SCHOOL OF PUBLIC HEALTH
COLLEGE OF HEALTH SCIENCES
UNIVERSITY OF GHANA

ACUTE FEBRILE ILLNESS: THE ROLE OF
RESPIRATORY SYNCYTIAL VIRUS AND
MALARIA IN AN URBAN PEDIATRIC
POPULATION IN GHANA

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FULFILMENT FOR THE AWARD OF A DOCTOR
OF PHILOSOPHY IN PUBLIC HEALTH
DECLARATION

I hereby declare that with the exception of cited references to other people’s work which has been duly acknowledged, this work is the result of my own research work done under supervision and has neither been presented elsewhere either in part or whole for another degree.

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Academic Supervisor

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Prof. John Gyapong
DEDICATION

This work is dedicated to my mum Mabel Arthur Hudson, my husband Andy Malm and my children Betsy, Brianna and Caleb.
ACKNOWLEDGEMENT

My sincere gratitude goes to Professor Fred Binka who encouraged me to start this PhD programme and also supported me financially and technically to finish it. I am also very grateful to Prof George Armah who helped me chose my topic and provided me with laboratory services. I appreciate very much Prof Kwadwo Koram, my primary supervisor who has been of enormous help, supporting me financially and technically. I am also grateful to Prof John Gyapong my secondary supervisor for his technical support and encouragement. I am also grateful to my other lecturers Prof Edwin Afari, Dr Samuel Sackey and Dr Moses Aikins for their contribution to this work. I appreciate greatly the National Malaria Control Programme Manager, Dr Constance Bart-Plange who has been of immense support to me.

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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>µL</td>
<td>microliter</td>
</tr>
<tr>
<td>ALRTI</td>
<td>Acute Lower Respiratory Tract Infection</td>
</tr>
<tr>
<td>ARI</td>
<td>Acute Respiratory Tract Infection</td>
</tr>
<tr>
<td>CCA</td>
<td>Chimpanzee Coryza Agent</td>
</tr>
<tr>
<td>CDC</td>
<td>Centre for Disease Control</td>
</tr>
<tr>
<td>cDNA</td>
<td>complimentary Deoxyribo-nucleic acid</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme Linked Immunosorbent Assay</td>
</tr>
<tr>
<td>GITC</td>
<td>Guanidine Isothiocyanate</td>
</tr>
<tr>
<td>GMT ratio</td>
<td>Geometric Mean Transmission ratio</td>
</tr>
<tr>
<td>HIV</td>
<td>Human ImmunoVirus Defficiency</td>
</tr>
<tr>
<td>ICU</td>
<td>Intensive Care Unit</td>
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<tr>
<td>IgA</td>
<td>Immunoglobulin-A</td>
</tr>
<tr>
<td>IgG</td>
<td>Immunoglobulin-G</td>
</tr>
<tr>
<td>IgM</td>
<td>Immunoglobulin-M</td>
</tr>
<tr>
<td>Immunoglobin</td>
<td></td>
</tr>
<tr>
<td>LRI</td>
<td>Lower Respiratory Tract Infection</td>
</tr>
<tr>
<td>LRTI</td>
<td>Lower Respiratory Tract Infection</td>
</tr>
<tr>
<td>Mths</td>
<td>months</td>
</tr>
<tr>
<td>MUAC</td>
<td>Mid Upper Arm Circumference</td>
</tr>
<tr>
<td>NaAc</td>
<td>Sodium Acetate</td>
</tr>
<tr>
<td>NHIA</td>
<td>National Health Insurance Authority</td>
</tr>
<tr>
<td>NMIMR</td>
<td>Noguchi memorial Institute for Medical</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
</tr>
<tr>
<td>pH</td>
<td>Percentage Hydrogen</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
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<tr>
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<td>--------------------------------------------------</td>
</tr>
<tr>
<td>RDTs</td>
<td>Rapid Diagnostic Tests</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribo-oxynucleic acid</td>
</tr>
<tr>
<td>RNaid</td>
<td>Ribonucleic Acid Extraction Aid</td>
</tr>
<tr>
<td>rpm</td>
<td>revolutions per minute</td>
</tr>
<tr>
<td>RSV</td>
<td>Respiratory Syncytial Virus</td>
</tr>
<tr>
<td>RSV-A</td>
<td>Respiratory Syncytial Virus-Subtype A</td>
</tr>
<tr>
<td>RSV-B</td>
<td>Respiratory Syncytial Virus-Subtype B</td>
</tr>
<tr>
<td>RSVIG</td>
<td>Respiratory Syncytial Virus Immunoglobulin</td>
</tr>
<tr>
<td>RT</td>
<td>Reverse Transcriptase</td>
</tr>
<tr>
<td>ssRNA</td>
<td>single strand Ribonucleic acid</td>
</tr>
<tr>
<td>URI</td>
<td>Upper Respiratory Tract Infection</td>
</tr>
<tr>
<td>USA</td>
<td>United States of America</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
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ABSTRACT

Introduction: Fever describes an increase in internal body temperature to levels that are above normal (commonly oral/axillary measurement of normal human body temperature is 36.8±0.7 °C or 98.2±1.3 °F) and is the most common response of the body to any insult to the body. The most common cause of fever in our setting is malaria and therefore children get treated for malaria once they have a fever whether the cause is proven (by laboratory confirmation) to be malaria or not. Other common causes of childhood fever, such as respiratory infections are not usually considered, although this has been found to be responsible for a high proportion of childhood illness in the West African Region in places such as The Gambia. Most of these respiratory infections are caused by viral agents including respiratory syncytial virus (RSV). Very little information exists on causes of fever in Ghana and therefore this study was conducted to determine the relative importance of RSV and malaria as causes of Acute Febrile Illness in children under 5 years presenting at an urban hospital in Accra.

Methods: The study recruited children under five years with an axillary temperature ≥ 37.5°C who presented to the out-patients department of the La General Hospital from February 2009 to February 2010. A questionnaire was administered to their parents/caretakers (after consenting to allow their children to be part of the study) to elicit the demographic and socioeconomic characteristics of the recruited children and a physical examination conducted all children. Nasopharyngeal aspirates and blood samples were taken for polymerase chain reaction (PCR) for RSV and microscopy for malaria parasites respectively. A case control study was also undertaken to determine the risk factors for RSV infection among acute respiratory infection cases. Cases were children with acute respiratory infection who were
positive for RSV and controls were children with acute respiratory infection who were negative for RSV.

**Results:** Malaria, defined as presence of malaria parasites on microscopy, was found in 11.2% of all acute febrile illness. RSV was seen in 15.6% and 17.5% of acute febrile illness and respiratory infections respectively. Seven children representing 1.5% of children with acute febrile illness were positive for both malaria and RSV. Overcrowding and exclusive breastfeeding were significantly positively associated with RSV infection (p-value<0.01), whilst male gender, young age of less than one year and prematurity were also positively associated with RSV infection though not statistically significant.

**Discussion and Conclusion:** The proportion of acute febrile illness due to malaria is lower than has been recorded routinely in children less than five years in this urban hospital. RSV is significantly prevalent in these children. Co-morbid infection with RSV and malaria was low. It is therefore important health practitioners support their diagnosis of malaria with laboratory confirmation, and also look out for other causes of fever such as RSV. The practice of treating almost all cases of fever as malaria needs to be examined critically.

Keywords: fever, respiratory syncytial virus, malaria, children
CHAPTER ONE: INTRODUCTION

A wide variety of illnesses can manifest as fever either alone or in combination with other symptom(s). The commonest medical condition presenting with fever in children under five years, in developing countries, are infections (Knockaert et al., 1993; Molina et al., 2004; Turkulov et al., 2011). Fever can also be the presenting symptom of quite a number of other non-infectious medical conditions. These include, cancers thromboembolic disorders, metabolic disorders, drug reactions and immunologic diseases (Farthing et al., 1994). There are however, cases of fever for which the causes or origins are unknown (Iikuni Y, 1994; Knockaert et al., 1993; Turkulov et al., 2011). In the Sub-Saharan region, the most important causes of fever in children less than five years are malaria, respiratory tract infections and diarrhoea. These diseases are estimated to account for about 54% of deaths in children less than five years (Bryce et al., 2005).

The prevalence of malaria is high in the African region accounting for 18% of deaths in children under five, but there are other additional important causes of mortality in children under five years. Pneumonia, neonatal causes and diarrhoea, as shown in Figure 1, occur frequently in Africa (Bryce et al., 2005). The importance of looking for these other diseases in children cannot be underestimated.
Figure 1: Causes of Death in Children Less than Five Years in the Six WHO Regions, 2000–2003

Source: Bryce et al., 2003

Key: Size of circle represents number of deaths in region. AFR=Africa. AMR=Americas. EMR=Eastern Mediterranean. EUR=Europe. SEAR=Southeast Asia. WPR=Western Pacific.

Some 10.8 million children under five years die each year (Black et al., 2003) and of these, the World Health Organization (WHO) estimates that about 2 million die of pneumonia each year (Bryce et al. 2005), 70 percent of them in Africa and Southeast Asia (Williams et al. 2002). Deaths from acute respiratory tract infections are due to lower respiratory tract infection, mainly pneumonia and bronchiolitis. The importance of lower respiratory tract infection as a major cause of childhood mortality was shown by Lopez et al. (2006) in their ranking of global burden of disease.
1.2 PROBLEM STATEMENT AND JUSTIFICATION

Acute febrile illness is the most common presentation of children under five years in hospitals in Ghana. Due to the fact that the Sub Saharan region of Africa is highly endemic for malaria, most often fever is diagnosed and treated as malaria to the neglect of other possible causes. This leads to mis-use of antimalarials, whilst other causes of fever are missed sometimes with fatal consequences (Evans et al., 2004; Reyburn et al., 2004 and Sievers et al., 2008). This behaviour puts unnecessary economic burden on both the parents/guardian who have to buy these medications as well as on the Government and other funding agencies who subsidize the medications.

Acute respiratory tract infection is among the top three diseases in children under five years of age in Ghana and antibiotics are the mainstay of treatment of acute respiratory infection in the country (Black et al, 2010). Once a child presents to a health facility with respiratory symptoms he/she is almost always treated with an antimalarial most often plus an antibiotic. Little effort is put into determining the underlying cause of the symptoms, some of which have been shown to be due to bacteria and viruses (Weber et al., 2002; Nokes et al., 2004; Medici et al., 2004; John et al, 1991; Forgie et al, 1992). Even though bacterial infection can be very severe and fatal, the importance of the viral causes can not be underscored. The literature on both bacterial and viral causes of acute respiratory infection in Africa and Ghana though poor, is worse for viruses. Bacterial causes of acute febrile and respiratory illnesses in Ghana have been better studied. Results from such studies supported the inclusion of vaccines against Hemophilus influenza B and pneumococcal bacteria as part of the immunization schedule of the country. With the inclusion of vaccines against the
major bacterial causes of childhood pneumonia (*Hemophilus influenza B* and pneumococcal bacteria) the search for other major aetiological agents of childhood pneumonia including viral causes needs to be intensified to offer better management of acute febrile illness in children. One of such virus is the respiratory syncytial virus (RSV) which has been shown to account for a significant proportion of acute respiratory tract infection in other developing countries (Weber *et al.*, 2002; Nokes *et al.*, 2004; Medici *et al.*, 2004). There is no published data on what proportion of acute respiratory infection is due to RSV in Ghana.

Generally, diseases can be tackled through proper case management, prevention through vaccination or breaking host-agent contact and/or modification of risk factors. Information on risk factors associated with RSV in Africa is not conclusive and in Ghana very little is known at all. The aim of the study therefore, was to provide information on the burden of RSV in children less than five years in relation to acute febrile illness and acute respiratory infections. It also gathered information on risk factors associated with RSV infection. Such information on the risk factors will help in the diagnosis of RSV in the sense that the risk factors will act as pointers for suspecting RSV infection in a child. With such information, the need to look for RSV among children who present with fever or acute respiratory tract infection could be advocated for; if not for all children at least for those at higher risk. Management of children with fever or acute respiratory infection will then not only focus on the blanket administration of antimalarials and antibiotics.
1.3 HYPOTHESES AND OBJECTIVES

The main objective of the study was to determine the proportion of acute febrile illness that is due to respiratory syncytial virus infection as well as the risk factors associated with respiratory syncytial virus in children less than five years in an urban area.

The specific objectives were

1. To determine the proportion of acute febrile illness due to RSV in children less than five years at the La General hospital.
2. To determine the proportion of acute respiratory tract infection due to RSV in children less than five years at the La General hospital.
3. To determine the risk factors of RSV in children less than five years of age at the La General Hospital.
4. To determine the proportion of acute febrile illness which is due to malaria in children less than five years at the La General Hospital.
5. To determine the genotype epidemiology of RSV in children less than five years at La General Hospital.

The study tested the hypothesis that there is no difference in the characteristics of children less than five years who have acute respiratory illness due to RSV and those with non-RSV respiratory illness
CHAPTER TWO: LITERATURE REVIEW

2.1 ACUTE FEBRILE ILLNESS

Acute febrile illness (AFI) is any illness characterized by an acute onset of fever. Fever also known as pyrexia is a common medical sign characterized by an elevation of temperature above the normal range of 36.5–37.5 °C (98–100 °F) (Karakitsos and Karabinis, 2008). Fever in itself is not a disease but a sign that the body’s immune system is fighting against an unwanted agent. It is one of the cardinal signs of inflammation. Normal body temperature may vary from one individual to another and even in the same individual at different times depending on host and environmental factors like age, sex, time of day and activity level (Wikipedia, 2011). Depending on which part of the body, the temperature is taken, the following temperature elevations are considered as fever: temperature in the anus (rectum/rectal) ≥37.5 °C (99.5–100.9 °F) (Axelrod and Diringer, 2008; Laupland, 2009), temperature in the mouth (oral) ≥37.7 °C (99.9 °F) (Barone, 2009), temperature under the arm (axillary) or in the ear (otic) ≥37.2 °C (99.0 °F)

Fever can be continuous, intermittent or remittent and the pattern of the fever maybe a pointer to the underlying disease. Continuous fever is when temperature remains above normal throughout the day and does not fluctuate more than 1 °C in 24 hours. This is common in lobar pneumonia, typhoid, urinary tract infection, brucellosis, or typhus. Intermittent fever is when the temperature elevation is present only for a certain period, and usually has a cyclical pattern. e.g. malaria, kala-azar, pyaemia, or septicemia. There are several cyclical patterns of intermittent fever. Quotidians fever, with a periodicity of 24 hours (typical of malaria due to plasmodium vivax and ovale),
tertian fever with a 48-hour periodicity and quartan fever with a 72-hour periodicity (Farthing et al., 1994). Remittent fever occurs when temperature remains above normal throughout the day and fluctuates more than 1 °C in 24 hours, this is common in infective endocarditis (Wikipedia, 2011).

The burden of AFI is significantly high sometimes accounting for more than 50% of all diseases presented at the out-patient department (Bryce et al., 2005; Lee et al., 2007). A study in Senegal, reported that 28% of mothers reported that their child had a fever in the two weeks preceding the study period.

**Pathogenesis of Fever**

Fever due to infections usually begins when pyrogens which induce fever, reach the anterior hypothalamus via the arterial blood supply (Prince, 1998). The hypothalamus, which acts a thermostat via the autonomic nervous system, then effects a heating mechanism. This mechanismis through increased muscle tone, shivering, release of hormones like epinephrine and/or vasoconstriction (Wikeapedia, 2011).

There are arguments for and against the usefulness of fever (Schaffner, 2006; Soszyński, 2003). Some studies believe fever is beneficial in the healing process because it enhances the mobility of leukocytes as well as their phagocytotic action, it also decreases the endotoxin effect and increases the proliferation of T cells, all of which are important aspects of the healing process in an infection (Wikeapidea, 2011). There is however a negative aspect of fever, for every 1°C rise in temperature, there is a 13% increase in the basal metabolic rate and oxygen consumption.
This therefore leads to increased energy requirements at a time when anorexia leads to decreased food intake (Farthing et al., 1994).

Causes of Febrile Illness
Causes of febrile illness are varied and may differ depending on the region and the country and therefore will need different diagnostic and treatment regimens (Kasper et al., 2012). It may also have seasonal pattern which differs across regions and countries (Abdou et al., 2005). Febrile illness in children may range from severe diseases which can be life threatening to mild illness (Hamilton and John, 2013). Quite a number of diseases may present as an acute febrile illness as stated earlier this maybe infectious (more commonly) or non-infectious in origin (Farthing et al., 1994; Prince, 1998). In a few cases, the causative organism cannot be identified and are classified as fever of unknown origin (Iikuni, 1994; Knockaert et al., 1993; Turkulov et al., 2011).

Infectious Causes of Fever
Infectious causes could be viral, bacterial or parasitological. In the Asian region a number of studies identified viruses as the leading cause of infectious febrile illness with the most common due to influenza (Kasper et al., 2012, Leelarasamee et al., 2004). It was estimated that in 2008, 41% of deaths in children under five years were due to malaria, diarrhoea, and lower respiratory infections (Black et al., 2010). All these diseases usually present with a fever as a frequent and early symptom. Diarrhoea is defined as the passage of three or more loose or watery stools per day.
Causes of diarrhoea are often infectious and involve a variety of pathogenic microorganisms that include viruses, bacteria and parasites (Stewein et. al., 1993 Abu-Elamreen et. al., 2008). Most diarrhoea is often associated with fever and abdominal pains.

In the Sub Saharan Africa, during the peak season for febrile illnesses, doctors are usually overwhelmed and are often replaced by auxiliaries such as nurses, midwives, pharmacists or any educated person in a village who often have expertise to provide only limited care and advice (Abdou et. al., 2005). Due to the fact that there is a close overlap between malaria and other causes of febrile illness, in some places algorithms have been developed for workers at the lower levels of health where laboratory diagnosis is not available. This algorithm is said to increase the sensitivity of identifying malaria cases by these lower level health worker to a level of 88% sensitivity and specificity as compared to diagnosis by peadiatrician (Bojang et. al., 2000)

**Non-Infectious Causes**

Non infectious causes of acute febrile illness are becoming more common and these include immunological diseases such as lupus erythematosus, sarcoidosis, inflammatory bowel disease; tissue destruction, which can occur in hemolysis hemolysis, surgery, infarction, crush syndrome, rhabdomyolysis, cerebral hemorrhage; reaction to incompatible blood products and medications; cancers, most commonly kidney cancer and leukemia and lymphomas; Metabolic disorders such as gout, porphyria ; and thrombo-embolic processes(Farthing et.al,1994).
Acute Fever of Unknown Origin

Fever of Unknown Origin (FUO) is defined as fever without an obvious source on initial clinical examination. FUO can be classified as acute (illness of $\leq$1 week's duration) or prolonged (>7 to 10 days' duration) (Akpede and Akenzua, 2001). Managing children with acute fever of unknown origin (FUO) is challenging. For children with fever of unknown origin or without source, it is suggested that urine analysis and culture be taken to help identify the possible cause (Brookman et. al., 2007). It is estimated that 10-20% of children with FUO will have serious bacterial infection. Due to the fact that no single clinical examination or laboratory examination can identify children at risk of serious bacterial infection (Gerviax et. al., 2001), the practice now is to look out for those who are less likely to be at risk of serious bacterial infection using criteria such as the Rochester criteria (Jaskiewicz et. al., 1994). Children who are found to be less likely to be at risk of serious bacterial infections can be observed for a while without antibiotics (Jaskiewicz et. al., 1994).

