AN EPIDEMIOLOGICAL STUDY OF PNEUMONIA IN MIGRANT INFANTS AND CHILDREN ON THE THAI-MYANMAR BORDER

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE BY RESEARCH IN PAEDIATRICS

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Abstract

Background: Pneumonia is the leading cause of death in children and the estimated incidence of childhood pneumonia in Myanmar is the third highest in the world. Effective interventions, including case-management and immunisations, have been established. However these focus on bacterial pneumonia and recent advances in molecular diagnostics have highlighted the substantial role played by viruses. An understanding of the local epidemiology and aetiology of childhood pneumonia is vital to ensure that current and future interventions are appropriate to the local population.

Objective: To study the severity and aetiology of childhood pneumonia, in migrant infants and children under five years old, presenting to a clinic on the Thai-Myanmar border.

Methods: A one-year prospective observational study was conducted in a rural clinic on the Thai-Myanmar border. All children attending the clinic, who were diagnosed with clinical pneumonia according to the World Health Organization definitions, were treated and investigated according to local paediatric guidelines.

Results: During the study period 175 episodes of clinical pneumonia were diagnosed. Of these 59% were male and half were infants (48%). The median age at presentation was 7.5 months (IQR 3 to 13). Clinically half (50%) had severe or very severe pneumonia. Using the WHO standardised method for interpretation, 38% of radiographs had primary end-point pneumonia.

At least one virus was identified in 49% of patients. The most commonly identified virus was adenovirus, which was identified in 24% of cases, followed by RSV (22%), then hMPV (6%) and Influenza A (3%). The cases of pneumonia peaked at the end of the rainy season and this closely followed the RSV activity.

Conclusion: The majority of children presented with severe or very severe pneumonia, suggesting that in this population patients may present later. The substantial burden in infants suggests that future interventions should focus on this vulnerable age group. Viruses were frequently identified. Adenovirus was commonly identified, however its presence in nasopharyngeal secretions is of uncertain significance, especially in the presence of other viruses. The persistence of adenovirus and its role in acute pneumonia needs further investigation. RSV is an important and commonly found pathogen and prevention of RSV through a safe and effective vaccine could have substantial effect on the reduction of childhood pneumonia on the Thai-Myanmar border.

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Dedicated to Tabo, whose memory I treasure.

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ABBREVIATIONS

ADV	Adenovirus
ALRI	Acute lower respiratory tract infection
ARI	Acute respiratory tract infection
CBC	Complete blood count
CHERG	Child Health Epidemiology Reference Group
CRP	C-reactive protein
DNA	Deoxyribonucleic acid
EV	Enterovirus
GAVI	Global Alliance for Vaccines and Immunisation
GAPP	Global Action Plan for Prevention and Control of Pneumonia
HBoV	Human bocavirus
HCoV	Human coronavirus
Hib	*Haemophilus influenzae* type B
HibCV	*Haemophilus influenzae* type B conjugate vaccine
HIV	Human immunodeficiency virus
hMPV	Human metapneumovirus
IF	Immunofluorescence
IMCI	Integrated Management of Childhood Illness
InfA	Influenza A
InfB	Influenza B
IQR	Inter-quartile range
LRTI	Lower respiratory tract infection

MDG	Millennium Development Goal
NSP	Non-severe pneumonia (WHO criteria)
OI	Other infiltrate
OR	Odds ratio
PCV	Pneumococcal conjugate vaccination
PCR	Polymerase chain reaction
PEP	Primary endpoint pneumonia
PIV	Parainfluenza virus
PR	Pulse rate
RNase P	Ribonuclease P
RNA	Ribonucleic acid
RR	Respiratory rate
RSV	Respiratory syncytial virus
RT-PCR	Reverse transcription polymerase chain reaction
RV	Rhinovirus
SD	Standard deviation
SMRU	Shoklo Malaria Research Unit
SP	Severe pneumonia (WHO criteria)
Under-5s	Children under the age of five years
UNICEF	United Nations Children's Fund
URTI	Upper respiratory tract infection
VSP	Very severe pneumonia (WHO criteria)
WHO	World Health Organization

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CHAPTER 1

INTRODUCTION

Pneumonia is a disease of the lungs, which causes cough, breathlessness and sometimes death. It affects all ages but young children and the elderly are at greatest risk. Pneumonia is usually caused by a viral or bacterial infection: *Streptococcus pneumoniae* (pneumococcus) and *Haemophilus influenzae* type-B (Hib) are the major bacterial pathogens, whilst respiratory syncytial virus (RSV) is the leading viral pathogen in childhood pneumonia. The presence of any pathogens in the lungs leads to inflammation. Fluid and cells fill the air spaces, which causes reduced oxygen exchange, and in severe cases hypoxaemia and death.

Pneumonia remains the leading cause of death in children under the age of five years (under-5s) despite the advances in antibiotic therapy, vaccine development and molecular diagnostics over the past fifty years. [1] Pneumonia is estimated to cause over 1.5 million deaths in children every year, nearly all of which are in the developing world. [1] Appropriate case management and infant vaccinations are recognised as fundamental approaches in reducing these childhood deaths. [2, 3]

Case management focuses on early detection and treatment of pneumonia. [4] In resource-limited settings doctors, radiography and laboratory facilities are often not available. Children are therefore treated empirically, and often care is provided by locally trained health workers. The World Health Organization (WHO) has developed an algorithm to aid the management of childhood pneumonia in such settings. [5] The WHO acknowledged that a significant proportion of childhood pneumonia was due to a bacteria infection and

recognised the high mortality associated with bacterial pneumonia. [6] The clinical diagnosis was therefore designed to be very sensitive and detect all children with a potential bacterial pneumonia, accepting a loss of specificity and therefore that a substantial number of children with a viral infection would receive unnecessary antibiotics. The ARI case-management strategy has proven to be successful in reducing both pneumonia mortality and overall mortality. [7, 8] There is however growing concern about the lack of specificity and the associated risk of antibiotic resistance.

Pneumonia pathogens vary between countries and regions, and knowledge of the regional aetiology can guide effective evidence-based treatment, appropriate vaccine development and vaccine deployment. [9] Further research is therefore needed to define the epidemiology of childhood pneumonia in areas where accurate information is currently lacking, particularly in developing countries. [10] The Thai-Myanmar border is one such area. Migrants from Myanmar working and living in rural areas along the Thai-Myanmar border have little or no access to healthcare in Myanmar and multiple barriers such as safety and financial costs, limit their access to Thai medical facilities. Myanmar itself is estimated to have the third highest incidence of childhood pneumonia in the world. [9] It is therefore likely that childhood pneumonia is a leading cause of morbidity and mortality in the migrant population along the Thai-Myanmar border. The aim of this current study is to improve the understanding of the epidemiology of childhood pneumonia in migrant infants and children living along the Thai-Myanmar border.

CHAPTER 2

LITERATURE REVIEW

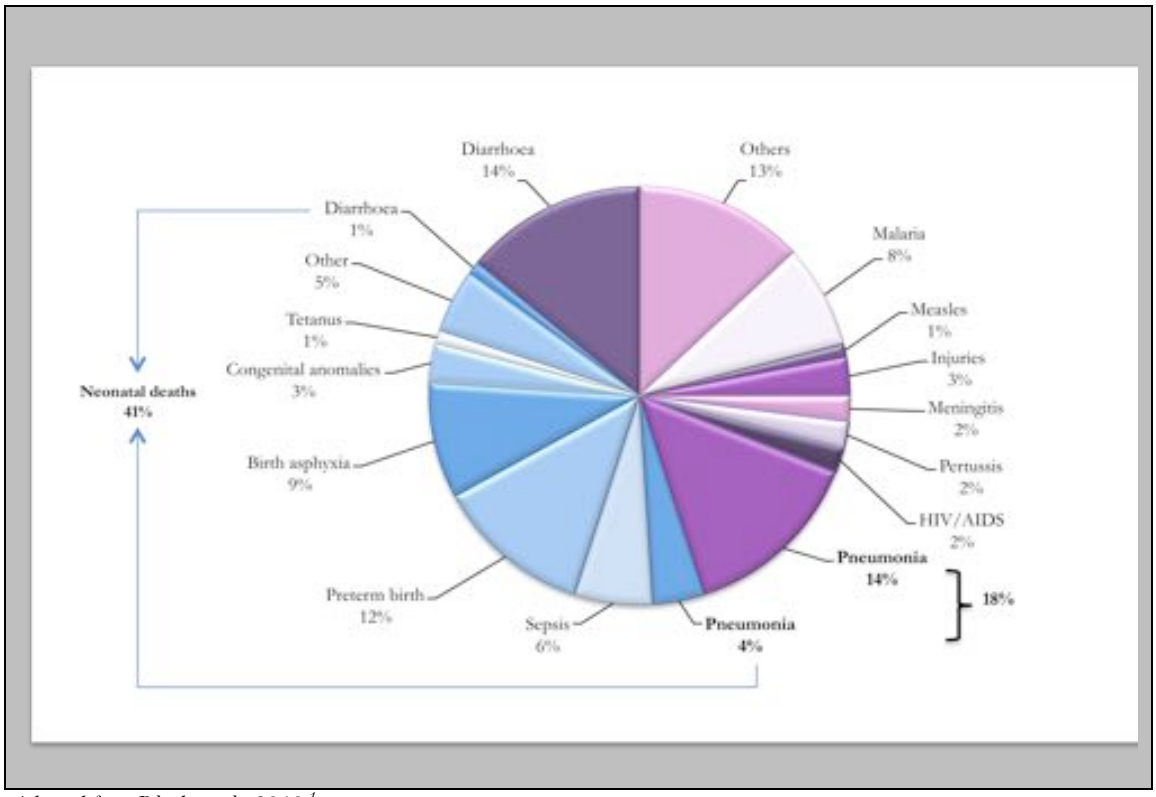
EPIDEMIOLOGY OF CHILDHOOD PNEUMONIA

GLOBAL CHILDHOOD MORTALITY AND THE CONTRIBUTION OF PNEUMONIA

Prior to the 1970s, it was widely believed that diarrhoea and malnutrition were the leading causes of childhood mortality in developing countries and they therefore received substantially more attention than acute respiratory infections (ARIs). It was not until 1973 that ARIs began to be recognised as an important cause of mortality in all age groups, especially in infants and particularly in developing countries. [11] Over the next two decades, research into childhood ARIs increased, which allowed mortality estimates to be refined. By the end of the twentieth century, an estimated ten million under-5s were predicted to be dying every year and 1.9 million (95% Confidence Interval (CI) 1.6–2.2 million) of these deaths were thought to be due to acute lower respiratory tract infections (ALRIs). [12] These unacceptable figures were acknowledged by the United Nations when they set their eight Millennium Development Goals (MDGs) in 2001. [13] With the ultimate aim of reducing poverty by 2015, the fourth of these goals (MDG4) called for a reduction in childhood mortality by two-thirds between the years of 1990 and 2015. WHO realised that to achieve MDG4, more accurate data on the causes of childhood mortality would be needed to allow interventions to be prioritised and their effectiveness to be assessed. Accordingly, the Child Health Epidemiology Reference Group (CHERG) was formed to improve estimates of childhood mortality worldwide. [12, 14, 15] Although childhood mortality has declined considerably from an estimated fifteen million deaths in 1980, the most recent review by

CHERG estimated that in 2008 there were still nearly nine million deaths in under-5s worldwide. [1] The majority of these deaths were due to only a short list of preventable and treatable conditions of which pneumonia was the leading cause (FIGURE 1).[1]

FIGURE 1: PRIMARY CAUSES OF ALL UNDER-FIVE MORTALITY, 2008



Adapted from Black et al., 2010 [1]

Although pneumonia is a disease that affects all paediatric age groups, the incidence tends to decrease with increasing age. [16] The highest incidence occurs in under-5s, especially those under twelve months old. [17-21] In addition, nearly all studies of children with pneumonia have shown a greater proportion (55% to 60%) of boys are affected by pneumonia than girls. [17-19] Based on estimates from community studies, up to 13% of children with clinically diagnosed pneumonia are sick enough to require admission to hospital. [16] The most severe cases of pneumonia occur in young infants and therefore the majority of hospitalisations occur during infancy particularly during the neonatal period. [19]

Nearly one-fifth of deaths in under-5s are now estimated to be due to pneumonia, and one-fifth of these deaths are thought to occur in the neonatal period (FIGURE 1). [1] A reduction in pneumonia-related deaths is vital if MDG4 is to be achieved. In 2006, the United Nations Children's Fund (UNICEF) and WHO acknowledged this, and outlined interventions to reduce the mortality from pneumonia in under-5s. [2] These interventions were recently reviewed in the Global Action Plan for Prevention and Control of Pneumonia (GAPP). [3] The key interventions highlighted were immunisation against pneumococcus and Hib, exclusive breastfeeding during the first six months of life, improved nutrition, reduction of low birth weight and expansion of pneumonia case-management both in clinics and in the community. [3]

The incidence of childhood pneumonia varies widely between countries and the countries with the highest incidence are also some of the poorest. However until the early nineteenth century when living conditions and nutrition began to improve in developed countries, pneumonia was a leading cause of childhood deaths worldwide. [10, 22] As the quality of medical care improved and the access to healthcare facilities grew, the mortality from childhood pneumonia in developed countries fell even further. [22] The further introduction of antibiotic therapy together with the development of vaccinations against some of the leading respiratory pathogens means that childhood deaths from pneumonia in developed countries are now rare. However, for the poorest children in the world malnutrition, lack of healthcare, poor living standards and inadequate vaccination programmes mean that the risk of pneumonia and death has hardly changed. It is estimated that over 97% of pneumonia cases in under-5s occur in developing countries. [9] This equates to 151 million episodes of childhood pneumonia in the world's poorest regions every year. [9] On average, the incidence of pneumonia in under-5s in developed countries is estimated to be only 0.05

episodes per child-year. [9] In contrast, a child in the developing world will experience nearly six times more episodes of pneumonia each year and estimates are particularly high in South-East Asia, where each child has 0.36 new episodes each year.[9]

CHILDHOOD MORTALITY AND PNEUMONIA ON THE THAI-MYANMAR BORDER

Myanmar, formerly known as Burma, has been affected by civil war for nearly five decades. Under the military regime, widespread human rights violations against ethnic minority groups, including the Karen, continue and many people have fled their homes to escape persecution and avoid conflict. Many of them remain in their own country as internally displaced people (IDP) whilst others have sought protection in refugee camps along the borders with neighbouring countries including Thailand. The refugee camps along the Thai-Myanmar border are now home to over 142,000 people. [23] In addition, a large number of undocumented migrant workers and their families, totaling as many as three million people, work and live along the border with Thailand. [24] Many of these migrants have little or no access to healthcare in Eastern Myanmar and numerous barriers limit their access to health programmes in Thailand including language, safety and financial costs. Children in particular have suffered and Myanmar's child health indicators are now among the worst in Asia. An estimated 123,500 under-5s die in Myanmar every year, nearly nines times more than in neighbouring Thailand (TABLE 1). [1] The infant and child mortality rates are also much higher in Eastern Myanmar compared to Myanmar overall (TABLE 2). [25, 26] In fact the under-5 mortality rate is almost double that reported in Myanmar.

TABLE 1: ESTIMATED NUMBERS OF CHILDHOOD DEATHS IN THAILAND AND MYANMAR

	Total number of deaths from all causes by age group			Number of pneumonia deaths	Percentage of pneumonia related deaths (%)
Area	0-27 days	1-59 months	<5 years	<5 years	<5 years
Myanmar	49,000	74,500	123,500	16,500	13.4
Thailand	10,000	4,000	14,000	1,500	10.7
Worldwide	3,575,000	5,220,500	8,795,500	1,575,500	17.9

Adapted from Black et al., 2010 [1]

TABLE 2: MORTALITY RATES IN MYANMAR, THAILAND AND EASTERN MYANMAR

	Thailand in 2009 [26]	Myanmar in 2009 [26]	Eastern Myanmar in 2010 [25]
Infant (<12 months) mortality rate (/1000 live births)	12	54	73
Child (<5 years) mortality rate (/1000 population)	14	71	138

Adapted from Diagnosis: Critical [25]

Myanmar has the third highest estimated incidence of childhood pneumonia in the world, second only to Sudan and Afghanistan. [9] In Myanmar a child under five will have an estimated 0.43 episodes of pneumonia each year, which translates into 1.8 million new episodes of pneumonia in under-5s in Myanmar every year. [9] It is estimated that for 16,500 of these children, their pneumonia will be fatal (TABLE 1). [1, 9] It is likely that childhood pneumonia is a leading cause of morbidity and mortality in the migrant population living along the Thai-Myanmar border. [27]

PATHOPHYSIOLOGY OF PNEUMONIA

Acute respiratory infection (ARI) is the term used to describe any infection of the upper or lower respiratory tract. [2] The upper respiratory tract is comprised of the airways from the nostrils through to the larynx. Infections of the upper respiratory tract include rhinitis (common cold), sinusitis, otitis media and pharyngitis. Most upper respiratory tract infections (URTIs) are self-limiting illnesses and are commonly caused by viruses. Bacterial URTIs and their complications account for only five percent of all childhood deaths from ARI. [28]

ALRIs affect the airways below the larynx including the trachea, bronchi, bronchioles and alveoli. [28] Bronchiolitis and pneumonia are severe ALRIs that affect the lungs, and they are the most common ALRIs to affect children. Pneumonia can be defined as inflammation of the lung parenchyma and is most frequently caused by bacteria and viruses, although fungi, yeast, rickettsiae and chemical inhalation are also implicated. Pneumonia usually follows an URTI. If the pathogens colonising the nasopharynx are inhaled into the lungs they become a pulmonary focus of infection. [28] Alternatively but less commonly, pneumonia can result from the spread of bacteria through the blood to the lungs. [28] In the case of neonatal pneumonia, pathogens can also be acquired from the birth canal either in-utero or by aspiration during delivery. [2]

The presence of a pathogen in the lungs triggers the immune response, which can injure the lungs and interfere with normal lung function. [29] Usually the thin walled alveoli are full of air, and their close proximity to capillaries allows easy exchange of oxygen and carbon dioxide into and out of the blood, respectively. In pneumonia, extravascular plasma fluid

and pus accumulate in the alveoli interfering with gas exchange. In addition, reactive oxygen species and proteases, generated by the neutrophils to kill the microbes, cause damage to host tissues and cells. [29] Together these processes interfere with oxygen absorption causing the patient's breathing to become harder. If the pneumonia is severe the patient will develop hypoxaemia, a major complication of pneumonia that is most prevalent in children hospitalised with pneumonia. [29] [30] Hypoxaemia can lead to tissue hypoxia, the major fatal complication of pneumonia. Hypoxaemia has been shown to increase the risk of mortality by 1.4 to 4.6 times compared to children with normal oxygenation. [31-33]

In addition to the pathogen, the development of childhood pneumonia depends on the presence of certain risk factors both in the host and the environment, which increase the risk of developing and dying from an ALRI. [9] Malnutrition, low-birth weight in term infants and non-exclusive breast-feeding are well-established risk factors for increased mortality in childhood ALRI. [9] Environmental factors, such as indoor air pollution and crowding, are also risk factors. [9]

Immunisations are an essential tool in the prevention of childhood pneumonia both through protection against respiratory pathogens and prevention of infections where pneumonia is an associated complication. [2] Vaccinations against two leading respiratory bacteria, *Haemophilus influenza* type b (Hib) and *Streptococcus pneumoniae (*pneumococcus), have proven highly effective in the prevention of pneumonia in developed countries. More recently they have been found to be effective in the reduction of pneumonia both in Africa and Asia. [34-37] Children infected with measles can develop pneumonia as a complication, either due to measles itself or to a bacterial superinfection. Pneumonia is the leading cause

of death in children with measles infection, and infants and children who are not immunised against measles therefore have a higher risk of developing pneumonia and its potentially fatal consequences. [9, 38, 39] Implementation of these immunisations and the development of vaccines for other pathogens, such as RSV, has the potential to dramatically reduce global childhood morbidity and mortality. Unfortunately the expense of vaccinations continues to limit their use. In many resource-limited settings a more realistic approach is to promote prevention and treatment of pneumonia, particularly at community level.

THE CLINICAL DIAGNOSIS OF PNEUMONIA

Cough and difficulty breathing are common reasons for children to seek medical attention but the underlying causes are varied and numerous. In resource-limited settings, simple and effective protocols for the management of these presentations are vital to help health workers differentiate the cause. The development of the current WHO guidelines for the case management of ARI in children has taken into account evidence from aetiological, clinical and interventional studies. The aetiological studies presented the reasons for treating all children with potential pneumonia with antimicrobials; the clinical studies provided the justification for the simplified diagnosis of pneumonia; and the interventional studies provided evidence of the viability of the case management strategy in resource-limited settings.

