal positive control for human nucleic acid. Negative template controls (NTC) and positive template controls (PTC) for all primer and probe sets were included in each run.

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All the samples that are positive on Influenza A primer and probe sets were then tested by separate primer and probe sets for influenza A(H3N2) and A(H1N1)pdm09. Twenty uL of Master Mix cocktail ingredients was prepared using the AgPath-ID™ One Step RT-PCR Kit (Applied Biosystems). The Master mix contained 12.5 µl of 2X RT-PCR Buffer (Ag-Path-ID™), 1 µl of reverse transcriptase/Taq mix (25X RT-PCR Enzyme Mix, Ag-Path-IDTM), 0.5 µl of each type specific primer and probe set. The Master mix was dispensed into the 96-well microtitre plates (RNA extracts and controls designated wells) in the Master Mix preparation bio-safety cabinet.

Five uL of samples and controls were added to the appropriate test reactions on the 96 well plate in the sample addition bio-safety cabinet. The plate was centrifuged for 5 seconds to collect the contents at the bottom of the tube and then placed on ice. Amplification was performed on the Applied Biosystems® 7300 Real-Time PCR instrument (Life Technologies, USA). Cycling conditions for all primer/probe sets consisted of a reverse transcriptase step of 50ºC for 30 min, followed by a *Taq* polymerase activation step of 95ºC for 2 min and then 45 cycles of 95°C for 15 sec (denaturing) and 55°C for 30 sec (annealing and extension step). Data was collected at the 55°C for 30 sec (annealing and extension) step. A unidirectional workflow technique was employed to prevent contamination and ensure integrity of all laboratory testing. This was archived by working from the reagent (master mix) preparation area through to the amplification area.

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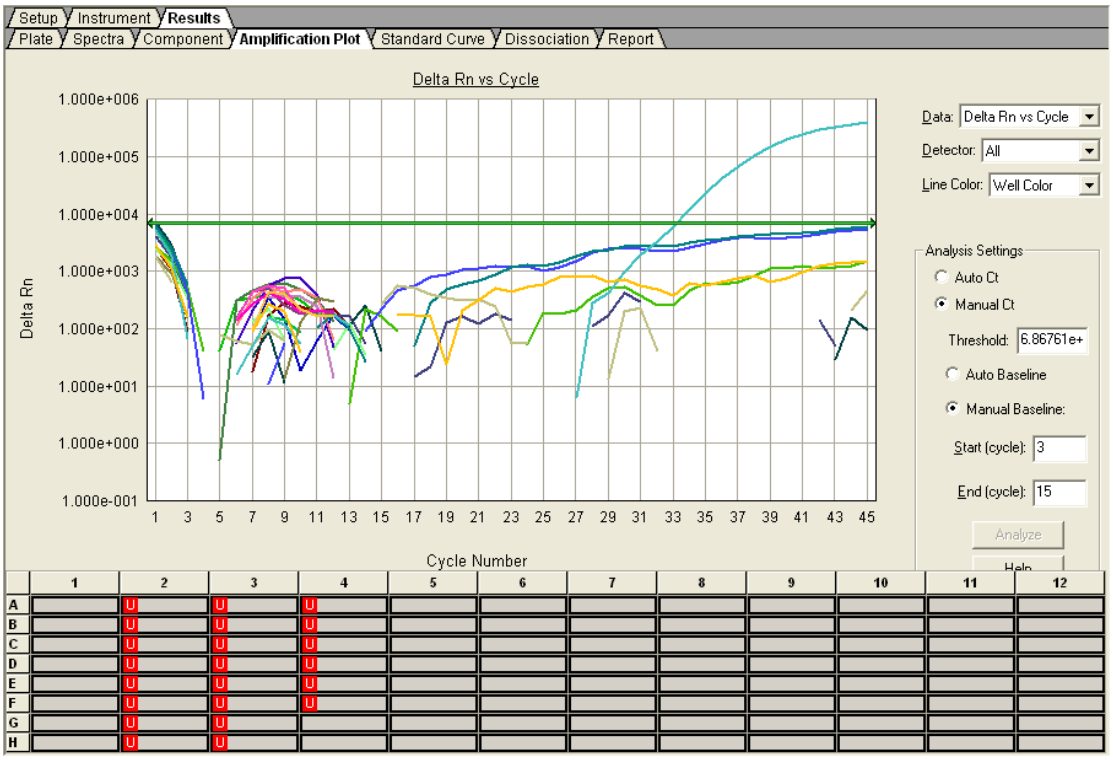
Interpretation of Results

With controls meeting all stated requirements, a specimen was considered positive for influenza A/B if the Influenza A/B reaction growth curves cross the threshold line within 37 cycles as shown in Figure 6 below. A specimen was considered positive for A (H1N1) pdm09 or A (H3N2) virus if both Influenza A and the respective subtype (A(H1N1)pdm09 or A(H3N2)) reaction growth curves cross the threshold within 37 cycles.

Figure 6: Real Time PCR amplification plot



3.9.3 Sample Quality Assurance and Quality Control

Human Specimen Control (HSC) was extracted with every batch of RNA extraction done for all samples. This was an internal control that gave an indication that the RNA extraction was done properly. This was confirmed by having amplification for only the RNP of the HSC.

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Separate workstations were used for RNA extraction, preparation of master mix and addition of samples to avoid contamination. PCR testing was done together with positive and negative controls for each run. It was expected and observed that there was no amplification for the negative controls since they had no template.

A Levey-Jennings graph which is used to monitor controls used for laboratory analysis was plotted for the Ct Values of all PCR done for the week. This was to ensure that all the Ct Values of the positive controls used were not more than 2 standard deviations away from the mean Ct Values for each control.

3.10 Data Analysis

Data was analysed using Statistical Package for the Social Sciences (SPSS; IBM Corporation, Armonk, NY) as per the study's specific objectives.

Data collected was grouped into patients with measured fever ≥37.5°C and influenza cases with history of fever. Univariate analysis was conducted to describe the proportion of influenza cases with measured fever ≥37.5°C and influenza cases with history of fever. Categorical variables were expressed in the form of frequencies and percentages. Appropriate measures of central tendency such as mean age and modes were calculated.

Bivariate analysis was used to assess antipyretic usage among ILI cases with measured fever ≥37.5°C and ILI cases with history of fever. Odds ratio and their corresponding 95% CI were used to assess association of antipyretic usage and history of fever with Influenza virus infection. The effect of antipyretic usage and history of fever on the case definitions for ILI was also determined using bivariate analysis (Contingency table). The accuracy of 3 case definitions was assessed based on their sensitivity and specificity.

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These case definitions were;

A. History of fever and Cough with onset of illness within the last 10 days

B. WHO case definition: Measured fever ≥ 37.5°C and cough with onset of illness within the last 10 days

C. Antipyretic use with history of fever and cough with onset of illness within the last 10 days

3.10.1 Data Management

Data was stored and managed using a Structured Query Language (SQL) 2008 database. Data was entered with checks created to ensure quality data for analysis.