2.2 MALARIA

2.2.1 Epidemiology of Malaria

Malaria is a disease caused by parasites of the plasmodium species and is spread from person to person through the bite of the female anopheles mosquitoes. There are five main types of parasites causing human malaria- Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae, and Plasmodium ovale and Plasmodium Knowlesi. (Cox-Singh et.al., 2008). P. falciparum is by far the most deadly type of malaria infection and most prevalent in Africa (WHO, 2007)
When an infected female anopheles mosquito takes a bloodmeal from man sporozoites are injected into the bloodstream of man. These circulating sporozoites then enter the liver of man and undergo schizogony within 5-16 days. This results in the release of merozoites and toxins into the bloodstream. The released merozoites invade the red blood cells and mature into schizonts (asexual forms) which are then released into the bloodstream repeatedly in a cyclical manner over 1-3 days depending on the species of malaria parasite. It is at this stage that fever and illness occur in the individual. Some of the merozoites develop into sexual forms, the male and female gametocytes which circulate in the bloodstream. Some of the sporozoites however enter the liver remaining dormant and causing relapse weeks to months later (in P. falciparum, P. ovale). When a female mosquito bites man to take a bloodmeal it
ingests these gametocytes, which develop in the guts of the mosquitoes going through several stages to produce the oocysts. These oocysts burst to release the sporozoites, which invade the salivary gland of the mosquito. The cycle of infecting man restarts when such a mosquito bites man.

Approximately, 50% of the world’s populations, mostly those living in the world’s poorest countries, are said to be at risk of malaria (WHO, 2013). Every year, more than 500 million people become severely ill with malaria. Over 90% of cases and deaths occur in sub-Saharan Africa with children under five years and pregnant women being affected mostly (WHO, 2007). What has become clear over the years is that most of these cases are diagnosed based on clinical symptoms and not many of these cases are confirmed by laboratory examination (Reyburn et.al., 2006). A number of factors have also been shown to increase the risk of malaria disease, these include low socioeconomic status (Mbando et.al., 2011; Messina et.al. 2011, Yusof et.al., 2010) males, poor housing and not sleeping in a mosquito net (Messina et.al. 2011, Haque et.al., 2011).

In Ghana, about 3.7 million cases of malaria were recorded in 2009 accounting for 32.5% of all out patient attendance (NMCP, 2009). Malaria admissions accounted for 35.8% of all admissions with the lowest proportions of admissions in Greater Accra. Only 26% of diagnosed cases were tested either by microscopy or rapid diagnostic test (NMCP, 2009). According to the Ghana Health Insurance Authority in 2008, cost of treatment of malaria topped their expenditure list accounting for 21.4% of all payments made by the authority (NHIA, 2008).
2.2.2 Clinical Presentation

The clinical presentation of malaria is so varied and non-specific it can easily be misdiagnosed. Malaria is classified as uncomplicated or severe depending on the symptoms that are presented. The early symptoms are usually non specific, these include fever, chills, sweats, headaches, muscle pains, nausea and vomiting, symptoms which can be the presentation of other bacterial (enteric fever) or viral diseases including acute respiratory infections. Likewise, the physical signs are often not specific eg. elevated temperature, perspiration (WHO, 2010a). Severe malaria can present as coma (cerebral malaria) severe anaemia, hypoglycaemia, acute renal failure, acute pulmonary oedema or metabolic acidosis (WHO, 2000a).

2.2.3 Diagnosis

Prompt and accurate diagnosis is a key to proper management of malaria. Since symptoms of malaria are so unspecific, as much as possible it should be confirmed by a laboratory test. The diagnosis of malaria is based on a clinical suspicion and a laboratory test confirmation. Currently the WHO recommends the confirmation of all cases of malaria by a laboratory test as much as possible (WHO, 2010b).

Laboratory confirmation is usually through the demonstration of malaria parasites in blood films through the use of light microscopy (Heyman et al., 2000). This however depends on the expertise of the microscopist, though it is not a difficult procedure to learn. Another routine test, which has been recently introduced, is the rapid diagnostic test that detects plasmodial antigens in the blood (Heyman et al., 2000). This test has a high sensitivity but because it tests for antigens, false positive results may occur for
about two weeks after treatment of malaria. The advantage of this test is that results are ready in a much shorter period and less expertise is needed to undertake the test. Diagnosis by the polymerase chain reaction is the most sensitive, but this is generally too expensive to be used routinely and is often used for research purposes. Antibodies determination is another laboratory method but it is not currently helpful because though antibodies appear after a week of infection, antibodies may persist for years (Heyman et.al., 2004).

2.2.4 Treatment of Malaria

The treatment of malaria remains a vital component in the control of malaria. The type of treatment given depends on whether the disease is uncomplicated or severe. The WHO recommends artemisinin-based combination therapy (ACT) as treatment of choice (WHO, 2010a) for uncomplicated malaria. In Ghana, the first line drug of choice for uncomplicated malaria is artesunate-amodiaquine. Arthemeter lumefantrine and dihydro-piperaquine serve as the alternative drugs (GHS, 2009a). WHO reviewed its treatment policy for severe malaria in April, 2010 and recommends intravenous artesunate as the drug of choice with quinine or arthermether as alternatives. The child with severe malaria should be given intravenous medication for at least 24 hours and then followed by a full course of an oral ACT if oral medication is possible (WHO, 2010a). In Ghana, the treatment policy is yet to be reviewed and the first line drug for the treatment of severe malaria is quinine with artesunate injection as the alternative (GHS, 2009a).
2.3 ACUTE RESPIRATORY TRACT INFECTIONS

Acute respiratory tract infections (ARIs) are infections of the respiratory tract and are classified into upper and lower respiratory tract infections. The upper respiratory tract consists of the airways from the nostrils to the vocal cords in the larynx, including the paranasal sinuses and the middle ear. The lower respiratory tract covers the continuation of the airways from the trachea and bronchi to the bronchioles and the alveoli. Acute respiratory tract infections are not confined to the respiratory tract and usually have systemic effects like fever because of possible extension of infection or microbial toxins, inflammation, and reduced lung function. It may be severe or mild; the proportion of mild to severe disease and mortality varies between high- and low income countries. This is because of differences in risk factors and accessibility to health care facilities (Lopez et.al., 2006). On the average children may have three to six episodes of ARIs annually worldwide (Bryce et.al., 2005).

2.3.1 Etiology of Acute Respiratory Tract Infections

Acute respiratory tract infections are mostly caused by viral and bacterial agents with a few caused by fungi, usually in the immunocompromised (Wu et.al., 2004). About 45%-55% of acute infections of the lower respiratory tract are viral in origin (John et.al., 1991; Yun et.al., 1995).

Bacteria Causes of Acute Respiratory Infection

The commonest bacteria responsible for ARIs are Streptococcus pneumoniae, Haemophilus influenzae and Mycoplasma pneumoniae (John et.al., 1991; Forgie et.al., 1992). Respiratory tract infections due to bacterial causes such as S.pneumoniae
and H Influenza are usually severe (Cutts et. al., 2005; Klugman et. al., 2003; Mulholland et. al., 1997). In children under five years in the developing world the most important cause of bacterial lower respiratory tract infection is Streptococcus pneumoniae.

**Streptococcus pneumoniae**

*Streptococcus pneumoniae*, or pneumococcus, is a Gram-positive, alpha-hemolytic, aerotolerant anaerobic member of the genus *Streptococcus* (Ryan and Ray, 2004a). Pneumococcus is the most commonly identifiable cause of community-acquired pneumonia and its infection is not only limited to the lungs (Nuarti, 2012). It can also be a major cause of severe diseases like meningitis, bactereamia, brain abscesses and milder but more common illnesses, such as sinusitis and otitis media (Siemieniuk et. al., 2011). Pneumonia due to *S. Pneumoniae* affects mostly the youngest and the oldest of the population in both developed and developing countries (Nuarti, 2012; Benavides, 2012). With the recent rise in the HIV in the developing world, there has been an increased impact of *S. Pneumoniae* in the developing world (Levine et. al., 2006). Pneumococcal disease is responsible for 1 million deaths annually, more than 800,000 of which occurs in children <5 years of age in the developing world (O’Brien et. al., 2009). The case fatality in severe invasive infections of *S. Pneumoniae* is about 25% (Caroll et. al., 2007 and 2009).

*Streptococcus pneumoniae* is transmitted directly from person to person through close contact via respiratory droplets. The organism frequently colonizes the nasopharynx of healthy people, particularly young children, without causing illness. Transmission
is thought to be common, but clinical illness occurs infrequently among casual contacts. The diagnosis is generally made based on clinical suspicion along with a positive culture from a sample from any part of the body (Siemieniuk et. al., 2011).

There are several pneumococcal conjugate vaccines available for the prevention of Streptococcus pneumonia. The choice of the conjugate vaccine to use depends on the type of \textit{S.pneumoniae} strains circulating in that particular area (Cornick et. al., 2011). Treatment is mainly by antibiotics usually parenteral or oral depending on the severity of the disease. The antibiotics that are used include penicillins, second and third generation cephalosporins and levofloxacin (John et. al., 2011).

\textbf{Haemophilus influenzae}

\textit{Haemophilus influenzae}, is a gram negative coccobacillibacterium of the Pasteurellaceae family, it is generally aerobic, but can grow as a facultative anaerobe (Kuhnert and Christensen, 2008). There are two major categories of \textit{H. influenzae}, the unencapsulated strains and the encapsulated strains. There are six generally recognized types of encapsulated \textit{H. influenzae}: a, b, c, d, e, and f (Ryan and Ray, 2004). Most strains of \textit{H. influenzae} are opportunistic pathogens but cause problems only when other factors (such as a viral infection, reduced immune function or chronically inflamed tissues, e.g. from allergies) create an opportunity (Wikipedia, 2013). Up to 15\% of children in non-immunized populations may harbour \textit{Heamophilus influenzaeb} in their nasopharynx (WHO, 2002). The pathogenesis of \textit{H. influenzae} infections is not completely understood, although the presence of the
capsule in encapsulated type b (Hib), is known to be a major factor in virulence (Wikipedia, 2011).

*H. influenzae* type b (Hib) is estimated to cause at least 3 million cases of serious disease and 400,000-700,000 deaths each year in young children. The disease burden is highest between the ages of 4 to 18 months and rarely occurs in infants less than three months and after age six years (WHO, 2002). Diagnosis of *H. influenzae* is by clinical diagnosis and bacterial culture or latex particle agglutinations. Diagnosis is considered confirmed when the organism is isolated from a sterile body site such as blood and cerebrospinal fluid. In this respect, *H. influenzae* cultured from the nasopharyngeal cavity or sputum would not indicate *H. influenzae* disease, because these sites are colonized in disease-free individuals.[Puri et. al., 1999]

The World Health Organization recommends a pentavalent vaccine, that combines vaccines against diphteria, tetanus, pertusis, hepatitis B and *Haemophilus influenzae* b (Hib) for the prevention of Hib in children under 5 years and asplenic patients (WHO, Bar-On Es et. al., 2012). In the treatment of severe cases of Haemophilus influenzae, cefotaxime and ceftriaxone are used parenteral, for the less severe cases, ampicillin and sulbactam, cephalosporins of the second and third generation, or fluoroquinolones are preferred (Chang et. al., 2010).
Mycoplasma pneumoniae

*Mycoplasma pneumoniae* is a very small bacterium in the class Mollicutes, it is the smallest free-living organism smaller than some viruses capable of self-replication. It causes disease of varying severity ranging from mild upper respiratory infection to severe mycoplasma pneumonia, a form of atypical bacterial pneumonia, and is related to cold agglutinin disease. Its infection is not limited to the respiratory tract infection, but includes non-pulmonary manifestations such as neurological, hepatic, cardiac diseases, hemolytic anemia, polyarthritis and erythema multiforme. Of the non-pulmonary manifestation, neurological manifestations are thought to be the most common (Razin et. al., 1998). Mycoplasma pneumoniae has been reported to be responsible for 24% cases of pneumonia in hospitalized children and about 15-27.4% of community acquired pneumonia (Shenoy et. al., 2005; Chaudry et. al., 1998).

Currently no primary prevention measures are available for mycoplasma pneumonia and vaccine research is on-going (Surender et. al., 2010). The clinical presentation is similar to ARTI due to other viruses, bacteria and other atypical pathogens. Laboratory diagnosis of M. pneumoniae infection is challenging due to the fastidious nature of the pathogen, the considerable sero-prevalence, and the possibility of transient asymptomatic carriage (Surender et. al., 2010). It is also hampered by the lack of standardized, sensitive and specific diagnostic methods (Daxboeck et. al., 2003). Antibiotic use in the treatment of mycoplasma pneumonia has been shown to reduce severity and duration of pneumonia and the use of broad spectrum antibiotics is advised (File et. al., 1998; Arnold, 2007).
Due to the severity of the bacterial causes of acute respiratory tract infection usually lower respiratory tract infection, there is a high misuse of antibiotics, with little attention to the viral causes especially in Africa. Algorithms and guidelines have been developed to improve the diagnosis of ARLTI

**Viral Cause of Acute Respiratory Infection**

The common viral culprits include human parainfluenza and influenza viruses (causing croup and pneumonia in children), adenovirus (pneumonia and pharyngoconjunctival fever in children) and respiratory syncytial virus. These are by far the leading viral cause of acute lower respiratory tract infections in early childhood (Yun et.al, 1995, Weigl et.al., 200, von Linstow et.al., 2004).

**Influenza Virus**

Influenza that cause human disease is an RNA virus of the family *Orthomyxoviridae* (Wright and Kawaoka, 2007) they make up three of the five genera of the Orthomyxoviridae family, influenza A, B and C. The type A viruses are the most virulent human pathogen among them. Influenza A subtypes that are important to humans are A(H3N2) and A(H1N1), of which the former is currently associated with most deaths (WHO, 2003). Wild aquatic birds are the natural reservoir of Influenza AVirus (Shinya et. al., 2010). Influenza B almost exclusively infect humans, the only other animals known to be susceptible to influenza B infection are the seal and the ferret. (Hay et. al., 2001; Fouchierr et. al., 2004; Osterhaus et. al., 2000; Jakeman et. al., 1994)
The pathogenicity of influenza virus is dependent on the function of viral proteins and on host immune responses, including innate and acquired immunity, indicating the importance of both viral factors and the host immune system for influenza pathogenesis (Fukuyama and Kawaoka, 2011). Influenza spreads around the world in seasonal epidemics, resulting in about three to five million yearly cases of severe illness and about 250,000 to 500,000 yearly deaths (WHO, 2009).

The virus is easily passed from person to person through the air by droplets and small particles excreted when infected individuals cough or sneeze (WHO, 2003). It can also be spread through contact with contaminated surfaces and bird droppings, though which means of transmission is most important is not absolutely clear (Brankston et al., 2007). People who contract influenza are most infective between the second and third days after infection (Carrat et al., 2006). Disease spreads very quickly among the population especially in crowded circumstances. Cold and dry weather enables the virus to survive longer outside the body than in other conditions and, as a consequence, seasonal epidemics in temperate areas appear in winter (WHO, 2003). Influenza pandemics occur when humans are introduced to Influenza A Virus with hemagglutinin (HA) to which they are immunologically naïve.

Respiratory illness caused by influenza is difficult to distinguish from illnesses caused by other respiratory pathogens on the basis of symptoms alone. However, during laboratory-confirmed influenza outbreaks, the majority of persons seeking medical advice for upper respiratory tract infections are likely to be infected by Influenza.
Laboratory confirmation will be required between annual influenza epidemics. Rapid diagnostic tests have recently become available to detect influenza viruses within 30 minutes. Despite the availability of rapid diagnostic tests, the collection of clinical specimens for viral culture remains critical to provide information regarding circulating influenza subtypes and strains.

Vaccination is the principal measure for preventing influenza and reducing the impact of epidemics. The World Health Organization recommends the influenza vaccine for high-risk groups, such as children, the elderly, health care workers, and people who have chronic illnesses such as asthma, diabetes, heart disease, or the immunocompromised (WHO, 2005a). Antiviral drugs such as adamantanes and zanamivir are available in some countries and effectively prevent and treat the influenza illness (WHO, 2009). Other reasonably effective ways to reduce the transmission of influenza include good personal health and hygiene habits such as: not touching your eyes, nose or mouth (CDC, 2010).

Human parainfluenza Virus

Human parainfluenza viruses belong to the family Paramyxoviridae, the same family as the RSV. These are enveloped viruses with a negative-sense single-stranded RNA genome (WHO, 2005a). Human parainfluenzaviruses types 1, 2 and 3 (PIV1, PIV2 and PIV3, respectively) are second only to RSV as important causes of viral LRI in young children (WHO, 2005a). It is responsible for 18% of upper respiratory
illnesses, 22% of lower respiratory illnesses and 64% of croup in young children (Heilman and WHO, 1990; Reed et. al., 1990)

PIV-1 and PIV-2 are the principal causes of croup, which occurs mostly in children from 6 to 48 months of age, whereas PIV-3 causes bronchiolitis and pneumonia predominantly in children less than 12 months of age (WHO, 2009). Parainfluenza viruses also cause a spectrum of respiratory illnesses, from upper respiratory infections, 30-50% of which are complicated by otitis media, to lower respiratory infections, about 0.3% of which require hospitalization (WHO, 2005a). Most children are infected by human parainfluenza virus type 3 (PIV-3) by the age of two years and by parainfluenza virus types 1 and 2 (PIV-1 and PIV-2) by the age of five years (Heilman and WHO, 1990). Parainfluenza illness just like other viral respiratory illnesses cannot be diagnosed based on clinical symptoms. HPIVs spread from person to person through close personal contact, through aerosols from coughing and sneezing, and through touching objects or surfaces that have HPIVs on them. It lasts only a few hours in the environment and it can be destroyed using common hygiene techniques and detergents, disinfectants and antiseptics. It can be confirmed by a number of laboratory tests which include isolation and detection in cell culture, antigen and antibody detection and polymerase chain reaction (CDC, 2012). Currently, there are no vaccines to prevent PIV though research is ongoing (CDC, 2012).