THE DEVELOPMENT OF THE GUIDELINES

In 1977 the high mortality from ARIs, particularly in children living in the developing world, prompted WHO to expand their tuberculosis programme to include other respiratory diseases. [40] Their approach focused on developing a simplified method to diagnose and manage ARIs in resource-limited settings and took into account the lack of diagnostic tests available in such settings. [6] Instead, the guidelines focused on using clinical symptoms and signs to identify cases of pneumonia. In addition, the guidelines were designed to be suitable for use by health workers with minimal training, thus the guidelines contain algorithms based on simple and clear clinical criteria. Furthermore, when WHO reviewed all available aetiological studies in the developing world, they found at least half of severe pneumonia cases were due to bacteria, and that pneumococcus and Hib accounted for at least two-thirds of these bacterial cases. [6] Based on this evidence, the WHO case-management strategy assumed a high proportion of fatal pneumonia to be of bacterial origin. The primary aim of the WHO case management strategy was therefore to administer timely antibiotic therapy to every child with a potential pneumococcal or Hib infection. Therefore WHO ensured that their clinical case definition of pneumonia was as sensitive as possible in order not to miss any patients with a bacterial pneumonia. [6]

THE EVIDENCE BEHIND THE CLINICAL DIAGNOSIS

At a WHO consultation on the clinical management of ARIs in children in 1980, a simple flowchart was proposed to aid the diagnosis and management of children presenting with an ARI. [41] The proposed flow-chart used the presence of clinical symptoms and signs

firstly to diagnose pneumonia and secondly to identify which cases required hospital admission. The flow-chart was based on work by Shann et al. in Papua New Guinea, where such flow-charts had been in use since 1974. [42] This landmark study used the presence of crepitations on auscultation to diagnose pneumonia. The study found that those children with cough and crepitations had a significantly faster respiratory rate than those children with cough but no crepitations. They concluded that a threshold respiratory rate of 50 breaths per minute could be used as part of a simple protocol to diagnose pneumonia, thereby identifying those children who required antibiotics. [42] Shann et al. also suggested that chest indrawing could be used to differentiate between those children requiring admission for intravenous antibiotics and those who could be treated at home. [42] This study became the foundation of the WHO case management strategy for ARI in children, which was first published in 1985. [43] Since then, further studies have been undertaken to evaluate and refine the clinical definition of pneumonia and its severity. Many of these subsequent studies suggested that the use of age-related thresholds for fast breathing could improve the sensitivity and specificity of the clinical diagnosis (TABLE 3). [44-46] Based on this evidence, WHO concluded the best combination of sensitivity and specificity were obtained if two different thresholds were used; one of 50 breaths per minute for children aged 2 months to 12 months and another of 40 breaths per minute for 12 months to 4 years old. [6]

TABLE 3: SUMMARY OF THE SENSITIVITY AND SPECIFICITY OF DIFFERENT THRESHOLDS FOR FAST BREATHING FROM STUDIES BY CHERIAN, CAMPBELL, MULHOLLAND [44-46]

Age group (Months)	Threshold for fast breathing (Breaths per minute)	Sensitivity (%)	Specificity (%)
2–11	50	77–89	81–98
	40	90–100	**51–62**
12–59	50	**52–64**	85–98
	40	71–87	72–87

DIAGNOSING PNEUMONIA IN YOUNG INFANTS

Neonates normally breathe at a rate of around 50 breaths per minute, therefore fast breathing needs to be defined differently. Furthermore, young infants can become sick very quickly and it can be hard to distinguish clinically between pneumonia, sepsis and meningitis. A multicentre study of young infants (less than 2 months old) showed that poor feeding, central cyanosis, convulsions, lower chest wall indrawing, failure to arouse with minimal stimulation, and reduced activity were all good indicators of serious infection in this age group. [47] The WHO therefore developed a different algorithm for the treatment of young infants into which these recommendations were incorporated. [6] This algorithm defines the threshold for fast breathing in young infants as 60 breaths per minute.

SEVERITY OF PNEUMONIA

The severity of clinical pneumonia must also be determined in order to aid further management decisions. Clinical studies have therefore tried to define signs that can be used to identify children who are at a higher risk of dying from pneumonia. As previously mentioned, Shann et al. suggested chest indrawing was the best indicator of severity. [42] In their 1984 paper, all the children admitted to hospital with pneumonia had chest indrawing and 6% of these children died. [42] In contrast only 2% of those children treated for pneumonia as outpatients had this sign. Chest indrawing is a subjective sign as it can vary greatly in severity, and can include both intercostal and lower chest wall indrawing. Recognising this, Mulholland et al. investigated chest indrawing during their work in Swaziland and the Philippines. [46] They defined chest indrawing as the inward movement of the bony structures of the lower chest wall with inspiration, and excluded intercostal indrawing and supraclavicular retractions from the definition. This study found that those children with *only* intercostal indrawing were significantly less likely to have severe pneumonia. [46] In a subsequent study undertaken by Shann et al., of over seven hundred children admitted with severe pneumonia, nine criteria were found to be significantly associated with mortality. [48] Of these criteria cyanosis and failure to feed were suggested as signs that health workers could be trained to identify reliably. [48]

Hypoxaemia is associated with an increased risk of mortality and therefore studies have attempted to identify reliable clinical predictors. [30] Central cyanosis has been found to be the most specific predictor of hypoxaemia. However central cyanosis is difficult to detect, particularly in patients with darker skin or if the hypoxaemia is not severe, thereby reducing the sensitivity of using it as a predictor of hypoxaemia. [32, 33, 49, 50] Other clinical predictors of

hypoxaemia, such as head nodding, inability to feed and grunting, are limited by low sensitivities and specificities. [51-54] A second method of evaluating hypoxaemia is by pulse oximetry. This provides a non-invasive method of measuring arterial oxygen saturation that is far more accurate than clinical signs in detecting hypoxaemia. [55] The use of pulse oximetry to guide oxygen therapy in children in the developing world has been shown to reduce mortality by over a third. [49] Provision of sufficient oxygen supplies and equipment for pulse oximetry can be sustainable and affordable in developing countries. [49] [30]

THE EFFECTIVENESS OF THE CASE MANAGEMENT STRATEGY

In addition to refining the clinical case definition of pneumonia, research has focused on establishing the effectiveness of the case management strategy in resource-limited settings. Between 1985 and 1989, community intervention trials were carried out in rural areas of Pakistan, Indonesia, Philippines, Tanzania, Bangladesh, India and Nepal. [56] A meta-analysis of six of these studies by Sazawal et al. demonstrated a significant reduction both in infant and under-five mortality of 20% and 25%, respectively. [57] The majority of these studies relied on community health workers with limited referral facilities, thus providing a convincing argument that it is possible to train community health workers in ARI case-management in a variety of resource limited settings. With the addition of supplementary data from the aforementioned studies and the completion of further studies, two subsequent meta-analyses of community-based intervention trials have been performed. [7, 8] Both meta-analyses found similar reductions in infant mortality of 20% and 21%, and similar reductions in under-five mortality of 21% and 24%. Theodoratou et al. also assessed the effect of the ARI case-management guidelines on children hospitalised with

pneumonia. Based on two before/after studies they found a 23% reduction in deaths after implementation of the guidelines. [8]

THE CASE MANAGEMENT STRATEGY

Following analysis of the available evidence described above, the WHO guidelines for the case-management of ARI in children were published in 1991. [6] Within 3 years, 59 out of 88 target countries (infant mortality > 40 per 1,000 live births) had implemented an ARI programme based on the WHO guidelines. [58] Later that decade, recognising the overlap between the clinical presentations of different diseases, WHO and UNICEF combined the ARI case management strategy with similar guidelines for other childhood illnesses to create the Integrated Management of Childhood Illness (IMCI) guidelines. [59] The aim of the IMCI guidelines was to improve the prevention, detection and treatment of all leading childhood illnesses. [60] Several studies in the Gambia, Ethiopia, Kenya and Uganda have evaluated the ability of the IMCI guidelines to diagnose pneumonia. [61-64] Their results showed that the ARI algorithm maintained a sufficient sensitivity and specificity in diagnosing pneumonia even when combined with the other IMCI guidelines (sensitivity 76-97%, specificity of 49-89%).

THE CURRENT CASE-MANAGEMENT STRATEGY

The current IMCI guidelines recommend that if fast breathing (as defined above) is present in a child with cough or difficult breathing they should be diagnosed with 'non-severe' pneumonia (NSP). [5] The additional presence of lower chest wall indrawing, stridor or any other danger sign in a child with cough or difficult breathing should lead to a diagnosis of 'severe' pneumonia (SP) or 'very severe disease' and the child should be referred urgently

to hospital. [5] WHO has published a Pocket Book with additional complementary guidelines for the management of common childhood illnesses by senior health workers in hospitals with limited resources. [65] In these guidelines, WHO further define the diagnosis and management of SP in children and older infants (aged over two months old). When lower chest wall indrawing is present in children with cough or difficult breathing, a diagnosis of SP should be given and intravenous antibiotics administered. [65] The additional presence of central cyanosis, convulsions, lethargy, unconsciousness, severe respiratory distress, vomiting of everything or the inability of the child to breastfeed or drink, should prompt a diagnosis of 'very severe' pneumonia (VSP). [65] In children with VSP, oxygen therapy should also be given if available. If pulse oximetry is accessible, it should be used to guide oxygen therapy and oxygen should be given to children whose saturation is <90%. [65]

The IMCI has a different algorithm for the management of young infants. [6] It recommends that any young infant with fast breathing (more than sixty breaths per minute), chest indrawing, other sign of pneumonia or any sign of serious infection should be classified as having a 'possible serious bacterial infection' and be referred to hospital for further assessment and intravenous antimicrobial therapy (TABLE 4). [5] The WHO Pocket Book again provides supplementary guidelines for the management of these young infants by senior health workers in hospitals with limited resources. [65] If further assessment reveals no obvious source of infection, the young infant should be treated as having bacterial sepsis and given ampicillin and gentamicin to cover both gram-positive and gram-negative organisms. If any signs of meningitis are present additional treatment with a cephalosporin is recommended. If however only signs of pneumonia are present the infant can be treated as such, also with ampicillin and gentamicin therapy. If fast breathing, chest indrawing, nasal flaring or grunting is present in a young infant with cough or difficult breathing; a

diagnosis of SP should be given. [65] As with older infants, a diagnosis of VSP should be given to any young infant with central cyanosis, convulsions, lethargy, unconsciousness or severe respiratory distress or if the infant is vomiting everything or unable to breastfeed. [65]

TABLE 4: SIGNS OF POSSIBLE SERIOUS BACTERIAL INFECTION IN YOUNG INFANTS

Signs	Classify as
- Convulsions **OR** - Respiratory rate greater than 60 breaths per minute **OR** - Severe chest indrawing **OR** - Nasal flaring **OR** - Grunting **OR** - Bulging fontanelle **OR** - Fever (37.5°C or higher) **OR** - Low body temperature (less than 35.5°C) **OR** - Movement only when stimulated or no movement at all - Umbilical redness extending to the skin **OR** - Many or severe skin pustules **OR** - Pus draining from the ear	**Possible serious bacterial infection**

Adapted from the IMCI guidelines.[5]

THE RADIOGRAPHICAL DIAGNOSIS

Following the clinical diagnosis of pneumonia based on symptoms and signs, chest radiography is often used to confirm the diagnosis. Chest radiographs are still generally accepted as the "gold standard" for diagnosing childhood pneumonia. [66-70] However the appearance of pneumonia on a radiograph can range from mild interstitial changes (FIGURE 2) through to lobar consolidation (FIGURE 3), and the aetiology of pneumonia cannot be reliably distinguished on the basis of radiographical findings. Some lung aspirate studies have shown that consolidation is associated with a bacterial aetiology (28% to 61%), predominantly pneumococcus. [71-74] However, studies of pneumonia in children under 2 years old in Chile and Nigeria isolated bacteria from 45% and 84% of children,

respectively, who had evidence of other infiltrates on their radiograph. [73, 75] This suggests that bacteria, including pneumococcus, can cause other appearances in addition to lobar consolidation.



FIGURE 2: AN EXAMPLE OF BILATERAL OTHER INFILTRATE ON CHEST RADIOGRAPH

(Authors collection)



FIGURE 3: A CHEST RADIOGRAPH SHOWING RIGHT UPPER LOBE CONSOLIDATION – PRIMARY ENDPOINT PNEUMONIA

(Authors collection)

The radiographic diagnosis of pneumonia is very subjective and there is great variation in inter-observer interpretations of chest radiographs. [68, 69, 76, 77] Usually radiographs are used in direct patient care, and therefore the clinical details often influence their interpretation. In general, clinicians are more likely to over treat patients with subtle changes on radiographs in order to be sure of treating all children with possible bacterial pneumonia.[78] Studies have found the best agreement is on the presence of alveolar consolidation but for other findings agreement is limited. Despite this substantial variation in interpretation, radiographs are frequently used to identify pneumonia as an outcome measure in epidemiological studies and vaccine trials. Many of these studies and trials are international, and a unified approach to interpretation is vital to ensure that any differences observed are indeed a true reflection of epidemiology or vaccine efficacy rather than due to variation in

interpretation. Consequently in 2001, WHO instigated the development of a standardised and simplified method for interpreting paediatric chest radiographs to help minimise inter-observer variation. [4] By 2005, the WHO working group had created standardised definitions for radiographic findings (**TABLE 5**). [79] This method was assessed for inter-observer and intra-observer variability and found to achieve good agreement in identifying radiographical pneumonia. The sensitivity and specificity of this method for identifying end-point pneumonia was 84%–87% and 87%–89%, respectively. There was also no significant difference between the interpretations by clinicians compared to radiologists, suggesting that with adequate training a variety of readers can be used. [79] The agreement in interpretation can be improved by using two independent readings from two different readers followed by a third independent reading if there are any discordant conclusions. [79]

These standardised radiographic definitions have since been used in a number of trials and studies. Results from pneumococcal conjugate vaccine (PCV) trials in children have reported a substantial reduction in 'primary end-point pneumonia' (PEP) in vaccinated children. In children who received PCV in the Gambia and South Africa, a 37% and 25% reduction in PEP were observed, respectively. [34, 36] In the Gambia an analysis of unvaccinated children also found bacteraemia to be significantly associated with PEP. [80] These observations suggest that there is a correlation between a bacterial aetiology and PEP. Unifying the interpretation of paediatric chest radiographs in epidemiological and interventional studies allows the comparison and amalgamation of data between studies. As more studies utilise this standardised method, the relationship between aetiology and radiographic findings will become clearer. [79]

TABLE 5: WHO STANDARDISED INTERPRETATION OF PAEDIATRIC CHEST RADIOGRAPHS. [79]

	Finding	Definition
Film quality	Uninterpretable	Not interpretable with respect to presence or absence of primary end-point pneumonia
	Suboptimal	Features allow interpretation of primary end-point pneumonia but not of other infiltrates or findings
	Adequate	Confident interpretation of primary end-point pneumonia and other infiltrates is possible
Classification of findings	Significant pathology	Presence of end-point pneumonia or other infiltrates
	End-point consolidation	A dense or fluffy opacity that occupies a portion of a lobe or a whole lobe or the entire lung. May contain air bronchograms
	Other infiltrate	Linear and patchy densities involving both lungs, peri-bronchial thickening, multiple areas of atelectasis Also small areas of atelectasis which are difficult to distinguish from consolidation
	Pleural effusion	Presence of fluid in lateral pleural space; usually at costo-phrenic angle; does not include fluid in horizontal or oblique fissures
Conclusion	Primary end-point pneumonia (PEP)	Presence of end-point consolidation as defined above Also pleural effusion associated with pulmonary infiltrate Also large pleural effusion that may obscure an opacity
	Other infiltrate (OI)	Presence of other infiltrate as defined above in absence of effusion
	No consolidation/no infiltrate/no effusion	Absence of end-point consolidation, other infiltrate, pleural effusion

THE AETIOLOGICAL DIAGNOSIS

OVERVIEW

Ideally, once a diagnosis of pneumonia has been made and confirmed by chest radiography, the causative pathogen should be identified to aid selection of the most appropriate treatment. However, achieving a true aetiological diagnosis of pneumonia in a child is challenging as sensitive and specific diagnostic techniques for identifying causative pathogens are lacking. Even in the developed world a definitive pathogen is only identified in about 50% of cases. [81] In developing countries, limited diagnostic facilities mean that the aetiology of the majority of cases of childhood pneumonia is never known. When the aetiology cannot be established, cases of childhood pneumonia are treated empirically. In order to make a rational choice of empirical therapy the most common pathogens in each region should be known. The surveillance systems that exist in developed countries make this easier but in less developed countries such surveillance is minimal or non-existent. Therefore research efforts must be made to improve the understanding of the aetiology of pneumonia in these regions in order to improve evidence-based empirical therapy and plan preventive strategies.

ACHIEVING A DIAGNOSIS OF BACTERIAL PNEUMONIA

Over time different methods have been used to determine the aetiology of childhood pneumonia in routine clinical care and in research settings. The first challenge in making an aetiological diagnosis is to obtain a representative specimen from the lower respiratory tract. Lung aspirates are considered the "gold standard" for the diagnosis of bacterial pneumonia. [82] A culture of lung aspirate has a very high specificity as the sample is taken

from a sterile area. It allows contamination from pharyngeal colonisation to be avoided, as neither the sample nor specimen has to pass the upper respiratory tract. Mimica et al. performed lung aspirates on 13 infants with no clinical or radiographical evidence of pneumonia and found all 13 aspirates to be sterile. [73] Conversely, bacteria were cultured from 45% of lung aspirates taken from infants with pneumonia. [73] The high specificity of this technique means that any positive cultures obtained provide strong evidence of bacterial aetiology. [83] Unfortunately, there are technical limitations to its use and potential risks associated with the technique. [83] Hence, lung aspirates have only been used in a limited number of studies in the developing world, [71, 72, 74, 84] and other suitable samples for bacterial cultures have been sought.

Blood cultures are relatively easy to obtain, and the isolation of bacteria from the blood of a child with a clinical diagnosis of pneumonia has a high specificity. [83] Unfortunately, blood cultures have a sensitivity of less than 25%. [83] This is partly because not all pneumonia cases are bacteraemic and partly because of antibiotic use in the community before seeking medical care. [85] In addition, some pathogens are difficult to culture and require laboratory facilities that are often not available in resource-limited settings. It is difficult to obtain sputum samples from children and any sputum obtained is frequently contaminated by normal upper respiratory tract flora. [85] For the same reason, pharyngeal cultures have limited specificity.

Alternative methods of identifying bacterial pathogens have been investigated. The detection of pneumococcal antigen in the urine has proven to be both sensitive and specific in the diagnosis of pneumococcal pneumonia in adults, however because children often carry pneumococcus in their nasopharynx, this method lacks specificity in children. [86,

[87] The same is true for Hib antigens, which although present in the urine of children with *Haemophilus influenzae* infections have also been found in the urine of healthy children who are asymptomatic nasopharyngeal carriers of Hib. [88] Antibody detection assays have been used to detect antibodies to bacterial pathogens including pneumococcus and Hib in patient's serum. [84, 89] The sensitivity and specificity of such techniques are not known and no such antibody assays are in routine use as the practical value of this technique is complicated by the need for paired serum samples; one taken during the acute and one taken from the convalescent period. [90, 91] In addition, antibody assays have a low sensitivity in young infants, as they do not always develop a serological response to bacterial pathogens. [88, 92, 93] Therefore antibody assays to both Hib and pneumococcus have a limited value in this age group.

Various efforts have been made to identify biomarkers of bacterial pneumonia. Levels of leukocytes and acute phase proteins such as c-reactive protein (CRP) and procalcitonin are elevated in pneumonia but currently the role of these markers remains controversial. [70, 94-96] Although many studies have observed a significant correlation between a raised CRP and bacterial pneumonia, the usefulness of the test is limited by a poor sensitivity. A recent meta-analysis of over 1000 children with pneumonia, found a CRP level of 40mg/dl or more to correlate diagnosis of bacterial pneumonia. [97] Similarly a pneumococcal vaccine trial in South Africa observed a greater vaccine efficacy against children with clinical pneumonia who also had a CRP level of at least 40mg/dl compared to children with a clinical diagnosis alone. [98] Therefore a raised CRP could eventually prove to be a useful adjunct in helping to differentiate between bacterial and viral pneumonia, however without an effective "gold standard" for the diagnosis of bacterial pneumonia it remains hard to establish its exact role.

Molecular diagnostic techniques are the latest development in establishing a bacterial aetiological diagnosis of childhood pneumonia. Bacterial DNA (deoxyribonucleic acid) from both viable and non-viable pathogens can be amplified using the polymerase chain reaction (PCR) using a PCR primer that targets a sequence of DNA unique to a particular bacterium. Various PCRs have been developed for the detection of pneumococcus and Hib. [99-102] Unfortunately, like with bacterial culture, the asymptomatic nasopharyngeal carriage of these bacteria limits the specificity of these techniques. [100, 101, 103, 104] The specificity can also be reduced by the presence of other closely related bacteria in the nasopharynx. The use of PCR is also limited by its sensitivity when compared to blood culture. [102] A number of limitations to the yield have been observed including the prior use of antibiotic therapy, absent and low levels of bacteraemia in children and the age of the child. [99, 103-105] In the case of pneumococcal PCR, the use of multiple blood fractions has been found to improve the sensitivity but this makes the process both more laborious and expensive and therefore limits clinical value of this test. [104] PCR performed on pleural fluid has proven more successful and improved the diagnostic yield of both Hib and pneumococcus, however the aforementioned risks of lung aspiration pertain and thus limit the use of such samples to severe cases. [104, 106] A further drawback of using PCR for the diagnosis of bacterial aetiology is the lack of information obtained on antibiotic susceptibility compared to culture. The potential for these molecular tests does however remain high, especially for pathogens whose isolation using culture is difficult.

Vaccine trials have provided an alternative method for estimating the disease burden attributable to bacterial pathogens. As described by Mulholland et al., the burden of pneumonia due to a specific pathogen can be estimated to equate to the reduction in the incidence of pneumonia cases in children who are vaccinated against a particular pathogen,

compared to those who are not. [107] Recent trials of pneumococcal and Hib vaccinations have provided information on their contribution to the burden of childhood pneumonia that might otherwise be missed by depending on positive cultures for diagnosis. [35-37] This will be discussed at greater length in "The bacterial aetiology of childhood pneumonia".