3.11 Data quality

Study sites were visited twice a week to monitor consenting, enrolment and specimen collection procedures. Hospital records of patients enrolled in the study were reviewed to determine if there were any mistakes in the enrolment process. Consent forms were reviewed to ascertain that proper consenting was done at the study site. Specimen collection and handling was monitored to ensure that the correct procedure was followed.

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CHAPTER FOUR

4.0 RESULTS

4.1 Demographic characteristics of study participants

Of the 321 participants enrolled into the study, 86(26.79%) were from Shai-Osudoku district hospital (SOD), 83(25.86) from Old-Ningo Health Centre (OHC), 65(20.25%) from Osudoku Health Centre (OSU) and 87(27.1%) from Prampram Health Centre (PHC).

Among the participants, 164(51.09%) were females and 157(48.91%) were males. Table 1 describes the age and sex distribution of study participants per study sites. The age range of the participants was 1 month to 76 years with a mean age of 10 years SD $\pm$ 13.24. The mean ages for male and female participants were 7 years SD $\pm$ 10.94 and 12 years SD $\pm$ 14.75 respectively.

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Table 1: Age and Sex Distribution of Study Participants from Study Sites, Shai-Osudoku and Ningo-Prampram districts, 2014

N=321	Dodowa Hospital		Osudoku Health Centre		Prampram Health Centre		Old Ningo Health Centre		Total		Overall total
Age Group	Male n(%)	Female n(%)	Male n(%)	Female n(%)	Male n(%)	Female n(%)	Male n(%)	Female n(%)	Male n(%)	Female n(%)	
< 5	27(8.41)	21(6.54)	22(6.85)	19(5.92)	24(7.48)	23(7.17)	30(9.35)	21(6.54)	103(32.09)	84(26.17)	187(58.26)
5 to 14	7(2.18)	12(3.74)	5(1.56)	6(1.87)	14(4.36)	4(1.25)	9(2.80)	6(1.87)	35(10.9)	28(8.72)	63(19.63)
15 to 24	2(0.62)	3(0.93)	1(0.31)	3(0.93)	3(0.93)	5(1.56)	1(0.31)	5(1.56)	7(2.18)	16(4.98)	23(7.17)
25 to 34	1(0.31)	6(1.87)	4(1.25)	1(0.31)	0(0.00)	8(2.49)	2(0.62)	3(0.93)	7(2.18)	18(5.61)	25(7.79)
35 to 44	0(0.00)	4(1.25)	1(0.31)	2(0.62)	0(0.00)	5(1.56)	0(0.00)	3(0.93)	1(0.31)	14(4.36)	15(4.67)
45 to 54	0(0.00)	1(0.31)	0(0.00)	0(0.00)	1(0.31)	0(0.00)	0(0.00)	1(0.31)	1(0.31)	2(0.62)	3(0.93)
55 to 64	2(0.62)	0(0.00)	1(0.31)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(0.31)	3(0.93)	1(0.31)	4(1.25)
≥ 65	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(0.31)	0(0.00)	1(0.31)	1(0.31)
Total	**39(12.15)**	**47(14.64)**	**34(10.59)**	**31(9.66)**	**42(13.08)**	**45(14.02)**	**42(13.08)**	**41(12.77)**	**157(48.91)**	**164(51.09)**	**321(100)**

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Table 2 shows demographic characteristics of participants in terms of educational status, marital status and occupation. Of the 321 participants enrolled 208 (64.80%) were children below 6 years and 10(3.12%) were illiterates. The same trend was observed in all four facilities. Thirty-five (10.97%) were married, 278 (87.15%) were single and 2 (0.63%) were divorced. The participants were predominantly children (61.99%) and students (18.69%). Among the participants, 172 (53.58%) and 149 (46.42%) were from Ningo-Prampram and Shai-Osudoku districts respectively.

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Table 2: General Demographic characteristics of participants, Shai-Osudoku and Ningo-Prampram Districts, 2014

Characteristics N=321	n(%)	95% CI
Educational Status		
Illiterate	10(3.12)	3.12 - 1.59
Junior High School (JHS)	21(6.54)	6.54 - 4.19
Pre-school	208(64.80)	64.8 - 59.27
Primary	54(16.82)	16.82 - 12.99
Senior High School (SHS)	16(4.98)	4.98 - 2.97
Tertiary and above	9(2.80)	2.8 -1.37
Unknown	3(0.93)	0.93 - 0.24
Marital Status		
Divorced	2(0.62)	0.11 - 2.48
Married	35(10.90)	7.81 - 14.96
Single	280(87.23)	82.96 - 90.58
Widow/widower	4(1.25)	0.4 - 3.38
Occupation		
Beautician	1(0.31)	0.02 - 2.00
Caterer	1(0.31)	0.02 - 2.00
Child	199(61.99)	56.41 -67.28
Driver	1(0.31)	0.02 - 2.00
Electrician	1(0.31)	0.02 - 2.00
Farmer	11(3.43)	1.81 - 6.23
Hairdresser	3(0.31)	0.24 2.94
House wife	1(0.31)	0.02 - 2.00
House help	1(0.31)	0.02 - 2.00
Mechanic	1(0.31)	0.02 - 2.00
Nurse	1(0.31)	0.02 - 2.00
Research assistant	3(0.31)	0.24 - 2.94
Seamstress	3(0.31)	0.24 - 2.94
Student	60(18.69)	14.67 23.48
Tailor	1(0.31)	0.02 - 2.00
Teacher	5(1.56)	0.42 - 5.38
Trader	21(6.54)	4.19 - 9.98
Unemployed	6(1.87)	0.76 - 4.23
Welder	1(0.31)	0.02 - 2.00

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4.2 Clinical Characteristics of study participants, Shai-Osudoku and Ningo-Prampram districts, 2014

Table 3 shows the clinical signs and symptoms presented by the study participants Aside cough which was the primary requirement for inclusion into the study, 229(71.34%) of the participants presented with runny nose, 236(73.52%) reported with a history of fever, 124(38.63%) had chills, 81(25.23%) had fever ≥ 37.5^{o}C at the point of enrolment followed by 70 (21.81) participants with chest pain.