Adenovirus
Adenoviruses are nonenveloped, double-stranded DNA viruses with a 35,000-bp genome belonging to the Adenoviridae family, genus Mastadenovirus. The viral nucleic acids and core proteins are enclosed in an icosahedral capsid, along with the major capsid protein, hexons, and pentons (Rux and Burnett, 2004; Sam and Burnett, 2003).

Adenoviruses in addition to causing respiratory illnesses cause, gastroenteritis, bladder infection (cystitis), or rash illness). The respiratory illness caused by Adenovirus is usually upper respiratory and not severe (CDC, 2011). Adenovirus infections clinically cannot be differentiated from other respiratory infections. It can be confirmed using antigen detection, polymerase chain reaction assay, virus isolation, and serology (CDC, 2011). Adenoviruses are unusually stable to chemical or physical agents and adverse pH conditions, allowing for prolonged survival outside of the body and water. There are no antiviral drugs to treat adenoviral infections, so treatment is largely directed at the symptoms (which as stated is mild) (CDC, 2011).
2.4 THE RESPIRATORY SYNCYTIAL VIRUS

2.4.1 The Origin and Structure of the Virus
Respiratory syncytial virus was discovered in 1956, when a group of chimpanzees in a colony outside of Washington, DC (USA) were noted to have developed cold-like illness. A cytopathic agent was recovered from one of these chimpanzees which was then named “chimpanzee coryza agent” (CCA) (Morris et al., 1956; Rhodes et al., 1962). The investigators examined the entire colony, and nearly 100% of the chimpanzees were infected with this same agent. An interesting observation was that the human contacts working with the chimpanzees were also infected and exhibited upper respiratory tract illness, though less severe than observed in the chimpanzees (Chanock et al., 1957).

Subsequent studies in humans identified two major isolates of this virus, the Long and the Schneider strains (Chanock and Finberg, 1957). Following further studies on the cytopathology of this virus, it was discovered to form syncytia on tissue culture and in addition had similarities to the isolates recovered from the chimpanzees. Chanock and colleagues in 1957 coined the term “respiratory syncytial virus” (RSV) to incorporate all available isolates, and provided a classic description of the disease in children (Chanock and Finberg, 1957).
RSV is of the genus *Pneumovirus*, which belongs to the family Paramyxoviridae (Fenner *et al.*, 1974). The Paramyxoviridae family also includes two other genera, *Paramyxovirus* (which includes parainfluenza virus types 1, 2 and 3 and mumps virus) and *Morbillivirus*. The virus (RSV) is a pleomorphic, enveloped, medium sized (200nm), cytoplasmic virus containing single-stranded, non-segmented negative-sense RNA. The RNA codes for a symmetrical helical nucleocapsid core that is encased within a lipid envelope. Complementary DNA (cDNA) cloning has identified ten different viral genes, each coding for a single protein. The characteristics of these genes differentiate RSV from the other members of Paramyxoviridae (Ruuskanen *et al.*, 1993).

The major antigenic determinants of the virus are the disulfide-bonded glycoprotein (F, fusion protein) and the large glycoprotein (G, attachment protein) which are
involved in the induction of protective antibodies (Fenner et.al., 1974). The G protein mediates viral attachment and the F protein is involved in viral penetration and syncytium formation (Fenner et.al., 1974). The F protein is relatively stable, which makes it a suitable target for therapeutic intervention in contrast to the G protein, which is more variable forming the basis for RSV classification into type A and type B (Simoes, 1999).

2.4.2 Genotypes of the Respiratory Syncitial Virus

Respiratory Syncytial Virus (RSV) has two major antigenic groups, A and B (Kuypers et.al., 2004; Scott et.al., 2006). Evidence shows that there is rarely any geographic clustering of RSV subtypes (Cane, 2007). It has been seen that the dominance of a genotype in the same area may vary over time giving way to the other subtype (Seki et.al., 2001; Kuroiwa et.al., 2005). In a study in South Africa subtype A was found to be more prevalent (Madhi et.al., 2003) whilst genotype B was seen to be prevalent in Japan in one season. During outbreaks both strains of the virus may circulate concurrently (Kuroiwa et.al., 2005).

Any genotype of RSV can affect either the lower or upper respiratory tract or both. The relationship between severity and genotype of RSV has been seen in children less than 6 months where subtype B was found to cause more severe infections (Imaz et.al., 2000; Neves et.al., 2001). The thinking that subtype B may be responsible for severe disease is however doubtful because in this same study it was
found that those children with subtype B who had severe infections were also less breastfed. This disparity may have resulted in this false relationship because breastfeeding is known to protect against infections or severity of infections which includes respiratory infections. There is controversy as to whether the subtype of the RSV has a relation to the severity of clinical disease. Whilst some studies support the notion that RSV-A-induced bronchiolitis is more severe than that induced by RSV-B (Imaz et al., 2000; Papadopoulos et al. 2004) others contradict this hypothesis (Bergsträsser, 1998; Imaz et al., 2000, Neves et al., 2001). There is however the need for more studies to determine the association between RSV subtypes and severity of disease if any.

Infection by one strain does not protect against infection from another strain. Although milder than a primary infection, re-infections are common and recur throughout life (Lee et al., 2007; Scott et al., 2006). It is suggested that antigenic variation between subgroups A and B may contribute to the ability of the virus to cause repeated infections (Sullender, 2000). Re-infections can arise with the same variant or with a different variant within intervals as short as 3 months. The frequency of re-infection however, decreases after age five (Lee et al., 2007; Scott et al., 2006).

2.4.3 Epidemiology of Respiratory Syncytial Virus Infection

The virus generally has a high morbidity infecting almost all children by two years of age (Medici et al., 2004). Many infants require hospitalization, usually for oxygen therapy, and a few require management in intensive care facilities (Weber et al., 2002; Iwane et al., 2004). As such, it is not only associated with higher hospitalizations but
also higher cost of care. The global annual infection for RSV is estimated to be 64 million cases (WHO, 2008). Although the infection rate is generally high, the course of disease is usually benign with a mortality rate of less than 1% in healthy children (and only accounting for an annual mortality figure of less than 200,000 worldwide Berner et al., 2001). However, life-threatening infections are common in immunocompromised patients and in patients with cardio-pulmonary abnormalities, in whom mortality is estimated to be about 37% and sometimes as high as 73% (Ogra, 2004). In the USA, 75,000 to 125,000 infants are hospitalized annually due to RSV associated bronchiolitis or pneumonia (McCarthy and Hall, 2003). On the average, it costs US$44 per outpatient visit; US$91 per emergency visit, US$800 for hospitalizations without Intensive Care Unit (ICU) stay and US$5313 for ICU stay for an episode of respiratory illness due to RSV in the United States of America (Lee et al., 2007).

A significant 18% of acute lower respiratory tract infections (ALRTI) were due to RSV in children less than five years in The Gambia (Weber et al., 2002). In Nigeria, the incidence of RSV-associated lower respiratory tract infections (LRTI) among children aged < 5 years, was 94/1000 child-years with the situation worsening in children under one year (Robertson et al., 2004). Indonesia, another developing country, had similar incidence (Robertson et al., 2004). RSV infection accounted for 15% of all incidence of ALRI, 22% of all severe LRTI in Kenya with similar incidence in other parts of Africa (CDC, 2007; Medici et al., 2004; Nokes et al., 2004; von Linstow et al., 2004; and Weigl et al., 2001). All these raise a suspicion that though
RSV infections are not widely studied in Africa as in the developed world it is prevalent in the region and must be looked at.

Respiratory syncytial virus has worldwide presence with predictable yearly outbreaks. There is variation in burdens of the infection among various populations and the differences may be attributed to intrinsic properties of the population, the burden of lower respiratory infections in the population and differences in methods of measuring (Nokes, 2007). Respiratory syncytial virus infection, as a problem in the developed world, has been well studied (CDC, 2007; Medici et.al., 2004; von Linstow et.al., 2004; and Weigl et.al., 2001). There is considerable evidence to support the presence of high RSV infection in some parts of Africa though RSV has not been studied in depth as in the developed world (Hussey et.al., 2000; Nokes et.al., 2004; Vardas et.al., 1999 and Weber et.al., 2002).

Activity of RSV is substantial when both ambient temperatures and absolute humidity are very high, perhaps reflecting greater stability of RSV in aerosols. There is regional seasonality of respiratory syncytial virus. In equatorial islands (e.g. Singapore, Fiji, Taiwan, Hawaii), RSV occurs year round, with some increases in the rainy season. In regions south of the equator, such as South Africa, Argentina and Brazil, RSV epidemics occur in the cool, dry season. North of the equator, in tropical and subtropical areas, such as India, Gambia and Kuwait, outbreaks of RSV predominate during the cool, rainy season. In temperate regions on both sides of the equator, RSV epidemics occur in the winter months (Stenballe et.al., 2003).
2.4.4 Severity of Disease

In infants and younger children, RSV infection causes disease of varying severity, ranging from a mild upper respiratory tract infection to severe lower respiratory tract infection and sometimes death (Berner et.al., 2001; Sampalis, 2003). Severity of RSV-ALRTI is measured by signs on examination, the duration of hospitalization, intensive care unit (ICU) admission, oxygen requirement and mechanical ventilation (Robertson et.al., 2004; Simoes, 1999). Although RSV hospitalization has become, by default, the basis for determining severity of illness and impact of either preventive or therapeutic interventions on the infection, mortality attributed to RSV remains the strongest decisive indicator of RSV severity.

2.4.5 Pathogenesis

Respiratory syncytial virus is spread by direct exposure to large droplet secretions through coughing and sneezing and by direct contact with contaminated surfaces (Hall et.al., 1981). Respiratory syncytial virus gains entry to the host through the mucous membranes in the eye, nose, throat and mouth (Hall et.al., 1981). Though it is temperature sensitive, it can stay viable for hours on contaminated surfaces and therefore be a major source for nosocomial transmission (McCarthy and Hall, 2003). Younger children of less than 6 months are of higher risk of acquiring infections in the hospitals (McCarthy and Hall, 2003).

The virus replicates in the nasopharynx, during the initial stages of infection, infecting the ciliated epithelial cells that line the nose as well as the large and small airways.
For most individuals, if epithelial-cell destruction is limited, RSV is restricted to the upper respiratory tract. However, in some individuals where large amounts of epithelial cells are destroyed, the infection may spread to the lower respiratory tract (van Schaik et al., 2000).

### 2.4.6 Clinical Presentation

The peak incidence of the infection is between 2-6 months of age. The incubation period for RSV infection is 2 to 8 days. Viral shedding in infants may last for about four weeks (Hall et al., 1975, McCarthy and Hall, 2003). Asymptomatic primary RSV infection in children is rare (Ogra, 2004). As stated earlier, RSV may stay localized to the upper airway, resulting in cough and rhinorrhea or pharyngitis. It has been suggested that common colds induced by RSV may be more prolonged and severe than those induced by other viruses (Ogra, 2004). About 50% or more of primary infections in infancy however spread several days later to the lower respiratory tract (Medici et al., 2004).

Lower respiratory tract infections frequently present as expiratory wheezing (referred to as bronchiolitis or wheezy bronchitis or asthma), pneumonia and acute otitis media. Distinguishing RSV from other acute lower respiratory tract infections clinically is difficult because of similarity in clinical presentation among children. The clinical picture of RSV infection however varies according to age (Ogra, 2004). In neonates, RSV infection differ from those in older children in the sense that neonates do not
often exhibit wheezing, and apnoea may be the only symptom of infection. In older children, pneumonia is the most common manifestation (Ogra, 2004).

2.4.7 Diagnosis

Clinically RSV does not present any differently from other respiratory tract infections therefore diagnosis cannot be based only on clinical symptoms. There are several laboratory methods for detecting RSV and when methods for RSV detection are being compared, defining a "gold standard" that works for daily routine is difficult. Some of the common laboratory techniques include culturing respiratory specimens in traditional cell cultures or using rapid shell vial methods and enzyme immunoassays (EIA). Others are the use of immunofluorescent antibodies and reverse transcription-polymerase chain reaction (RT-PCR) amplification assays that detect RSV RNA and distinguish between subtypes have also been developed.

Isolation in cell culture has been proven to be sensitive and specific. In contrast to the other methods, it does not target a single virus. Poor specimen quality and/or inappropriate specimen storage and transport, however, severely decrease the sensitivity of cell culture, giving rise to false-negative results (Minnich and Ray, 1980). In addition, isolation in cell cultures is not sufficiently rapid to influence patient management and therefore cannot be used routinely in the management of patients (Engler and Preuss, 1997).
Analysis of nasopharyngeal aspirate by EIA technology represents an alternative method for virus detection combining high specificity and moderate sensitivity with time requirements shorter than those of culture techniques. However, the merely moderate sensitivity of the antigen capture enzyme immunoassays (EIA) restricts the method to acute-phase samples of children who shed significantly higher amounts of respiratory viruses (Hall et al., 1975).

Based on EIA technology, a rapid test for RSV detection has been developed and shown to have satisfying sensitivity (96.3 to 97%) and specificity (97 to 99%) in comparison with cell culture isolation (Krilov et al., 1994, Olsen et al., 1993), immunofluorescence assays (Dominguez et al., 1993; Miller et al., 1993), and EIAs (Obel et al., 1995). This rapid test is quite affordable and is largely used for testing for RSV at admission to the hospital and for bedside testing.

Immunofluorescence assays is yet another diagnostic method but it is labour intensive (Miller et al., 1993) and the use of microscopy in this method makes its sensitivity very dependent on the expertise of the microscopist and therefore results can be quite subjective and thus makes comparisons between different laboratories difficult.

Several publications have reported the use of RT-PCR for the detection of RSV just as for several other viruses including influenza A and B viruses, and the parainfluenza viruses (Fan et al., 1998; Stockton et al., 1998). The main benefit of molecular
methods is their extreme sensitivity and a high specificity depending on appropriate primer selection. One of their drawbacks has been that PCR is expensive and requires high technical laboratory standards. Therefore, the PCR method is not used routinely in clinical diagnostic laboratories but mainly for research purposes.

2.4.8 Prevention
Prevention of RSV infection is mainly by reducing environmental risk factors such as exposure to smoking, proper hand washing techniques and overcrowding. As at now, there is no effective vaccine available although various attempts for developing a vaccine are ongoing (Dudas and Karon, 1998). There is also no proven method for active immunity. Various immunoglobulins are available for acquiring passive immunity against RSV infection. In the United States of America where these are used, they are recommended only for children at high risk of acquiring RSV infection (Hasmi, 2000; Romero, 2003; Sandritter, 1999). Aerosolized ribavirin has been used to prevent RSV infections but it was not successful in its use as for treatment of RSV infection (Seo et. al., 2013; Chamaly et. al., 2010). Administering it as an intermittent dosing schedule is preferred to the continuous dosing schedule because of the high cost and inconvenient administration of the latter (Chamaly et. al., 2012).

2.4.9 Treatment
The treatment of RSV bronchiolitis is mainly supportive and symptomatic (Simoes, 1999). The treatment involves gentle handling, moderate fluid supply and maintaining
oxygenation at arterial oxygen (O\textsubscript{2}) saturation (S\textsubscript{a, O2}) ≥95% through administration of humidified O\textsubscript{2} in severe cases. Though Respiratory Syncytial Virus Immunoglobulin (RSVIG) has proven effective in preventing severe RSV LRI, it may not be capable of treating the condition once established (Rodriguez \textit{et al.}, 1997a; Law \textit{et al.}, 1997). A study in children at high risk for severe RSV disease because of congenital heart disease, lung disease, or extreme premature gestation failed to show any RSVIG efficacy in treatment of children hospitalized with RSV infection. (Rodriguez \textit{et al.}, 1997b).

\textbf{2.4.10 RSV and Other Viral Infections}

Dual respiratory viral infections occur in as high as 30% of infants with a lower respiratory tract (LRT) infection, (Aberle \textit{et al.}, 2005) though the incidence of dual respiratory viral infections varies widely across geographical locations. RSV can co-infect an individual together with other viruses like adenovirus, rhinoviruses and metapneumoviruses (Al-Sonboli \textit{et al.}, 2005; Cuevas \textit{et al.}, 2003; von Linstow, 2004). Clinical characteristics differ significantly in single and cases of respiratory viral co-infection with other viruses. The characteristics depend on the nature of the viruses involved. Clinical presentation has been shown to be more severe in dual infections involving RSV for example obstructive airway disease was more prevalent and severe in such dual cases (Aberle \textit{et al.}, 2005). Infants with dual RSV infections have been shown to be more hypoxic and have higher fever (Aberle \textit{et al.}, 2005).
A review of literature shows there is little data on the relationship between HIV status and RSV infection. Existing data, though limited, have shown that pneumonia rather than bronchiolitis is more common in HIV-infected children than in HIV-uninfected children (King, 1997). In South Africa, HIV-infected children had a higher case fatality rate than HIV-uninfected children when they developed RSV infection (Madhi et al., 2001). In contrast, in a study in the same country but in an area where HIV prevalence was low, there was no association between HIV and RSV in children less than two years (Hussey et al., 2000). HIV-positive children have been seen to shed the RSV virus for much longer periods, even as long as 199 days, and this has epidemiological implications on the persistence of the RSV virus in communities (King et al., 1993, Stensballe et al., 2003). There is evidence to suggest that respiratory viral infection may result in changes in HIV replication and, theoretically, HIV disease progression (Kumar et al. 2008; Reuters et al., 2010 and King, 1997).