ACHIEVING A DIAGNOSIS OF VIRAL PNEUMONIA

The diagnosis of viral pneumonia also uses a variety of techniques for sampling and pathogen identification. Suitable samples can be acquired from throat swabs, nasopharyngeal swabs, nasal washes and nasopharyngeal aspirates (NPA). [20] Traditionally cell culture was used to diagnose viral pneumonia, and it remained the "gold standard" for the detection of viral pathogens until the development of molecular diagnostic techniques. Most viruses can be cultured but the process is slow and has a poor sensitivity. Due to the labile nature of many viral pathogens the success of this method is greatly affected by the sampling techniques, transport methods and cell culture conditions. [20, 21]

Viral pathogens can also be detected by alternative methods. Serology can been used to detect antibodies to viral pathogens, however the same disadvantages as described for bacterial pathogens persist. The sensitivity of viral detection can be enhanced by the use of assays, such as immunofluoresence and enzyme immunoassays, to detect viral antigens from clinical samples. [20, 21] Newer molecular diagnostic tests, use PCR to detect viral pathogens. The use of PCR has improved the sensitivity and speed of viral detection from clinical specimens compared to traditional methods. [108, 109] This increased sensitivity has led to the detection of multiple viruses in single patients, and the detection of viral pathogens in healthy children; however, the significance of these findings requires further

investigation. [110] Again, quantitative PCR is likely to play a vital role in improving the utility of molecular diagnostics.

AETIOLOGY OF CHILDHOOD PNEUMONIA

OVERVIEW

Childhood pneumonia is caused by a variety of pathogens, either alone or in combination. Bacterial infections have been implicated in the majority of hospital admissions for pneumonia, and WHO estimates that over half of the pneumonia deaths in under-5s in the year 2000 were due to pneumococcus and Hib. [111] Viruses are also important causes of childhood ALRIs in developing countries, and have been identified in 30% to 40% of cases. [17, 18, 20, 21] Before the widespread introduction of the measles vaccination, measles was the leading viral pathogen responsible for childhood pneumonia. [38] As the coverage of measles vaccination has increased, RSV has become the most important viral pathogen. However, in many developing countries, the lack of surveillance systems and diagnostic facilities mean the aetiology of childhood pneumonia remains poorly understood. [112]

THE BACTERIAL AETIOLOGY OF CHILDHOOD PNEUMONIA

Although early studies in developing countries used only small numbers of children with severe pneumonia, they were still able to identify pneumococcus and Hib as the most common bacterial pathogens in childhood pneumonia. [71, 72, 74, 84, 113, 114] Using traditional diagnostic techniques of blood culture, and in some studies lung aspiration, these studies found pneumococcus in 20% to 53% of children hospitalised with pneumonia. In addition Hib was isolated from 7% to 40% of these children.

Vaccine trials of PCV in developing countries have improved the estimates of the burden of pneumococcal pneumonia. In a trial of the nine-valent PCV in the Gambia, the vaccination prevented 37% of radiographical pneumonia but only prevented 7% of clinical pneumonia. [34] Similar results were found with this vaccine in a South African trial, which demonstrated a 25% reduction in radiographical pneumonia and a 17% reduction in clinically diagnosed severe pneumonia in vaccinated children. [115] Only one PCV trial has been carried out so far in Asia. This large trial of the 11-valent PCV in the Philippines, found a 22% efficacy against radiographic pneumonia. [35] A recent meta-analysis of these three studies concluded that PCV reduced clinical pneumonia by 7%, clinically severe pneumonia by 7% and radiographically confirmed pneumonia by 26%. [116] In all the studies, the number of culture negative cases prevented was far higher than the number of culture positive cases prevented, confirming what has always been suspected, that the contribution of pneumococcus in childhood pneumonia has previously been underestimated due to diagnostic limitations.

Prior to the development of a conjugate vaccine against Hib (HibCV) that was both safe and effective in infants, Hib was a leading cause of serious childhood illness worldwide. [117] The most common manifestations of Hib infection are pneumonia and meningitis. Since the introduction of the HibCV, Hib infections have become rare in both developed and developing countries that have incorporated the vaccine into their national immunisation schedules. [118] However, in many developing countries where the vaccine is not used, Hib infections remain a significant threat to child health. The contribution of Hib to childhood pneumonia in these countries is still not fully understood due to the lack of sensitive and specific diagnostic tests for Hib. However, studies of the HibCV are not reliant on diagnostic tests and have revealed a more accurate picture of Hib's contribution to

pneumonia. By measuring the reduction in pneumonia episodes in Hib vaccinated children compared with unvaccinated children, the proportion of pneumonia cases that are attributable to Hib can be estimated. [107] There are relatively few HibCV studies that assess its effect on clinical and radiographical pneumonia in the developing world but overall their results suggest that Hib accounts for at least one-fifth of radiographically confirmed pneumonia. [119] The first evaluation of the Hib vaccination in a developing country was an open-label trial in Chile. [120, 121] This study observed an 80% reduction in Hib bacteraemic pneumonia and a 22% reduction in radiographic consolidation in children who received HibCV. A similar study by Mulholland et al. in the Gambia demonstrated comparable results. Infants in this study that were fully vaccinated with HibCV had 22% fewer episodes of radiographic pneumonia, although there was no effect on the incidence of clinical pneumonia. [37] Case-control studies of Hib vaccination in Brazil, Colombia and Bangladesh have provided additional evidence. [122-124] These studies found a 15% to 55% reduction in culture-negative, radiographic pneumonia in children who received HibCV. These results suggest that like pneumococcus, the difficulties in culturing Hib have led to the burden of Hib pneumonia being underestimated. However, more studies are needed, as the burden of Hib pneumonia is likely to vary between regions and countries as demonstrated by a more recent randomised controlled trial of HibCV in 55,000 children in Indonesia. [125] This was the first trial to evaluate the role of HibCV in reducing the burden of childhood pneumonia in Asia. Although the study found a small reduction in clinical pneumonia (4%), no effect was demonstrated on the presence of radiographically confirmed pneumonia in HibCV-vaccinated children (-12%, $P>0.05$). [125] This implies that Hib may not play as greater role in childhood pneumonia in Asia as it does in Africa, but further studies in other areas of Asia are needed to confirm this.

If the results of the Hib and pneumococcal vaccine studies are considered together, they suggest that, depending on the serotypes vaccinated against, at least half of radiographically confirmed pneumonia in children is due to Hib and pneumococcus. In addition to Hib and pneumococcus, other important although less common bacterial pathogens have been identified in childhood pneumonia, including *Staphylococcus aureus, Klebsiella pneumoniae, Moraxella catarrhalis, Escherichia coli, Streptococcus viridans,* Acinetobacter species, non-*typhi salmonellae*, *Bordetella pertussis* and non-typeable *H. influenzae.* [10] These bacterial pathogens will become more important in the aetiology of pneumonia if the burden of disease attributable to Hib and pneumococcus is substantially reduced by vaccination.

Some pathogens are a greater problem in immunocompromised children. For example, *Mycobacterium tuberculosis* has been found in the lung aspirates and sputum of children with acute pneumonia, [71] but it is a particularly important cause of severe pneumonia in children with human immunodeficiency virus (HIV). [126] Similarly, the fungus *Pneumocystis jiroveci* has been found in cases of severe pneumonia in immunocompetent children but it is more commonly identified in cases of severe pneumonia in children with HIV, where it is has been found in over a third of such cases. [127-129]

THE VIRAL AETIOLOGY OF CHILDHOOD PNEUMONIA

Viral infections have long been recognised as an important cause of ALRIs in developing countries. [19-21, 130] Early studies that used traditional diagnostic techniques including culture, serology and antigen detection were able to identify a virus (excluding measles) in up to half of under-5s admitted to hospital with severe ALRIs. [21, 71, 131-133] Using the same techniques, community-based studies that have followed children longitudinally, have

identified viruses in up to one-third of under-5s with an ALRI. [89, 134, 135] To date, few studies in resource-limited settings have utilised PCR to aid aetiological diagnosis, but those that have, have shown that viral pathogens contribute to a greater proportion of childhood pneumonia than previously thought. A hospital-based study in Nepal that used PCR, identified at least one virus in 40% of children under 3 years old with clinical pneumonia; nearly all cases were of NSP. [18] At least one virus was identified by PCR in over two-thirds of children admitted to hospital with ALRI in two studies in Vietnam. [136, 137] A hospital-based study in Kenya identified at least one viral pathogen by PCR in 56% of children under 12 years old admitted with SP, [17] while a study in Mozambique found a virus in 49% of under-5s hospitalised with pneumonia. [138]

The most common viral respiratory pathogens identified in children with ALRI are RNA viruses. RSV has long been recognised as the predominant viral pathogen in under-5s with ALRI. [19] Influenza viruses also cause substantial morbidity amongst children and are beginning to be recognised as important viral pathogens in ALRI in infants and children living both in temperate and tropical climates. [139-141] Since its recent discovery, human metapneumovirus (hMPV) has been shown to be an important worldwide pathogen in childhood ALRIs. [142, 143] At present Adenovirus (ADV) is the only DNA virus commonly found in children with an ALRI. [20]

RESPIRATORY SYNCYTIAL VIRUS

RSV is an RNA virus of the paramyxovirus family, which was first isolated from children with a diagnosis of ALRI over fifty years ago. [144] It was estimated that in 2005, around 34 million new cases of RSV-associated infections occurred in under-5s worldwide. Three

million of these cases were severe enough to require hospital admission and up to 200,000 of these children are estimated to have died. [145] Over 90% of RSV infections and nearly all the RSV-related deaths occurred in the developing world. [145]

In children and infants it is not uncommon for RSV to infect the lower respiratory tract and cause bronchiolitis or pneumonia, although the majority of RSV infections cause mild URTIs. [146] For some infants and children RSV associated ALRIs can become severe enough to cause respiratory failure and require ventilation and intensive care. [147] It also appears that children can be infected with RSV more than once, however these subsequent infections tend to be less severe than the first. [148, 149] Early studies in developing countries using combinations of viral culture, antigen detection and serology identified RSV as the most important viral cause of ALRI in children, finding RSV in up to 37% of all children with an ALRI. [19, 21, 74, 150-152] The proportion of ALRIs attributable to RSV appears to increase with severity as shown by a recent study in four different developing countries; Indonesia, Mozambique, Nigeria and South Africa. [153] The study found RSV in up to 36% of infants and up to 35% of under-5s with an ALRI and in up to 64% of infants and up to 45% of under-5s with severe ALRI. [153] Cohort studies in India and Kenya also observed the proportion of RSV to be higher in severe ALRI. [154, 155]

More recently molecular diagnostic tests have been used to identify RSV in children with ALRIs. In a large study of mainly NSP cases in over two thousand Nepalese children under 3 years old, RSV was the most frequently identified virus and was present in 15% of cases. [18] Other studies using PCR have focused on children with severe ALRIs. Two recent prospective studies in Vietnam found RSV in 23% (n=1,000) and 24% (n=300) of children hospitalised with ARIs. [136, 137] In a study of 759 children under 12 years old admitted to

hospital with SP and VSP in Kenya, RSV was identified in 34% of all children and 42% of infants. [17] A second African study in Mozambique of 800 under-5s admitted to hospital with SP found RSV in 11% of children. [138] Together these studies confirm that RSV is a leading pathogen in childhood ALRI and suggest that the proportion of ALRIs attributable to RSV is highest in severe cases and in infants.

Mortality from RSV infection in developed countries is rare, occurring in less than 1% of patients infected with RSV, with the majority of these deaths occurring in children with underlying disease. [156-158] Studies in the developing world have found higher rates of mortality, even in infants and children without underlying illnesses. [159, 160] The role of RSV as a causative or contributing factor in childhood deaths from ALRI was also exemplified by a study in Mexico where RSV was detected in the postmortem lung tissue of one-third of children under 2 years old who died from pneumonia. [161]

In general the male predominance in all childhood ALRIs is also reflected in the proportion of males with RSV-associated ALRIs. [17, 18, 21] The first infection with RSV usually occurs before the age of 2 years old and the majority before the age of one. [148, 149] The age distribution of RSV observed in developing countries is similar. One community-based cohort study in Colombia that followed children from birth to 17 months of age, found that 86% of RSV cases occurred in the first 12 months of life and 40% of cases in the first 6 months. [162] Similarly, hospital-based studies in the developing world have found around two-thirds of RSV infections occur during infancy. [21, 132] Not all children with an RSV infection develop ALRI, and the risk of developing an RSV-associated ALRI decreases with increasing age. [155] The risk of developing severe disease or dying is highest in infants, particularly those under six months old and those born prematurely. [147-149, 163, 164]

In temperate regions of the world, it is known that RSV infections occur in well-defined epidemics in the cold winter months. [21] However, in sub-tropical and tropical regions where rainfall is seasonal, the pattern of RSV infection has been shown to vary between regions. [21, 153] A relationship between increased RSV infections and increased rainfall has been demonstrated in many countries in Asia, [136, 137, 153, 165] South America [166] and Africa. [153, 167, 168] This association of RSV infections with the rainy season is by no means a universal pattern. [20, 153] Other studies in Africa have observed peaks of RSV infection during the dry season. [153] Whilst in Bangladesh and India, RSV infections have been found year-round with peaks in the winter months. [154, 169] In Nepal two peaks of activity were observed, the first at the end of the rainy season and a second smaller peak during the winter. [18]

Despite a variety of approaches, there is currently no safe and effective RSV vaccination available and until there is, RSV is likely to remain the most common viral pathogen in childhood pneumonia. [170] Unfortunately, early trials of RSV vaccinations increased the severity of disease, and in some cases resulted in death when children were later naturally infected with the virus. [171, 172] Since these disastrous results, alternative vaccines against RSV have been in development and although some vaccines have been found to be safe in clinical trials, none have yet been found to protect children against ALRI. [173-175] Therefore there is promise for an RSV vaccine, and as other vaccines begin to reach more and more children with the support of GAVI, the future implementation of an RSV vaccine in the developing world is not improbable. [176]

INFLUENZA

Influenza virus is a highly contagious respiratory pathogen with three immunological types: A, B and C. Influenza viruses particularly influenza A (InfA), are highly variable and undergo continuous antigenic variation in their surface antigens leading to frequent epidemics and pandemics. Influenza is known to be a leading cause of morbidity and mortality worldwide and WHO has estimated that up to 500,000 deaths from influenza occur every year. [177]

Influenza viruses cause significant childhood morbidity. [139] Although the majority of influenza infections in children are mild, self-limited URTIs, pneumonia is now recognised as an important complication in children with influenza infections. Hospitalisation for influenza infections is principally for influenza-associated ALRIs and has been shown in temperate climates to be more common in children younger than 2 years of age, particularly those under 6 months. [139, 178] Studies in Europe and the USA have reported pneumonia in 8% to 36% of children hospitalised with influenza. [179-182] Knowledge of the epidemiology of influenza in childhood ALRIs in developing countries is limited. However, the data that are available suggest that influenza also contributes substantially to childhood pneumonia in these countries. One study in Thailand identified influenza in 13% of children admitted with pneumonia and the majority of these cases were in under-5s. [183] Similarly, a recent longitudinal study in a poor urban area of Bangladesh cultured influenza from nearly 10% of pneumonia cases in under-5s. [141] In addition the study demonstrated that 28% of children with influenza infections developed pneumonia and showed that those who developed pneumonia were significantly younger. In fact two-thirds of influenza-associated pneumonia occurred before 2 years of age. A recent case-control study

in Nepal found a strong association between InfA and clinical pneumonia in children under 3 years old. [184] Influenza was six times more likely to be detected in children with pneumonia than in controls.

In temperate countries there are clear seasonal trends in influenza infections. As with RSV, the occurrence of influenza in temperate countries is more common in the cold winter months, occurring in well-defined outbreaks lasting two to three months. However, in tropical and sub-tropical areas the seasonality is less clear. In Thailand and Myanmar influenza infections have been found to occur mainly during the rainy season. [185, 186 187, 188] Observations in various other countries in the tropics, including Senegal, Brazil and India have also demonstrated a seasonal pattern of influenza infection that coincides with the rainy season. [189-191] Conversely, in Nepal influenza infections were detectable most of the year, with the largest peaks occurring in the winter. [18] In addition, studies in Brazil, Kenya and Taiwan have found no clear link between influenza infection and season. [167, 192, 193] There is therefore no firm consensus regarding the seasonality of influenza in the tropics.

Another interesting factor relating to influenza, is that it appears to act synergistically with other pathogens particularly pneumococcus. [194] Studies indicate that several mechanisms may exist to allow influenza to interact with pneumococcus thereby exacerbating the burden and severity of pneumonia. [195] Although most studies of childhood pneumonia have been limited by the poor sensitivity of blood cultures when searching for mixed infections, vaccine trials have provided a new insight into the interaction between bacterial and viral pathogens. A South African trial found that immunisation with a 9-valent PCV prevented 31% of all viral-associated pneumonia and 45% of InfA-associated pneumonia.

[196] This is strong evidence of the important role that viruses, particularly influenza, play in childhood pneumonia.

Currently, influenza is the only respiratory viral pathogen associated with pneumonia that can be prevented by vaccination. In regions where the burden of influenza is found to be substantial, influenza immunisations could play a vital role in reducing the burden of childhood pneumonia.

HUMAN METAPNEUMOVIRUS

HMPV is a recently discovered respiratory pathogen that is now known to be an important cause of childhood ALRI. [197] This RNA virus was first isolated from respiratory secretions of children with respiratory symptoms in the Netherlands in 2001. [143] The newly identified pathogen was found to be a paramyxovirus, closely related to RSV. HMPV has now been identified in many other regions including Asia, [198, 199] North America, [200] Australia [201] and Africa [202] suggesting a global distribution. [142]

Studies have found hMPV in patients of all ages with acute respiratory tract infections and accumulating evidence shows young children are particularly susceptible to infection with this pathogen. Following its discovery, several studies have used PCR to identify hMPV in viral specimens of previously unknown etiology. These studies identified hMPV in patients of all ages both in the community and in hospital, although primarily they identified hMPV in under-5s. [201, 203] A retrospective study that investigated respiratory infections in under-5s, found that up to 20% of patients with a previously negative viral culture actually had an hMPV infection. [204] The average age of children with an hMPV infection appears to be

older than for RSV, with reports ranging from ten to thirty-two months. [198, 204-207] All studies have observed a male predominance, with between 52% and 75% of children reported to be male. [137, 198, 205, 207, 208]

The clinical picture first observed by Van den Hogen showed hMPV to cause a spectrum of disease ranging from mild upper respiratory symptoms through to bronchiolitis and pneumonia. [143] Further studies have also shown hMPV to cause a similar range of clinical presentations. [143, 198, 199, 201, 203, 204, 206, 209, 210] A recent hospital-based study in Israel identified hMPV in 8% of children with radiographically confirmed pneumonia suggesting that this pathogen does indeed contribute significantly to the burden of childhood pneumonia. [207] This is supported by a recent case-control study in Nepal, where hMPV was four times more likely to be found in children with pneumonia, than healthy age-matched controls. [184]

Studies looking at the prevalence of hMPV in temperate countries, have found hMPV in 5% to 7% of children hospitalised for respiratory symptoms. [197] Comparable results have been seen in Vietnam, India, South Africa, Hong Kong and Mexico where hMPV was detected in 3% to 13% of children admitted to hospital with respiratory symptoms. [137, 198, 202, 205, 208, 211] Bronchiolitis and pneumonia are the main clinical syndromes associated with hMPV infection in hospitalised children and on some occasions the disease is severe enough to require intensive care. [198, 202, 203, 210, 212] In addition there are also data to suggest that hMPV can cause severe morbidity and mortality in premature infants and immunocompromised patients. [203, 205, 210]

Both early retrospective studies and more recent prospective data suggest that hMPV has a similar seasonal pattern to other respiratory viruses such as RSV and influenza. Studies

carried out in temperate regions found the majority of hMPV infections occurred during the winter or early spring. [204, 206, 210, 212-214] Limited data are available from the developing world but, like other viruses, hMPV also appears to have different seasonal patterns in different regions. For example in Mexico and South Africa hMPV activity peaks in the dry season. [205, 215] Similarly data from two studies of under-5s in India found the peak of activity to occur in the cold months. [208, 211] Conversely in subtropical Hong Kong, hMPV activity appears to coincide with the rainy season. [198] Most of these studies were over short periods of time and involved only small numbers of patients. Larger prospective studies over multiple seasons are needed to explore the seasonality of hMPV further.