Other symptoms recorded were sore throat, myalgia, diarrhea, headache, breathing difficulty, shortness of breath, abdominal pain and vomiting. A bivariate analysis to assess the association of the clinical symptoms with laboratory confirmed influenza infection showed that patients with laboratory confirmed influenza are more likely to present with headache (OR: 3.1, CI: 1.39 – 6.93, P-value: < 0.01) and a measured axillary body temperature ≥ 37.5^{o}C (OR: 2.82, CI: 1.61 – 4.95, P-value: < 0.001). One participant had a history of smoking. Six of the participants indicated that they received influenza vaccine in the year 2010 during the influenza A(H1N1)pdm09 outbreak

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Table 3: Clinical symptoms from cases with Influenza Laboratory testing, Shai-Osudoku and Ningo-Prampram Districts, 2014

Symptoms	Response	Total Tested	Influenza negative		Influenza positive		OR	95% CI	P - Value
			No.	%	No.	%			
	All	321	252		69				
Sore throat	Yes	67	49	19.44	18	26.09	0.94	0.48-1.85	0.86
	No	114	82	32.54	32	46.38			
	Unknown	140	121	48.02	19	27.54			
Runny Nose	Yes	229	179	71.03	50	72.46	1.03	0.57-1.97	0.92
	No	89	70	27.78	19	27.54			
	Unknown	3	3	1.19	0	0.00			
Chills	Yes	124	90	35.71	34	49.28	1.48	0.83-2.66	0.19
	No	128	102	40.48	26	37.68			
	Unknown	69	60	23.81	9	13.04			
Myalgia	Yes	67	45	17.86	22	31.88	1.71	0.88-3.35	0.11
	No	122	95	37.70	27	39.13			
	Unknown	132	112	44.44	20	28.99			
Diarrhoea	Yes	61	55	21.83	6	8.70	0.34	0.14-0.83	0.01
	No	256	194	76.98	62	89.86			
	Unknown	4	3	1.19	1	1.45			
Headache	Yes	28	16	6.35	12	17.39	3.1	1.39-6.93	<0.01
	No	293	236	93.65	57	82.61			
	Unknown	0	0	0.00	0	0.00			
Breathing Difficulty	Yes	26	22	8.73	4	5.80	0.64	0.21-1.91	0.42
	No	292	227	90.08	65	94.20			
	Unknown	3	3	1.19	0	0.00			
Shortness of breath	Yes	13	11	4.37	2	2.90	0.65	0.14-2.99	0.57
	No	305	238	94.44	67	97.10			
	Unknown	3	3	1.19	0	0.00			

(*The unknown responses were not included in the contingency table analysis).*

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Table 3 continued…

Symptoms	Response	Total Tested	Influenza negative		Influenza positive		OR	95% CI	P - Value
			No.	%	No.	%			
	All	321	252		69				
vomiting	Yes	55	47	18.65	8	11.59	0.56	0.25-1.26	0.16
	No	263	202	80.16	61	88.41			
	Unknown	3	3	1.19	0	0.00			
Abdominal Pain	Yes	66	47	18.65	19	27.54	1.14	0.57-2.28	0.72
	No	99	73	28.97	26	37.68			
	Unknown	156	132	52.38	24	34.78			
Chest pain	Yes	70	48	19.05	22	31.88	1.3	0.66-2.57	0.45
	No	96	71	28.17	25	36.23			
	Unknown	155	133	52.78	22	31.88			
Measured Fever	Yes	85	51	20.24	30	43.48	2.82	1.61-4.95	<0.001
	No	236	201	79.76	39	56.52			
	Unknown	0	0	0.00	0	0.00			
History of Fever	Yes	236	201	79.76	39	56.52	0.33	0.19-0.58	<0.001
	No	85	51	20.24	30	43.48			
	Unknown	0	0	0.00	0	0.00			
Cough	Yes	321	252	100.00	69	100.00			
	No	0	0	0.00	0	0.00			
	Unknown	0	0	0.00	0	0.00			

(*The unknown responses were not included in the contingency table analysis).*

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4.3 Demographic characteristics of laboratory confirmed influenza cases

Figures 7 shows the distribution of laboratory confirmed influenza subtypes among study participants. Out of 321 Nasopharyngeal and Oropharyngeal samples processed, influenza virus was detected in 69 (21.50%) samples of which 4(4.36%) were A (H1N1) pdm09, 2(0.62%) were A(H3N2) and 53(16.51%) were influenza B virus.

Figure 7: Distribution of Influenza sub-types, Shai-Osudoku and Ningo Prampram districts

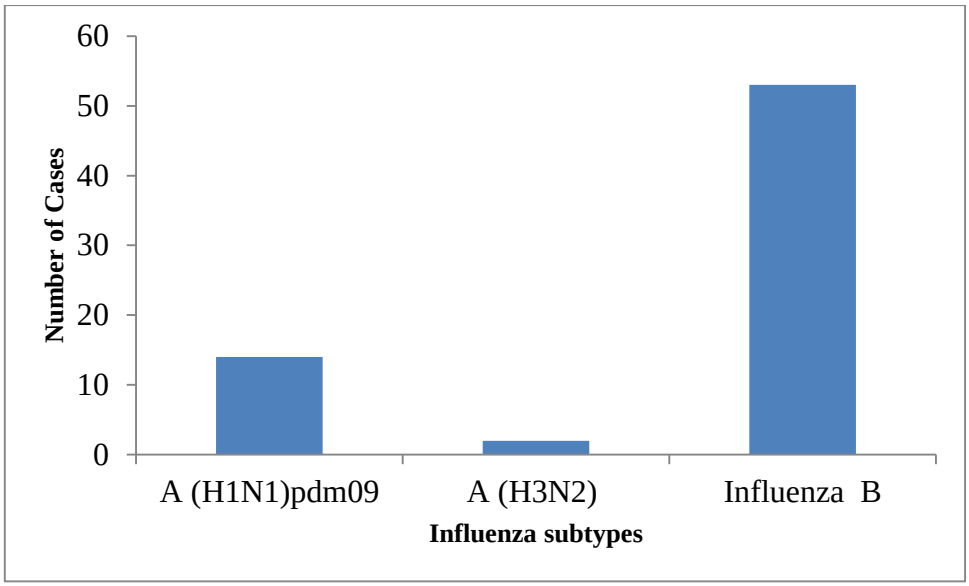

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Table 4 shows the distribution of influenza cases by age and sex. Females recorded the highest number of Influenza positives 38(55.07%), however, no significant difference was observed between males and females (P=0.46).

Children under five had the highest number of samples testing positive for influenza virus with 31(44.93%), followed by children within the age group 5-14 years with 24(34.78%) . This was statistically significant (P= 0.04). It was also observed from the bivariate analysis that children within the age groups 5 – 14 years (OR: 2.9, CI: 1.58 – 5.30, P value: <0.001) were more likely to develop an influenza infection.

Table 4: Distribution of Influenza viruses by age and sex, Shai-Osudoku and Ningo Prampram districts

Variable	**Characteristic**	ILI Cases		Influenza Positive		**P – Value**
		No.	**%**	**No.**	**%**	
Sex	Male	157	48.91	**31**	**44.93**	0.46
	Female	164	51.09	**38**	**55.07**	
Age Groups	< 5	187	58.26	**31**	**44.93**	0.04
	5 to 14	63	19.63	**24**	**34.78**	
	15 to 24	23	7.17	**4**	**5.80**	
	25 to 34	25	7.79	**6**	**8.70**	
	35 to 44	15	4.67	**3**	**4.35**	
	45 to 54	3	0.93	**1**	**1.45**	
	55 to 64	4	1.25	**0**	**0.00**	
	≥ 65	1	0.31	**0**	**0.00**	

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4.4 Proportion of ILI cases with measured fever ≥37.5°C and ILI cases with history of fever

Table 3 also shows the distribution of ILI cases with measured fever ≥37.5°C and ILI cases with history of fever. Among 321 participants, 236 (73.52%) had a history of fever and 85 (26.48%) had measured fever ≥ 37.5ºC at the time of enrolment. Out of 236 participants who had history of fever, 39(16.52%) were positive for influenza virus infection. Among 85 participants who had measured fever ≥ 37.5ºC, 30(35.29%) were positive for influenza virus infection.