2.4.11 Impact of RSV Infection on Respiratory System

There is considerable evidence that having RSV infection in the first year of life has a positive correlation with future problems in the respiratory system especially during the subsequent years till about the fifth year of life (Kneyber et al., 2000; Poulsen et al., 2006). Wheezing is common after RSV bronchiolitis in infancy. It may persist for up to 5 years. According to Kneyber, in the first 5 years there is increased risk of recurrent wheezing in children who had RSV bronchiolitis in infancy. These children may have more airway-related health problems in the first five years of life (Kneyber et al.2000; Poulsen et al., 2006). Despite all these present evidence is not conclusive.
that RSV bronchiolitis is a cause of atopic asthma in later life (Kneyber et al. 2000). It can currently be stated that RSV bronchiolitis constitutes a risk factor rather than a cause for the development of infantile asthma. This risk is increased in children with familial or personal antecedents of atopy (Mateos, 2001; Sznajder et al., 2005).
2.5 RISK FACTORS FOR RESPIRATORY SYNCYTIAL VIRUS INFECTION AND SEVERITY OF DISEASE

A number of factors have been known to be associated with RSV infection and severity of RSV disease in both developed and developing countries. The findings however, are variable from one location to another. In Africa in particular, very few risk factor studies have been conducted and findings are therefore not conclusive. The factors that have been found to be associated with the risk of RSV disease and/or its severity include prematurity, young age, seasonality, day care attendance, chronic heart or lung disease, exposure to smoking, overcrowding, socioeconomic status and male gender (Berner et.al., 2001; Madhi et.al., 2003; Simoes et.al., 2003; Weber et.al., 1999).

Prematurity is a proven risk for severe RSV infection. In a prospective study conducted during 1993–1994, in Canada prematurity was independently associated with an increased likelihood of apnea, admission to intensive care, the need for mechanical ventilation following RSV infection. Other studies have also confirmed these findings (Berner et.al., 2001; Carbonell-Estrany et.al., 2004; Figueras-Aloy et.al., 2004, Simoes et.al., 2003). The explanation that has been given to this observation is that normally efficient transfer of maternal neutralizing antibodies across the placenta occurs in the late stage of pregnancy (26-32 weeks). In premature babies however there is inadequate transfer of maternal antibodies and this therefore increases such a child’s risk to infections including RSV (Suara et.al., 1996). In addition, premature babies have narrow airways which are easily obstructed (Jones, 2009).
Young age less than 1 year has been shown to be associated with RSV infection (Weber *et al.*, 1998, Madhi *et al.*, 2003). In a study which identified risk factors that most likely may lead to development of RSV-related respiratory infection and subsequent hospital admission among premature infants, children 10 weeks and less were associated with a higher risk of lower respiratory illness, specifically RSV infection, and higher hospital admissions (Figueras-Alloy *et al.*, 2004; Madhi *et al.*, 2003). It has also been found that children less than three months were likely to stay for longer periods in the hospital than those aged 4 months to 4 years, although the younger children exhibited less fever (Berner *et al.* 2001).

Younger children are thought to have immature respiratory tract system. There is a school of thought that younger children may have laryngeal mucosa which is hypersensitive (Lee *et al.*, 2007). The exact cause of the hypersensitivity however has not been explained. It has also been observed that, during the first 6 months of life, children with chronic heart disease or prematurity were two-to-threefold more likely to be hospitalized with RSV infection (Boyce *et al.*, 2000; Lee *et al.*, 2007).

A male preponderance has generally been observed to be associated with RSV infection as observed for other acute lower respiratory tract infections (Weber *et al.*, 1998, Madhi *et al.*, 2003; Nagayama *et al.*, 2006). This observation seems more prominent with the lower age groups i.e. less than six months (Weber *et al.*, 1998, Madhi *et al.*, 2003; Nagayama *et al.*, 2006 a and b). It has been demonstrated that laboratory responses characterized by WBC counts and serum CRP levels at acute
stage were predominant in girls. Such observed gender differences could potentiate the predominance of asthma onset in male (Nagayama et al., 2006b). Current information on inflammatory response is not conclusive. Generally, circulating testosterone levels in boys are high during the early months and there is the need for the roles of sex hormones in the risk of acquiring of such diseases in this critical period to be investigated further. Most probably, immunologic influence could not be ignored because male hormones are generally immunosuppressive (Nagayama et al., 2006b).

Overcrowding has also been documented to significantly increase the risk of RSV infection and its severity as seen in other respiratory tract infections (Figueras-Aloy, 2004). Having more than ten people in a household increases the risk of severe respiratory syncytial virus infection as compared to living in a household of less than ten people (Weber et al., 1999). More importantly, having two or more children under five years in a household is associated with a higher hospital admission for RSV infection (Weber et al., 1999)]. This observation is not surprising because the mode of transmission of RSV is by direct contact or droplet infection. This risk factor may be very pertinent to Ghana because the country has large proportion of its population living in poor conditions and that includes overcrowding.

In a prospective study involving infants with RSV infection in the developed world, children exposed to second-hand maternal cigarette smoke during pregnancy had a significantly lower oxygen saturation during hospitalization i.e. more severe RSV
infection than those not exposed: (Bradley et.al., 2005). Maternalsmoking during pregnancy may impair in-utero airway development or alter lung elastic properties accounting for such higher rates of severe infection in children exposed to maternal smoking. In a developing country like Gambia, however maternal exposure to smoking was rather inversely associated with risk of severe RSV infection (Weber et.al., 1999). The researchers were of the view that this observation occurred because smoking was not common generally in the country where the study was done as compared to women in the developing world (Weber et.al., 1999). This is a plausible explanation because even in the developed world, in areas where smoking had reduced it was seen that there was no association between smoking and RSV infection or severity of infection.

There is controversy as to whether the nutritional status of the child affects the risk of RSV infection. In some studies RSV infection was more prevalent in well nourished children (Adegbola et.al., 1994; Nokes et.al., 2004; Nwankwo et.al., 1994) whilst the reverse has been seen in other studies (Simoes, 1999). In Ghana, 28% of children less than five years are malnourished and therefore the importance of establishing the relationship between the nutritional status of a child and RSV infection cannot be underscored (GSS amd MI, 2008).

Breastfeeding for at least the first two months of life has been seen to protect against RSV infection (Figueras-Aloy, 2004; Holdberg et.al., 1991 and Bulkow et.al., 2002).
This is thought to be so because maternal antibodies are transferred during breastfeeding. In Ghana, breastfeeding in the first few months of life is common and may therefore help reduce RSV infection in these children if this observation about breastfeeding is true.

In Gambia, where an extensive risk factor study has been done, increased socioeconomic status of the child did not decrease the risk of acquiring RSV infection (Weber et.al., 1999). People from lower socioeconomic status seem to be protected. This finding is not in line with what is known about socioeconomic status and its relationship with respiratory tract infection. This finding was however thought to have occurred because this study recruited children admitted from hospitals and because those in the higher socioeconomic group are more likely to take their children to the hospital they were thought to have been overrepresented in the case-group.

Day care attendance has also been seen as a risk factor for RSV infection in children less than five years. Day care attendance was higher in cases of RSV infection as compared to controls (Madhi et.al., 2003). This finding is supported by the fact that RSV is transmitted by direct contact and in the day care centres, mobility is high and contacts among children is enhanced.

There is controversy surrounding climate as a risk factor for RSV infection. The number and timings of RSV epidemics vary considerably within the same particular
geographical region and may only be crudely linked to climatic patterns (Nokes, 2007). Even locations with the same climatic pattern but not far apart may exhibit different patterns with respect to RSV seasonality (Robertson et.al., 2004). In the tropical regions where RSV infections increase in the rainy season it is believed that it is because mothers who take care of children could be busy on their farms and therefore do not give the children the needed attention giving rise to increased in incidence around the season (Weber et.al., 1999). In the rainy season in Gambia for instance, vegetables and fruits which have been found to be protective against respiratory tract infections are scarce (Weber et.al., 1999).

2.6 RESPIRATORY SYNCYTIAL VIRUS INFECTION AND MALARIA
There are some commonalities between RSV and malaria infection giving rise to misdiagnosis of the two diseases. Respiratory syncytial virus infection is most prevalent among children under five years of age, the same age group most affected by malaria. Even amongst children less than five years of age RSV is more prevalent in the lower age group, just like malaria (Madhi et.al., 2003).

There is high overlap in the clinical symptoms between malaria and pneumonia, the commonest and most severe presentation of RSV, in children under 5 years. Both of these illnesses can present as a febrile illness with other nonspecific symptoms. Malaria can present solely with pulmonary symptoms and may be taken as a case of pneumonia (Taylor, 2006). There is evidence to suggest that a child presenting with fever in the dry season is more likely to be suffering with an infection other than malaria, specifically pneumonia (O'Dempsey et.al., 1993; Rougemont et.al., 1991).
The clinical overlap between malaria and pneumonia due to RSV has important implications for case management strategies and evaluation of disease-specific interventions in regions like ours in which both pneumonia and malaria are prevalent.

Though only few studies have been done on the relationship between malaria and RSV, what is available is suggestive of a negative or no association between malaria and RSV. In Mozambique, malaria was independently related to a lower risk of RSV infection, and this remained so after adjusting for age group (Loscertales et.al., 2002). In a study in children in Kenya where malaria is endemic, 5% (16/335) of children with all LRTI had concurrent malaria. Malaria co-occurred in 3 of 133 infants with RSV infection (Nokes et.al., 2004). Another study has suggested the suppression of malaria in children infected with other respiratory influenza or measles virus but RSV was not looked at (Rooth and Bjorkman, 1992). In a trial in Gambia, there was no difference in the occurrence and mortality of severe lower respiratory tract infection between two groups of children, one of which received a malaria preventive therapy and the other which did not (Greenwood et.al., 1989). These studies however are too few to be conclusive.
CHAPTER THREE: METHODS

Country Profile
Ghana is located in the West African coast with a land area of 238,537 square kilometers. It is bordered on the north by Burkina Faso, the west by Cote d’ivoire, the east by Togo and the south by the Gulf of Guinea. Ghana is generally a lowland country with the highest point above sea level, Mount Afajato at 884 meters. It has a tropical climate with temperatures and rainfall varying according to distances from the coast and elevation. The average annual temperature is about 26°C. With the exception of the northern part of Ghana where there is one main rainy season, there are two distinct rainy seasons, April to June and September to November. In the north, the rainy season starts from March and ends in September. Annual rainfall ranges from 1,015 millimetres in the north to about 2,030 millimetres in the southwest (GDHS, 2008).
Figure 3: Map showing the study area in the context of Ghana-West Africa

Source: Ghana Web, 2011
There are ten administrative regions in the country namely Greater Accra, Central, Western, Eastern, Volta, Ashanti, Brong Ahafo, Northern, Upper West and Upper East. The regions are subdivided into 170 districts with Accra in the Greater Accra region as the capital of the country. Agriculture is the main economic activity of the country contributing to 34% of the gross domestic product (GSS and MI, 2008) followed by services. The leading export commodities are cocoa, gold and timber (GSS and MI, 2008). From the provisional results from the 2010 census, Ghana is estimated to have a population of 24,233,431 with an annual growth rate of 2.4% and a sex ratio is 95 males per 100 females (GSS, 2011).

3.1 STUDY AREA

The study was conducted in the La General Hospital located in the La Sub-metro in the Accra Metropolis of the Greater Accra Region. The hospital serves mainly the La Sub-metro in the Accra Metropolis (which includes people from La, Teshie and Nungua) and some patients from surrounding sub-metros. The sub-metro is mainly urban and had a population of 233,210 in 2009 with an annual growth rate of 4.4%. Children under five years account for 12.8% of this population (GHS, 2009). People living in this sub-metropolis are mainly of the middle to lower social class, with a minority in the upper class. They are mostly traders and the language commonly spoken is Ga.
3.1.1 La General Hospital

La General Hospital was established in the year 1963 as a polyclinic and upgraded to a district hospital in the year 2004. It is the only government health institution overseeing the work of both private and quasi government health facilities in the sub-metro. It also serves as a model institution for the National Health Insurance Scheme. La General Hospital is the major public hospital in the sub-metro in addition to other quasi-government facilities like the Police Hospital and Aviation clinic and several private clinics dotted around the sub-metro. The hospital in addition to serving the people of the district serves the Osu Children’s Home, which caters for orphans, so the information related to parents of such orphans could not be determined in the course of the study.

The hospital offers 24-hour Out Patient Department(OPD), In-patient and emergency services for children and adults. The hospital offers general medicine, general surgery, obstetric and gynecological care, maternal health/ family planning, eye, dental, community psychiatry, public health, ear-nose-throat and radiology services. It also has a good laboratory, pharmacy and mortuary to support the services. The laboratory is able to perform malaria microscopy and biochemical tests. It however does not have the facilities to perform bacteriological and virological tests. The hospital therefore collaborates with a private laboratory which performs the bacteriological tests (GHS, 2009b).

A Medical Director who is assisted by a Deputy Director of Nursing Services manages the hospital. The hospital has a staff strength of 278 which includes 10
specialist doctors, 6 general practitioners and 5 medical assistants. The specialists included one paediatrician. The staffing situation as against the catchment population in the hospital is as shown below in Table 1 (GHS, 2009b).

Table 1: Staff and Bed Situation of La General Hospital, 2009-2010

<table>
<thead>
<tr>
<th>Indicator</th>
<th>2009</th>
<th>2010</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doctor/Population Ratio</td>
<td>1:14,576</td>
<td>1:14,322</td>
</tr>
<tr>
<td>Nurse/Population Ratio</td>
<td>1:1515</td>
<td>1:1449</td>
</tr>
<tr>
<td>Pharmacist/Population Ratio</td>
<td>1:77737</td>
<td>1:1551</td>
</tr>
<tr>
<td>Bed/Population Ratio</td>
<td>1:2011</td>
<td>1:2318</td>
</tr>
</tbody>
</table>

Source: GHS, 2009b

Out-Patient Department (OPD) attendance

The per capita OPD attendance which was 0.2 in 2007 increased to 0.6 in 2008, an increase attributed to the commencement of the national health insurance in the hospital (GHS, 2009b). The per capita OPD attendance however declined slightly in 2009 and 2010 to 0.5 because more private health facilities had been constructed in the sub-metro (GHS, 2009b). In children under five years, malaria was the most frequent disease seen, followed by ARI and diarrhea from 2008 to 2010. Malaria and ARI together constituted about 75% of OPD attendance among children under five years in 2008, 2009 and 2010 (Table 2).
### Table 2: Most frequent Diseases Seen at the Out-patient Department Among Children Under Five Years, 2008-2010

<table>
<thead>
<tr>
<th>Rank</th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Disease (%)</td>
<td>Disease (%)</td>
<td>Disease (%)</td>
</tr>
<tr>
<td>1</td>
<td>Malaria (48.8)</td>
<td>Malaria (44.2)</td>
<td>Malaria (40.1)</td>
</tr>
<tr>
<td>2</td>
<td>ARI (18.6)</td>
<td>ARI (23.7)</td>
<td>O ARI (21.8)</td>
</tr>
<tr>
<td>3</td>
<td>Diarrhoea (6.0)</td>
<td>Diarrhoea (5.8)</td>
<td>Diarrhoea (10.1)</td>
</tr>
<tr>
<td>4</td>
<td>Acute Eye Infection (2.1)</td>
<td>Skin Diseases + Ulcers (4.5)</td>
<td>Skin Diseases + Ulcers (8.5)</td>
</tr>
<tr>
<td>5</td>
<td>Skin Diseases and Ulcers (2.8)</td>
<td>Acute Eye Infection (4.4)</td>
<td>Acute Ear Infection (3.2)</td>
</tr>
<tr>
<td>6</td>
<td>Acute Ear Infection (2.5)</td>
<td>Acute Ear Infection (2.7)</td>
<td>Acute Eye Infection (3.1)</td>
</tr>
<tr>
<td>7</td>
<td>Aneamia (1.8)</td>
<td>Acute Urinary Tract Infection (0.9)</td>
<td>Pneumonia (1.3)</td>
</tr>
<tr>
<td>8</td>
<td>Dental Caries (1.2)</td>
<td>Aneamia (0.7)</td>
<td>Acute Urinary Tract Infection (1.2)</td>
</tr>
<tr>
<td>9</td>
<td>SickleCell Diseases (0.5)</td>
<td>Dental Caries (0.6)</td>
<td>Aneamia (1.2)</td>
</tr>
<tr>
<td></td>
<td>All other Diseases (16.7)</td>
<td>All other Disease (12.4)</td>
<td>All other Diseases (9.5)</td>
</tr>
</tbody>
</table>

*ARI- acute respiratory tract infection excludes tuberculosis, asthma, ear infection
Source: Records Department, La General Hospital

Of the suspected malaria cases seen at the hospital 39.8%, 51% and 44.7% were confirmed by laboratory testing in 2008, 2009 and 2010 respectively. Unfortunately more often, diagnosis is given and prescription is given even before the test result comes in.
3.2 STUDY DESIGN

A hospital based surveillance system was set up to recruit children less than five years who reported at the children’s out-patient department of the La General Hospital with fever defined as axillary temperature $\geq 37.5^\circ$C from February 2009 to February 2010. An unmatched case-control study from within the children from the hospital surveillance was conducted to determine the risk factors of RSV.

Parents/guardians of the children with fever were approached to enrol their kids/wards into the study after explaining to them what it entailed. Their consent was sought and those who agreed were recruited. The recruitment process was such that it did not affect the care for the child in the hospital. Health practitioners as all other children attended to a child whose parent/guardian refused to take part in the study. Where the parent/guardian gave his/her consent a questionnaire was administered to the parent/guardian. This questionnaire was used to elicit basic socio-demographic and medical information of the child. After answering the questionnaire (usually done when they are waiting for their turn to see the physician) the sick child was examined by the physician –on-duty who then filled the examination form. After the examination, the child was taken to the ward where a nasopharyngeal aspirate was taken. In addition, capillary blood sample was taken through a finger prick. The blood was smeared on a frosted slide and allowed to dry.

**Inclusion Criteria for Hospital Based Surveillance**

Children were selected into the hospital based surveillance based on the following criteria

- Children who were less than five years old at the time of reporting to the hospital with an axillary temperature of $\geq 37.5^\circ$C during the study period.
- Consent from the parent or the guardian to participate in the study.

Exclusion criteria; children who were too sick for samples to be taken i.e unconscious, convulsing, severely lethargic children

**Case- Control for Risk Factor of Respiratory Syncytial Virus Infection**

A standard questionnaire, which elicited information on potential risk factors, was given to all parents/guardians of children who were enrolled. Cases and controls were selected during analysis using the case definition for the cases and controls. The unmatched case-control study involved children who had acute respiratory infection by clinical diagnosis. Cases were children with ARI who were positive for RSV by the PCR and controls were children with ARI but were negative for RSV.