ADENOVIRUS

ADV is a DNA virus that was first isolated from adenoidal tissue over 50 years ago. [216] Over 50 serotypes have been identified, [217] however only a limited number of serotypes are known to be associated with clinical disease, and particular serotypes have a predilection to infect different sites. ADV can cause a wide spectrum of illnesses in adults and children including respiratory, gastrointestinal, urinary tract and ocular illnesses. ADV is an important cause of respiratory infections in children, which range from common cold, otitis media and pharyngitis, [218, 219] to pneumonia, croup, bronchiolitis and a syndrome almost identical to pertussis. [220, 221] ADV can also cause severe infections and can even result in death, both in immunocompromised and healthy individuals. [222-226] More severe infections have been associated with immunodeficiency, certain serotypes and younger age. [224, 227-230] The serotypes most frequently identified in children with ARIs are serotypes 1, 2, and 5, [231, 232] while serotypes 3 and 7 have been associated with epidemics of severe ALRI in children. [220, 229, 233]

The distribution of adenoviral infections appears to be worldwide. ADV has been identified in studies of childhood ALRI in Europe, [234-237] Asia, [134, 136, 137, 220, 238] [131, 132, 135, 183, 239] America, [231, 240] South America [150, 192, 229, 241, 242] and Africa [17, 84, 138] Early studies used conventional methods of viral identification, such as immunofluorescence and culture, to identify ADV in children with ALRI. Such studies of children presenting with ALRI to clinics in Columbia, India and Pakistan identified ADV in 1.5% to 6.5% of children. [131, 150, 239] Similar results were observed in community-based studies in Thailand, Philippines and the Gambia where ADV was found in 3% to 4% of children with ALRI. [89, 134, 135]

PCR has improved the sensitivity of ADV detection in clinical specimens compared to traditional methods. [243, 244] Studies using PCR have focused on hospitalised children, and recent studies in the developed world that have used PCR have identified ADV infections in up to 12% of children hospitalised with an ALRI. [235, 237, 245] Studies in the developing world have also used PCR to identify adenoviral infections in children admitted to hospital with ALRI. Two studies in Vietnam and one in Kenya found ADV in up to 5% of children hospitalised with ALRI. [17, 136, 137] A fourth study in Mozambique found a much higher prevalence (21%) of ADV in under-5s admitted with severe pneumonia. [138]

The majority of ADV-associated ALRIs occur in under-5s with the most severe disease occurring in children under 2 years of age. [220] Some studies have reported the highest incidence of ADV infections between six and 12 months old and suggested that infections under 6 months of age are rare. [218] [232] Other studies however have found the majority of ADV infections to occur during the first 6 months of life and deaths from ADV infections in neonates have been reported. [227, 229] A male predominance has been observed in ADV-associated ALRI, with up to two-thirds of infections reported in male patients. [137, 220, 229]

Adenoviral infections do not seem to have the seasonal variation that is associated with other respiratory viral pathogens. Most studies have reported sporadic infections all year-round, [17, 150, 198, 229, 232, 239] although several studies have described additional one-off peaks of ADV infections, some of which have been linked to certain serotypes. [132, 135, 220]

SUMMARY

Globally, pneumonia is the leading cause of death in children and the estimated incidence of childhood pneumonia in Myanmar is the third highest in the world. [1, 9] Previous research has already established effective interventions that can help reduce these childhood deaths, including the WHO case-management strategy and infant immunisations. [2, 3, 176] However, these current strategies focus primarily on bacterial pneumonia, namely Hib and pneumococcus, which are known to have a high associated mortality. Recent advances in molecular diagnostics have highlighted the substantial role that is played by viral pathogens, particularly RSV, in childhood pneumonia. As more aetiological studies employ PCR to detect viral pathogens in children with pneumonia, our understanding of their contribution will increase. This is vital to ensure that the existing case-management guidelines are as effective as possible and that immunisation programmes and development of vaccines are appropriate for the geographical location in which they are being used. [9]

CHAPTER 3

OBJECTIVES

OVERALL OBJECTIVE

To study the severity and aetiology of childhood pneumonia, in migrant infants and children under five years old, presenting to a clinic on the Thai-Myanmar border.

SPECIFIC OBJECTIVES

In migrant children under five on the Thai-Myanmar border:

1. Describe the clinical features and severity of pneumonia in children with a clinical diagnosis of pneumonia.
2. Describe the clinical features of pneumonia in children with a radiographical diagnosis of pneumonia.
3. Describe the aetiology of childhood pneumonia over a one-year period.

CHAPTER 4

SETTING, POPULATION AND CASE DEFINITIONS

STUDY DESIGN

A prospective observational study of pneumonia in migrant children under five years of age was conducted over a one-year period in a rural clinic on the Thai-Myanmar border.

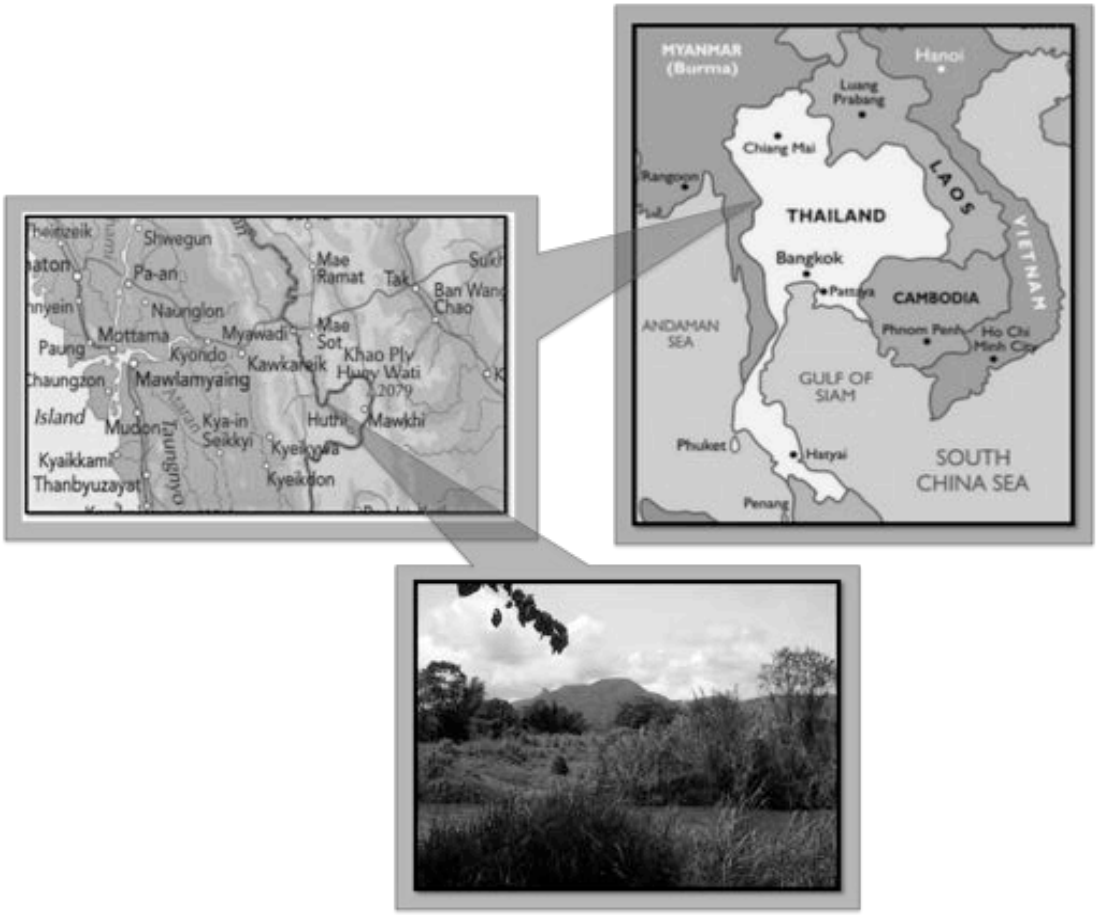
STUDY AREA AND POPULATION

The study was conducted in Mawker Thai (FIGURE 4), a small Thai village situated on the western border of Thailand with Myanmar, formerly Burma (16°N, 99°E). The village is located alongside the river that separates the two countries in a fertile lowland area bordered by mountains on both sides. Mawker Thai lies around 80km south of Mae Sot at an altitude of 253 metres above sea level. The climate here is tropical with a six-month rainy season running from May to early October. Rainfall is the major seasonal variable as the study area has year-round temperatures of between 24°C and 32°C. The area has a mean annual rainfall of 1,400mm, most of which falls in the rainy season and an annual relative humidity of above 75%.

The region around Mawker Thai is one of many areas along the Thai-Myanmar border that, due to ongoing conflict in Myanmar, hosts an uprooted population of migrant workers predominantly of the Karen ethnic group. This population has limited, if any, access to healthcare within Myanmar and is often prevented from accessing Thai healthcare

due to cost, language and safety. In Mawker Thai village, Shoklo Malaria Research Unit (SMRU) runs a clinic to provide free outpatient and in-patient care for migrants living both within Thailand and across the border in Myanmar. Locally trained medical assistants, nurses and home visitors run the clinic under the supervision of expatriate clinicians. The clinic sees approximately 13,000 patients a year.

FIGURE 4: MAPS SHOWING GEOGRAPHICAL LOCATION OF MAWKER THAI VILLAGE



CLINICAL DEFINITIONS OF PNEUMONIA

The medical assistants and nurses working in Mawker Thai clinic were trained to diagnose pneumonia in under-5s based on WHO classification shown in FIGURE 5. As part of routine care, all children who presented to the outpatient department with cough or difficulty breathing were screened for a fast respiratory rate in relation to their age and were assessed for the presence of additional signs of respiratory distress. Based on their assessment, a clinical diagnosis of pneumonia and its associated severity was made. For those children in whom a wheeze was heard on auscultation, a trial of rapid-acting inhaled bronchodilator was given (Appendix 3). A diagnosis of pneumonia was only made in those children with wheeze in whom signs of pneumonia persisted after salbutamol was given. Children whose signs of pneumonia resolved after salbutamol administration were excluded from the study.

According to the local paediatric guidelines based on the IMCI and WHO guidelines, a diagnosis of NSP was given to children with a history of cough or difficult breathing combined with a fast respiratory rate (more than 50 breaths per minute for children aged 2 months to 11 months old and more than 40 breaths per minute for children aged 12 months to 5 years old). [5, 65, 246] A diagnosis of SP was given to any children with cough or difficult breathing together with lower chest wall indrawing but no signs of VSP. [65] The presence of at least one of: central cyanosis, an oxygen saturation <90% by pulse oximetry, inability to breastfeed or drink, vomiting everything, convulsions, reduced consciousness or severe respiratory distress led to a diagnosis of VSP as described in FIGURE 5.

FIGURE 5: CLASSIFICATION OF PNEUMONIA IN CHILDREN FROM 2 MONTHS TO 5 YEARS OF AGE.

Signs	Classify as
Cough or difficult breathing **PLUS**: Fast breathing as per following criteria according to age: -- Age 2–11 months: > 50 breaths/minute -- Age 12 months to 5 years: > 40 breaths/minute	Non-severe pneumonia (P)
Cough or difficult breathing **PLUS**: -- Lower chest wall indrawing	Severe pneumonia (SP)
Cough or difficult breathing **PLUS**: -- Central cyanosis -- Inability to breastfeed or drink -- Vomiting everything -- Convulsions, lethargy or unconsciousness -- Severe respiratory distress e.g. head nodding All or some signs of SP may be present	Very severe pneumonia (VSP)

Adapted from the Pocket book of hospital care for children: guidelines for the management of common illnesses with limited resources. ISBN 92 4 154670 0 (NLM classification: WS 29)

Young infants (less than 2 months old) with signs of pneumonia were defined differently in accordance with WHO guidelines for hospital care. [65] Due to the increased risk of mortality in young infants a diagnosis of NSP was not given. For young infants, SP was defined as the presence of fast breathing (>60 breaths per minute) with or without chest wall indrawing. The additional presence of central cyanosis, inability to breastfeed, apnoeic episodes or severe respiratory distress led to a diagnosis of VSP as described in FIGURE 6.

FIGURE 6: CLASSIFICATION OF PNEUMONIA IN YOUNG INFANTS

Signs	Classify as
Cough or difficult breathing PLUS: -- Fast breathing >60 breaths/min **OR** -- Lower chest wall indrawing **OR** -- Grunting **OR** -- Nasal flaring	Severe pneumonia (SP)
Cough or difficult breathing PLUS: -- Apnoeic episodes **OR** -- Central cyanosis **OR** -- Inability to breastfeed or drink **OR** -- Vomiting everything **OR** -- Convulsions, lethargy or unconsciousness **OR** -- Severe respiratory distress e.g. head nodding All or some signs of severe pneumonia may be present	Very severe pneumonia (VSP)

Adapted from the Pocket book of hospital care for children: guidelines for the management of common illnesses with limited resources. ISBN 92 4 154670 0 (NLM classification: WS 29)

TREATMENT

All children were routinely treated following local paediatric protocols based on WHO guidelines. [5, 65] Children with NSP received oral antibiotic treatment with amoxicillin for seven days. Children with SP and VSP were admitted to the in-patient department for the administration of parenteral antibiotics. Children and older infants diagnosed with SP received first-line treatment with ampicillin. Children with VSP received ampicilllin together with gentamicin. Young infants received both ampicillin and gentamicin for SP and VSP. Children with oxygen saturations less than 90% were given oxygen therapy until they were able to maintain their oxygen saturations above 90% in room air as recommended by WHO. [65]

METEOROLOGICAL DATA

The environmental factors, namely rainfall (cm) and temperature (°C) were acquired from the Mae Sot Meteorological Station.

STATISTICAL ANALYSIS

Statistical analyses were performed using IBM® SPSS® Statistics Version 19 for Macintosh. Normally distributed continuous variables were expressed as a mean and standard deviation (SD) whilst all other continuous variables were expressed as a median with an inter-quartile range (IQR). All categorical variables were expressed as a percentage. Comparisons between groups were made using Mann-Whitney's *U,* Student's *t,* Fisher's exact and χ^2 tests as appropriate. Two-tailed *P*-values <0.05 were considered statistically significant.

Logistic regression analysis was used to assess the independent association of clinical and laboratory features with a diagnosis of pneumonia.

ETHICS

This observational study was conducted on behalf of SMRU, with the primary aim of improving the knowledge of childhood pneumonia in this population and therefore the clinical management provided by SMRU. Currently the SMRU paediatric guidelines are based upon the WHO criteria and this study assessed the utility of the WHO clinical

criteria in this population. Routine clinical care was provided to all under-5s presenting with a clinical diagnosis of pneumonia and no additional interventions were undertaken. Although only routine clinical care was provided, verbal informed consent was still obtained from the parent or guardian responsible for each child whose clinical information was included in this study.

The data collection was undertaken as part of a larger project on acute respiratory tract infections in children on the Thai-Myanmar border, which was approved by the Ethics Committee of the Faculty of Tropical Medicine, Mahidol University, Thailand (MUTM 2009-306 and MUTM 2010-404).

All patients treated at Mawker Thai clinic, including those children in this study, received treatment free of charge. Participants were informed that inclusion in the study did not affect the routine management and treatment offered by the clinic.

LITERATURE SEARCH

A literature search was conducted of EMBASE and Medline. Databases were searched on 14th January 2011 using the following terms: pneumonia, ARI, ALRI, infant, child, paediatric, aetiology and epidemiology.

CHAPTER 5

THE CLINICAL AND RADIOGRAPHICAL CHARACTERISTICS OF PNEUMONIA IN MIGRANT INFANTS AND CHILDREN LIVING ON THE THAI-MYANMAR BORDER

INTRODUCTION

Myanmar has one of the highest child mortality rates in Asia and the figures reported for children living along the border with Thailand are higher still. [1, 25] Globally, pneumonia is the leading cause of death in under-5s and Myanmar has the third highest incidence of childhood pneumonia worldwide. [9] Given the limited access to healthcare along the Thai-Myanmar border, it is likely that pneumonia is a leading cause of morbidity and mortality in migrant children.

With the aim of reducing the number of childhood deaths, WHO has developed ARI case-management guidelines to aid the diagnosis and treatment of childhood pneumonia in resource-limited settings. [5, 6, 43, 247] In such settings, the main providers of healthcare are often local staff with minimal training, accordingly the guidelines have been designed to be as simple as possible. [42] The use of the ARI case-management strategy in resource-limited settings is now well established and has been highlighted by UNICEF and WHO as one of three key interventions in their Global Action Plan for Prevention and Control of Pneumonia (GAPP). [3]

Although the role of radiography in distinguishing between bacterial and viral aetiology currently remains elusive, chest radiographs are still the most effective method for defining the presence of childhood pneumonia. [70, 248] Whilst this remains the case, the utility of radiographs must be maximised. The WHO has developed guidelines to standardise the interpretation of paediatric chest radiographs and limit the variability in interpretation. [79] These radiographic definitions have been successfully employed in vaccine trials, the results of which suggest a strong correlation between PEP and bacterial aetiology. [34, 35, 80, 115] Although further studies are required to fully determine the relationship between the aetiology and the radiographical definitions, the results from the vaccine trials suggest that the WHO radiographic definitions are a useful tool in helping refine the clinical diagnosis of pneumonia. A few studies have already sought to correlate these definitions with the clinical presentation of childhood pneumonia. [80, 249-251]

This current study aims to describe the clinical features, the disease severity and the radiographic characteristics of migrant infants and children under five years old who are diagnosed with clinical pneumonia as defined by the WHO at this rural clinic in South-East Asia.

METHODS

The study was conducted at the Shoklo Malaria Research Unit (SMRU) clinic in Mawker Thai village on the Thai-Myanmar border. All migrant infants and children less than five years old presenting between 12th October 2009 and 12th October 2010 with clinical pneumonia as defined by WHO were studied. Local medical assistants and nurses were trained to diagnose clinical pneumonia using local paediatric guidelines based on the IMCI

and WHO guidelines. [5, 65, 246] According to these guidelines, any child with wheeze was given a trial of rapid-acting bronchodilator before being classifed as pneumonia (Appendix 3). The severity of each case was also established and a diagnosis of NSP, SP or VSP was given. [5, 65] In line with the Paediatric Guidelines, if the caregiver consented, a complete blood count (CBC) and CRP level were requested for all children with clinical pneumonia. As per the SMRU protocol, a blood film was taken for microscopic examination for malaria from infants and children with a history of fever or with a documented fever at presentation and from all unwell infants less than two months old. Chest radiographs were requested for all children diagnosed with clinical pneumonia. The radiographs were performed at the nearest Thai hospital in Phop Phra and transportation to the hospital for the patient and their caregiver was provided on a daily basis. Children with NSP attended for a radiograph as was convenient for the patient and their family, as long as it was within seven days of presentation. For those children with SP and VSP, chest radiographs were postponed until the patient no longer required parenteral antibiotic therapy, intravenous fluids or oxygen therapy.

All chest radiographs were interpreted using WHO definitions and classified as PEP, OI, 'nil' or 'uninterpretable'. [79] Three clinicians undertook training in WHO standardised method for interpretation. [79] Two clinicians then read each radiograph independently and recorded their interpretation on a specifically designed case report form. Where the two reports disagreed, the third clinician was invited to read the radiograph. Where all three reports disagreed, the radiograph was reported as uninterpretable.

The eligibility and classification of the different severities of clinical pneumonia cases were clarified from the original Paediatric Clinical Questionnaires. Statistical analysis was

performed using IBM SPSS Statistics Version 19 for Macintosh. Continuous variables were expressed as a mean with a standard deviation or a median with an inter-quartile range (IQR) as appropriate, whilst all categorical variables were expressed as a percentage. Demographic, clinical, laboratory and radiographic features were compared using Mann-Whitney's *U*, Student's *t,* Fischer's exact and $\chi 2$ tests as appropriate.

RESULTS

STUDY POPULATION

During the one-year study period, over 3000 under-5s were seen in Mawker Thai clinic. Of these children, 180 were diagnosed with clinical pneumonia. Upon review of the paediatric clinical questionnaires, 5 patients did not fulfill the WHO clinical case definition and were excluded from the analysis. Nearly three-quarters of the children with pneumonia lived on the Myanmar side of the border (123/175, 70.3%). The homes of the patients had a mean of 6 ± 2 occupants during the previous month, and nearly all patients were the youngest member of their household. Most homes consisted of less than three rooms (2 ± 1), and 96.0% used an indoor fire. The most common fuel burnt was charcoal, which was used in nearly three-quarters of homes (73.7%). The remaining families with indoor fires used wood as their fuel. There were no demographic differences in subgroup analysis by age group (TABLE 6). As shown in FIGURE 7, of the 175 children who were diagnosed with clinical pneumonia, nearly half of the children were diagnosed with NSP (87/175, 49.7%). A quarter of the children were diagnosed with SP (44/175, 25.1%) and a quarter with VSP (44/175, 25.1%). A chest radiograph was performed in 94.9 % (166/175) of children with clinical pneumonia. The demographic characteristics of this sub-group were not

statistically different from the entire study population described in TABLE 6. Using the WHO standardised method for interpretation, 38.0% (63/166) of radiographs had PEP of which only two patients had a complete lobar pneumonia and none had evidence of pleural effusion (FIGURE 8). Over one-third of patients (57/166, 34.3%) had OI, whilst 29/166 (17.5%) had no infiltrate or pneumonia. The remaining 10.2% (17/166) of the radiographs were reported as uninterpretable.