However, a significant association was observed among patients with measured fever ≥ 37.5ºC (OR: 2.82, CI: 1.61 – 4.95, P-Value: < 0.001) and influenza virus, that is, patients with measured fever ≥ 37.5ºC were 3 times more likely to have had an influenza infection compared to patients with history of fever as shown in Table 5.

Table 5: Association of Measured fever ≥ 37.5ºC with Influenza

	Measured fever ≥ 37.5ºC		
	Yes	**No**	**Total**
Influenza			
Yes	30	39	69
No	54	198	252
Total	85	236	321

OR: 2.82, CI: 1.61 – 4.95, P<0.001

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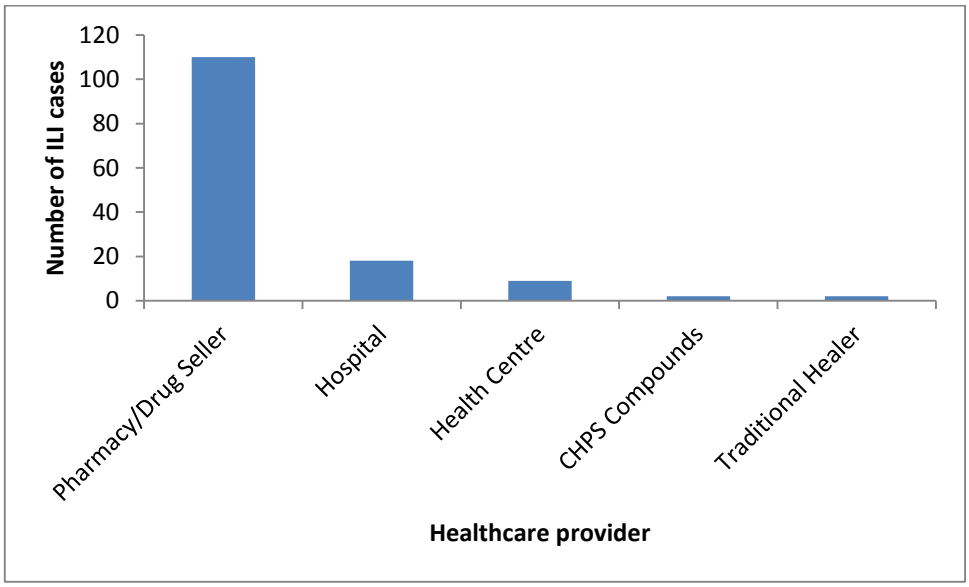
4.5 Assessment of antipyretic usage among ILI cases with measured fever ≥37.5°C and ILI cases with history of fever

Figure 8 shows the distribution of healthcare providers visited by study participants prior to their visit to the study site. Out of 321 participants enrolled into the study, 142 (44.24%) visited a healthcare provider for their illness. Among those who visited a healthcare provider prior to their visit to the health facility, 110 (34.27%) visited the pharmacy followed by 18(5.61%) who visited a hospital facility.

Figure 8: Healthcare providers visited by participants, Shai-Osudoku and Ningo Prampram districts, 2014



The data showed that 166 (51.71%) out of 321 participants took some medication for their illness before visiting the study facility. Figure 9 describes the medications by participants prior to visiting the study site. Antipyretics were the highest recorded with 121(37.69%) followed by cough syrup, 32 (9.97%). A total of 95(29.60%) participants took antipyretics before visiting the facility. Out of these 95 participants, 62(65.26%) had measured body temperature < 37.5°C.

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Figure 9: Medications by participants prior to visiting the study site, Shai-Osudoku and Ningo Prampram districts, 2014

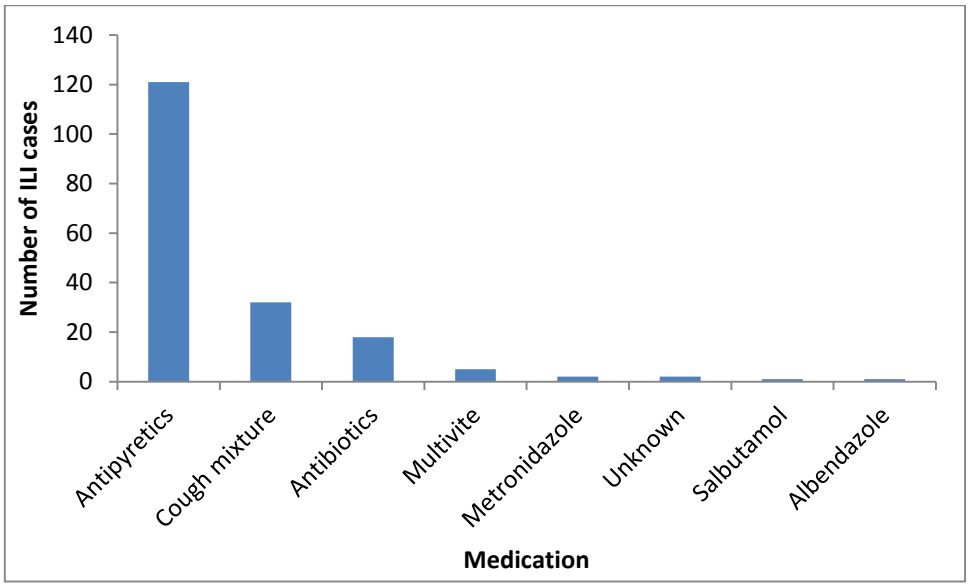

Table 6 shows that participants who took antipyretics before reporting to the facility were more likely to have a history of fever (OR: 1.78, CI: 1.05 – 3.00, P-Value: 0.03) compared to those who did not take any antipyretics.

Table 6: Association of antipyretics and history of fever

	History of Fever		
	Yes	**No**	**Total**
Antipyretic			
Yes	33	62	95
No	52	174	226
Total	85	236	321

OR: 1.78, CI: 1.05 – 3.00, P=0.03

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4.6 Effect of antipyretic usage and history of fever on sensitivity and specificity of ILI case detection

Out of 321 participants enrolled into the study, 236 had a history of fever and cough, 85 had measured fever ≥ 37.5ºC and cough and 62 had taken antipyretics with history of fever and cough prior to their visit to the facility. Among the 69 samples that were positive for influenza virus infection, 39(56.52%) had a history of fever and cough, 30(43.48%) had measured fever ≥ 37.5ºC and cough and 8(11.59%) had taken antipyretics with history of fever and cough.