A child was defined as having an ARI if she or he had symptoms of upper or lower respiratory tract infection. Upper respiratory tract infection may manifest as a cold or nasal discharge or cough whilst lower respiratory tract infection is defined as any of the above plus one or more of the following:

- Difficulty in breathing
- Nasal flare
- Fast breathing was defined as ≥ 60 breaths per minute in children aged < 2 months, ≥ 50 per minute in children aged 2-11 months, and as ≥ 40 per minute in children aged 12-59 months
- Lower chest wall indrawing, stridor
- Wheezing
- Apnoea
- Cyanosis
- Source: (WHO, 2005b)

Severity of ARI was defined as the presence of one or more of the following on admission fast breathing, lower chest wall indrawing, stridor, wheezing, apnoea or cyanosis, in addition in admission was used as a proxy for severity (Loscertales et.al., 2002; Robertson et.al., 2004)

**Sample Size for the Unmatched Case-Control study**

Based on the following assumptions

Ratio of case: control= 1:3

Exposure among controls: 20% this was guided by Weber’s study in Gamabia where the prevalence of the most important risk factor for severe RSV infection was 29%(this risk factor was number of people living with the child in the household (Weber et. al., 1999)

Minimum detectable odds ratio= 2[2 was chosen due to the expected high prevalence of RSV among cases of acute respiratory infection as shown from literature review (CDC, 2007; Medici et.al., 2004; Nokes et.al., 2004; von Linstow et.al., 2004; Weber et.al, 2002 and Weigl et.al., 2001)]

Confidence level= 95%

Power= 85.4%

The sample size used were= **70 cases: 210 controls**
Exposure variables in the Case-Control Study

A number of exposure variables were examined to determine any possible association with RSV infection. The host factors examined were age, sex, prematurity, exclusive breastfeeding, congenital abnormality and whether the child had had any past similar respiratory illness. Whilst the parental factors were highest educational level attained by mother and father, whether the child was taken care of by a single parent as well as whom the usual caregiver was. The household/environmental factors were day care attendance, presence of anybody who smokes in the child’s house, the socioeconomic status of the household.

Age was measured in months and a child’s age was the number of months completed by the child at the time of study visit.

Nutritional status was determined using the Epi Info 3.4.1 Nutritional Programme to calculate weight-for-age indicator. This is an indicator for both acute and chronic nutritional status. Children whose weight-for-age was below -2SD were considered as underweight (GSS amd MI, 2008)

Socioeconomic status estimation: the socioeconomic status was estimated using the availability and type of toilet in the household as well as the type of fuel used in the household. For fuel use, a household that used gas/electric stove was given a value of 3; a household that used kerosene a value of 2 and a household, which used firewood/charcoal, a value of 1. In weighting the availability and type of toilet used, a household without toilet had a value of 0; household with a flush toilet was assigned the value of 3, a household with KVIP was assigned a value of 2 and a household with
a pit/pan latrine was assigned 1. The values for the variables (i.e. toilet and fuel used by household) were aggregated to estimate a composite score. These scores were ranked and divided into five equal quintiles from lowest to highest to represent the poorest to richest socioeconomic status respectively.

The study definition for malaria was a child who presents a fever $\geq 37.5^\circ$C with malaria parasite on microscopy.

In making the comparison of diagnosis made by clinicians and what was found from the research, the children were grouped into cases of malaria, acute respiratory tract infection, gastroenteritis and others. For the purpose of this study, gastroenteritis was defined as a child with diarrhoea i.e. the passage of three or more loose or watery stools per day in a child with fever with no malaria parasites on examination.

3.3 SPECIMEN COLLECTION AND LABORATORY METHODS

Nasopharyngeal Aspiration

Nasopharyngeal specimens were collected by the use of a mucus trap with an attached catheter that was connected to a pressure suction machine. Through the nose the catheter was passed down to the pharynx of the child. With applied suction an aspirate was taken into the trap and 2ml buffered saline solution was then added to the aspirate. This was kept on ice in an ice-chest in the ward till close of day when it was sent to Noguchi Memorial Institute for Medical Research (NMIMR) for further storage.
and analysis. At the laboratory the samples were kept in the freezer at -20ºC until it was ready to be worked on.

**Respiratory Syncytial Virus Detection**

Respiratory Syncitial Virus detection was by Polymerase Chain Reaction (PCR). Initially the plan was to use rapid test kits of enzyme immunoassays to test. The PCR was chosen over the EIA because the study was not set to test laboratory results daily on the samples and after a few trials of using the RSV EIA rapid tests, it was realized that it will be cost efficient to batch the samples together and examine them in batches for the RSV. From literature it was shown that the yield of RSV using EIA after storage more than two weeks (Krilov *et al.*, 1994) was low and therefore it was not the most appropriate method to use, this necessitated the storage at -20ºC and the use of PCR. The processes were as follows: detection of RSV involved extraction of RSV genome (genetic material), reverse transcription of RSV single-stranded (ss)RNA into complementary DNA (cDNA), Amplification of the RSV cDNA, RSV Genotyping and Agarose Gel Electrophoresis. The PCR was chosen over the Antigen-Antibody test because though antigen-antibody test is sensitive enough for use in clinical conditions the PCR was genotype specific and more sensitive.

The samples, which were stored at -20 ºC, were thawed in the Biosafety cabinet II at room temperature. The single stranded RNA RSV was extracted by adding 50 µl of 1M sodium acetate (NaAc) containing 1% Sodium duodecylsulphate to 500 µl of nasopharyngeal suspension. An equal volume (500 µl) of phenol/chloroform (5:1)
was then added, vortexed for 1 minute and incubated at 56°C for 15 minutes. The suspension was vortexed for 1 minute and centrifuged for 3 minutes at 12,000 rpm, carefully to remove the supernatant (upper aqueous phase) containing the RNA, which was placed in a clean eppendorf tube. 500 µl of 6 M Guanidinium thiocyanate (GITC) was added to the recovered supernatant, vortexed for 30 seconds and centrifuged at 12,000 rpm for 5 minutes (Ushima et.al.,1994). This was done for each nasopharyngeal sample.

The RNaid matrix, which comes with the RNaid extraction kit, was vortexed very well after which 10 µl of the matrix was added to each recovered supernatant, vortexed for 10 seconds and incubated on a rocker at room temperature for 15 minutes. This was then centrifuged at 5,000 rpm for 20 seconds and the supernatant discarded. What was left was washed with 400 µl of RNaid wash buffer (also supplied with the RNaid extraction kits), centrifuged again for 30 seconds at 12,000 rpm. The supernatant containing the extracted single stranded RNA was put into a sterile eppendorf tube and stored at -20°C until needed. (Annex1: Standard Operating Procedures for RSV RNaid Extraction)(Ushima et.al.,1994).

After the extraction, the single stranded RNA was reverse transcribed into complimentary DNA (cDNA) at a temperature of 48°C using the Avian Myeloblastosis Virus (AMV) reverse transcriptase and RSV gene specific primers: RSV-AB1: GTCTTACAGCCGTGATTAGG (20mer, forward primer) and RSV-AB2: GGGCTTTCTTTGGTTACTTC (20mer, reverse primer)(Abels et.al.,2001).
The first run Polymerase Chain Reaction (PCR) was done to amplify the RSV cDNA. The PCR conditions were as follows: Initial Denaturation at 95ºC for 2 minutes and 35cycles of in-cycle denaturation at 95ºC for 1 minute. In-cycle annealing at 50ºC for 1.5 minutes, in-cycle extension at 72ºC for 2 minutes and a final extension at 72ºC for 7 minutes (Stockton et.al., 1998)

The second run PCR which was a Genotyping PCR was undertaken on the products which were positive for RSV after the first run of PCR using primers specific for RSV genotype A and B, the PCR conditions were as follows: 35cycles of in-cycle denaturation at 95ºC for 1 minute. In-cycle annealing at 50ºC for 1.5 minutes, in-cycle extension at 72ºC for 2 minutes and a final extension at 72ºC for 7 minutes. (Annex 2: RSV RT-PCR CONDITIONS)( Stockton et.al., 1998)

The amplicons were electrophoresed on a 2% Hi-Res Standard Agarose gel (Bioproducts Limited). The gel was viewed under ultraviolet light and documented using AlphaDigDoc™ RT2 imaging system (Alpha Innotech Coorporation, USA) digital photography. The expected fragment length for RSV A and B were 334 and 183 base pairs (Abels et.al.,2001).
**Malaria Parasite Detection**

Using a sterile lancet, the third finger was punctured. Pressure was applied to the finger to express blood; the first drop of blood was wiped off with a dry piece of cotton wool. Then, still applying gentle pressure 2-3 drops of blood was dropped onto a clean slide. This was evenly spread with the help of another slide to make a circular thick film of about 1cm in diameter. The film was allowed to dry at room temperature in a flat, level position protected from flies (WHO, 2000b).

At the end of the day, the slides were transported to NMIMR in slide boxes and stained. The slides were placed in a staining trough and a 3% Giemsa solution in buffered distilled water of pH 7.2 was used to fill the trough to cover the slides. It was left in the trough for 30-45 minutes. Clean water was then poured gently into the trough to float the iridescent scum off the surface of the stain. Afterwards, clean water was used to rinse rapidly. The slide was removed from the trough and placed in a slide rack with the film side down to drain making sure that film does not touch the rack (WHO, 2000b). After 15-20 minutes of drying, the slide was read with a magnification of 1000 underoil emersion. Trophozoites were examined and the number of trophozoites seen in 100 fields was counted against 200 white blood cells and recorded (WHO, 2000c).
3.4 DATA MANAGEMENT AND STATISTICAL ANALYSIS

Data Quality

Data was entered into Epi Info version 3.4.1 (from Centres for Disease Control and Prevention) and transferred into SPSS 12.0 (a product of SPSS Inc) for analysis. The quality of the data collected was ensured by monitoring for the following that:

- there was adequate and proper sample collection by using trained nurses and laboratory technicians to take the samples
- samples were transported in sealed traps in saline carried on ice in an ice chest to the laboratory where it was stored in the freezer at -20°C until it was ready to be worked on
- the reagents for testing was in good state by storing them in accordance with manufacturer specifications
- Ten percent of the blood film slides (negative and positive slides separately) were sampled and slide reading validated by three different microscopist.

To ensure that the quality of data collected using the questionnaire was good the following was undertaken:

The nurses who recruited the children and the research assistants who interviewed the guardians/parents were properly trained. The questionnaire was pretested in a similar urban health facility. All anomalies detected during the pretesting were corrected before the actual data collection started. During data collection I randomly selected some questionnaires filled by research assistant (whilst the parents/guardian is still in the hospital) to ensure completeness and accuracy of data collected, where gaps were detected complete the questionnaire properly. To ensure that no child with fever was
missed during the period of data collection, all children who came into the Out Patients department for care had their temperature taken and the parent/guardian of every child who met the criteria was consented.

**Data Analysis**

First, the demographic characteristics of all cases of fever were determined. The percentage of patients with RSV infection was determined for the whole study period. Distribution of factors (age, sex, highest educational level attained by mother and father, socioeconomic status) between RSV negative and RSV positive children were compared by a Chi-square test.

The following analyses were also done:

- The percentage of febrile cases due to respiratory syncytial virus in children less than five years
- The percentage of febrile illness due to malaria parasites in children less than five years
- The percentage of children less than five years with both respiratory syncytial virus and malaria infection and the relation of co-morbidity to presentation of illness
- Bivariate analysis was used to determine the risk of each factor for cases (odds ratios and 95% confidence intervals were calculated). All the factors were then entered into a multivariate logistic regression model. Adjusted odds ratios were calculated within the multivariate logistic regression to estimate the association of each factor with RSV infection after controlling for the
effect of other factors in the model, since all these factors tend to act together in real life.

**Outcome Variables**

The outcome variables were:

- Proportion of acute febrile illness due to RSV infection in children less than five years
- Proportion of acute febrile illness due to malaria in children less than five years
- Proportion of acute respiratory illness due to RSV in children less than five years
- Proportion of children less than five years with RSV and malaria co-infection
- Specific risk factors of RSV infection.

### 3.5 ETHICAL CONSIDERATIONS

Ethical clearance was sought from the Ghana Health Service and the Noguchi Memorial Institute of Medical Research Ethical Review Committees. Consent was sought from the guardians/parents. Privacy and Confidentiality of the study participants was ensured by the following measures:

- Interviewing and examination were done in private and conducive environment as far as possible.
- Information collected was secured and accessible only to the Principal investigator and her supervisors.
• The patients were coded and data, which contained the names and code, were separated from the rest of the database.

• Individual study participants will not be referred to in any publications.

Limitations

• There were periods when kits for collection of nasopharyngeal samples ran out and replacement stocks delayed leading to periods where only blood samples and not nasopharyngeal samples were collected. This same problem resulted in periods where entire sample collection was put on hold.

• Reagents for testing for presence of RSV also were unavailable for long periods resulting in delays in laboratory analysis of some samples, about eight months after collection of samples. These samples were however stored at -20°C. Due to this long waiting period ELISA which was initially planned to be undertaken before PCR was abandoned as the yield from ELISA was low and unreliable.

• Though efforts were made to ensure that the quantity of nasopharyngeal aspirate taken from the children were adequate, there were a few situations where due to children being uncooperative, samples taken were not adequate and therefore could not be used for RSV extraction at the time of laboratory analysis.

• Though proper storage was ensured as much as possible, during the transport of nasopharyngeal samples, a few samples volumes diminished due to improper sample container closure.

• The use of PCR for the detection of RSV may have led to a situation where the RNA form past RSV infection which may not be accounting for the current
disease condition maybe detected. This is likely to be in the minority because it has been shown that asymptomatic infection is rare (Ogra, 2004).
CHAPTER FOUR: RESULTS

4.1 BACKGROUND CHARACTERISTICS OF CHILDREN

During the one year period of data collection from February 2009 to February 2010, 7042 new patients under five years reported at the Out Patients Department (OPD) of the La Hospital with an average of 631 children per month, with the peak attendance in May and June. Out of this total OPD attendance, 613 presented with fever (axillary temperature $>37.5^0{\text{C}}$) at time of attendance and were recruited representing 8.7% of out-patient cases and an average of 55 children per month, the highest recruitment was in February 2010. Figure 3 below shows the proportion of children out of monthly out-patient cases who were recruited for the study.

Figure 4: Proportion of New Patients at the Out Patients Department Recruited for the Study from February 2009 to February 2010

![Proportion of New Patients at the Out Patients Department Recruited for the Study from February 2009 to February 2010](image)
4.1.1 Demographic Characteristics of the Children studied

Children recruited were aged between less than one month and 59 months with a median age of 17 months, majority of the children (36.5%) were less than 12 months, and the least (6.4%) of the children belonged in the age group 48-59 months old. Fifty three percent representing three hundred and twenty seven of the children were males, and the rest females (See Table 3).

Table 3: Demographic Characteristics of Children

<table>
<thead>
<tr>
<th>Variable</th>
<th>Number of children</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Agegroup (mths)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-11</td>
<td>224</td>
<td>36.5</td>
</tr>
<tr>
<td>12-23</td>
<td>164</td>
<td>26.8</td>
</tr>
<tr>
<td>24-47</td>
<td>186</td>
<td>30.3</td>
</tr>
<tr>
<td>48-59</td>
<td>39</td>
<td>6.4</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>327</td>
<td>53.3</td>
</tr>
<tr>
<td>Female</td>
<td>286</td>
<td>46.7</td>
</tr>
<tr>
<td>Usual Caregiver</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mother</td>
<td>561</td>
<td>93.2</td>
</tr>
<tr>
<td>Father</td>
<td>8</td>
<td>1.3</td>
</tr>
<tr>
<td>Grandmother</td>
<td>24</td>
<td>3.9</td>
</tr>
<tr>
<td>Others</td>
<td>9</td>
<td>1.5</td>
</tr>
<tr>
<td>Highest educational level attained by mother</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No education</td>
<td>69</td>
<td>11.4</td>
</tr>
<tr>
<td>Primary</td>
<td>393</td>
<td>64.2</td>
</tr>
<tr>
<td>Secondary</td>
<td>106</td>
<td>17.6</td>
</tr>
<tr>
<td>Tertiary</td>
<td>35</td>
<td>5.8</td>
</tr>
<tr>
<td>Highest educational level attained by father</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No education</td>
<td>35</td>
<td>5.9</td>
</tr>
<tr>
<td>Primary</td>
<td>277</td>
<td>46.7</td>
</tr>
<tr>
<td>Secondary</td>
<td>200</td>
<td>33.7</td>
</tr>
<tr>
<td>Tertiary</td>
<td>81</td>
<td>13.7</td>
</tr>
<tr>
<td>Socioeconomic Status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q1 (poorest)</td>
<td>23</td>
<td>3.8</td>
</tr>
<tr>
<td>Q2</td>
<td>199</td>
<td>33</td>
</tr>
<tr>
<td>Q3</td>
<td>117</td>
<td>19.4</td>
</tr>
<tr>
<td>Q4</td>
<td>102</td>
<td>16.9</td>
</tr>
<tr>
<td>Q5 (richest)</td>
<td>162</td>
<td>26.4</td>
</tr>
</tbody>
</table>
Majority (64.2%) of the mothers of the children had attained primary education whilst only 5.8% had attained tertiary education; 11.4% had no formal education as shown in Table 3 above. Of the fathers, 46.7% had primary education and 13.7% had tertiary education. Though the proportion of those who had tertiary level education was low it was an improvement on the proportions of mothers who had attained higher education as was to be expected. The percentage of fathers who had not attained any formal education was 5.9% which was lower than that for the mothers (11.4%).

From Table 3, only 3.3% of the children were of the poorest socioeconomic status (SES) majority (33%) were in the second SES, the highest SES was occupied by 26.4% of the children. Thus the study population had a fair representation of both the rich and poor.

4.1.2: Type of symptoms presented by children

The temperature of the children at presentation (OPD) ranged from 37.6 °C to 39.9 °C with a median temperature of 38.0 °C. Some (14.5%) children were brought to the hospital within 24 hours of developing the fever, whilst some caretakers/parents managed the child for as long as 14 days before bringing the child to the hospital, the median duration of fever before presenting to the hospital was three days (Table 4). In addition to having fever, the children presented with one or more of the following symptoms: cough (reported in 65.7% of children), difficulty in breathing (reported in 52.5% of children), nasal discharge (reported in 68.1% of children) and diarrhoea (reported in 63.6% children).
Table 4: Duration of Fever before Reporting to the Hospital

<table>
<thead>
<tr>
<th>Time</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within 24 hours</td>
<td>87</td>
<td>14.5</td>
</tr>
<tr>
<td>&gt;2 day to 3 days</td>
<td>337</td>
<td>56.2</td>
</tr>
<tr>
<td>4 days to 7 days</td>
<td>160</td>
<td>26.7</td>
</tr>
<tr>
<td>&gt;7 days</td>
<td>16</td>
<td>2.7</td>
</tr>
</tbody>
</table>

It was also realized that a number of children had taken one medication or the other before being brought to the hospital, the commonest being paracetamol which was taken by 80% of the children either alone or in combination with other drugs. Other medications included antibiotics, anti-malaria drugs and cough syrup as shown in Table 5 below; only 6 caretakers representing 1% admitted giving the child herbal medication.