TABLE 6: DEMOGRAPHIC CHARACTERISTICS OF THE STUDY POPULATION OVERALL AND BY AGE GROUP

Characteristic	**Overall**	**Cases <2 months**	**Cases 2 -11 months**	**Cases 12-59 months**
	n= 175	**n=9**	**n=75**	**n= 91**
	Number (%)			
Live on Burmese side	123 (70.3)	5 (56)	57 (76)	61(67)
Live on Thai side	52 (29.7)	4 (44)	18 (24)	30 (33)
	Mean (SD)			
No. people in the home	5.8 (1.9)	5.7 (1.7)	5.7 (1.8)	5.9 (2.0)
No. adults in the home	3.1 (3.6)	3.2 (1.2)	3.1(2.6)	3.1 (4.4)
No. older children in the home	1.9 (1.4)	1.6 (1.6)	1.8 (1.4)	2.1 (1.4)
No. younger children in the home	0.1 (0.3)	0.0 (0)	0.0 (0.1)	0.2 (0.4)
No. rooms in the home	1.9 (1.4)	2.4 (1.8)	1.8 (0.8)	2.0 (0.9)
No. smokers in the home	1.3 (0.9)	1.1 (0.9)	1.2 (0.9)	1.4 (0.8)
	Number (%)			
Use of indoor fire	168(96.0)	8(88.9)	71(94.7)	89(97.8)
- Charcoal as fuel	129 (73.7)	7(77.8)	58(77.3)	64(70.3)
- Wood as fuel	39 (22.3)	1(11.1)	13(17.3)	25(27.5)

FIGURE 7: DISTRIBUTION OF CLINICAL SEVERITY OF PNEUMONIA AT PRESENTATION

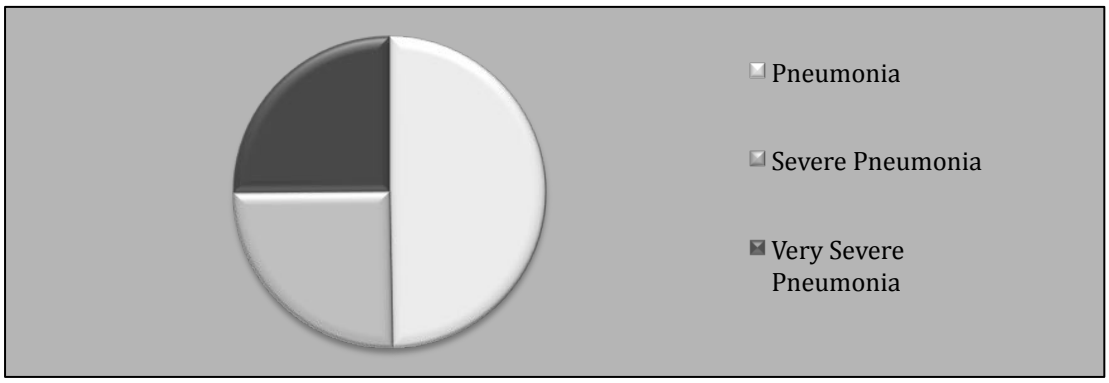
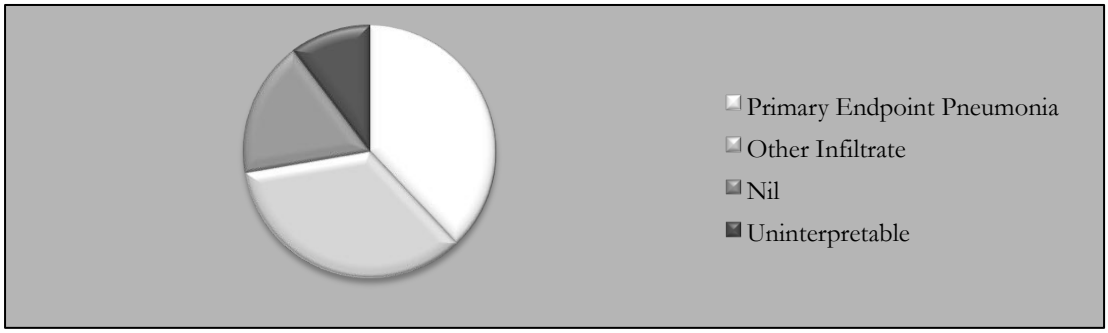


FIGURE 8: INTERPRETATION OF CHEST RADIOGRAPHS USING THE WHO STANDARDISED METHOD



AGE AND GENDER DISTRIBUTION

Of the 175 children included, the median age was 7.5 months (IQR 3 to 13) as shown in TABLE 7. The median age decreased with increasing severity of pneumonia: The median age of children with P was 15.0 months (IQR 7.0-23.9), with SP was 12.6 months (IQR 6.3-26.9) and with VSP was 7.5 months (IQR 3.0 – 14.0) (TABLE 7). The median age of those with VSP was significantly lower (P=0.013) (TABLE 7). Nearly half of the children were infants (48.0%, 84/175) but only 5.1% (9/175) were young infants (TABLE 8).

TABLE 7: AGE AND GENDER OF PATIENTS COMPARED TO THE SEVERITY OF THE CLINICAL DIAGNOSIS

	Clinical Presentation				
	Non-severe pneumonia cases n=87	**Severe Pneumonia cases n=44**	**Very Severe Pneumonia cases n=44**	**Total, n= 175**	**P value**
Number of male, (%)	47/87 (54.0)	26 (59.1)	31 (70.5)	104 (59.4)	0.082
Median Age, (IQR)	15.0 (7.0-23.9)	12.6 (6.3-26.9)	7.5 (3.0-14.0)	7.45 (3-13)	**0.013**

TABLE 8: AGE AND GENDER DISTRIBUTION OF CLINICAL PNEUMONIA CASES

Age group	No. patients (%)	No. male patients (%)
0 to 59 months	175	104 (59.4)
<12 months	84/175 (48.0)	55/84 (65.5)
< 2months	9/175 (5.1)	8/9 (88.9)
2 to 11 months	75/175 (42.9)	47/75 (62.7)
12 to 59 months	91/175 (52.0)	49/91 (53.8)

The distribution of clinical severity according to the WHO age groups is shown in TABLE 9. Infants were twice as likely to be diagnosed with SP or VSP (unadjusted odds ratio 2.26, P=0.01, CI=1.22 to 4.18) (TABLE 10). According to the WHO guidelines young infants cannot be diagnosed with NSP and this largely accounts for the observed difference. If these young infants are excluded from the analysis, the odds ratio drops to 1.86 (CI=0.99 to 3.49) (TABLE 10).

TABLE 9: THE CLINICAL SEVERITY OF PNEUMONIA BY WHO AGE GROUP

WHO age groups	Non-severe pneumonia (NSP) cases n= 87	Severe pneumonia (SP) cases n=44	Very severe pneumonia (VSP) cases n=44
Number of children < 2 months n= 9 (%)	n/a	1 (11.1)	8 (88.9)
Number of children 2 to 11 months n=75 (%)	33 (44.0)	20 (26.7)	22 (29.3)
Number of children 12 to 59 months n=91 (%)	54 (59.3)	23 (25.3)	14 (15.4)

TABLE 10: THE CLINICAL SEVERITY OF PNEUMONIA COMPARED TO AGE

Age groups	Severe pneumonia or very severe pneumonia cases n=88 (79[a])	Non-severe pneumonia cases n= 87	Unadjusted Odds Ratio (95% Confidence Interval)
Number of children 0 to 11 months *(infants)* n=84	51 (60.7)	33 (39.3)	2.26 (1.22 to 4.18)
Number of children 2 to 11 months n= 75	42	33	1.86 (0.99 to 3.49)

[a] Excluding children under 2 months old

As shown in TABLE 11, patients with radiographic changes, either OI or PEP, on their radiograph were significantly older (median age 15.0 months, IQR 7.8–24.8) than those children with nil on their radiograph (median age 5.7 months, IQR 3.4–9.9), (P <0.001).

TABLE 11: MEDIAN AGE AND GENDER OF PATIENTS WITH DIFFERENT RADIOGRAPHICAL DIAGNOSES

	Radiographical Diagnosis				
	PEP and OI n=120	**PEP n=63**	**OI n=57**	**Nil n=29**	***P value***
Male gender (%)	67 (55.8)	40 (63.5)	27(47.4)	21(72.4)	0.141
Median age (IQR)	15.0 (7.8–24.8)	13.3 (5.4–20)	16.8 (8.8–28.0)	5.7 (3.4–9.9)	<0.001[a]

[a] Comparing PEP plus OI to Nil. PEP, primary endpoint pneumonia. OI, other infiltrate.

Children over 12 months old with a clinical diagnosis of pneumonia were significantly more likely to have radiographical changes (73/79, 92.4%) than infants (47/70, 67.1%), P<0.001. Indeed children over 12 months old were nearly 6 times more likely to have OI or PEP than infants (unadjusted odds ration 5.95, P<0.001, CI=2.21–16.03), as shown in TABLE 12.

TABLE 12: THE RADIOGRAPHICAL DIAGNOSIS COMPARED TO AGE GROUP

	Radiographical Diagnosis		
	PEP and OI n=120	**Nil n=29**	**Unadjusted Odds Ratio (95% Confidence Interval)**
Number of children 0 to 11 months (infants) n=70 (%)	47 (67.1)	23 (32.9)	0.17 (0.06 to 0.45)
Number of children 12 to 59 months n= 79 (%)	73 (92.4)	6 (8.2)	5.95 (2.21 to 16.03)

Overall, the majority of children diagnosed with pneumonia were male (59.4% 104/175) and the proportion of male patients was greater than 50% in all three age groups (TABLE 8). The proportion of male patients was higher in the younger age groups but this did not reach statistical significance. There was no significant difference in the proportion of male cases with increasing severity of the clinical pneumonia (TABLE 7). Neither did the proportion of male patients differ significantly between the three radiographical diagnoses (TABLE 11).

CLINICAL HISTORY

No significant differences were observed in the illness history reported by the caregiver when compared to the severity of the clinical diagnosis. Both the reported duration of illness and the presence of complaints associated with respiratory illness were similar in children with NSP, SP and VSP (TABLE 13). When comparing the clinical history of children with different radiographic diagnoses, the reported duration of illness prior to presentation was longer for those patients with PEP or OI (median 3 days, IQR 2–4) than for those patients with nil on radiograph (median 2 days, IQR 2–3). No other aspects of the clinical history were found to be significantly different between the three radiographic diagnoses (TABLE 14).

TABLE 13: THE CLINICAL HISTORY COMPARED TO THE SEVERITY OF THE CLINICAL DIAGNOSIS

	CLINICAL DIAGNOSIS			
	Non-severe pneumonia cases n=87	**Severe pneumonia cases** n=44	**Very severe pneumonia cases** n=44	*P value*
HISTORY				
Median no. of days to presentation (IQR)	3.0 (2.0-4.0)	2.0 (2.0-3.8)	3.0 (2.0-4.0)	0.697
History of cough (%)	87/87 (100)	44/44 (100)	43/44 (97.7)	1.000
History of runny nose (%)	76/87 (87.4)	37/44 (84.1)	37/44 (84.1)	0.657
History of fever (%)	72/87 (82.8)	40/44 (90.9)	40/44 (90.9)	0.136

TABLE 14: THE CLINICAL HISTORY COMPARED WITH THE RADIOGRAPHICAL DIAGNOSES

	RADIOGRAPHICAL DIAGNOSIS				
	PEP and OI n=120	**PEP** n=63	**OI** n=57	**Nil** n=29	*P value*
HISTORY					
Median no. of days to presentation (IQR)	3.0 (2-4)	3.0 (2-4)	3.0 (2-4)	2.0 (2-3)	**0.011**[a]
History of cough (%)	120 (100.0)	63 (100)	57(100)	29(100)	1.000[a]
History of runny nose (%)	106 (88.3)	57(90.5)	49(86.0)	22(75.9)	0.132[a]
History of fever (%)	108 (90.0)	57(90.5)	51(89.5)	23(79.3)	0.121[a]

[a] Comparing PEP plus OI to Nil. PEP, primary endpoint pneumonia. OI, other infiltrate.

VITAL SIGNS AND PHYSICAL EXAMINATION

At presentation, there were no differences in the mean temperature or the proportion of patients with a fever ≥38.0°C between the three levels of severity (TABLE 15). Children with SP and VSP had a higher heart rate than those with NSP (P=0.003), and overall those children with SP and VSP also had a significantly higher respiratory rate than those with NSP (TABLE 15). However, at subgroup analysis, no differences were found in the respiratory rate of infants compared to severity. For children over 12 months old, those with a diagnosis of SP or VSP had a significantly higher mean respiratory rate (59 breaths per minute, SD 11) than those with NSP (49 breaths per minute, (SD 6), P value <0.001). As expected, children with a diagnosis of VSP were more likely to have lower oxygen saturations and require oxygen therapy than those with NSP or SP (P<0.001). Only three children were noted to have central cyanosis on clinical examination, all of these children had VSP and had oxygen saturations of 77%, 80% and 92% recorded on pulse oximetry. Of the 18 children with saturations less than 90% at presentation only two of these children were noted to have central cyanosis clinically. This suggests that central cyanosis is not a reliable sign for the administration of oxygen in this population.

TABLE 15: THE VITAL SIGNS COMPARED TO THE SEVERITY OF THE CLINICAL DIAGNOSIS

	CLINICAL DIAGNOSIS				
	Pneumonia cases	**Severe pneumonia cases**	**Very severe pneumonia cases**	**Severe PLUS very severe cases**	***P value***
	n=87	**n=44**	**n=44**	**n=88**	
VITAL SIGNS					
Mean triage temperature, ºC (SD)	37.5 (0.9)	37.4 (0.9)	37.6 (1.0)	37.5 (0.9)	0.710[b]
No. of patients with temperature ≥38.0 (%)	25 (28.7)	10 (22.7)	16 (36.4)	26 (29.5)	1.000[b]
Mean oxygen saturation, % (SD)	94.3 (1.8)	93.9 (2.0)	89.1 (7.5)	91.5 (5.9)	**0.001[a]**
No. of patients with oxygen saturation <90% (%)	0 (0)	0 (0)	18 (40.9)	18 (20.5)	[c]
Mean heart rate, beats per minute (SD)	137.1 (16.6)	143.2 (19.6)	146.6 (16.1)	144.9 (17.9)	**0.003[b]**
Mean respiratory rate, breaths per minute (SD)	53.6 (8.4)	56.9 (9.1)	63.1 (17.0)	60.0 (13.9)	**<0.001[b]**
<2months	- -	62.0 (0)	62.6 (32.2)	62.6 (30.2)	0.986
2 to 11 months	60.6 (6.8)	57.8 (7.1)	62.3 (13.8)	60.1 (11.2)	0.825[b]
12 to 59 months	49.4 (6.1)	55.9 (10.8)	64.7 (9.2)	59.2 (11.0)	**<0.001[b]**

[a] Comparing P plus SP to VSP. [b] Comparing P to SP plus VSP, [c] clinical feature predefined as VSP

The associated severity of many clinical signs is predefined by WHO, including head-bobbing, cyanosis, grunting, stridor and chest indrawing (TABLE 16). In this current study, tracheal tug was associated with increased severity and was significantly more likely to be found in patients with VSP than SP (70.5% vs. 41.0%, *P*=0.01). Five patients (5.7%) with tracheal tug had no additional signs of SP or VSP and were treated as NSP. Nasal flaring

was also associated with increased severity. Nasal flaring was found in significantly more children with VSP than SP, 25.0% and 4.5% of cases, respectively (P=0.01), and it was observed in only a single patient diagnosed with NSP who had no additional signs of severity. Those children with a diagnosis of SP or VSP were significantly more likely to have crepitations on auscultation than those with NSP (85.2% vs. 60.9%, P<0.001). Similarly, those children with SP or VSP were also significantly more likely to have auscultatory wheeze than those children with NSP (34.1% vs. 18.4%, P=0.025).

TABLE 16: THE PHYSICAL EXAMINATION COMPARED TO THE SEVERITY OF THE CLINICAL DIAGNOSIS

	CLINICAL DIAGNOSIS				
	Pneumonia	**Severe pneumonia**	**Very severe pneumonia**	**Severe PLUS very severe**	***P* value**
	n=87	**n=44**	**n=44**	**n=88**	
EXAMINATION					
Patients with cyanosis (%)	0	0	3 (7.1)	3	[a]
Patients with grunting (%)	1 (1.1)	0	6 (13.6)	6 (6.8)	[a]
Patients with stridor (%)	0	0	1 (2.4)	1	[a]
Patients with tracheal tug (%)	5 (5.7)	18 (40.9)	31 (70.5)	49 (55.7)	*P*=0.01[b]
Patients with nasal flare (%)	1 (1.1)	2 (4.5)	11 (25.0)	13 (14.8)	*P*=0.01[b]
Patients with chest indrawing (%)	0	43 (97.8)	44 (100)	87 (98.9)	[c]
Patients with head-bobbing (%)	0	0	25 (56.8)	25 (28.4)	[a]
Crepitations on auscultation (%)	53 (60.9)	37 (84.1)	38 (86.4)	75 (85.2)	*P*=1.0[b] *P*<0.001[d]
Wheeze on auscultation (%)	16 (18.4)	13 (29.5)	17 (38.6)	30 (34.1)	*P*=0.5[b] *P*=0.025[d]

[a] Clinical feature predefined as VSP, [b] comparing SP to VSP, [c] clinical feature predefined as SP, [d] comparing P to SP plus VSP

At presentation, there were no differences in the mean temperature, mean heart rate, mean respiratory rate or mean oxygen saturations for the different radiographic diagnoses (TABLE 17). On examination nasal flare was significantly more likely to be found in patients with nil on their radiograph (5/29, 17.2%) than those with PEP or OI (6/120, 5.0%), (TABLE 17). The presence of chest indrawing and other signs of respiratory distress were not associated

with radiographical diagnosis. However, crepitations on auscultation were significantly more likely to be found in patients with PEP or OI than those with nil on their radiograph.

TABLE 17: THE VITAL SIGNS AND PHYSICAL EXAMINATION COMPARED TO THE RADIOGRAPHICAL DIAGNOSES

	RADIOGRAPHICAL DIAGNOSIS				
	PEP and OI n=120	**PEP n=63**	**OI n=57**	**Nil n=29**	***P value***
VITAL SIGNS					
Mean temperature ºC (SD)	37.5 (0.9)	37.6 (0.9)	37.4 (0.9)	37.4 (0.9)	0.416[a]
Mean oxygen saturations (SD)	92.9 (5.1)	92.2 (6.4)	93.8 (2.8)	92.8 (4.0)	0.917[a]
Mean heart rate (SD)	140.2 (17.9)	143.4 (17.5)	136.7 (17.8)	147.8 (13.4)	0.033[a]
Mean respiratory rate (SD)					
<2months	50.4 (21.8)	50.4 (21.8)	*No patients*	60.7 (13.4)	0.482[a]
≥ 2 to < 12 months	61.1 (10.6)	61.7 (10.2)	60.3 (11.3)	58.7 (8.8)	0.379[a]
≥12 months	53.4 (9.8)	55.2 (10.1)	51.7 (9.3)	58.0 (12.3)	0.277[a]
EXAMINATION					
No. patients with grunting (%)	4 (3.3)	3(4.8)	1(1.8)	1(3.4)	1.000[a]
No. patients with stridor (%)	1 (0.8)	1(1.6)	0	0	1.000[a]
No. patients with tracheal tug (%)	40 (33.3)	17(27.0)	23(40.4)	8(27.6)	0.660[a]
No. patients with nasal flare (%)	6 (5.0)	3(4.8)	3(5.3)	5(17.2)	**0.039**[a]
No. patients with chest indrawing (%)	60 (50.0)	31(49.2)	29 (50.9)	17(58.6)	0.418[a]
No. patients with head-bobbing (%)	15 (12.5)	9(14.3)	6(10.5)	6(20.7)	0.248[a]
No. patients with crepitations on auscultation (%)	97 (80.8)	50(79.4)	47(82.5)	15(51.7)	**0.002**[a]
No. patients with wheeze on auscultation (%)	30 (25.0)	13(20.6)	17(29.8)	8(27.6)	0.814[a]

[a] Comparing PEP plus OI to Nil

INVESTIGATIONS

The study achieved good coverage with each investigation being performed in more than 94% of children. A CBC was taken from 169 children (96.6%), CRP from 166 children (94.9%). Neither the CRP level, lymphocyte count, leukocyte count nor neutrophil count were associated with severity (TABLE 18). Neither was there a difference in the levels of these inflammatory markers between those patients diagnosed with PEP, OI and nil on chest radiography (TABLE 19).

TABLE 18: THE LABORATORY CHARACTERISTICS OF CHILDREN COMPARED TO THE SEVERITY OF THE CLINICAL DIAGNOSIS

	CLINICAL DIAGNOSIS			
	Non-severe pneumonia cases n=87	**Severe pneumonia cases n=46**	**Very severe pneumonia cases n=42**	**All cases n=175**
Haemoglobin ($/10^3/\mu l$), mean (SD)	10.2 (1.3)	10.4 (1.3)	11.2 (2.0)	10.5 (1.6)
Leukocyte count ($/10^3/\mu l$), mean (SD)	15.2 (6.3)	16.3 (6.8)	14.5 (5.1)	15.3 (6.1)
Neutrophil count ($/10^3/\mu l$), mean (SD)	7.4 (5.3)	8.1 (6.1)	7.3 (5.0)	7.6 (5.4)
Lymphocyte count ($/10^3/\mu l$), mean (SD)	6.5 (2.8)	6.4 (3.0)	5.9 (2.4)	6.3 (2.8)
CRP mg/L, Median (IQR)	19.8 (9.5-50.5)	27.9 (11.7–49.5)	22.5 (10.4-54.9)	22.5 (10.8-50.5)
No. patients with CRP > 40mg/L (%)	22/81 (27.2)	12/43 (27.9)	13/42 (31.0)	47/166 (28.3)

The mean haemoglobin level of the children was 10.5 g/dl (±1.6) reflecting the high prevalence of anaemia in this population. [252] There was no difference between the levels of anaemia compared to the severity of pneumonia. A blood film was examined for malaria in 84.6% (148/175) of pneumonia cases and was found to be negative in all but one of the children. This case had *Plasmodium falciparum* diagnosed on microscopic examination of the blood film and had a second clinical diagnosis of SP associated with a negative blood culture and negative NPA but a CRP of 160.