Table 7 describes the accuracy of ILI case detection in relation to sensitivity, specificity, predictive values and likelihood ratios. History of fever and cough had the highest sensitivity (55.07%). Measured fever ≥ 37.5ºC and cough and antipyretic use with history of fever and cough had the highest specificity (78.57%). Measured fever ≥ 37.5ºC and cough had the highest PPV (36.47%) and NPV (83.09%). Participants with measured fever ≥ 37.5ºC and cough had the highest accuracy of 71.34%.

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Table 7: Accuracy of case definitions for ILI case detection

Case Definition	Sensitivity	Specificity	PPV	NPV	LR +	LR -	CDA
	%(CI)	%(CI)	%(CI)	%(CI)	%(CI)	%(CI)	%(CI)
A. History of fever and Cough	56.52 (43.38- 66.23)	21.43 (16.81-26.9)	16.53 (11.96- 21.33)	63.53 (52.92- 72.97)	0.70 (0.66 - 0.74)	2.01 (1.72 - 2.55)	28.66 (23.99-33.84)
B. Measured fever ≥ 37.5°C and cough	43.48 (33.77-56.62)	78.57 (73.1-83.19)	35.29 (27.03- 47.08)	83.09 (78.67- 88.04)	2.10 (1.87 - 2.35)	0.70 (0.66 - 0.74)	71.34 (66.16-76.01)
C. Antipyretic use with history of fever and cough	11.59 (5.99- 21.25)	78.57 (73.1- 83.19)	12.90 (6.686- 23.45)	76.45 (70.92-81.21)	0.54 (0.081 - 3.63)	1.13 (1.09 - 1.17)	64.17 (58.79-69.22)

PPV: Positive Predictive Value; NPV: Negative Predictive Value; CDA: Case Definition Accuracy;

LR+: Likelihood Ratio Positive; LR-: Likelihood Ratio Negative CI: Confidence Interval

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CHAPTER FIVE

5.0 DISCUSSION

The symptoms of Influenza infection are non-specific and make it difficult to make presumptive diagnosis based on clinical symptoms (Aguilera et al., 2002). The only way to confirm an influenza infection is by laboratory confirmation as it shares symptoms with other diseases with malaria being the most common in sub-Saharan Africa (McBratney, 2011). In furtherance to this, the WHO has set in place a case definition to help narrow the scope of case detection based on the most predictive symptoms. However, the purchase of over the counter drugs and self-medication, which appears to be a common practice in Ghana, has the tendency of relieving patients of these symptoms. This reduces the likelihood of them being detected by a surveillance system which has a case definition solely dependent on these predictors. This brings about the need to establish a sensitive and specific case definition for ILI case detection which takes into account such factors.

5.1 Clinical characteristics of study participants

Fourteen respiratory symptoms were assessed in this study. Although all participants presented with one or more of these symptoms, a bivariate analysis to assess the association of these clinical symptoms with laboratory confirmed influenza infection showed that patients who present with headache (OR: 3.1, CI: 1.39 – 6.93, P-value: < 0.01) are 3 times more likely to be diagnosed of influenza infection.

Studies have shown that, sore throat, breathing problems, aching muscles and abdominal pain are also significantly associated with influenza virus infection (Huang et al., 2012; Bonney et al, 2012). However, our data did not show any significant association with these indicators and this may be due to the fact that, it was difficult to

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assess some of these symptoms namely sore throat, in children under five who accounted for a greater fraction of the enrolled participants.

5.2 Demographic characteristics of laboratory confirmed influenza cases

Out of 321 samples analyzed in the lab, 69 samples are positive for influenza virus of which 4(4.36%) were A (H1N1) pdm09, 2(0.62%) were A(H3N2) and 53(16.51%) were influenza B. The circulating strains detected are similar to the ones circulating in other parts of sub-Saharan Africa (World Health Organization, 2014a). Although females recorded the highest number of positives, there was no significant difference in positivity compared to males (P=0.46) as seen in a study conducted by Haung et. al (2012). This indicates that gender is not a risk factor for influenza virus infection.

The analysis showed that children under five and those between the ages of 5 to 14 are the most affected with 44.93% and 34.78% respectively. Our data confirmed the WHO facts and a study conducted by Bonney et al (2012), which found these groups of people to be the population at higher risk of influenza infection. It has also been established that the very elderly are also at risk of influenza infection (Huang et al., 2012; World Health Organization, 2004). However, the findings from this study data did not confirm this finding. A possible explanation could be the fact that most of the participants were children less than 15 years.

5.3 Proportion of ILI cases with measured fever ≥37.5°C and ILI cases with history of fever

Out of 321 participants enrolled, 236(73.52%) has a history of fever and 85(26.48%) had a measured fever $\geq 37.5^{o}$C at the time of enrolment. Similar results were achieved

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in a study conducted by Babcock, Merz, & Fraser, 2006. Among 69(21.5%) total positives out of 321 participants, 39(56.52%) were detected among participants with history of fever and 30(43.48%) among participants with measured fever ≥ 37.5ºC. This shows that 39 out of the 69 (57%) patients with influenza will be missed if the WHO case definition is strictly applied.

The positivity rate among participants with history of fever is 16.53% and that of participants with measured fever ≥ 37.5ºC is 35.29%. This is similar to findings by Thursky, Cordova, Smith, & Kelly, et al.(2003). The positivity rate among participants with history of fever maybe under estimated. This is based on the fact that assessment of history of fever was based on the responses given by the participants with no clear evidence. Secondly, responses given are likely to be influenced by recall bias. All these factors may inflate the total number of participants with history of fever, thereby reducing the positivity rate.

Although a larger proportion of participants with influenza infection had a history of fever, our analysis suggests that participants with measured fever ≥ 37.5ºC (OR: 2.82, CI: 1.61 – 4.95, P-Value: < 0.001) are 3 times more likely to have had an influenza infection compared to those with a history of fever.

5.4 Assessment of antipyretic usage among ILI cases with measured fever ≥37.5°C and ILI cases with history of fever

In sub-Saharan Africa, purchase of over the counter drugs is a common practice which may increase the use the antipyretics prior to visiting a healthcare provider (Adu-Sarkodie, 1997). Among those who sought healthcare prior to their visit to the study site, 34.27% visited the pharmacy. This might be due to the fact that antipyretic

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medication can be purchased over the counter without prescription. Although fever is a natural immunological response to an infection, it raises some form of anxiety among participants who see it as sign of severity (Sullivan & Farrar, 2011). The data collected showed that 29.60% of the total participants took some medication for their current illness with antipyretics being the highest medication recorded with 37.69% (Walsh, Edwards, & Fraser, 2007). The bivariate analysis indicated that participants who took antipyretics before reporting to the facility were more likely to have a measured body temperature < 37.5ºC.