Table 5: Medications taken by Children before Reporting at the Hospital

<table>
<thead>
<tr>
<th>Medication</th>
<th>N</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibiotic only</td>
<td>10</td>
<td>1.6</td>
</tr>
<tr>
<td>Anti-malarial only</td>
<td>9</td>
<td>1.5</td>
</tr>
<tr>
<td>Paracetamol only</td>
<td>295</td>
<td>48.4</td>
</tr>
<tr>
<td>Cough mixture only</td>
<td>17</td>
<td>2.8</td>
</tr>
<tr>
<td>Two or three medications</td>
<td>202</td>
<td>33.2</td>
</tr>
<tr>
<td>Four different medications</td>
<td>5</td>
<td>0.8</td>
</tr>
<tr>
<td>No medication</td>
<td>71</td>
<td>11.7</td>
</tr>
</tbody>
</table>

The clinical diagnosis made by clinicians was analysed against case definitions established for the study (based on clinical presentation and laboratory findings). Clinical diagnosis by clinicians was available for 80% (492/613) of the children seen and of these 87%(429/492) be specific had a clinical diagnosis of malaria alone or in
combination with another illness. The other most frequent diagnosis included gastroenteritis and ARI. Figure 5 below shows the three main diagnostic categories of malaria, gastroenteritis and acute respiratory infections recorded by the clinicians at the hospital. A few children had other diseases like meningitis, skin rash urinary tract infection etc and are represented in the box outside the venn diagram (Figure 5) below. About 4.6% children were admitted or referred whilst the rest were seen on out-patient basis.

**Figure 5: Clinical Diagnosis of Children with Acute Febrile Illness made by Clinicians**

![Venn Diagram](http://ugspace.ug.edu.gh)

Please note as stated earlier on, the clinical diagnosis was available for 492 of patients, so this diagram represents a total of 492 children

Using the study case definitions, the major diagnosis made were malaria, acute respiratory infection and gastroenteritis. Figure 6 shows that the numbers of cases due to malaria were considerably few, with a higher number of the children falling into
the ARI group; 53 children did not fall into any of the categories below and are represented in the box outside the venn diagram.

Figure 6: Diagnosis Based on Research Findings

Please note: these numbers covers the 492 cases in Figure 5 whose clinical diagnosis by clinicians were available.

Table 6 further compares the diseases of children based on clinical diagnosis and the study definition, the proportions of acute febrile illness due to malaria is considerably reduced from about 87% to 11%.
Table 6: Comparison of Proportion of Diseases Diagnosed based on Method of Diagnosis

<table>
<thead>
<tr>
<th>Disease</th>
<th>Clinical Diagnosis (%)</th>
<th>Study Definition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malaria only</td>
<td>56.3</td>
<td>2.1</td>
</tr>
<tr>
<td>Malaria + other disease</td>
<td>30.9</td>
<td>9.0</td>
</tr>
<tr>
<td>ARI</td>
<td>8.3</td>
<td>54.9</td>
</tr>
<tr>
<td>Gastroenteritis</td>
<td>1.6</td>
<td>5.0</td>
</tr>
<tr>
<td>ARI/Gastroenteritis</td>
<td>0.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Others</td>
<td>2.8</td>
<td>8.8</td>
</tr>
</tbody>
</table>
4.2: MALARIA AS A CAUSE OF ACUTE FEBRILE ILLNESS

4.2.1: Proportion of Febrile Illness Due To Malaria
Using microscopy as the method for confirming malaria 605 cases were tested, out of which only 68 were positive for malaria, giving an overall positivity rate of 11.2%. Seven of these children presented with severe symptoms and were either admitted or detained.

Twenty five percent of the admissions among the children studied was due to malaria (positive microscopy for malaria parasites). There were relatively more admissions in the children with positive malaria microscopy (18.5%) than among the children with negative malaria microscopy (10%) though not significant (Fisher’s exact test, p-value = 0.12).

Of the 605 children mentioned above, 490 had their clinical diagnosis recorded making comparison possible. Using the study definition of fever and microscopy results as the gold standard and comparing the cases diagnosed based on clinical judgment, a significant 404/490 (82.4%) were false positives and 0.6% (3/490) were false negatives, only 16.9% were true cases either true positives or true negatives giving a positive predictive value of clinical diagnosis as 11.6% (Chi-square = 18.3, df = 4, p-value = 0.001).

4.2.1: Characteristics of children with Malaria according to Study Definition
Just like the other children in the study, there was multiple symptom presentation among the malaria positive cases. In addition to fever, the most common symptom complaint was difficulty in breathing seen in 55.9% (38/68) of cases with the least
being diarrhoea which was present in 23.5% of cases. Seventeen percent of the children had used some form of antimalarial either correctly or incorrectly before coming to the hospital. There were significantly more children with negative microscopy who had taken some form of antimalarial ($X^2 = 9.114$, df=3, p-value=0.012).

Malaria was equally distributed among males and females (50% male and 50% female). The age characteristic of the children with respect to their malaria positivity is as shown below in Figure 7. Overall, the proportion of malaria cases increased as age increases. Children aged 48-59 months were mostly affected by malaria with those 0-11 months least affected as shown in Figure 8 below.

**Figure 7**: Proportion of children with malaria in each Age group at La General Hospital from February, 2009 to February, 2010
Among the various quintiles, the most affected quintile was the second quintile whilst the fifth quintile was the least affected. The poorest quintiles (first and second) had about 18% each of malaria (Figure 9). Malaria was more commonly seen among those of the lower socioeconomic class.

**Figure 8**: Socioeconomic Status of Parents/Guardian of Children with malaria during the Study Period from February 2009 to February 2010
4.3: RESPIRATORY SYNCYTIAL VIRUS AS A CAUSE OF ACUTE FEBRILE ILLNESS

4.3.1: Proportion of Acute Febrile Illness Due to Respiratory Syncytial Virus

Four hundred and eighty one children representing 78.4% were tested for RSV out of which 75 were positive for RSV accounting for 15.6% of febrile illnesses tested. Some people were not tested because at a point in the study there were no kits for nasopharyngeal aspiration whilst a few were not tested because of loss of sample solution leading to insufficient sample for RSV extraction; there was no deliberate selection as to who gets tested and who does not. Thirteen percent of the children (recruited for this study) who were admitted were positive for RSV. The proportion of RSV cases seen by month as against all febrile cases seen and those tested is as shown in Figure 10 below. The highest proportion was in February 2009 where 23% of all cases tested were RSV positive. There were also smaller peaks seen in April and November, 2009. There was no recruitment in September and October 2009 due to lack of kits for nasopharyngeal aspiration.
The children less than 12 months old were the most affected (19.8%) as compared to the other age groups. There were slightly more males than females who were positive for RSV infection though this difference was not significant (p-value=0.748). A higher proportion of children who had more than 7 people sleeping with them were positive for RSV and this finding was significant (see table 5).
Table 7: Characteristics of RSV Positive cases among Acute Febrile Illness

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total</th>
<th>RSV positive</th>
<th>p-value for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Count</td>
<td>%</td>
</tr>
<tr>
<td><strong>Agegroup(months)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-11</td>
<td>177</td>
<td>35</td>
<td>19.8</td>
</tr>
<tr>
<td>12-23</td>
<td>123</td>
<td>21</td>
<td>17.1</td>
</tr>
<tr>
<td>24-47</td>
<td>155</td>
<td>17</td>
<td>11</td>
</tr>
<tr>
<td>48-59</td>
<td>26</td>
<td>2</td>
<td>7.7</td>
</tr>
<tr>
<td><strong>Highest educational level attained by mother</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No education</td>
<td>53</td>
<td>14</td>
<td>26.4</td>
</tr>
<tr>
<td>Primary</td>
<td>303</td>
<td>45</td>
<td>14.9</td>
</tr>
<tr>
<td>Secondary</td>
<td>89</td>
<td>13</td>
<td>14.6</td>
</tr>
<tr>
<td>Tertiary</td>
<td>28</td>
<td>2</td>
<td>7.1</td>
</tr>
<tr>
<td><strong>Highest educational level attained by father</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No education</td>
<td>29</td>
<td>7</td>
<td>24.1</td>
</tr>
<tr>
<td>Primary</td>
<td>215</td>
<td>35</td>
<td>16.3</td>
</tr>
<tr>
<td>Secondary</td>
<td>154</td>
<td>24</td>
<td>15.6</td>
</tr>
<tr>
<td>Tertiary</td>
<td>67</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td><strong>Number of people who sleep in the same room with child</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-3</td>
<td>267</td>
<td>31</td>
<td>11.6</td>
</tr>
<tr>
<td>4-6</td>
<td>195</td>
<td>39</td>
<td>20</td>
</tr>
<tr>
<td>≥7</td>
<td>8</td>
<td>3</td>
<td>37.5</td>
</tr>
<tr>
<td><strong>Socioeconomic status</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q1(poorest)</td>
<td>19</td>
<td>5</td>
<td>26.3</td>
</tr>
<tr>
<td>Q2</td>
<td>162</td>
<td>23</td>
<td>14.2</td>
</tr>
<tr>
<td>Q3</td>
<td>88</td>
<td>17</td>
<td>19.3</td>
</tr>
<tr>
<td>Q4</td>
<td>74</td>
<td>9</td>
<td>12.2</td>
</tr>
<tr>
<td>Q5(richest)</td>
<td>129</td>
<td>20</td>
<td>15.5</td>
</tr>
<tr>
<td><em>Sex ratio</em></td>
<td>613</td>
<td>1.27</td>
<td></td>
</tr>
</tbody>
</table>

*This is a ratio so has no count or percentage and the p-value is for a X² test

Seventy two children presented with severe symptoms and were either admitted, detained or referred. Of these 15 (20.8%) were among the group whose RSV status could not be tested, whilst 12 (16.6%) and 45 (62.6%) were RSV positive and negative respectively. This difference in severity between the RSV positive and negative groups was not significant (z-test p-value=0.482).
4.3.2 Genotypes of Respiratory Syncitial Virus Seen Among the Children Tested

Majority 89.3 % (67/75) of RSV positive cases were of the B genotype, 6 (8.0%) were of genotype A and 2 (2.6%) were of mixed infection AB. Among the RSV positive cases, all the children with severe illness were of the genotype B. Figure 10 above shows a picture of agarose gel of the PCR products. The period over which the data was collected does not make possible for any conclusions to be made about the seasonality of the RSV genotypes.

Figure 10: Agarose gel showing RSV A and B

M: Molecular Marker, 1-7: PCR products, NC: negative control
Fifty six out of the 75 RSV cases (74.6%) were diagnosed by clinicians either as malaria or malaria in combination with gastroenteritis or ARI with only 3 as purely ARI, this is shown in figure 11 below.

**Figure 11:** Clinical Diagnosis of RSV Positive Children made by Clinicians During study period of February, 2009 to February, 2010

*The diagram above shows the kind of diagnosis made by the clinicians with respect to the RSV cases detected by the study.*
4.3.3 Malaria and RSV Co-infection

Seven of the 75 RSV positive cases (9.3%) were co-infected with malaria (positive for malaria parasites on microscopy) and all the co-morbid cases were of type B genotype. Five of the co-morbid cases were females and two were male. Two of the cases were less than 12 months and five were aged 12-24 months. None of these children presented with severe symptoms and were all treated on out-patient basis.

4.4 RESPIRATORY SYNCYTIAL VIRUS AS A CAUSE OF ACUTE RESPIRATORY TRACT INFECTION

Eighty three percent (509/613) of the children had upper respiratory symptoms whilst 6.8% of the children had lower respiratory infection according to symptoms. Altogether 83.4% (511/613) of children had respiratory tract symptoms. In addition to fever which all the children had, nasal discharge was the most common (81.6%) symptom among the children with ARI whilst diarrhoea was the least (26.2% of ARI cases). Almost 15% of children with acute respiratory tract infection had taken some antibiotics prior to their coming to the hospital as against 12% of those without symptoms of ARI, though this finding was not significant ($X^2 = 0.613, df=1, p-value = 0.434$).

Of the children with acute respiratory tract infection (according to the study criteria) 401 out of the 511 were tested for RSV. Of these, 17.5% (70/401) were positive for RSV; only five children were positive for RSV but did not have...
respiratory tract symptoms. In other words RSV infection was significantly higher in those with respiratory tract infections as against those without respiratory tract infections ($X^2=6.364$, p-value=0.012).
4.5 RISK FACTORS ASSOCIATED WITH RESPIRATORY SYNCTIAL VIRUS INFECTION

In all 70 cases and 210 controls were recruited. The male: female ratio for the cases was 1.25 whilst that for the controls was 1.12. The median (inter-quartile range) age for the cases was 12months(16.5) and 18months(14.5) for the controls.

4.5.1 Host Factors Associated with RSV Infection

Five hundred and eleven children had acute respiratory tract infection, out of which 401 were tested for RSV. As shown in Table 8 more cases were of younger age and the proportions of RSV infection decreased as age increased. When analyzed alone this observation was significant (p-value=0.001) but when confounding factors are controlled for it was no more significant (p-value=0.27). There was no difference with respect to sex among the cases and controls. All RSV positive cases were term babies as against 5.3% of non-RSV controls who were born premature and this difference was significant (p-value=0.04). There were relatively more controls with congenital disease than cases but this difference was not significant whilst there was a fairly equal proportion of cases and controls with history of a past similar illness (See Table 8). Initially when the effect of exclusive breastfeeding was analysed individually, being exclusively breastfed was a risk factor for acquiring RSV infection but after the multivariate logistic regression, exclusive breastfeeding became a strong protective factor. RSV infection was more prevalent in healthy children than those who were underweight (Table 8).
### Table 8: Relationship between Host Factors and RSV Infection at La General Hospital from February 2009 to February 2010

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cases</th>
<th>controls</th>
<th>Crude estimates</th>
<th>Adjusted estimates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Odds ratio (95% CI)</td>
<td>p-value</td>
</tr>
<tr>
<td><strong>Agegroup (mths)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-11</td>
<td>34</td>
<td>70</td>
<td>3.89 (0.92-16.53)</td>
<td>0.001</td>
</tr>
<tr>
<td>12-23</td>
<td>19</td>
<td>50</td>
<td>3.04 (0.67-13.79)</td>
<td></td>
</tr>
<tr>
<td>24-47</td>
<td>15</td>
<td>74</td>
<td>1.62 (0.34-7.73)</td>
<td></td>
</tr>
<tr>
<td>48-59</td>
<td>2</td>
<td>16</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male: female</td>
<td>1.25</td>
<td>1.12</td>
<td>1.12 (0.60-2.00)</td>
<td>0.67</td>
</tr>
<tr>
<td><strong>Prematurity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Premature</td>
<td>0</td>
<td>11</td>
<td>0.037 (0.00-1.36)</td>
<td>*0.04</td>
</tr>
<tr>
<td>Term</td>
<td>70</td>
<td>195</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Congenital disease</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>1</td>
<td>7</td>
<td>0.42 (0.05-3.44)</td>
<td>0.38</td>
</tr>
<tr>
<td>Absent</td>
<td>69</td>
<td>209</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>History of past similar illness</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>27</td>
<td>61</td>
<td>1.52 (0.83-2.79)</td>
<td>0.14</td>
</tr>
<tr>
<td>Absent</td>
<td>43</td>
<td>148</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Exclusive breastfeeding</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>2</td>
<td>21</td>
<td>0.26 (0.04-1.2)</td>
<td>0.057</td>
</tr>
<tr>
<td>Yes</td>
<td>68</td>
<td>187</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Nutritional Status of child</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Underweight</td>
<td>16</td>
<td>56</td>
<td>0.81 (0.41-1.61)</td>
<td>0.54</td>
</tr>
<tr>
<td>Healthy</td>
<td>54</td>
<td>154</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Fisher’s Exact Test was used*

### 4.5.2 Parental Factors Associated with RSV Infection

From Table 9, there was an inverse association between the educational level attained by the child’s parents (mother and father) and RSV infection when each factor was analysed alone. The trend reversed when the effect of the other factors were considered. The risk of RSV infection was lower for those who were taken care of by single parents. These findings however were not significant. Having the mother as the
usual caregiver was associated with a higher risk of RSV infection than having the father as the usual caregiver, though they were both not significant.