TABLE 19: THE LABORATORY CHARACTERISTICS OF CHILDREN COMPARED TO THE RADIOGRAPHICAL DIAGNOSIS

	RADIOGRAPHICAL DIAGNOSIS		
	Primary endpoint pneumonia n=63	**Other infiltrate n=57**	**Nil n=29**
Leukocyte count (/10^3/μl), mean (SD)	15.6 (5.1)	15.7 (8.0)	13.6 (4.7)
Neutrophil count (/10^3/μl), mean (SD)	7.8 (4.9)	8.3 (7.1)	6.1 (3.9)
Lymphocyte count (/10^3/μl), mean (SD)	6.1 (2.1)	6.0 (2.8)	6.4 (2.9)
C-reactive protein (/10^3/μl), mean (SD)	42.5 (48.4)	40.7 (47.6)	43.7 (50.9)
No. patients with CRP>40 mg/l (%)	17/60 (28.3)	15/54 (27.8)	9/28 (32.1)

RELATIONSHIP BETWEEN SEVERITY AND RADIOGRAPHICAL DIAGNOSIS

Those patients with PEP on their chest radiograph were not more likely to have clinically severe disease. The proportion of children with PEP did not differ significantly between the three levels of clinical severity or from the overall proportion of children with PEP

(63/166, 38.0%) as shown in TABLE 20. The same was true for a radiographical diagnosis of OI; there was no significant difference in the proportion of patients with OI between P, SP and VSP. Therefore neither PEP nor OI were associated with the clinical severity of disease.

TABLE 20: THE CLINICAL SEVERITY OF PNEUMONIA COMPARED TO THE RADIOGRAPHICAL DIAGNOSES

	CLINICAL DIAGNOSIS			
RADIOGRAPHICAL DIAGNOSIS	Non-severe pneumonia n=83 (%)	Severe pneumonia n=45 (%)	Very severe pneumonia n=38 (%)	***P value***
Primary endpoint pneumonia n= 63	32 (38.6)	14 (31.1)	17 (44.7)	0.368
Other infiltrate n=57	28 (33.7)	17 (37.8)	12 (31.6)	0.192
Nil n=29	11 (13.3)	8 (17.8)	10 (26.3)	0.172

TREATMENT AND OUTCOMES

All of the patients received antibiotic therapy for pneumonia, and nearly all of the patients (93.7%, 64/175) received antibiotics in line with local paediatric protocols. Ten patients with a clinical diagnosis of SP were unable to stay in the IPD due to social circumstances or lack of beds in the clinic. These patients were treated at home with amoxicillin and followed-up as outpatients. All these patients recovered completely. Two patients with NSP required treatment with the second-line antibiotic, erythromycin, to which they responded, whilst five patients with VSP required treatment with the second-line antibiotic ceftriaxone (5/41, 12.2%).

Oxygen therapy was administered to patients with an oxygen saturation <90% and was given to 10.3% (18/175) of all patients and 20.5% (18/88) of patients admitted with pneumonia. The mean duration of oxygen therapy was 70 hours (±50). Intravenous fluids were given to those children who were dehydrated or unable to tolerate oral fluids. They were administered to 8.6% (15/175) patients for a mean duration of 28 hours (±14).

There were two deaths during the study, giving an overall mortality rate of 1.1%. Both deaths were in infants with a diagnosis of VSP. The first death was in a 3 day old male neonate who presented in severe respiratory distress with a respiratory rate of 129 per minute, saturations of 86% on air, chest indrawing, tracheal tug, nasal flaring and head bobbing. His chest was clear on ausculation and his CRP only $8x10^3/\mu l$ but he had a neutrophilia of $15.3x10^3/\mu l$. Neither an NPA nor chest radiograph was performed but the blood culture was negative. Despite treatment with oxygen, intravenous fluids, ampicillin, gentamicin and cefotaxime he deteriorated and following active resusitation he died 24 hours after admission.

The second mortality was in a 3-month-old male infant who presented 4 days into his illness and died within an hour of admission. He had a respiratory rate of 63 per minute, saturations of 88% on air, was mottled and moderately dehydrated. He also had severe respiratory distress with chest indrawing, grunting and head bobbing. His bloods showed a CRP of only $8x10^3/\mu l$ and a neutrophil count of only $3.2x10^3/\mu l$. Adenovirus was detected in his NPA, his blood culture was negative and again a chest radiograph was not undertaken. He was given oxygen, intravenous fluids, ampicillin and gentamicin but arrested within an hour of admission and could not be resuscitated.

PREDICTORS OF CLINICAL SEVERITY

A stepwise elimination logistic regression was used to identify the independent predictors of SP and VSP. Chest indrawing is used as a diagnostic criterion for SP; it is therefore not unexpected that it was a significant factor for severe disease at univariate analysis. As it is not possible to have chest indrawing with a diagnosis of NSP, there are zero children in this category and it is therefore not possible to include chest indrawing in the multivariate analysis. A similar situation arises with head bobbing which is a sign of severe respiratory distress and therefore corresponds to a diagnosis of VSP. All other factors identified as significant ($P<0.05$) at univariate analysis were entered into the model together with the patient's age. The goodness-of-fit test showed the model to be a good approximation (Hosmer-Lemeshow χ^2 test = 0.792).

Three factors were identified as significant independent predictors of severe disease in children with pneumonia (TABLE 21). Children with crepitations on auscultation were 4.78 times more at risk of SP or VSP than those without (95%CI=1.77-12.88). Children with lower oxygen saturations and faster respiratory rate were also significantly more likely to have SP or VSP.

TABLE 21: INDEPENDENT PREDICTORS OF SEVERE DISEASE IN CHILDREN WITH CLINICAL PNEUMONIA

Factor	Odds Ratio	95% Confidence interval
Crepitations	4.78	1.77, 12.88
Respiratory rate	1.07	1.02, 1.11
Oxygen Saturations	0.85	0.75, 0.96

PREDICTORS OF RADIOGRAPHIC CHANGES

A stepwise elimination logistic regression was used to identify the independent predictors of changes on chest radiograph. All factors identified as significant ($P<0.05$) at univariate analysis were entered into the model together with RR and age. The goodness-of-fit test showed the model to be a good approximation (Hosmer-Lemeshow χ^2 test = 0.060). Three factors were identified as significant independent predictors of changes on chest radiograph; absence of nasal flaring, crepitations on auscultation and increasing age (TABLE 22). Children with crepitations on auscultation were 4.84 times more likely to have PEP or OI than those without crepitations (95% CI=1.89-12.39). When nasal flaring was present, children were 0.16 times less likely to have PEP or OI than when nasal flaring was absent (95% CI=0.04-0.66).

TABLE 22: INDEPENDENT PREDICTORS OF RADIOGRAPHICAL CHANGES

Factor	Odds Ratio	95% Confidence interval
Nasal flaring	0.161	0.039, 0.659
Crepitations	4.838	1.888, 12.393
Age	1.060	1.011, 1.111

DISCUSSION

OVERVIEW

The IMCI guidelines for the case-management of children with cough or difficult breathing were developed by WHO for use by health workers with minimal training in resource-

limited settings. [5] They aim to help health workers identify and treat children with pneumonia, whilst the Pocket Book of Hospital Care for Children provides additional guidance on the treatment and management of more severe disease by senior health workers in small hospitals. [65] The local SMRU paediatric guidelines used in Mawker Thai clinic for the case-management of ARI are based upon these WHO and IMCI guidelines. [246] Mawker Thai clinic is overseen by an expatriate clinician but is run by locally trained nurses and medical assistants, who have been trained to use the ARI case-management guidelines, administer oxygen therapy and give intravenous antibiotics. Over the one-year study period, 175 migrant infants and children were diagnosed and treated for clinical pneumonia according to the WHO definitions. Over 97% of children diagnosed with clinical pneumonia were correctly diagnosed. In addition, over 93% of these children received antibiotic therapy in line with the local protocols, nearly all children had their oxygen saturations recorded, and all children with an oxygen saturation lower than 90% were given oxygen therapy. The overall mortality rate was 1.1% and both deaths were in children with VSP. Together, these results demonstrate that with training, local staff in this rural South-East Asian clinic were able to effectively diagnose and manage children presenting with clinical pneumonia of all levels of severity, according to the WHO guidelines.

Although the number of children included in this study is relatively small, the cases of childhood pneumonia are well characterised using WHO standardised clinical and radiographical definitions, which allows the data to be easily compared to other studies using the same methodology. Of the 175 cases of clinical pneumonia in this current study, half of the cases were diagnosed with NSP and half were diagnosed with either SP or VSP. A similar hospital-based study of 300 hundred under-5s in urban India also included cases

of NSP, SP and VSP, and 57% of cases were diagnosed with NSP. [211] A hospital-based study of children aged between 2 months to 35 months in Nepal observed a much higher proportion of NSP (94%). [253] However, unlike this current study and the study in India, both active and passive surveillance were used in Nepal to identify cases of pneumonia. The earlier detection of cases by trained fieldworkers is likely to account for the large difference in severity. A meta-analysis of the implementation of community-based ARI case-management strategies has already demonstrated the effectiveness of such an approach, with an associated reduction in mortality from pneumonia of up to 40%. [7] The high proportion of severe disease in this current study compared to that observed in Nepal, highlights the valuable role that community health workers could play in the reduction of morbidity and mortality from childhood pneumonia in this population. However implementation of a community-based strategy in a migrant population could pose a particular challenge due to the mobile nature of the population.

In this current study, over one-third (38%) of the children with a clinical diagnosis of pneumonia also had PEP as defined by the WHO. Given the association between PEP and bacterial pneumonia observed in the pneumococcal vaccine trials, it is likely that a high proportion of these cases were bacterial in aetiology. [34, 35, 115] Although these vaccine trials have not observed a similar reduction in OI, previous studies have isolated bacteria from children with infiltrates on their radiograph. [73, 75] Until further studies can better clarify the relationship between PEP, OI and aetiology it remains impossible to reliably distinguish between bacterial and viral aetiology by radiography. [70, 248] In this current study, nearly three-quarters (72%) of patients with a WHO clinical diagnosis of pneumonia had radiographic changes consistent with a possible bacterial pneumonia (PEP and OI) and over half of these children had radiographic changes suggestive of a likely bacterial

pneumonia (PEP). This implies that in this rural South-East Asian setting, the WHO case definition is a valuable tool in predicting children with a possible bacterial pneumonia.

Other similar studies that have employed the standardised radiographic definitions have found varying results. A large study of nearly 2000 children aged 2 months to 5 years old presenting to 6 hospitals in Pakistan with NSP used an early version of the WHO radiographic standardisation. [249] This study found only 14% of children diagnosed with NSP had radiographical evidence of either PEP or OI and 82% of children had a completely normal radiograph. This is much lower than found in this current study and may be due to the inclusion of only NSP cases, however as discussed below this current study found no relationship between radiographical diagnosis and severity. The results from this current study are more comparable to a recent retrospective study of children aged 1 month to 5 years old in Fiji. [250] Here, although the proportion of OI was not reported, 34% of Fijian children with a diagnosis of SP or VSP had PEP. Similarly in over 600 episodes of SP in Mozambican children under 2 years of age, 43% of them had PEP, whilst another 17% had OI. [251] In a Gambian vaccine trial, data from nearly 4000 cases of SP in unvaccinated children aged between 6 weeks and 30 months old found PEP in 17% and OI in 28%. [80]

AGE DISTRIBUTION

The incidence of pneumonia has long been observed to be highest in infants. [19] Although this current study was not designed to estimate the incidence of pneumonia it did find nearly half of the clinical pneumonia cases (48%) were in infants and 58% of children admitted with pneumonia were infants. This current study also observed a negative

correlation between the patient's age and the severity of the pneumonia diagnosis (37.9% of NSP, 45.7% of SP and 71.4% of VSP). Cases of VSP were significantly younger than children who were diagnosed with NSP or SP (median age in months, 7.5 vs. 14.2; P <0.05). A similar observation was made in Nepal where cases of NSP and SP were studied. [253] Here, children with SP were significantly younger than those with NSP (median age in months, 8.6 vs. 13.7).

In contrast to other similar studies, this current study found that children with a normal chest radiograph were significantly younger than those with either PEP or OI (median age in months, 5.7 vs. 15.0). Infants were actually six times less likely to have any changes consistent with an ALRI on their radiograph than children older than 12 months. The study of Pakistani children diagnosed with NSP found no difference in the age of children with and without PEP. [249] Similar observations were made in both the study of Fijian children aged 1 month to 5 years old with SP and VSP and the study of children with SP in Mozambique. [250, 251] A vaccine study in the Gambia also utilised the standardised radiographic diagnoses to describe chest radiographs taken during episodes of SP in children up to 30 months old. [80] This study observed no significant difference in the median age of children diagnosed with PEP, OI and nil.

GENDER DISTRIBUTION

In this current study, 59.4% of children with clinical pneumonia were male, and similar results were observed in all levels of severity. Nearly all previously published studies have found boys to suffer from pneumonia more often than girls. An early multi-centre study carried out in ten different developing countries found that in most countries between 55%

and 60% of cases of ALRI in under-5s were male. [19] This study also observed the male predominance to persist in hospitalised cases of ALRI. More recent studies using the WHO criteria for a diagnosis of clinical pneumonia have found between 57% and 72% of children to be male. [17, 211, 249, 251, 253] This current study also found no significant difference in gender distribution between each of the three WHO radiographical diagnoses. Similar observations have been made in studies in Pakistan, Fiji and Mozambique. [249-251]

SEVERITY AND RADIOGRAPHIC CHARACTERISTICS

One of the strengths of this current study is the inclusion of cases of clinical pneumonia with all three levels of severity. This current study found no evidence that the severity of the clinical presentation was related to the presence of either PEP or OI on radiography. Of the aforementioned studies that have used the WHO radiographic definitions, only the study in Fiji included more than one clinical category: SP and VSP cases. [250] This study found no significant difference in the proportion of PEP in children with VSP compared to children with SP.

CLINICAL CHARACTERISTICS

This current study explored the relationship of the clinical history, vital signs and physical examination findings to both the severity of the clinical diagnosis and the radiographical diagnosis. Many clinical indicators, such as chest indrawing, are already included in the WHO clinical definitions so their presence or absence in levels of severity is predefined. Of the additional clinical indicators considered in this current study, at multivariate analysis the only significant associations with increased severity were a higher respiratory rate

(accounted for by children aged over twelve months), lower oxygen saturation and crepitations on auscultation. None of the potential clinical indicators were identified as beneficial in differentiating between the presence and absence of PEP. However, significant differences were observed between those children with and without radiographic changes consistent with ALRI (OI plus PEP). At multivariate analysis, the absence of nasal flaring and the presence of crepitations on auscultation remained significant predictors of radiographic changes (PEP and OI vs. nil). Given that the relationship between the aetiology and radiographic changes remains to be proven, if the WHO clinical diagnosis could be refined to include only those children with radiographic evidence of PEP or OI, this would offer a substantial improvement. Other symptoms and signs that have been observed to be significantly associated with the presence of PEP in previous studies include hypoxaemia, fever and time to presentation.

CREPITATIONS

Crepitations were significantly associated with a diagnosis of SP or VSP compared to those children with NSP (85% vs. 61%, P<0.001). At multivariate analysis children with SP or VSP were five times more likely to have crepitations on auscultation. In addition, although crepitations were present on auscultation in similar proportions of children with PEP and OI, there was a significant correlation between radiographic changes and crepitations on auscultation. Those children with crepitations were five times more likely to have radiographic changes consistent with ALRI than those without crepitations (OR 4.8, 95%CI 1.9 to 12.4). In Mozambique a similar observation was made. [251] In this study of children under two years old with SP, children diagnosed with PEP were nearly twice as likely to have crepitations than those without PEP (P=0.001). Again in the Gambian PCV

trial, in nearly four thousand cases of SP in children who had not received PCV, crepitations were present in significantly more children with PEP or OI than in children with nil on their radiograph ($P<0.001$). [80]

Before the availability of radiography, auscultation was the main technique used by clinicians to diagnose pneumonia. Despite the subjective nature of auscultation, it is still used by clinicians as an adjunct to radiography to facilitate the diagnosis of childhood pneumonia in developed countries. In resource-limited settings radiography is rarely available and the diagnosis of pneumonia still heavily relies upon clinical signs and symptoms. The current ARI case-management strategy does not include auscultatory findings in its clinical diagnosis as it is has been suggested that to identify crepitations by auscultation requires substantial training and expertise. [42] Many of the early studies carried out during the development of the ARI case-management strategy actually used the presence of crepitations on auscultation as equivalent to a diagnosis of pneumonia. [42, 44, 45, 48] Subsequent studies have observed a varying association between crepitations and radiographic consolidation and others have observed significant inter-observer variability in the detection of crepitations. [82, 254-257] However, given the association of crepitations both with increased severity and with radiographic changes consistent with ALRI, which were observed in this current study, maybe it is time to reconsider the possibility of standardising the technique of auscultation and the possibility of training health workers in the skill of auscultation.

HYPOXAEMIA

Hypoxaemia is a known risk factor for increased mortality in childhood pneumonia. [31-33] The presence of hypoxaemia (SpO_2 <90%) corresponds to a diagnosis of VSP and if available the need for oxygen therapy. In a recent review, the median prevalence of hypoxaemia (<90%) in children hospitalised with SP or VSP was 13.3% (IQR 9.3-37.5%). [30] In this current study, the prevalence of hypoxaemia in children admitted with SP and VSP was 20.5%, which although comparable, lies at the higher end of this range. This is in keeping with previous observations that the prevalence of hypoxaemia in childhood pneumonia is higher in Asia than in Africa. [30]

In many rural resource-limited settings, oxygen saturations are rarely measured and oxygen therapy is often not available. In these settings sick and hypoxic children are often referred to urban hospitals, but it has been found that less than a third of such children complete referral due to cost, transport and home responsibilities. [258] A retrospective study in Fiji found that even when pulse oximetry was available, oxygen was not administered in up to 30% of hypoxaemic patients. [250] However in this current study, oxygen was administered to all children with documented hypoxaemia, which suggests that with training local health workers can administer oxygen therapy appropriately. In this current study, of the eighteen children with hypoxaemia, only two had central cyanosis and therefore sixteen children would not have received oxygen therapy if pulse oximetry had not been used systematically for screening.

In this current study, no significant difference was observed in the presence of either cyanosis or the median oxygen saturation between radiographical diagnoses. However in

contrast, both in Mozambique and Fiji there was a significant association between hypoxaemia and PEP. [250, 251] In Mozambique, a study of children with SP found the presence of hypoxaemia was twice as likely in children with PEP than without (OR 1.74, P=0.002). [251] Similarly in nearly two hundred Fijian children with either SP or VSP, the rate of hypoxaemia was significantly higher in those children with PEP (P<0.05). [250] In contrast, a large study of nearly 4000 children with SP in The Gambia found no significant difference in the proportion of children with hypoxaemia between the three radiographical diagnoses. [80]

Although it remains uncertain whether hypoxaemia is related to the radiographic appearance, it is clear that a significant proportion of infants and children with clinical pneumonia have hypoxaemia. Given also that access to radiography in these settings is limited and that no accurate clinical indicators to identify hypoxaemia are available, screening for hypoxaemia using pulse oximetry and providing oxygen therapy should be both supported and encouraged. There is now growing evidence, including this current study, that this approach is both sustainable and affordable in resource-limited settings. [30, 49]

WHEEZE

The original IMCI and WHO guidelines did not differentiate between children with and without wheeze. [5] However, in response to a growing concern surrounding the case-management of children with wheeze, WHO recently supported a large studies in Pakistan and Thailand to assess the role of salbutamol in under-5s diagnosed with NSP or SP that also had wheeze at presentation. [259-261] These studies found between a 62% and 85% response to salbutamol in children with NSP and between a 27% and 71% response in

children with SP, and at least two-thirds of these patients continued to improve on salbutamol alone. The IMCI guidelines have since been revised to recommend a trial of salbutamol in any child with fast breathing and/or chest indrawing where wheeze is heard on auscultation before a diagnosis of pneumonia is given. [262, 263] In this current study the nurses and medical assistants were trained to identify wheeze by auscultation and give a trial of salbutamol to all those children in whom wheeze was present. Over one-quarter (26%) of children with a final diagnosis of clinical pneumonia at presentation were documented to have wheeze on auscultation. In addition the presence of wheeze was found to be significantly associated with increased severity. This highlights the need to encourage and support the use of salbutamol in resource-limited settings in children with clinical pneumonia and wheeze as this could reduce the morbidity from childhood pneumonia. In this current study, children with a radiographic diagnosis of PEP, OI and nil were equally as likely to present with wheeze. Although it is suggested that wheeze is associated with viral infections, over one-third of children (34%) with wheeze on presentation actually had radiographic evidence of PEP, which is associated with bacterial aetiology. In Pakistan, the study of children with NSP also found no difference in the presence of wheeze between the different radiographic diagnoses and similarly to the current study, one-quarter of children with wheeze had PEP on radiography. [249] Given the association of PEP with bacterial aetiology, the presence of wheeze does not therefore exclude a bacterial co-infection. The data from this current study support the use of the revised IMCI guidelines in this setting and therefore following a trial of salbutamol, all children with wheeze and clinical pneumonia should receive both salbutamol and antibiotics. [262, 263]

NASAL FLARING

This current study found the presence of nasal flaring to be significantly associated with increased severity of clinical pneumonia. Although none of the recent studies using the WHO clinical definitions have described the prevalence of nasal flaring, older studies found nasal flaring to be significantly associated with hypoxaemia. [32, 50, 256] Interestingly this current study also found nasal flaring to be significantly more likely in patients with nil on their radiograph (17.2%) compared to those with PEP or OI (5.0%). This suggests that nasal flaring could be a useful sign in discriminating those with and without bacterial pneumonia. However conflicting results have been published in Mozambique where a study of children under 2 years with SP found no difference in the prevalence of nasal flaring in children with PEP compared to children with OI or nil. [251] Given that very few studies have included this clinical sign in their assessment, further aetiological studies with larger numbers are needed to establish the role of nasal flaring in the diagnosis of childhood pneumonia.