5.5 Effect of antipyretic usage and history of fever on sensitivity and specificity of ILI case detection

Influenza is an epidemic prone disease and therefore highlights the need to have an effective case definition that takes into account factors that will strengthen the surveillance system for early detection of outbreaks. Based on the different classifications of the case definitions (A, B and C), it is observed that sensitivity is higher in case definition A (56.52%) compared to case definition B (43.48%) and C (11.59%). This indicates that case definition A has a higher tendency of picking up true positives. For an influenza surveillance system which aims at early detection of outbreaks, there is the need to have a sensitive case definition to avoid losing a significant proportion of cases. This makes case definition A suitable for surveillance purposes.

On the contrary, case definition B and C has the highest specificity of 78.57% compared to A (21.43%) indicating that case definition B and C have a higher

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probability of picking up true negatives compared to case definition A. Case definition B has the highest PPV (35.29%), NPV (83.09%) and likelihood positive ratio (2.01). This makes it appropriate for presumptive diagnosis of influenza infection.

After calculating the accuracy of the case definitions based on the indicators specified earlier, case definition B (WHO case definition for ILI) had the highest accuracy of 71.34% followed by case definition C with a percentage accuracy of 64.17%.

5.6 Limitations

Although findings from this study will go a long way to impact public health action, the following limitations must be taken into consideration in interpreting the results;

- Identification of patients with history of fever was dependent on what the participant said which may be influenced by recall bias.

- The study focused on symptomatic illness and did not obtain data on asymptomatic infections, which may be an important group to consider.

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CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

A larger proportion of patients with ILI present to the health facility with a history of fever compared to those with measured fever ≥ 37.5^{o}C. A greater proportion of the patients with history of fever takes in antipyretics prior to seeking care at the health facility which results in a measured body temperature < 37.5^{o}C.

For an influenza surveillance system which aims at early detection of outbreaks, there is the need to have a sensitive case definition to avoid losing a significant proportion of cases. Sampling patients with measured axillary temperature ≥ 37.5^{o}C and patients with history of fever after antipyretic use will increase sensitivity of influenza case detection by an ILI surveillance system.

However, for presumptive diagnosis of influenza infection, the WHO case definition should be applied based on its case detection accuracy.

6.2 Recommendations:

To enhance ILI surveillance and improve the health of the people of Ghana, the following recommendations need to be implemented.

Ministry of Health

The ministry of health should broaden the current case definition for ILI by incorporating the use of antipyretics and history of fever into the WHO ILI case

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definition. This will enhance early outbreak detection and interventions to control and manage the spread of influenza outbreak.

Medical officers

In the absence of laboratory confirmation, clinicians should use the WHO case definition for presumptive diagnoses.

Patients who present with ILI symptoms without a measured axillary temperature

$\geq 37.5^{o}C$ but admits taking antipyretics to reduce fever should also be considered in the presumptive diagnoses of Influenza.

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APPENDIX 1: ADULT INFORMED CONSENT FORM

ADULT INFORMED CONSENT FORM

THE EFFECT OF ANTIPYRETICS USAGE ON THE ACCURACY OF WORLD HEALTH ORGANISATION (WHO) CASE DEFINITION FOR INFLUENZA LIKE ILLNESS (ILI)

Age of Participant: .....

Organizations: University of Ghana- School of Public Health (UG-SPH), Ghana Health Service (GHS), Noguchi Memorial Institute for Medical Research (NMIMR), U.S. Centers for Disease Control and Prevention (CDC).

Principal Investigator: Mr. Joseph Asamoah Frimpong

Supervisor: Dr. Kofi Mensah Nyarko

Reason for Research

Noguchi Memorial Institute for Medical Research, in partnership with Ghana Health Services and the U.S. Centers for Disease Control and Prevention, is doing a research study to understand the causes of respiratory illness in the Shai-Osudoku and Ningo-Prampram Districts. As part of this study we would also like to know the effect of antipyretics on the WHO case definition for influenza like illness. We are asking you to be part of this study because you are showing signs of respiratory illness. As part of this study, you will be asked to answer questions about your illness and to provide a specimen for testing. You may decline to answer any question and/or provide any sample. After we collect the sample, your participation in the study will be complete. Answering the questions and collecting the samples should only take a few minutes.

Possible Risks/Discomfort

If you agree to participate in this study, you will be asked to provide a throat and nose swabs. These samples will be collected using a thin plastic stick wrapped with polyester (Dacron) at one end to swab the back of your throat and nose. This is a routine clinical procedure. There will be little discomfort and you might gag during the collection of the sample.

Possible Benefits

Your participation in this research may help the Ghana Health Service find better ways to take care of people with respiratory infections in the future.

Confidentiality

Information about you will be kept confidential. All records will be stored securely and will only be accessed by researchers working on this study. Findings from the study may be published but we will not use your name or identification information.

Storage and future use of Biological Specimen:

You will be asked if we could store leftover of your specimen for future studies and testing for other respiratory viruses for 10 years. Your name will not be recorded on the specimen. You can still participate in this study even if you do not want us to store your specimen.

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Voluntary Participation
You participation in this study is completely voluntary. The health care provider will treat you with the same quality of care even if you decide not to participate. You are free to withdraw your participation in this study with no explanation at any time.

Contacts
If you have questions about the study, feel free to contact the Principal Investigator, Mr. Joseph Asamoah Frimpong, through mobile number 0262944332.

Your Rights as a Participant
This research has been reviewed and approved by the Institutional Review Board of Noguchi Memorial Institute for Medical Research (NMIMR-IRB). If you have any questions about your rights as a research participant you can contact the IRB Office between the hours of 8am-5pm through the landline 0302916438 or email addresses: nirb@noguchi.ug.edu.gh or HBaidoo@noguchi.ug.edu.gh.

PARTICIPANT AGREEMENT
The above document describing the risks, benefits and procedures for this research has been read and explained to me. I have been given an opportunity to ask questions about the research study and they have been answered to my satisfaction. I acknowledge that my participation is completely voluntary.

Can specimen be stored for future studies and testing? □Yes □No

_____ _____
Date Signature (thumbprint) of participant

If patient cannot read the form themselves, a witness must sign here:
I was present while the risks, benefits and procedures were read and explained to the patient. All questions were answered and the patient has agreed to take part in this research study.

_____ _____
Date Signature of witness

I certify that the nature and purpose, the potential benefits, and possible risks associated with participating in this research have been explained to the above individual.

_____ _____
Date Signature of person who obtained consent

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APPENDIX 2: PARENT/LEGAL GUARDIAN INFORMED CONSENT FORM

PARENT/LEGAL GUARDIAN INFORMED CONSENT FORM

THE EFFECT OF ANTIPYRETICS USAGE ON THE ACCURACY OF WORLD HEALTH ORGANISATION (WHO) CASE DEFINITION FOR INFLUENZA LIKE ILLNESS(ILI)

Age of Participant: .....