Table 9: Relationship between Parental Factors and RSV Infection at La General hospital from February 2009 to February 2010

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cases</th>
<th>Controls</th>
<th>Crude estimates</th>
<th>Adjusted estimates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases</td>
<td>Controls</td>
<td>Odds Ratio(95%CI)</td>
<td>p-value</td>
</tr>
<tr>
<td><strong>Maternal education</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No educ</td>
<td>14</td>
<td>25</td>
<td>6.72(0.30-147.0)</td>
<td>0.07</td>
</tr>
<tr>
<td>Primary</td>
<td>42</td>
<td>132</td>
<td>3.89(1.35-110.00)</td>
<td></td>
</tr>
<tr>
<td>Secondary</td>
<td>12</td>
<td>37</td>
<td>3.82(2.2-6.)</td>
<td></td>
</tr>
<tr>
<td>Tertiary</td>
<td>1</td>
<td>12</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><strong>Paternal education</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No educ</td>
<td>7</td>
<td>10</td>
<td>3.15(0.79-47.72)</td>
<td>0.08</td>
</tr>
<tr>
<td>Primary</td>
<td>34</td>
<td>92</td>
<td>1.34(0.77-2.33)</td>
<td></td>
</tr>
<tr>
<td>Secondary</td>
<td>22</td>
<td>74</td>
<td>1.66(0.29-9.61)</td>
<td></td>
</tr>
<tr>
<td>Tertiary</td>
<td>6</td>
<td>27</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><strong>Parenting</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>single</td>
<td>4</td>
<td>19</td>
<td>0.41(0.11-1.35)</td>
<td>0.11</td>
</tr>
<tr>
<td>non single</td>
<td>66</td>
<td>129</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Usual caregiver</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mother</td>
<td>64</td>
<td>187</td>
<td>0.11(0.01-0.77)</td>
<td>0.87</td>
</tr>
<tr>
<td>father</td>
<td>1</td>
<td>4</td>
<td>0.08(0.01-1.94)</td>
<td></td>
</tr>
<tr>
<td>grandmother</td>
<td>1</td>
<td>13</td>
<td>0.03(0.01-2.49)</td>
<td></td>
</tr>
<tr>
<td>others</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

4.5.3 Household Factors Associated with RSV Infection

As shown in Table 10, among household and environmental factors, increased number of people sleeping in a room with a child, as well as not attending a day-care centre were significantly associated with RSV infection. The risk of acquiring RSV infection was lowest among the children of the fourth quintile. The risk was higher among the fifth than the fourth quintile. The risk of RSV increased as the socioeconomic status decreased from the fourth quintile.
Table 10: Relationship between Household/ Environmental Factors and RSV Infection at La General Hospital from February, 2009 to February, 2010

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cases</th>
<th>controls</th>
<th>Crude estimates</th>
<th>Adjusted estimates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Odds ratio</td>
<td>p-value</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No of people sleeping with child</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-3</td>
<td>27</td>
<td>126</td>
<td>0.14(0.02-1.12)</td>
<td><strong>&lt;0.001</strong></td>
</tr>
<tr>
<td>4-6</td>
<td>39</td>
<td>77</td>
<td>0.34(0.04-2.63)</td>
<td></td>
</tr>
<tr>
<td>≥7</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Socioeconomic status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q1(poorest)</td>
<td>5</td>
<td>7</td>
<td>2.35(0.56-9.82)</td>
<td>0.26</td>
</tr>
<tr>
<td>Q2</td>
<td>22</td>
<td>65</td>
<td>1.11(0.51-2.46)</td>
<td></td>
</tr>
<tr>
<td>Q3</td>
<td>17</td>
<td>40</td>
<td>1.4(0.59-3.30)</td>
<td></td>
</tr>
<tr>
<td>Q4</td>
<td>8</td>
<td>38</td>
<td>0.69(0.24-1.93)</td>
<td>0.94</td>
</tr>
<tr>
<td>Q5(richest)</td>
<td>17</td>
<td>56</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>11</td>
<td>41</td>
<td>0.75(0.36-1.55)</td>
<td>0.28</td>
</tr>
<tr>
<td>Absent</td>
<td>59</td>
<td>165</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day-care attendance</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>15</td>
<td>85</td>
<td>0.375(0.19-0.71)</td>
<td><strong>&lt;0.001</strong></td>
</tr>
<tr>
<td>No</td>
<td>55</td>
<td>117</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

After adjusting for the effects of the factors on each through a multivariate logistic regression model, exclusive breastfeeding, and the number of people who sleep in a room with a child remained significantly associated with RSV infection and this is shown in tables above (tables 8-10). Whilst being exclusively breastfed turned to be strongly protective, a child with RSV is more likely to be sleeping with 4 or more people, the risk increasing as the number of people increase. These findings goes to
reject the null hypothesis that there is no difference between the children with acute respiratory illness due to RSV and those who have non-RSV acute respiratory illness.
CHAPTER FIVE: DISCUSSION

5.1 Respiratory Syncytial Virus as A Cause of Acute Febrile Illness

Fifteen percent of children with febrile illness were found to have RSV infection. As shown from the study majority (74.7%) of the cases detected by this study to have RSV were diagnosed as having malaria by clinicians because as is well known (WHO 2010b), the symptoms of malaria are non-specific and so many other diseases can present with similar symptoms. This finding compares with Taylor’s (2006) assertion that there is a high overlap of the clinical symptoms of malaria and respiratory tract infections. If the malaria confirmation test is negative, one major factor that we might have to look at as a potential risk factor is whether the person lives in a crowded home or not to help children who are more likely to have RSV infection rather than malaria.

Seven percent of the RSV cases were admitted, this finding is lower than what Medici et al. (2004) found in Italy where 40.4% of RSV positive children were admitted. This disparity is likely to be due to the fact that Medici et al. used children from emergency departments whilst this study looked at children from the general OPD. The children who were recruited by Medici et al. were children who had been seen by general practitioners and referred either due to severity of symptoms or need for expert care at the emergency department. As such, these children were generally more sick than the children from my study who were recruited from the OPD. This argument is substantiated by Carbonell-Estrany et al., (2004) who recruited children from the community and therefore had a lower admission rate of 3.6%, a figure lower than what was seen in this study.
There were more RSV cases of the genotype B than A. This compares with findings by Imaz et al. (2000) and Neves et al. (2001) but contradicts findings in other areas where subtype A was found to be more dominant (Carballal et al., 2000; Rajala et al., 2003; Fodha et al., 2004; Kuypers et al., 2008; Scott, 2008). Several studies have shown that there is a lot of variation in the dominance of a particular subtype in an area. Even in the same area, the dominance of a subtype may change from year to year (Choi and Lee, 2000; Kuroiwa et al., 2005; Sekhi et al., 2001). In the United Kingdom, a triennial pattern in groups A and B had been observed (Cane, 2001). In Finland, the subtype that dominates alternates every two years (Waris, 1991) with similar cycles of different lengths observed in other areas (Cane and Pringle, 1992; Cane et al., 1994; Cane, 2001).

The mechanism responsible for this observed variation in dominance of RSV subtype is unknown, but a number of reasons have been speculated. It may possibly be due to the fact that the persistence of a particular subtype in an area leads to gradual development of some form of immunity against that subtype, leading to gradual takeover of dominance by the other subtype (Cane, 2007). It may also be due to accumulation of maternal immunity in the population after an epidemic of a particular strain of virus leading to subsequent decrease in prevalence and severity of infection by that particular strain (Cane, 2007).

Children with genotype B were found to have more severe symptoms than those with genotype A, a similar finding to what Imaz et al. (2000), and Neves
et al. (2001) reported in Argentina and Kenya respectively. There is little evidence of subtypes related to the severity of the infection even when molecular differences in the subtypes are considered (Cane, 2007). For this reasoning, it is difficult to conclude that RSV type B is more associated with severity of disease. It was not possible to make any conclusive statement on the seasonality of the genotype of RSV from this study because the data was collected over a period of a year. As has been shown in some areas the dominance of a genotype may vary over time, and in some situations a cyclical trend have been observed, with the dominant genotype changing every three to four years. There will be the need for more in-depth studies into the genotype epidemiology of RSV infection.

Malaria-RSV co-infection was low in this study and this is similar to what was found in Mozambique and Kenya by Loscertales (2002) and Nokes (2004) respectively. This group of children did not present with more severe symptoms than those affected by only RSV or malaria alone. This supports the finding by Rooth and Bjorkman (1992) where they showed suppression of malaria in children who are infected by influenza or measles. There is therefore the possibility that RSV infection may also be suppressing malaria but it is not conclusive from the study and more work needs to be done in this area. There were more admissions in the malaria positive children than the RSV positive children.
5.2 Malaria as a Cause of Acute Febrile Illness

Based on presumptive diagnosis, more than 80% of the children with fever were diagnosed as malaria and therefore treated with antimalarials. However, based on clinical suspicion confirmed by microscopy, the proportion of febrile illness due to malaria was found to be 11.2%. Treatment based mainly on clinical diagnosis leading to gross abuse of antimalarials has been similarly seen in other parts of Africa like Mozambique and Tanzania where malaria is endemic (Hume et al., 2008; Mwanziva et al., 2008). Clinicians, generally treat patients based on presumptive diagnosis because of influence from peers and the pressure to conform to perceived expectations from colleagues as well as patients (Chandler et al., 2008). There is enough evidence to show that when diagnosis of febrile illness is based on clinical symptoms, more than 50% of cases treated as malaria will not be malaria (Makani, 2003; Nankabirwa, 2009) as was seen from the reports of the National malaria Control Programme Report in 2009 where about 35% of all OPD cases were due to malaria because most diagnosis are presumptive (NMCP, 2009). The treatment of febrile cases based solely on clinical symptoms has been shown to be less cost effective than confirming the diagnosis with a laboratory test (Uzochukwu et al., 2009) and also promotes the occurrence of drug resistance (Hamer et al., 2007).

Even in some cases where laboratory result are negative clinicians have still gone ahead to prescribe antimalarials (Hamer et al., 2007) because of the assertion that malaria is much more easier and acceptable to diagnose than other illnesses and is unpardonable to miss a case of malaria (Chandler et al., 2008). This practise could
explain the high proportion of funds the National Health Insurance Authority (NHIA) spends on the treatment of malaria alone (NHIA, 2008).

The finding from this study that the proportion of fever due to malaria is low compares to what was reported in other studies that not all fever is malaria even in the endemic regions (Berkley et.al., 2006; Reyburn et.al., 2004, Wang et.al., 2006). Even if those who took anti-malarials prior to coming to the hospital in this study were considered as false negatives (WHO, 2010b), the proportion of febrile illness due to malaria is still low. Presumptive diagnosis could be associated with improper management of fevers (Reyburn et.al., 2004) sometimes leading to fatal consequences (Makani, 2003).

From the findings of this study, together with the findings of other mentioned studies (Berkley et.al., 2006, Reyburn et.al., 2004; Makani, 2003) the need for confirmation of malaria diagnosis by a laboratory test cannot be overemphasized. In order for clinicians to be able to do this however there is the need for an effective, easy to use and quick laboratory test. Microscopy is the gold standard of laboratory diagnosis for routine clinic (Bell et.al., 2006; Reyburn, 2006), but in a resource challenged country like Ghana making microscopes available throughout the country can be difficult. Sometimes the expertise to handle these microscopes is limited (Bell et.al., 2006; WHO, 2004).
In the light of the recent introduction of affordable artemisinin-based combination therapy into the country with the support of the Global Fund, it is even more important to improve the diagnosis and management of febrile illness in the country. This is where the recently introduced malaria rapid diagnostic tests are important. In areas where clinicians adhere to the results of the test, it has been seen to be cost effective in the diagnosis of malaria. It can reduce drastically the false positives that occur when presumptive diagnosis is used (Thiam et al., 2011).

Evidence from Senegal shows that massive scale up of RDTs led to reduction in ACT consumption because clinicians generally followed guidelines (Thiam et al., 2011). Though some critics caution against the rapid adoption of the policy to test for malaria before treatment (Graz et al., 2011; Reyburn et al., 2007), introduction of RDTs nationwide at affordable prices supported by adherence to guidelines on management by clinicians i.e clinical diagnosis supported by laboratory confirmation will help reduce the burden of malaria as well as promote the proper treatment of non-malarious febrile illness and reduce avoidable death due to other causes (Bell et al., 2006; Berkley, 2005; Kyabayinze et al., 2008). The importance of adherence to guidelines such that clinicians don’t still treat for malaria even when malaria tests are negative (Hume et al., 2008; Mwanziva et al., 2008) as was seen in some areas must be emphasized if this is to be achieved.

Twenty five percent of recorded admissions were due to malaria. This figure is lower than admissions of 35.8% recorded by the National Malaria Control Programme.
(2009) nationally. This disparity may be due to the recruitment criteria used for this study where only children with fever of 37.5°C or more at presentation were recruited. This recruitment criterion might also have accounted for the relatively lower numbers of acute febrile illness (i.e. about 8% of OPD attendants in children under five years) seen in this study. Twenty five percent however is still significant and efforts must be increased to encourage people to present to the appropriate health facility early to decrease the numbers who get admitted due to malaria.

In this study, males and females were equally affected by malaria. This finding is similar to what was shown in other studies where there was no difference in malaria prevalence among males and females (Haque et.al., 2009; Stefani et.al., 2011). This however, contradicts what has been shown in a number of studies (Haque et.al., 2011 and Yusof et.al., 2010) where males were more affected. In both studies where males were at higher risk, older people were studied and occupational exposure among males might have increased their risk of exposure to mosquito bites.

The finding of the people affected by malaria being mostly of the lower socioeconomic group compares well with what was found by Mbando et.al., 2011, Messina et.al.(2011) and Haque et.al. (2011). Such an observation has been linked to the fact that people from high socioeconomic status have better housing which is less likely to allow mosquitoes to enter and stay in. In addition they generally have surroundings, which are less conducive for breeding of mosquitoes (Mbando et.al.).
The age group mostly affected were children 48 to 59 months, though this contradicts what had been said in past literature (Madhi et.al., 2003; WHO, 2007) where the children less than one year were mostly affected. A more recent study support this finding where the incidence of malaria peaked at aged 2-3 years (Stefani et.al., 2011). It was postulated that the younger children may still be benefiting from maternal antibodies and that interventions maybe focused more at the younger children (Stefani et.al., 2011).

5.3 Respiratory Syncytial Virus as a Cause of Acute Respiratory Tract Infection

The proportion of acute respiratory tract infections due to RSV was 17.5% in this study and this is similar to findings in Gambia (Weber et.al., 2002) and in the USA (CDC, 2007). This finding is however slightly higher than findings in Kenya (Nokes et al, 2004, Nokest et.al. 2009) but lower than findings in Italy by Medici et.al. (2004). Differences in burden of RSV has been attributed to a number of reasons including intrinsic susceptibility of the population, differences in burden of ARI and differences in methods for surveillance and case ascertainment (Nokes, 2007). Medici et.al. had higher proportions because the recruitment was done during an epidemic season for about seven months and the study children were from emergency departments.

The proportion of ARI due to RSV found in this study is significant given the fact that this is a hospital based study and the surveillance was passive. Even though there maybe a bias in this regard, correction of the bias may lead to slightly lower
proportion, that is if the surveillance was community based and active, though the numbers are likely to be higher, the population base will also be higher. The method used for collecting the samples was nasopharyngeal aspiration which usually gives a higher but more accurate yield as compared with estimates using other methods such as nasal wash or swab. This method is the gold standard for sample collection but may be inappropriate for routine sampling. The Polymerase Chain Reaction (PCR) method, which was used to detect the RSV genome has a high yield and might have accounted for such higher proportions. However this is much more accurate than the other methods such as enzyme immunoassay and immunofluorescence (Fan et al., 1998, Stockton et al., 1998). This finding indicates the importance of RSV as a cause of respiratory illness in children in Ghana making it important that we take a closer look at the treatment of children with fever and respiratory symptoms.

The fact that only 5 out of the 75 positive cases did not have respiratory symptoms shows that RSV is mainly respiratory in nature and it is not surprising quite a number of studies in determining the burden of RSV usually recruit patients with respiratory symptoms as the entry point (Weber et al., 2002; Madhi et al., 2003). This study however used febrile illness as its starting point because of the question that it had to answer as to which proportions of the febrile illnesses are due to RSV.

In this study, the highest proportion of RSV cases was seen in February 2010 when the weather is relatively cool just before the rainy season. Not much was seen in around the same period in 2009 in this study because, data collection started in the last...
week of February 2009. Data is not complete from this study to make an emphatic statement on seasonality.
5.4: Risk Factors associated with RSV Infection

A higher proportion of cases had increased number of people sleeping with them in the same room than controls. Children sleeping in a room with more people had significantly more cases of RSV than those who slept in a room with less people. This factor had the strongest association with RSV infection even after controlling for the effect of other factors. This compares with what was found in other studies by Figueras-Aloy et al. (2004) and Weber et al. (2002 and 1999). This is observation maybe due to the mode by which RSV infection spreads i.e. overcrowding increases the risk of contact with formites or droplets from other infected persons. It is therefore important as a country efforts are made to ensure that less people are crowded in a room. One major way of achieving this might be reduction in poverty supported by appropriate education.

The risk of RSV was shown to increase with younger age. Children less than 12 months were mostly affected. This finding although significant in the bivariate analysis was not significant when other factors were taken into account in the multivariate logistic regression model. The trend in the risk with young age is similar to what was found in a number of studies both in the developed and developing world (Figuera-Aloy et al., 2004; Madhi et al., 2003; Medici et al., 2004; Nokes, 2004 and Weber et al., 1998). It has been suggested that younger children are at higher risk because of immature respiratory tract system (Lee et al., 2007). This has a lot of implication on designing an immunization schedule in the future if a vaccine becomes available. A possible immunization should be undertaken early in a child’s life to protect the child against RSV infection. Whilst these other studies cited above showed...
that among the young children those aged 2-6 months are most at risk, in this study, it was those aged 7-11 months that were most at risk. There is a possibility that the effect of exclusive breastfeeding that was seen to be highly protective in the multivariate logistic model could have accounted for this difference. This is because exclusive breastfeeding in Ghana is promoted for the first six months of life and the maternal antibodies transferred during that period might have protected the children (Suara et al., 1996).

This study showed that there were relatively more males among cases than controls though this finding was not significant it is still maybe a pointer to the increased risk of infection among males as seen in studies in Italy by Medici (2009), and in Gambia by Nagayama (2006), and Weber et al. (2002). It also supports what was found from the review of fourteen studies by Weber (1998) where he found 12 out of 14 studies to have a male preponderance. Not much information is known about why this is so, though the effect of the sex hormones and differential anti-inflammatory response in the sexes have been suggested (Nagayama et al., 2006b). Since females are also affected, though to a less extent, it will be appropriate to focus resources on preventing or managing infection in both sexes especially in resource challenged areas.

All the cases were term as against 11 controls that were preterm. This is in contradiction to what have been seen in other studies (Carbonell-Estrany et al., 2004; Figueras- Aloy et al., 2004). This difference in observation maybe because these other studies looked at children less than six months old as against this study which looked
at children from day1 to 59 months. It is possible that the effect of prematurity might have been greatly diminished in the older children in this study, therefore the effect of prematurity in terms of being a risk factor may not be observed. For the reason of having small number of cases and no case being preterm, it was not possible for prematurity to be stratified by age to in order to study the effect of prematurity in younger children less than two years.

A higher percentage of the cases were found to have been exclusively breastfed than in controls. After adjusting for the effect of the other factors, exclusive breastfeeding became strongly protective. This compares to what was found in developed countries (Bulkow et.al., 2002; Carbonell-Estrany et.al., 2004; Figueras-Aloy, 2004 and Holdberg et.al., 1991) where breastfeeding was found to be protective. Weber (1999) in Gambia, however, did not find any difference in breastfeeding between cases and controls because almost all children were breastfed. In this study, healthy children as compared to those underweight were at a higher risk of developing RSV infection. This is similar to what was found by in Kenya (Nokes et.al., 2004); in Gambia (Adegbola et.al., 1994) and in Nigeria (Nwankwo et.al., 1994).

There was no difference in the proportion of cases and controls with history of a similar illness. This variable was used as an attempt to find a proxy for past RSV-ARI infection to detect any pattern in recurrence of infection. This study did not show that there is any pattern of recurrence for RSV infection in children less than five years.
Day care attendance was initially seen to be protective against RSV infection though it was no more significant after the multivariate logistic regression analysis. This is in contradiction to what was shown by Madhi et.al. in 2003 in the developed world. This observation maybe because in Ghana, people tend to send their children at a much later age to daycare center than in the developed world. Children who attend daycare are generally older and since young age have been shown to have a higher risk for RSV infection; it confounded the relationship between daycare attendance and RSV infection making daycare attendance protective when it was analyzed alone with RSV infection. This association was however not seen after adjusting for other factors including age.