FEVER

Although the presence of fever is not currently included in the WHO diagnosis of pneumonia, it has been suggested that fever could be used to improve the specificity of the clinical diagnosis of pneumonia. A previous study of bronchiolitis based in the United Kingdom found that children with fever were more likely to have radiographical evidence of pneumonia. [264] The data from this current study do not support this observation, as there was no significant difference in the mean temperature at presentation between the different radiographical diagnoses. This may in part be due to the high rate of self-

treatment in this region and many children may have already received anti-pyretic medication at home. [265] Similarly, the study of children with NSP in Pakistan and that of children with SP and VSP in Fiji both observed no difference in the presence of fever at presentation between children with and without PEP. [249, 250] In contrast, the study of children with SP in the Gambia did find that the temperature at presentation was significantly higher in those children with PEP than with OI or 'nil' but the difference was however minimal (median temperature 38.6°C vs. 38.2°C or 38.4°C, P<0.001). [80] Also in the study of Mozambican children with SP, the presence of fever (≥37.5°C) was associated with PEP compared to other radiographical diagnoses (P=0.02). [251]

TIME TO PRESENTATION

Radiographic changes of pneumonia may not be detected early on in the illness, therefore the time to presentation has been suggested as a reason for a low prevalence of PEP in children with a WHO clinical diagnosis of pneumonia. In this current study, children with 'nil' on their radiograph had a slightly, but significantly shorter time to presentation compared to those with either OI or PEP (median days to presentation 2 vs. 3; P<0.05). It is therefore possible that these children presented too early in their illness to detect any radiographic changes. A similar observation was made in The Gambia where in unvaccinated children with SP, the proceeding duration of illness was slightly longer in children with PEP. [80] However to the contrary, in Pakistan no difference in the time to presentation between radiographical diagnoses was observed. [249]

INVESTIGATIONS

Although the current study observed a trend towards a higher leukocyte count and neutrophil count in children with PEP or OI compared to children with 'nil' on radiography, the difference was not significant. In a large study of nearly 4000 children in the Gambia, the median leukocyte count was significantly higher in children with PEP than in children with either OI or nil on radiography (median count per $10^3/\mu l$, 16 vs. 12). [80] The other studies that have employed the WHO standardised radiographic diagnoses have not sought to correlate them to inflammatory markers levels. No significant difference in the level of CRP was observed between the three radiographic diagnoses in this current study. A recent meta-analysis found a high CRP (>40mg/l) to correlate with a diagnosis of bacterial pneumonia. [97] Supporting this observation, a study in Africa found PCV to have a greater efficacy against cases of clinical pneumonia in children with a CRP>40mg/l. [36] This current study found 28.3% (47/166) of children with clinical pneumonia had a CRP>40mg/l. At this level the aetiology is unlikely to be solely viral but only 36% (17/47) of these patients had PEP on radiography and only two grew a possible pathogen from their blood culture. This current study also found similar proportions of patients with a CRP>40mg/l in patients with PEP, OI and nil.

LIMITATIONS

Through basing the study at a clinic there is a risk that the results are biased towards the inclusion of children with more severe symptoms. It is possible that not all cases of NSP presented to the clinic, as self-treatment without medical assessment remains a widespread practice in this population. [265] There is also a possibility of survivor bias, as any children

who died at home without seeking medical treatment would not have been included. A cohort study with active surveillance would help capture both these sets of children and provide a more accurate representation of the severity of illness in this population. However such a study in this migrant population is challenging due to the mobile nature of such a population.

Although this current study sought to include young infants (under two months old), only nine were actually included. Neonates are estimated to contribute to 22% of the burden of pneumonia in under-5s and therefore an improved understanding of pneumonia in this age group is vital. [1] It is therefore important that this age group is captured in any future studies of pneumonia in this region. Longitudinal follow-up of a birth cohort could enhance the information collected from episodes of pneumonia in young infants.

In addition to the lack of radiology facilities and the financial barriers to acquiring chest radiographs in resource limited settings, the radiograph quality is highly dependent on the radiographer. In this current study, over one-third of the radiographs were classified as suboptimal which emphasises the difficulty in obtaining good quality paediatric radiographs in resource-limited settings. This high proportion of suboptimal radiographs may have affected their interpretation.

CHAPTER 6

THE VIRAL AETIOLOGY OF CHILDHOOD PNEUMONIA IN MIGRANT INFANTS AND CHILDREN LIVING ON THE THAI-MYANMAR BORDER

INTRODUCTION

Every year nearly 9 million children continue to die before reaching their fifth birthday and pneumonia is the leading cause, accounting for nearly one-fifth of these deaths. [1] The countries with the highest incidence of childhood pneumonia are also some of the poorest. Estimates are particularly high in South-East Asia and Myanmar has the third highest incidence of childhood pneumonia in the world. [9] The main objective of this study was to define the viral aetiology of clinical pneumonia, as defined by WHO, in migrant infants and children living on the Thai-Myanmar border. In addition the study sought to compare the aetiology with the clinical severity and radiographic changes.

Infant vaccinations and case management are two key interventions available to reduce childhood morbidity and mortality from pneumonia in resource-limited settings. [2] For effective implementation of these interventions, the aetiology of childhood pneumonia needs to be understood. Achieving a true aetiological diagnosis for individual children is challenging, even in the developed world. [81] In resource-limited settings, the lack of diagnostic facilities means that cases of childhood pneumonia are often treated empirically. In such settings research can improve the knowledge of the local aetiology and the form the basis of effective treatment and appropriate immunisation programmes.

A wide variety of pathogens are known to cause pneumonia in children but bacterial and viral pathogens account for the majority of cases. If vaccinations against Hib and pneumococcus can be implemented globally, the role of viral pathogens will become more important. Aetiological studies using traditional techniques identified viruses in up to a third of children with ALRI in the community. [89, 134, 135] Whilst studies of children hospitalised with ALRI found viruses in up to half of children. [21, 71, 131-133] An increasing number of studies in the developing world are now using PCR to identify viruses in children with pneumonia and have found viral pathogens in an even greater proportion of children. [17, 18, 136-138]

Chest radiography still remains the best method for defining the presence of pneumonia in children but unfortunately the changes observed do not correlate fully with the aetiology. [71-75, 248] WHO has recently developed guidelines for the standardised interpretation of paediatric chest radiographs. [79] Their use in vaccine trials has demonstrated a correlation between bacterial aetiology and PEP. [34, 36, 37] The relationship between the viral aetiology of childhood pneumonia and the radiographical interpretation is even less well understood. Since the introduction of molecular diagnostic tests, the detection of viral pathogens in children with pneumonia has improved. However as of yet no studies have sought to correlate the detection of viral pathogens with the WHO standardised radiographic diagnoses.

The lack of diagnostic tests and surveillance systems in many resource-limited settings means that the aetiology of childhood pneumonia in many of these areas remains poorly understood. The Thai-Myanmar border is no exception and no previous aetiological studies of childhood pneumonia either in Myanmar itself or in the migrant population living along

the border with Thailand have been published. Because the aetiology of childhood pneumonia can vary between countries and regions, it is vital to gain knowledge of the local aetiology to plan effective future interventions. This current study aimed to establish the viral aetiology of clinical pneumonia as defined by WHO in migrant infants and children presenting to a rural clinic on the Thai-Myanmar border.

METHODS

A prospective study of the viral aetiology of clinical pneumonia in migrant infants and under-5s was conducted at the SMRU clinic in Mawker Thai village on the Thai-Myanmar border between the 12th October 2009 and 12th October 2010. Each child had a clinical history and physical examination undertaken by a medical assistant or nurse. A standardised paediatric clinical questionnaire (Appendix 1) was completed for each patient to document the presence and duration of symptoms commonly associated with childhood illness. A standard set of vital signs were recorded for each child; pulse rate (PR), respiratory rate (RR), oxygen saturations and temperature. Oxygen saturations were recorded from the finger or toe of the child using a hand-held pulse oximeter (Respironics/Novametrix oximeter, model 512) with a paediatric sensor (Y-sensor). For infants, a rectal temperature was recorded using a mercury thermometer inserted into the anus for 1 minute. Whilst for those children one year and older, a tympanic temperature was recorded (Braun IRT-4520 Thermoscan Ear Thermometer). The standard clinical examination comprised of clinical assessment of conscious level, colour, level of hydration, observation for respiratory distress, chest auscultation, heart auscultation, ENT examination and palpation of liver and spleen. The examination findings were recorded on

the same paediatric clinical questionnaire. A short demographic questionnaire (Appendix 2) was also completed to document details of the child's home environment.

The local medical assistants and nurses were trained to diagnose clinical pneumonia using local paediatric guidelines based on the IMCI and WHO guidelines. [5, 65, 246] According to these guidelines, any child with wheeze was given a trial of rapid-acting bronchodilator before being classifed as pneumonia (Appendix 3). The severity of each case was also established and a diagnosis of NSP, SP or VSP was given. [5, 65]

After completion of a full history and examination, if the caregiver consented, a CBC, CRP, NPA and a blood culture were requested for each child with clinical pneumonia as part of routine clinical care. All children with a history of fever or a fever at presentation had a blood film taken for on-site microscopic examination for malaria in line with local protocols.

Venous blood was collected using an aseptic technique, cleaning the skin twice with povidine-iodine solution and a third time with alcohol before collecting blood (FIGURE 9). Blood collected was introduced directly into the BacT/ALERT PF blood culture bottle (1-3mL) and EDTA tube (0.5mL). The samples were mixed and stored at room temperature or refrigerated respectively until transfer to the central SMRU laboratory in Mae Sot. Blood cultures were incubated for five days using a BioMerieux BacT/Alert system and cultured organisms were identified using standard bacteriological techniques.

FIGURE 9: VENEPUNCTURE IN A YOUNG INFANT WITH PNEUMONIA (left) **NASOPHARYNGEAL ASPIRATION SPECIMEN COLLECTION** (centre, right)



A sterile 8-French naso-gastric tube connected to a 20 mL syringe was used to collect an NPA specimen from each child (FIGURE 9). [186] The naso-gastric tube was inserted horizontally via the child's nostril to the nasopharynx, using the distance from the child's nostril to its earlobe to estimate how far to insert the tube. Suction was then applied to the nasogastric tube using the syringe and the nasogastric tube was withdrawn. The nasopharyngeal secretions were transferred into a 1mL tube of viral transport medium together with the tip of the naso-gastric tube. The NPA specimens were transferred in a cool box to the central SMRU microbiology laboratory where they were stored at -80°C prior to testing for viral pathogens.

All PCR assays were performed at the SMRU microbiology laboratory in Mae Sot. The viral nucleic acids were extracted from the NPA specimens using a QIAamp viral RNA mini kit (Qiagen, Germany) following the manufacturer's instructions. Real-time reverse transcription-polymerase chain reaction (rRT-PCR) reactions were performed on all NPA specimens using assays for the following viruses: InfA, [266] InfB (CDC assay), RSV (CDC assay), ADV (CDC assay) and hMPV. [186, 267] In addition, all NPA specimens that were

positive for influenza A were also H-typed using separate rRT-PCR assays for seasonal H1N1, seasonal H3N1 and pandemic H1N1. [266]

To monitor the adequacy of each specimen and detect the presence of PCR inhibitors, a control for the human Ribonuclease P (RNase P) gene was run for each sample. [186] Each PCR run also included a negative control to ensure there was no contamination and a positive control to ensure the assay was working correctly. If the results of these control assays confirmed the PCR had run as expected and the viral PCR cT value was less than 40, the NPA specimen was considered positive for that particular virus. If a viral PCR cT value was 35 -39 then the sample was run a second time and only if the second run also had a cT value less than 40 was the NPA specimen considered positive for that virus.

To assess the presence of radiographic pneumonia, a chest radiograph was also requested for each child at the local Thai hospital in Phop Phra. All radiographs were read independently by two physicians trained in the WHO standardised method for interpretation. If these first two reading were in disagreement, a third independent reading of the radiograph was undertaken by another physician trained in the same method. [79] The final interpretation was reported as the majority or when all three readings differed, the radiograph was reported as uninterpretable.

Treatment for pneumonia and other illnesses was provided free of charge and in accordance with the local SMRU Paediatric Guidelines.

RESULTS

During the study period 175 infants and children with a clinical diagnosis of pneumonia were treated and an NPA was performed in 171 (98%) of them. Of these 171 children, 59% of them were male and their median age was 13.0 months (IQR 5.9 to 21.6). 94/175 (53.7%) were treated in the Outpatient Department (OPD) and 81/175 (46.3%) were admitted to the Inpatient Department (IPD) of Mawker Thai clinic. The demographic profile of the children was not significantly different to the overall study population described in TABLE 6.

At least one virus was identified in 49.1% (84/171) of all patients with an NPA (TABLE 23) and a total of 94 viruses were isolated from the 171 specimens. Multiple viruses were identified in 10.7% (9/84) of the positive specimens and in 5.3% of all patients (9/171). The most common combination to be identified was RSV with ADV, which was found in five cases. In one patient, three different viruses were identified, the combination of which was RSV, ADV and hMPV.

TABLE 23: THE NUMBER OF VIRUSES IDENTIFIED IN ALL CASES AND ALL POSITIVE ISOLATES

Number of viruses	No. of isolates	Percentage of all cases, n=171 (%)	Percentage of positive isolates, n=84 (%)
At least 1 virus	84	49.1	
1 virus	75	43.9	89.3
2 viruses	8	4.7	9.5
3 viruses	1	0.6	1.2

In addition to an NPA, a blood culture was performed in 98.2% (168/171) of the patients. Potentially pathogenic bacteria were cultured from four patients: *Moraxella catarrhalis, Acinetobacter baumannii*, *Staphylococcus aureus* and *Salmonella* sp. Cultures remained negative after 5 days in 72.0% (121/168) of the samples, whilst 25.6% (43/168) of the samples grew likely contaminants. None of the patients with a positive blood culture had a virus identified in their NPA.

VIRAL AETIOLOGY

The most commonly identified virus was ADV, which was identified in 48.8% (41/84) of positive NPAs, followed by RSV in 44.0% (37/84) then hMPV (13.1%) and InfA (6.0%) (FIGURE 10). Influenza B (InfB) was not isolated from any of the patients in this study (TABLE 24).

FIGURE 10: THE DISTRIBUTION OF VIRUSES IDENTIFIED IN ALL PATIENTS

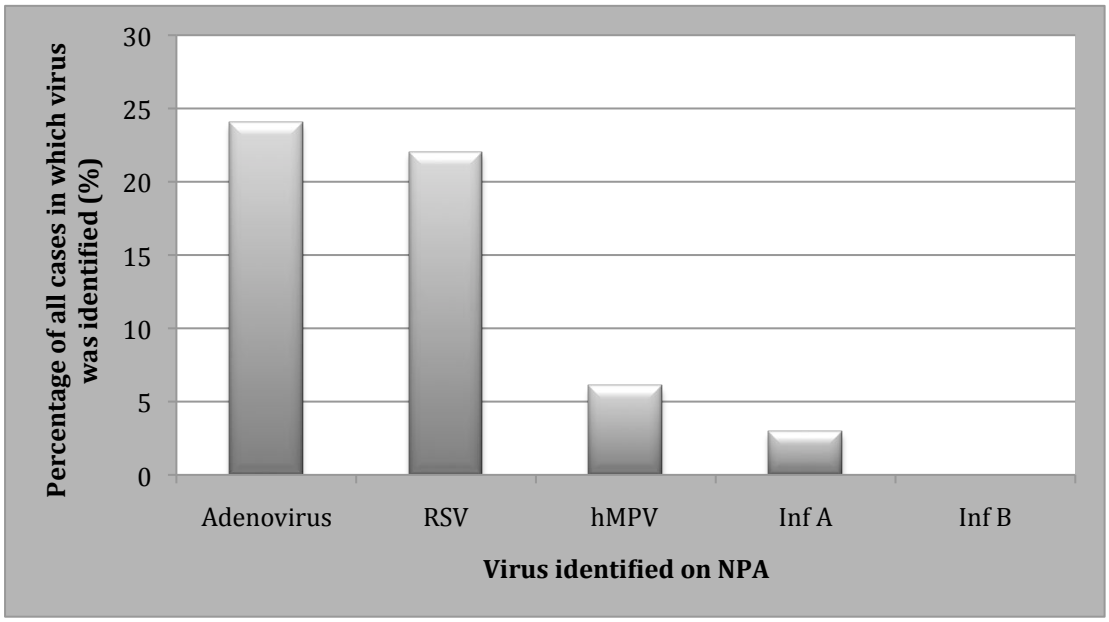


TABLE 24: VIRUSES IDENTIFIED IN ALL PATIENTS AND ALL POSITIVE ISOLATES

Virus identified	% of all cases, n=171	% of positive isolates, n=84
Adenovirus	41/171 (24.0)	41/84 (48.8)
RSV	37/171 (21.6)	37/84 (44.0)
hMPV	11/171 (6.4)	11/84 (13.1)
Influenza A	5/171 (2.9)	5/84 (6.0)
Influenza B	0	0

SEASONAL VARIATION

Cases of childhood pneumonia were observed year-round with the largest number of cases observed during the winter months of September to December. Rainfall is the main seasonal variable in this area and this peak of pneumonia cases coincided with the end of the rainy season (**FIGURE 11**). The number of cases of pneumonia with RSV, ADV, InfA and hMPV isolated per month is shown in **FIGURE 12**. The total number of pneumonia cases can be seen to closely follow the activity of RSV (**FIGURE 12**). The number of cases of RSV was greatest in the winter months after the rainy season had ended (September to December) and peaked in October. The cases of hMPV peaked at a similar time in September and October and adenoviral infections appear to contribute to a small peak of pneumonia cases in May during the rainy season. The number of cases of InfA did not vary throughout the year.

FIGURE 11: TOTAL NUMBER CHILDHOOD PNEUMONIA CASES SEEN PER MONTH IN RELATION TO THE TOTAL RAINFALL PER MONTH (FROM 12TH OCTOBER 2009 UNTIL 12TH OCTOBER 2010)

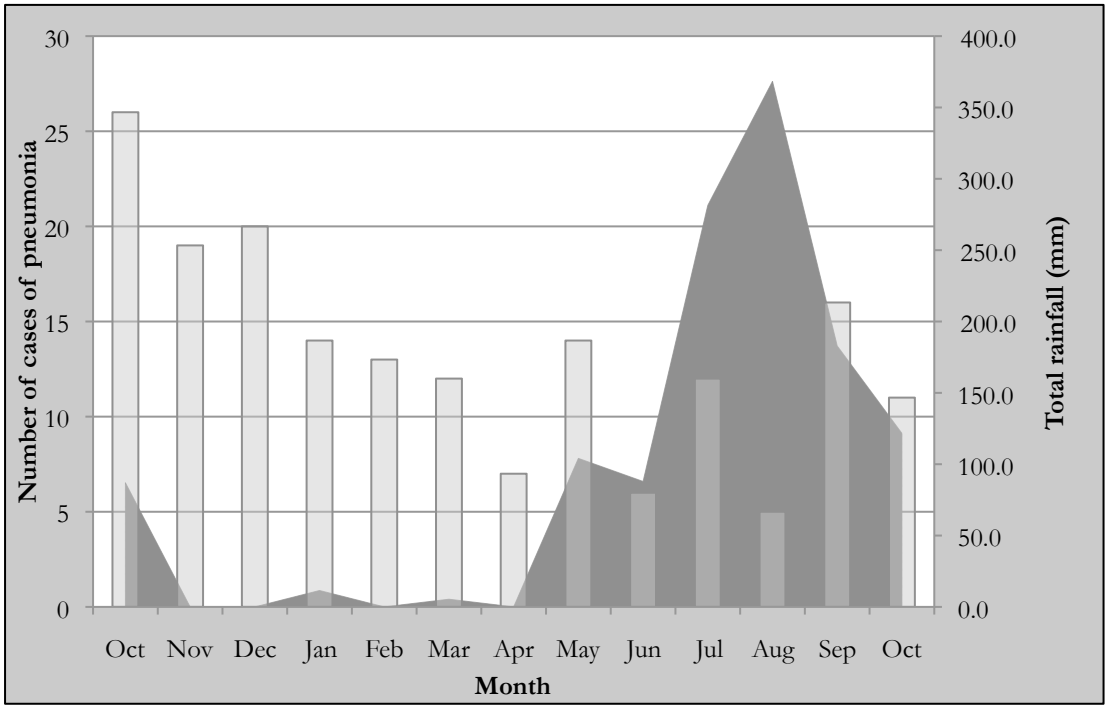
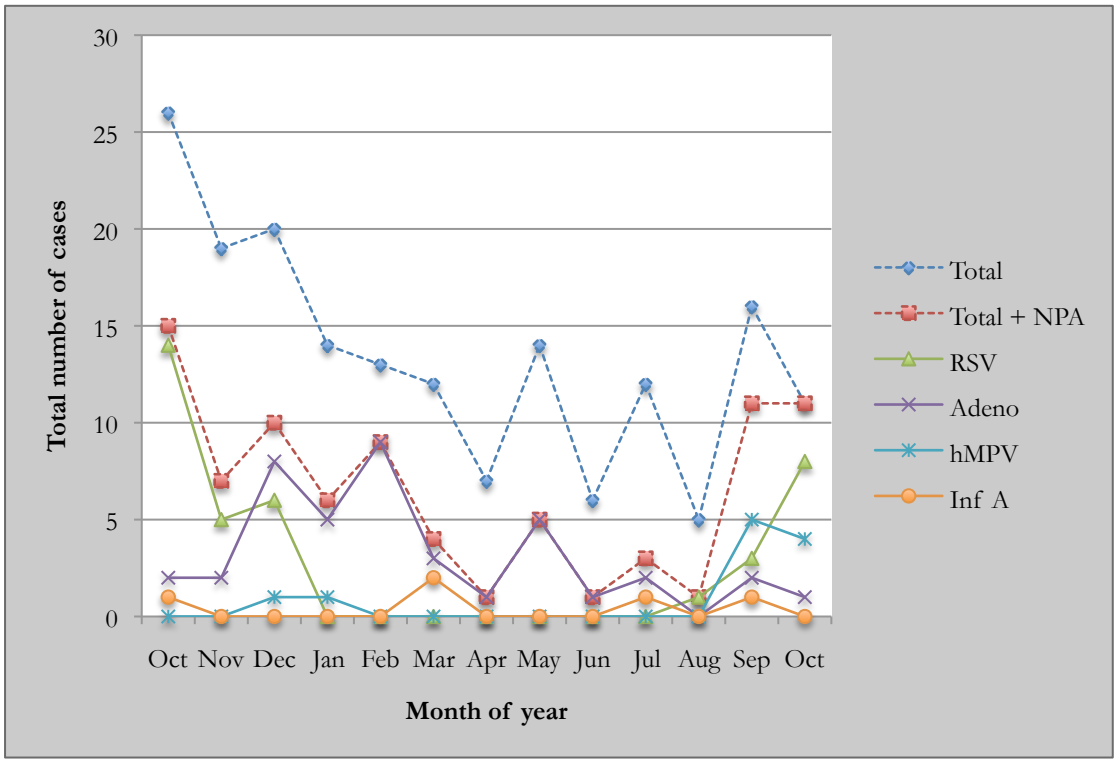


FIGURE 12: GRAPH SHOWING THE MONTHLY DISTRIBUTION OF VIRUSES DETECTED DURING THE STUDY (12TH OCTOBER 2009 UNTIL 12TH OCTOBER 2010)



Children with at least one respiratory virus detected had a median age of 9.9 months (IQR 5.1 to 21.3) and were younger than those with no virus detected (median age of 14.2 months (IQR 6.3 to 21.6) (TABLE 25). Among those infected with at least one virus, over half (56.0%) were infants (less than 12 months old), compared to only 40.2% of patients with a negative NPA. Therefore those patients with a positive NPA were more likely to be infants (P=0.047). There was no significant difference in the proportion of male patients with and without viruses isolated on NPA.