Organizations: University of Ghana- School of Public Health (UG-SPH), Ghana Health Service (GHS), Noguchi Memorial Institute for Medical Research (NMIMR), U.S. Centers for Disease Control and Prevention (CDC).
Principal Investigator: Mr. Joseph Asamoah Frimpong

Supervisor: Dr. Kofi Mensah Nyarko

Reason for Research
Noguchi Memorial Institute for Medical Research, in partnership with Ghana Health Services and the U.S. Centers for Disease Control and Prevention, is doing a research study to understand the causes of respiratory illness in the Shai-Osudoku and Ningo-Prampram Districts. As part of this study we would also like to know the effect of antipyretics on the WHO case definition for influenza like illness. We are asking your child to be part of this study because your child is showing signs of respiratory illness. As part of this study, your child will be asked to answer questions about his/her illness and to provide a specimen for testing. Your child may decline to answer any question and/or provide any sample. After we collect the sample, your child's participation in the study will be complete. Answering the questions and collecting the samples should only take a few minutes.

Possible Risks/Discomfort
If your child agrees to participate in this study, your child will be asked to provide a throat and nose swabs. These samples will be collected using a thin plastic stick wrapped with polyester (Dacron) at one end to swab the back of your child's throat and nose. This is a routine clinical procedure. There will be little discomfort and your child might gag during the collection of the sample.

Possible Benefits
Your child's participation in this research may help the Ghana Health Service find better ways to take care of people with respiratory infections in the future.

Confidentiality
Information about your child will be kept confidential. All records will be stored securely and will only be accessed by researchers working on this study. Findings from the study may be published but we will not use your child's name or identification information.

Storage and future use of Biological Specimen:
You will be asked if we could store leftover of your child's specimen for future studies and testing for other respiratory viruses for 10 years. Your child's name will not be recorded on the specimen. Your child can still participate in this study even if you do not want us to store his/her specimen.

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Voluntary Participation
The participation of your child in this study is completely voluntary. The health care provider will treat your child with the same quality of care even if your child decides not to participate. Your child is free to withdraw his/her participation in this study with no explanation at any time.

Contacts
If you have questions about the study, feel free to contact the Principal Investigator, Mr. Joseph Asamoah Frimpong, through mobile number 0262944332.

Your Rights as a Participant
This research has been reviewed and approved by the Institutional Review Board of Noguchi Memorial Institute for Medical Research (NMIMR-IRB). If you have any questions about your child's rights as a research participant you can contact the IRB Office between the hours of 8am-5pm through the landline 0302916438 or email addresses : nirb@noguchi.ug.edu.gh or HBaidoo@noguchi.ug.edu.gh .

PARENT/LEGAL GUARDIAN'S AGREEMENT
The above document describing the risks, benefits and procedures for this research has been read and explained to me. I have been given an opportunity to ask questions about the research study and they have been answered to my satisfaction. I acknowledge that my child's participation is completely voluntary.

Can specimen be stored for future studies and testing? □Yes □No

_____ _____
Date Signature (thumbprint) of parent or legal guardian

If parent/legal guardian cannot read the form themselves, a witness must sign here:
I was present while the risks, benefits and procedures were read and explained to the parent/legal guardian of the patient. All questions were answered and the parent/legal guardian of the patient has agreed to allow his/her child to take part in this research study.

_____ _____
Date Signature of witness

I certify that the nature and purpose, the potential benefits, and possible risks associated with participating in this research have been explained to the above individual.

_____ _____
Date Signature of person who obtained consent

Administer child assent to children between the ages of 5 and 17 (inclusive).
If the child does not provide assent to participate in the study, all study procedures must stop at this time.

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APPENDIX 3: CHILD ASSENT FORM

CHILD ASSENT FORM

My name is Mr. Joseph Asamoah Frimpong and I am a student from University of Ghana, School of Public Health. I am conducting a research study entitled **"The Effect of antipyretics usage on the accuracy of WHO case definition for Influenza like illness.**

I am asking you to take part in this research study because I am trying to learn more about influenza as causes of ill-health in Shai-Osudoku and Ningo-Prampram Districts. Your participation will take a few minutes.

If you agree to be in this study, you will be asked to provide throat and nose swabs.

Your participation in this study will help the Ghana Health Service find better ways to take care of people with respiratory infections in the future.

However, the risks associated are little discomfort and you might gag during the collection of the sample.

You can stop participating at any time if you feel uncomfortable. No one will be angry with you if you do not want to participate.

Your information will be kept confidential. No one will be able to know how you responded to the questions and your information will be anonymous.

You may ask me any questions about this study. You can call me at any time through mobile number 0262944332 or talk to me the next time you see me.

Please talk about this study with your parents before you decide whether or not to participate. I will also ask permission from your parents before you are enrolled into the study. Even if your parents say "yes" you can still decide not to participate.

By signing below, it means that you understand and know the issues concerning this research study. If you do not want to participate in this study, please do not sign this assent form. You and your parents will be given a copy of this form after you have signed it.

This assent form which describes the benefits, risks and procedures for the research titled **"The Effect of antipyretics usage on the accuracy of WHO case definition for Influenza like illness"** has been read and or explained to me. I have been given an opportunity to have any questions about the research answered to my satisfaction. I agree to participate.

Child's Name:..... **Researcher's Name:**.....

Child's Signature/ Thumbprint:..... **Researcher's Signature**.....

Date:..... **Date:**.....

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APPENDIX 4: DATA COLLECTION FORM

SARI / ILI DATA COLLECTION FORM

Fill in the Blank Space or Tick √ the box □ as appropriate

STUDY ID

DISTRICT □ Shai-Osudoku □ Ningo-Prampram

Health Facilities

□ SOD Hospital □ Battor Hospital □ Akuse Hospital □ Prampram H/C

□ Old Ningo H/C □ Osudoku H/C □ Agomeda CHPS □ Agotor CHPS □ Lekpongunor CHPS

□ **ILI Case** □ **SARI Case**

Date of Interview:____/____/_____(dd/mm/yyyy) Date of Admission:_____/_____/_____

Time of Interview: _____

Demographic Details

Hospital ID: _____ **DSS**_____

Sex □Male □Female **Date of birth** ____/____/_____

Age **Years** _____ **OR** **Months** _____ **Name of Village/Suburb/Town**_____

House No:_____ **Occupation**_____

Brief Direction to House:_____

Tel: _____ **Source of Information:** □ Patient □ Parent/Guardian

Marital status: □ Single □ Married □ Divorced □ Separated □ Widow/widower

Educational Status:

□ Pre-school □ Primary □ Junior High School (JHS) □ Senior High School (SHS)

□ Tertiary and above □ Illiterate □ Unknown

Pre-existing Medical Conditions

□ Asthma □ Chronic heart disease □ Diabetes □ Chronic liver disease □ Chronic lung disease (COPD)

□ Cancer □ Immunosuppression □ HIV □ TB □ Malnutrition □ Unknown

□ None □ Other(specify)_____

Pregnancy Status: □ Pregnant □ Not Pregnant □ Unknown □ Not applicable

History of Smoking

□ Yes, regularly □ Yes, irregularly □ Yes, in the past □ No

Vaccination

Previously vaccinated against influenza? □ Yes □ No □ Unknown

If Yes, specify most recent year of vaccination_____

Illness Onset and Presenting Symptoms

Date of symptom onset:_____/_____/_____

Within the last 10 days, have you/the patient experienced any of the following symptoms?