Single parenting was much more common among cases as compared to controls and having the mother as the usual caregiver was associated with a higher risk of RSV infection than having the father or others as the usual caregiver, though they were both not significant. The finding of having the mother as the usual care giver being higher among cases is similar to what was found in Gambia (Weber et.al., 1999). Since this is hospital based study, this observation might have occurred because the children who were taken care of by their mothers were more likely to be brought to the hospital when they fall sick than when they are taken care of by other relatives i.e. these children had better care. The children who were taken care of usually by grandmothers had the highest risk of RSV infection after adjusting for confounding by other factors. It is possible that this might be linked to overcrowding in the sense that they are more people in the homes that have the grandmothers than the other homes.
There was relatively no difference in the socioeconomic status as well as paternal and maternal education among cases and controls. There was a relatively higher proportion of both cases and controls with parents who had attained primary education. This finding contradicts what has been found in other places where an association was detected (Weber et al., 1999; Bradley et al., 2005). Weber et al. (1999) found that there was increased risk of RSV infection among those from the richer homes. The observation in this study maybe a reflection of the educational levels of the underling study population in which majority had attained primary education.

Smoking in the household was seen to be more prevalent among controls than cases; this is in contradiction to what was found by Bradley et al. (2005). There is the possibility that this was masked by other factors like the wealth of the people. It could also be that this study because of the small numbers of people who smoked in the households of the children could not determine the actual relationship between smoking and RSV infection.
CHAPTER SIX: CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The perception that almost every fever is malaria in malaria endemic country should be dismissed. Respiratory syncytial virus was found in 15.6% of febrile children and malaria was found in 11.2% of children less than five years with acute febrile illness. Clinicians treated majority of these children who presented at the hospital with fever as malaria. This study however showed that most febrile illnesses are not due to malaria. There is the need for clinicians should be looking out for other causes of fever like RSV.

There were very few cases of malaria and RSV co-morbidity and these co-morbid cases were not associated with severe disease. Children aged 48-59months were those mostly affected by malaria. People from the lower socioeconomic group were more affected by malaria, but there was no difference in the proportion of males and females affected by malaria.

The most important factor that was found to be associated with RSV infection in this study was a child living in crowded rooms; exclusive breastfeeding was also seen to be protective. Young age i.e less than one year old, male sex, prematurity and not attending a day care centre were also associated with RSV infection though the finding was not significant after adjusting for the effect of other factors.
6.2: Recommendations

To health practitioners
Since not all fever is malaria, health practitioners should as much as possible follow the WHO recommendation of confirming suspected malaria cases. In diagnosing they should look for other possible causes of fever. Pointers like overcrowding, male sex and young age of less than one year can be used as pointers in looking out for possible viral causes of fever like RSV.

The Ministry of Health
The ministry should intensify its efforts to promote confirmation of suspected malaria before treatment, by making available laboratory facilities like microscopy and rapid diagnostic tests for malaria diagnosis.

To the research community
There will be need for community based studies as well studies in a well defined population setting for example at the demographic sentinel sites of Ghana for more information on RSV in Ghana as well as study into other causes of non-malaria acute febrile illness in children.

To the Government of Ghana/Health Partners
The government must provide resources to support research into important but neglected diseases like RSV. Health partners are also encouraged to allocate a portion of their funds to support research.
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APPENDICES

HOSPITAL SURVEILLANCE QUESTIONNAIRE

FORM NUMBER [__|__|__|__|__|] FORMNUM

ELIGIBILITY: CHILD \( \leq 60 \) MONTHS WITH FEVER \( > 37.5^\circ C \)

1.0 BASIC DATA:

1.1 Date of visit [__|__|__|__|__|] DATE

1.2 Study child ID (=SAMPLE NO.) [__|__|__|__|__|] SC_ID

1.3 Hospital folder No. [__|__|__|__|__|__|__|__|] HOSP_ID

1.4 Study child name ____________________________________ SCNAM

1.5 Date of Birth [__|__|__|__|__|__|__|__|] DOB

   Age (in months): [___] AGE

   Sex: [1. M 2. ] SEX

2.0 HISTORY OF CURRENT ILLNESS:

2. Please describe your child's condition today

   Cough [1. Yes 2. No] COUGH

   Difficulty in breathing [1. Yes 2. No] DIFBREAT

   Fever [1. Yes 2. No] FEVER

   Nasal Discharge [1. Yes 2. No] NASCHARGE

   Diarrhoea [1. Yes 2. No] DIARR1

   Other [1. Yes 2. No] OTHER

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If yes specify: ____________________________________________

2.2. DURATION: For those symptoms that the parent/guardian responded yes in previous question, ask for duration in days and fill appropriately. If the symptom is not present, enter 88

Cough began  [ ] [ ] COUGHSTART

Fever began  [ ] [ ] FEVERSTART

Breathing difficulty begun  [ ] [ ] DIFBRESTART

3.0. MEDICATION

3.1 Has the child had any of these medicine for this illness before coming here?

Antibiotics  1. Yes  2. No  8. NA  9. NK ANTI

Antimalarials  1. Yes  2. No  8. NA  9. NK ANTIMAL

Cough mix/antihistamin  1. Yes  2. No  8. NA  9. NK COUGHMIX

Herbal remedies  1. Yes  2. No  8. NA  9. NK HERBAL

Paracetamol  1. Yes  2. No  8. NA  9. NK PARA

CASE-CONTROL QUESTIONNAIRE
1.0 **PAST MEDICAL HISTORY**: Has the child had the following in the past?

- **Heart Disease**: 1. Yes 2. No 3. NK  HTDIS
- **Diarrhoea**: 1. Yes 2. No 3. NK  DIARR2
- **Similar illness**: 1. Yes 2. No 3. NK  SIMILAR
- **Congenital Abnormality**: 1. Yes 2. No  CONABNOM
  Specify______________________________________________________________________

2.0 **BREASTFEEDING HISTORY**: Was the child breastfed or is the child being breastfed?

- **Breast Fed**: 1. Yes 2. No  BFED
  If yes, **Duration (months)**: [ ] [ ] BFEDDUR

2.1 Was the child exclusively breastfed or is the child being exclusively breastfed?

- **Exclusive Breast Feeding**: 1. Yes 2. No  EXBFED
  If yes, **Duration (months)**: [ ] [ ] EXBFEDDUR

3.0 **SOCIO ECONOMIC STATUS**

3.1 **Does child sleep alone**: 1. Yes 2. No  SLEEPALONE
  If No, No in room [ ] [ ] NUMROOM

3.2 **Highest educational level attained by mother**: 1. Prim 2. Sec. 3. Poly/Trg 4. Uni 5. Nil  MODEDU

3.3 **Single parent**: 1. Yes 2. No  SIPARENT
3.4 Usual caregiver | 1. Mother | 2. Father | 3. Grand'M | 4. other | CAREGIV

3.5 What kind of fuel do you usually use at home? (Please tick)
   1. Firewood
   2. Charcoal
   3. Kerosine
   4. Gas cooker
   5. Electric cooker | FUEL

3.5.1 Do you use a toilet facility that is owned and used by your household alone?
   1. Household toilet
   2. Toilet in another household
   3. Community toilet | TOIFAC

3.6 What kind of toilet do you use at home?
   4. Pan latrine
   5. Pit latrine
   6. KVIP
   7. Flush watercloset | TOIKIND

4.0 SMOKING HISTORY

Does anybody smoke in home of child | 1. Yes | 2. No | SMOKE

5.0 GESTATIONAL AGE HISTORY

5.1 Was the child born at term? | 1. Yes | 2. No

5.2 If premature, what was the gestational age
   ___ <28 weeks, ___ 28 - 37 weeks | GESTAGE

6.0 DAY CARE HISTORY

6.1 Child attending crèche | 1. Yes | 2. No | CRECHE

8.0 OUTCOME OF THIS VISIT:

8.1 Died | 1. Yes | 2. No | OUTCOME

8.1 Admitted | 1. Yes | 2. No | ADMI
8.3 Discharged  

EXAMINATION RESULTS

1.1 Mental status:  

<table>
<thead>
<tr>
<th>Normal</th>
<th>Irritable</th>
<th>Drowsy/lethargic</th>
<th>Unconscious</th>
</tr>
</thead>
</table>

MENTAL

1.2 Temperature  

|       | °C | TEMP |

1.3 Respiratory rate  

|       | bpm | RESRATE |

1.4 Weight (kg)  

|       | WEIGHT |

1.5 Height (cm)  

|       | HEIGHT |

1.6 Strydor  

| Yes | No | STRYDOR |

1.7 Chest wall indrawing  

| Yes | No | CHESTWID |

1.8 Wheezing  

| Yes | No | WHEEZE |

1.9 Apnoea  

| Yes | No | APNOEA |

1.8 Cyanosis  

| Yes | No | CYNOSIS |

1.9 Nasal Flare  

| Yes | No | NFLARE |

1.10 Dehydration  

| None | Mild | Moderate | Severe | DEHYD |
## SAMPLE COLLECTION FORM

<table>
<thead>
<tr>
<th>Nasopharyngeal aspirate:</th>
<th>1. Yes</th>
<th>2. No</th>
<th>SAMPLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample code number</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Blood</th>
<th>1. Yes</th>
<th>2. No</th>
<th>BLOOD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample code number</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
CONSENT FORM

Dear Parent/Guardian,

Acute febrile illness is an acute illness that manifest as fever. The commonest presenting symptom/sign of ill children less than five years is fever. Fever can be the presenting symptom of quite a number of medical conditions. But the commonest medical condition presenting with fever especially in developing countries like Ghana is infectious diseases. Acute respiratory tract infection, malaria and diarrhoea are the commonest infections plaguing children less than five years. Except for the children one month and below, acute respiratory tract infections (ARI) account for the highest morbidity and mortality in children less than five years worldwide. About half of all respiratory infections are viral and the majority of such viral respiratory infections is caused by an agent called the respiratory syncytial virus. We however do not know the extent of this infection in Ghana and how it relates to malaria which is also very common in Ghana. We at the University of Ghana (School of Public Health) are undertaking this study, **Acute Febrile Illness The Role Of Respiratory Syncytial Virus Infection And Malaria In An Urban Paediatric Population**, to find out how many children in La are affected by respiratory syncytial virus and malaria infection.

In connection with this exercise we will also be asking you the parent or guardian to provide information on health and other characteristics about your child and your family.

In addition, we will take 2-3 drops of blood from the finger of your ward or child and a little saliva from the throat of ward/child. The process of taking the blood may be painful but not harmful to your child, whilst the process of taking the saliva may be uncomfortable but neither painful nor harmful. Please be assured that all information you give us in this study will be kept confidential. Furthermore, should the results of this study ever be reported in medical journals or at medical meetings, you or your child/ward will not be identified by name.

The information we ask about your child and family may make you or your family feel uncomfortable. Also note that there may be no personal benefit to your child by taking part. However, your child taking part in the study will help us know the size of the problem in La. This information may help us determine what to do about the problem. It is, however, possible that we may find out that your child/ward has malaria or respiratory syncytial virus infection. If that should prove to be the case, you will be treated according the treatment guidelines of the hospital.

You have every right to refuse to allow your child/ward to participate in the study without any one objecting. You may also decide after agreeing to allow us to examine your child change your mind and withdraw your child from the study without any penalty. You are assured that refusing to participate in this study will not in anyway affect the treatment of your child/ ward in this facility. If you wish to have some matters relating to this study clarified, do not hesitate to contact Dr Keziah Malm on 0244237564.

If you agree to allow your ward/child to participate in the study please write your name and sign in the spaces below.

______________________
Signature of parent/guardian
ANNEX 1: EXTRACTION OF RSV ssRNA FOR RT-PCR

PHENOL/CHLOROFORM - RNAID® KIT EXTRACTION METHOD

i. Detailed method

1. Remember to place the 1M NaAc containing 1% sodium dodecyl sulphate (SDS), pH 5.0 in the 37°C water bath.

2. Add 50 µl of 1M NaAc containing 1% SDS to 800 µl of Nasopharyngeal aspirate, vortex for 10 seconds and incubate for 15 minutes at 37°C.

3. Add an equal volume (500 µl) of phenol/chloroform (1:1), vortex for 1 minute and incubate at 56°C for 15 minutes.

4. Open and immediately reseal tubes prior to vortexing to release built in pressure and prevent the tube from popping open when vortexing.

5. Vortex for 1 minute and centrifuge for 3 minutes at 12,000 rpm, carefully remove the supernatant (upper aqueous phase) containing the dsRNA, and place in a clean eppendorf tube.

   Note: avoid any interface material as this contains proteins and DNA that will contaminate your extraction and potentially degrade your RNA.

6. Repeat steps 3 – 5 using 250 µl of phenol/chloroform (5:1).

7. Add 500 µl of 6 M GITC to the recovered suspension and vortex for 30 seconds. Centrifuge at 12,000 rpm for 5 minutes.

8. Transfer solution into clean eppendorf tube if pellet forms.

   NB: Do not inhale GITC – work in safety cabinet.

9. Vortex RNaid matrix (comes with RNaid extraction kit) very well and again just prior to addition to each sample. Add 10 µl of the matrix to each sample, vortex for 10 seconds and incubate on a rocker at room temperature for 15 minutes.

10. Centrifuge at 5,000 rpm for 20 seconds. Discard the supernatant.

11. Add 400 µl of RNaid wash buffer (also supplied with the RNaid extraction kit) to the pellet and gently resuspend with a pipette. Centrifuge for 30 seconds at 12,000 rpm and discard the supernatant.

12. Repeat step 10 again this time using 10 µl of RNaid wash buffer. Centrifuge at 12,000 rpm for 60 seconds. Discard the supernatant. Blot well to get rid of excess ethanol.
13. Resuspend pellet in 40 µl of DEPC treated water (supplied with the RNaid extraction kit). Incubate at 65°C in a water bath for 10 minutes to elute the RNA from the beads.

14. Centrifuge at 12,000 rpm for 3 minutes.

15. Carefully transfer the supernatant containing the extracted RNA to a sterile eppendorf tube. Store at -20°C until needed for RT – PCR reaction.

ii. PREPARATION OF REAGENTS

a. Phenol: chloroform (1:1)
   Add 1 part of phenol to 1 part of chloroform and mix well. Place solution in a dark or foil-covered bottle as the solution is light-sensitive.

b. 1% sodium dodecyl sulphate (SDS)
   10% sodium dodecyl sulphate (SDS) stock
   Add 10 g of SDS in 100 ml of distilled water. Dissolve in a 65°C water bath.

c. 1M NaAc with 1% sodium dodecyl sulphate (SDS), pH 5.0
   Dissolve 8.2 g sodium acetate in 60 ml distilled water. Add 1 ml of 10% SDS stock and mix. Adjust pH to 5.0 with glacial acetic acid and make up to 100 ml with distilled water.

d. 6M Guanidine Isothiocyanate (GITC)
   Add 7.09 g of GITC in 4 ml of distilled water to make up to 10 ml. Heat at 56°C in a water bath to dissolve the GITC crystals.
   **NB: Prepare solution just prior to use. Do not inhale!!**
ANNEX 2: RSV RT-PCR CONDITIONS

References:

• REVERSE TRANSCRIPTION
RSV-AB1: GTCTTACAGCCGTGATTAGG (20mer, forward primer)
RSV-AB2: GGGCTTTCTTTTGTTACTTC (20mer, reverse primer)

*Expected fragment length: 836 bp – 838 bp (1.5% - 2.25% agarose gels)

1x Reaction (Master Mix)
0.2 μl 10mM dATP
0.2 μl 10mM dCTP
0.2 μl 10mM dGTP
0.2 μl 10mM dTTP
0.5 μl AMV RTase (10U/μl)
2 μl 5X AMV RTase Buffer
1.7 μl dH$_2$O

Incubate at 48°C for 2 hours 30 minutes

1st ROUND PCR
0.2 μl 10mM dATP
0.2 μl 10mM dCTP
0.2 μl 10mM dGTP
0.2 μl 10mM dTTP
10 μl 5X Green GoTaq Buffer (10U/μl)
23.95 μl dH$_2$O
0.25 μl 5X GoTaq Polymerase (5U/μl)

Denaturation: 95°C, 1 min
Annealing: 50°C, 1 min 30 secs
Extension: 72°C, 2 min

Final Extension: 72°C, 7 mins
2nd ROUND (GENOTYPING) PCR

RSV-A1: GATGTTACGGGAGTCT (20mer, forward primer)
RSV-A2: GTACACTGTAGTTAATCACA (20mer, reverse primer)

*Expected fragment length: 334 bp (1.5% - 2.25% agarose gels)

RSV-B1: AATGCTAAGATGGGGAGTTC (20mer, forward primer)
RSV-B2: GAAATTGAGTTAATGACAGC (20mer, reverse primer)

*Expected fragment length: 183 bp (1.5% - 2.25% agarose gels)

1x Reaction (Master Mix)

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 pmol/μl RSV-A1</td>
<td>1.0 μl</td>
</tr>
<tr>
<td>20 pmol/μl RSV-A2</td>
<td>1.0 μl</td>
</tr>
<tr>
<td>20 pmol/μl RSV-B1</td>
<td>1.0 μl</td>
</tr>
<tr>
<td>20 pmol/μl RSV-B2</td>
<td>1.0 μl</td>
</tr>
<tr>
<td>10mM dATP</td>
<td>0.2 μl</td>
</tr>
<tr>
<td>10mM dCTP</td>
<td>0.2 μl</td>
</tr>
<tr>
<td>10mM dGTP</td>
<td>0.2 μl</td>
</tr>
<tr>
<td>10mM dTTP</td>
<td>0.2 μl</td>
</tr>
<tr>
<td>5X Green GoTaq Buffer (10U/μl)</td>
<td>10 μl</td>
</tr>
<tr>
<td>dH₂O</td>
<td>26.95 μl</td>
</tr>
<tr>
<td>5X GoTaq Polymerase (5U/μl)</td>
<td>0.25 μl</td>
</tr>
</tbody>
</table>

8.0 μl RSV cDNA
42.0 μl PCR Master Mix

Denaturation: 95°C, 1 min
Annealing: 50°C, 1 min 30 secs
Extension: 72°C, 2 min

35 cycles

Final Extension: 72°C, 7 mins
ANNEX 3: PICTURES SHOWING RESEARCH ACTIVITIES

Figure 12: Investigator taking a sample (nasopharyngeal aspirate) from a child

Figure 13: Investigator working in the laboratory