Symptom	Yes	No	Unknown
History of fever	□	□	□
Measured fever ≥37.5°C (Axillary)	□	□	□
Cough	□	□	□
Sore throat	□	□	□
Runny nose	□	□	□
Chills	□	□	□
Myalgia	□	□	□

Symptom	Yes	No	Unknown
Diarrhoea	□	□	□
Abdominal pain	□	□	□
Vomiting	□	□	□
Chest pain	□	□	□
Shortness of breath	□	□	□
Difficulty breathing	□	□	□
Mental status change	□	□	□

Other(Specify):_____

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Treatment history prior to visit to health facility

Did you/the patient visit any health care provider for the illness? □Yes □No

What type of health care provider did you visit?

□ Hospital □ Health Centre □ CHPS Compounds □ Traditional Healer

□ Pharmacy/Drug Seller □ Other(Specify)_____

Did you/the patient take any medicine for the illness? □ Yes □ No

What were the medicines? A._____ B._____ C._____ □Unknown

Travel history prior to onset of symptoms

Have you/the patient travelled anywhere 2 weeks prior to onset of symptoms? □ Yes □ No

□ Local □ International □ By road □ By air □ By water □ By rail

If Yes, state travel destination_____

Physical Examination

Temperature(°C): _____(Axillary) Blood pressure (mmHg):_____

Height(cm):_____ Pulse rate:_____

Weight(kg):_____ Oxygen saturation:_____

Respiratory rate:_____ Supplemental oxygen? □Yes □No

SARI case

Inability to drink: □ Yes □ No

Inability to be breastfed: □ Yes □ No

Chest indrawing (<5 years): □ Yes □ No

Stridor in a calm child (<5 years): □ Yes □ No

Unconscious: □ Yes □ No

Breath sounds: □ Vesicular □ Bronchial □ Vesicular with prolonged expiration □ Difficult to comment

Rhonchi: □ Present □ Absent □ Difficult to comment

Crepitation: □ Present □ Absent □ Difficult to comment

Final Clinical Diagnosis

Please write in the final diagnosis: _____

Final Outcome

□ Recovered / Discharged □ Deceased □ Lost to follow-up □ Transferred Out □ Still under treatment

Date final outcome established ____/____/_____ **If person died, date of death** ____/____/_____

Length of Stay at the Health Facility

Please write how long patient stayed at the health facility: _____

Contact details of Interviewer

Name of Interviewer:_____ Tel. No.:_____

Title: _____

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APPENDIX 5: LAB FORM

NP and OP SWABS LAB FORM: SEVERE ACUTE RESPIRATORY INFECTION (SARI), INFLUENZA-LIKE-ILLNESS (ILI)

Health Facilities *(Kindly tick your facility)*

☐SOD Hospital ☐Battor Hospital ☐Akuse Hospital ☐Prampram H/C ☐Old Ningo H/C

☐Osudoku H/C ☐Agomeda CHPS ☐Agotor CHPS ☐Lekpongunor CHPS

Consent Details *(To be completed at Health facility by person who obtained consent)*

Date consent obtained: ☐☐-☐☐-☐☐☐☐

Study Label

Initials of person who obtained consent: ☐☐☐

Did patient consent to future use of specimen O Yes (1) O No (0)

Sample Collection Details *(To be completed at Health facility by person who collected clinical Specimen)*

Initial of person collecting specimen: ☐☐☐ Date Collected: (dd mm yyyy) ☐☐ ☐☐ ☐☐☐☐ Time collected: ☐☐ ☐☐ O am O pm

Swab collected: O OP O NP O None

Time in fridge: ☐☐ ☐☐ O am O pm

If none, did patient refuse to provide sample? O Yes O No

Time in Liquid Nitrogen: ☐☐ ☐☐ O am O pm

If yes, stop all study procedures and return this form to surveillance staff

Pick up date: ☐☐ ☐☐ ☐☐☐☐ Pick up time: ☐☐ ☐☐ O am O pm

SPECIMEN ANALYSIS *(To be completed at NMIMR laboratory personnel)*

PCR Analysis	NMIMR Results	Subtype Results	Viral Culture Results	NMIMR Comments
FLU A	☐ Positive(+) ☐ Negative(-)	☐ H1 ☐ H3 ☐ H5 ☐ (H1N1)pdm09 ☐ Not able to subtype ☐ Subtyping not performed	☐ H1 ☐ H3 ☐ H5 ☐ (H1N1)pdm09 ☐ Not able to subtype ☐ Subtyping not performed	
FLU B	☐ Positive(+) ☐ Negative(-)	☐ Not able to subtype ☐ Subtyping not performed	☐ Victoria ☐ Yamagata ☐ Undetermined ☐ Not performed	
Date Received:____/___/_____		Date of Analysis:____/___/_____		

Results reported back to facility:

Date: ☐☐-☐☐-☐☐☐☐

Name of Personnel receiving results: ☐

Initials of personnel reporting results: ☐☐☐

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APPENDIX 6: ETHICAL CLEARANCE

NOGUCHI MEMORIAL INSTITUTE FOR MEDICAL RESEARCH
Established 1979
A Constituent of the College of Health Sciences
University of Ghana

INSTITUTIONAL REVIEW BOARD

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Legon, Accra
Ghana

My Ref. No: DF.22
Your Ref. No:

11th November, 2013

ETHICAL CLEARANCE

FEDERALWIDE ASSURANCE FWA 00001824 **IRB 00001276**

NMIMR-IRB CPN 049/13-14 **IORG 0000908**

On 11th November 2013, the Noguchi Memorial Institute for Medical Research (NMIMR) Institutional Review Board (IRB) conducted expedited review and approved your protocol titled:

TITLE OF PROTOCOL : **The effect of antipyretic usage on the accurancy of the World Health Organisation (WHO) case definition for Influenza Like Illness (ILI)**

PRINCIPAL INVESTIGATORS : **Joseph Asamoah Frimpong, MPhil Cand.**

Please note that a final review report must be submitted to the Board at the completion of the study. Your research records may be audited at any time during or after the implementation.

Any modification of this research project must be submitted to the IRB for review and approval prior to implementation.

Please report all serious adverse events related to this study to NMIMR-IRB within seven days verbally and fourteen days in writing.

This certificate is valid till 10th November, 2014. You are to submit annual reports for continuing review.

Signature of Chair: .....

Mrs. Chris Dadzie
(NMIMR – IRB, Chair)

cc: Professor Kwadwo Koram
Director, Noguchi Memorial Institute
for Medical Research, University of Ghana, Legon