TABLE 25: AGE AND SEX OF PATIENTS WITH AND WITHOUT VIRUSES ISOLATED ON NPA

	≥1 virus isolated from NPA **n=84**	**No virus isolated from NPA** **n =87**	***P value***
Median age, months (IQR)	9.9 (5.1-21.3)	14.2 (6.3-21.6)	0.13
No. children 0 to 11 months (%)	47 (56.0)	35 (40.2)	0.047
No. male patients (%)	47 (56.0)	54 (62.1)	0.44

RSV and hMPV were identified in younger children with a median age of 9.0 months (IQR 4.2 to 17.8) and 8.0 (IQR 5.5 to 25.0) respectively (TABLE 26). Two-thirds of patients infected with RSV (62.2%) or hMPV (63.6%) were infants. Conversely, ADV was identified in older children with median age of 14.2 months (IQR 8.3 to 29.8) and only 41.5% of adenoviral positive isolates were from infants.

TABLE 26: THE AGE DISTRIBUTION OF PATIENTS IN RELATION TO VIRUSES DETECTED ON NPA

Virus isolated	Median age, months (IQR)	No. of infants with virus isolated (%)
Adenovirus n=41	14.2 (8.3 – 29.8)	17/41 (41.5)
RSV n=37	9.0 (4.2 – 17.8)	23/37 (62.2)
hMPV n=11	8.0 (5.5 -25.0)	7/11 (63.6)
Influenza A n=5	15.7 (3.4 – 36.0)	2/5 (40.0)

Of the 171 cases of pneumonia in which a NPA was collected a clinical diagnosis of NSP, SP and VSP was given to 86 (50.3%), 43 (25.1%) and 42 (24.6%) children respectively. Viruses were identified in approximately half of the cases of NSP, SP and VSP (TABLE 27). There was no evidence of an association between the presence of one or more viruses on NPA and severity of disease.

TABLE 27: THE SEVERITY OF CLINICAL PNEUMONIA COMPARED TO THE VIRUSES DETECTED

	Non-severe pneumonia (P) n=86	Severe pneumonia (S) n=43	Very severe pneumonia (VSP) n=42	Total n=171
No. NPA positive isolates (%)	42/86 (48.8)	23/43 (53.5)	19/42 (45.2)	84 (49.1)
No. RSV positive isolates (%)	15/86 (17.4)	9/43 (20.9)	13/42 (31.0)	37 (21.6)
No. Adenovirus positive isolates (%)	26/86 (30.2)	9/43 (20.9)	6/42 (14.3)	41 (24.0)
No. hMPV positive isolates (%)	6/86 (7.0)	5/43 (11.6)	0/42 (0.0)	11 (6.4)
No. Influenza A positive isolates (%)	2/86 (2.3)	2/43 (4.7)	1/42 (2.4)	5 (2.9)

RSV was isolated from 21.6% of all pneumonia cases and was the most common virus identified in cases of VSP. VSP was diagnosed in 42 children and 13 (31.0%) of these had an RSV infection. Although the proportion of RSV positive isolates increased with severity of pneumonia this was not statistically significant. ADV was isolated from 24.0% (41/171) of all pneumonia cases (TABLE 27). Although not statistically significant, the proportion of ADV positive isolates was much higher in patients with NSP compared to SP and VSP.

Of the 171 children with an NPA collected, 164 (95.9%) of these children had a chest radiograph taken. As previously described these were interpreted using WHO standardised method. 37.8% (62/164) of radiographs had evidence of PEP, 34.8% (57/164) had evidence of OI and 17.7% (29/164) had no infiltrate or pneumonia. In addition, 9.8% (16/164) of the radiographs were reported as uninterpretable, and no patients had evidence of a pleural effusion.

Of those children with PEP, 51.6% (32/62) had a virus identified, whilst a virus was indentified in 49.1% (28/57) of children with OI and 37.9% (11/29) of children with nil (TABLE 28). Therefore children with changes reported on their radiograph (PEP plus OI) were more likely to have a virus detected (60/119, 50.4%) than those with nil (11/29, 37.9%) (TABLE 29). As shown in FIGURE 13, the most commonly identified virus in children with PEP was RSV (17/62, 27.4%), followed by ADV (14/62, 22.6%). RSV itself was more frequently identified in patients with PEP (17/62, 27.4%), than in patients with OI (11/57, 19.3%) or nil on their radiograph (3/29, 10.3%) (TABLE 28).

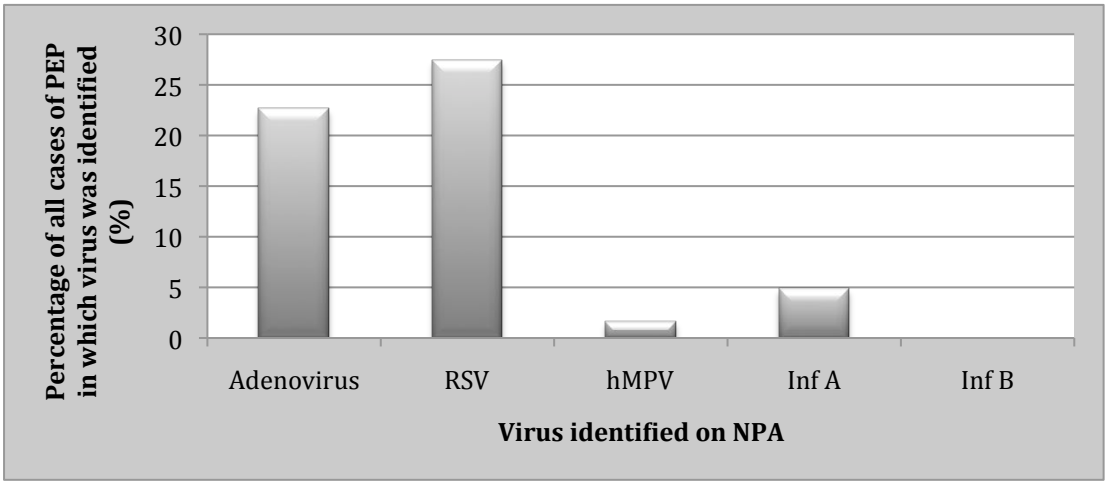
TABLE 28: THE DISTRIBUTION OF VIRUSES COMPARED TO THE RADIOGRAPHICAL DIAGNOSES

	RADIOGRAPHICAL DIAGNOSIS			
	Primary endpoint pneumonia	**Other infiltrate**	**Nil**	**Uninterpretable**
	n=63	**n=57**	**n=29**	**n=17**
Total no. NPA collected	62	57	29	16
No. NPA positive isolates (%)	32/62 (51.6)	28/57 (49.1)	11/29 (37.9)	10/16 (62.5)
No. RSV positive isolates (%)	17/62 (27.4)	11/57 (19.3)	3/29 (10.3)	5 (29.4)
No. ADV positive isolates (%)	14/62 (22.6)	12/57 (21.1)	8/29 (27.6)	4 (25.0)
No. hMPV positive isolates (%)	1/62 (1.6)	7/57 (12.3)	0	1 (6.3)
No. InfA positive isolates (%)	3/62 (4.8)	2/57 (3.5)	0	0

TABLE 29: THE DISTRIBUTION OF VIRUSES COMPARED TO RADIOGRAPHICAL CHANGES

	Primary endpoint pneumonia PLUS other infiltrate **n=120**	**Nil** **n=29**	***P value***
Total no. NPA collected	119	29	
No. NPA positive isolates (%)	60/119 (50.4)	11/29 (37.9)	0.300
No. RSV positive isolates (%)	28/119 (23.5)	3/29 (10.3)	0.135
No. ADV positive isolates (%)	26/119 (21.8)	8/29 (27.6)	0.623
No. hMPV positive isolates (%)	8/119 (6.7)	0	0.356
No. InfA positive isolates (%)	5/119 (4.2)	0	0.583

FIGURE 13: THE DISTRIBUTION OF VIRUSES DETECTED IN CASES OF PRIMARY END-POINT PNEUMONIA



There was no significant difference observed between the clinical presentation of those with a virus isolated in their NPA compared to those with no virus isolated (TABLE 30). Both the triage observations and the clinical examination were similar between the two groups (P value > 0.05).

TABLE 30: THE CLINICAL PRESENTATION OF PATIENTS WITH AND WITHOUT VIRUSES

	Virus isolated from NPA n=84	**Negative NPA n =87**	***P values***
Observations			
Mean triage temperature ºC (SD)	37.6 (0.9)	37.4 (0.93)	0.092
Mean triage oxygen saturations (SD)	92.8 (5.2)	93.7 (4.6)	0.664
Mean heart rate (SD)	141.7 (19.1)	139.7 (16.1)	0.472
Mean respiratory rate (SD)	57.3 (11.4)	55.5 (9.9)	0.289
Examination			
Patients with cyanosis (%)	2 (2.4)	1 (1.1)	0.616
Patients with grunting (%)	3 (3.6)	3(3.4)	1.000
Patients with stridor (%)	0 (0)	1 (1.1)	1.000
Patients with tracheal tug (%)	23 (27.4)	28 (32.2)	0.509
Patients with nasal flare (%)	4 (4.8)	8 (9.2)	0.371
Patients with chest indrawing (%)	42 (50)	42 (48.3)	0.879
Patients with head-bobbing (%)	9 (10.7)	15 (17.2)	0.273
Crepitations on auscultation (%)	64 (76.2)	62 (71.3)	0.492
Wheeze on auscultation (%)	18 (21.4)	26 (30.7)	0.225

DISCUSSION

During the one-year study period, this current study demonstrated a high prevalence of respiratory viruses among infants and children with clinical pneumonia in this rural resource-limited setting in South-East Asia. Using PCR, at least one virus was detected in 49% of childhood pneumonia cases. It is not easy to compare this prevalence directly to that of other aetiological studies of childhood pneumonia due to the variation in study settings, diagnostic techniques, age groups, viruses screened for and case definitions employed. This current study is most comparable to other hospital-based studies looking at both ambulatory and hospitalised cases of childhood ALRI in Asia. Early studies of this nature used traditional techniques and found viral pathogens in between 36% and 45% of children with ALRI. Firstly in urban Bangkok, a two-year study of nearly 600 under-5s used serology and immunofluorescence (IF) to screen for InfA, InfB, RSV, PIV1, PIV3, ADV. [268] Viral pathogens were found in 45% of children with ALRI, half of which were ambulatory cases. A second large study of both ambulatory and hospitalised under-5s was carried out over seventeen months at two hospitals in Pakistan and again half of the cases were ambulatory. [131] Using culture and IF to screen for the same viral pathogens as in Bangkok, viruses were found in 39% of in-patients and 36% of out-patients with ALRI. A smaller study of three hundred children in southern India also used culture and IF to screen for the same pathogens and detected viruses in 49% of children under six years old with ALRI. [132] More recently PCR has been used in two studies of ambulatory and hospitalised children however despite the increased sensitivity of PCR, similar proportions of viral pathogens to the earlier studies using traditional techniques were detected. This may be because neither study using PCR screened for ADV. The first study was in Delhi and screened for RSV, PIV1-3, hMPV, InfA and InfB in under-5s with ALRI. [211] Again

around half of cases studied had NSP (57%) and viral pathogens were detected in 35% of children overall. [211] Another recent three-year hospital-based study of over two thousand Nepalese children aged two months to three years of age, screened for the same viruses as in Delhi and identified at least one virus in 40% of clinical pneumonia cases. [18] Although this study included cases of SP the majority of cases studied were NSP. Apart from these two studies by Mathisen et al. and Bharaj et al., other studies that have used PCR in the diagnosis of viral-associated childhood pneumonia in Asia have focused on hospitalised children. Therefore it is possible that any differences observed may be due to the increased severity of the hospitalised cases, although as discussed in more detail below this is unlikely. The differences observed in these studies of hospitalised children are more likely to be due to the wider diagnostic panels used compared to the aforementioned studies and in this current study. Two such studies of hospitalised children have screened for the same viruses as this current study together with PIV, coronavirus (HCoV), bocavirus (hBoV) and rhinovirus (RV) and in the second study, Do et al. also screened for enterovirus (EV). [136, 137] In the study by Yoshida et al. in Vietnam, at least one viral pathogen was found in 70% of Vietnamese under-5s. Whilst in the study by Do et al., viral pathogens were detected in 72% of Vietnamese children aged between two months to fifteen years old. Yoshida et al. observed a substantial contribution by RV (27%) which was not screened for in this current study. [137] Although Do et al. only found RV in 4% of cases, the proportion of HBoV, EV and HCoV cases were much higher and were identified in 16%, 9% and 8% of cases respectively. [136] None of these viruses were screened for in this current study and this in itself may account for the higher proportion of viral-associated pneumonia found in these studies of hospitalised children. The overall high prevalence of viral pathogens detected in these studies of children with clinical pneumonia in Asia, supports the findings

of this current study and suggests that viruses play a fundamental role in the development of childhood pneumonia in this region.

Other studies using molecular diagnostic techniques have been carried out in Africa, where the proportions of viral-associated pneumonia were considerably lower taking into consideration the wider panel of viral pathogens they screened for. A study in Mozambique of under-5s admitted with SP, used PCR to screen for twelve viruses including RSV, InfA, InfB, PIV, RV, EV, ADV, hMPV. [138] However despite this wide diagnostic panel, this study only found a virus in 49% of cases and a large proportion of these were due to RV (41%) and ADV (21%). In Kenya, a study of children under twelve years old admitted with SP and VSP also used PCR to screen for a wide panel of viruses (RSV, InfA, InfB, PIV, ADV, hMPV, hBoV and hCoV) but only found a virus in 56% of cases. [17] It is not inconceivable that the viral aetiology could be different between Africa and Asia and that these viral pathogens play a more pertinent role in Asian regions. However, even the studies within Asia show variation between countries; this highlights the need for localised aetiological studies to aid the planning and development of future interventions.

AGE DISTRIBUTION OF VIRAL AETIOLOGY

Viral-positive cases of pneumonia were more likely to be observed in infants than viral-negative cases were (P = 0.05) and children with a viral-associated pneumonia were younger (median 9.9 months, IQR 5.1 to 21.3) than those with no virus detected (median 14.2 months, IQR 6.3 to 21.6). Other studies in Asia have not observed any significant difference in the age of viral-positive and viral-negative pneumonia cases. In India, the median age of children with viral-positive ALRI was 12 months, whilst in viral-negative

cases it was 10 months. [211] The median age in both viral-positive and viral-negative cases of ALRI in hospitalised Vietnamese children was 17 months. [137] A second study in Vietnam also found similar median ages in viral-positive (22 months, IQR 14-35 months) and viral-negative (24 months, IQR 14-36 months) cases. [136] Similar observations to this current study have however been observed in children hospitalised with ALRI in Kenya. [17] In this study, the median age of children with viral-associated pneumonia was significantly lower (7.5 months, IQR 2.7 to 18) than viral-negative cases (11months IQR 3.8 to 24). More studies are needed to explore this relationship between age and viral aetiology, as it will be important to focus future interventions, particularly immunisations at the correct age group.

THE CLINICAL FEATURES AND SEVERITY OF VIRAL-ASSOCIATED PNEUMONIA

This current study found no difference between the clinical features of children with viral-positive ALRIs compared to those children with viral-negative ALRIs. Similar observations have been made in other studies of viral aetiology that have attempted to differentiate viral positive and viral negative cases based on clinical signs alone. A study of children hospitalised with ALRI in Vietnam found no difference in the frequency of fever, fast breathing, cyanosis, chest indrawing, stridor, wheeze or crepitations between children with and without viral pathogens. [136] A hospital-based study of both ambulatory and hospitalised children in India compared both signs and symptoms of respiratory illness between viral positive and viral negative cases and found no significant difference between the two groups. [211] In Kenya, only the presence of wheeze, hypoxaemia and prolonged capillary refill were compared and no association between their presence and the detection of a viral pathogen was observed. [17]

This absence of differences in the clinical presentation of viral-positive and viral-negative cases of ALRI in children may be due to a variety of reasons. Firstly, in this current study it is possible that the viral-negative cases may be due to viruses not screened for. Secondly, as suggested by the vaccine trials, a large proportion of both viral-positive and viral-negative cases may have an associated bacterial aetiology creating an overlap in presentation.

SEVERITY OF VIRAL AETIOLOGY

Viral pathogens were identified in around half of cases of clinical pneumonia in all levels of severity in this current study. The lack of association between the presence of viral pathogens and the severity of the disease has also been described in Vietnam, where viruses were detected in 71% of children hospitalised with an ALRI compared to 73% of children who required intensive care for an ALRI. [136] The same observation was made in the hospital based study in India, where the proportions of viral pathogens detected by PCR in NSP, SP and VSP were 35%, 36% and 38% respectively. [211] The hospital-based study in Pakistan of both ambulatory and hospitalised patients did not classify the severity of the pneumonia but identified viral pathogens in similar proportions of children treated as outpatients compared to in-patients, 64% and 61% respectively. More data are needed to confirm this observation, however if it is indeed the case that the prevalence of viruses in childhood pneumonia is the same regardless of severity, then the role of additional factors in the development of disease needs further investigation.

CO-DETECTION OF VIRAL PATHOGENS

This current study sought to identify 5 different viruses by PCR and multiple viruses were identified in 5.3% of cases. In Nepal PCR was used to screen for a similarly limited panel of only 7 viruses, and identified multiple viruses in 3.3% of children with pneumonia. [18] In Vietnam and Mozambique between 12 and 15 different viral pathogens were screened for and multiple viruses were found in between 12% and 21% of cases. [136-138]

The most common combination of viral pathogens identified in this current study was RSV with ADV. Other studies using PCR have found different combinations to be more common. The most frequent co-detection identified in Nepalese children was PIV with hMPV but this study did not screen for ADV. [18] In Vietnam, although ADV was screened for, hBoV was the most common virus involved in co-detection. [136] In Mozambique, ADV and EV had the highest rates of co-detection. [138]

The proportion of viral co-detections and the nature of the combinations vary widely between the studies. This is likely to be due to the differences in the number of pathogens screened for. [269] It may also be related to severity, as much higher proportions of ambulatory patients were included in this current study and the study of Mathisen et al. than in Vietnam and Africa. The clinical significance of viral co-detections remains unclear. [270] It is probable that the presence of multiple pathogens plays a role in the pathogenesis and severity of pneumonia as has previously been observed in the case of influenza and pneumococcus. [195] It is also possible that when more than one virus is detected, only one virus is pathogenic and additional viruses may be present due to persistent nasal carriage. [244]

Larger longitudinal studies of naso-pharyngeal carriage of viral pathogens in children, in relation to acute episodes of pneumonia could help to clarify this.

RESPIRATORY SYNCYTIAL VIRUS

In this current study RSV was the second most commonly identified viral pathogen after ADV and was detected in the NPA of 22% of all clinical pneumonia cases. Although most studies in this region have found RSV to be the primary pathogen, the prevalence of RSV-associated cases that have been reported, are comparable to this current study. In Thailand, a two-year study of nearly six hundred ambulatory and hospitalised under-5s identified RSV in 20% of children using serology and IF. [268] Two smaller hospital-based studies in India used culture and IF to identify viral pathogens in children with ALRI. The first study in southern India detected RSV in 32% of children under the age of six over a two-year period. [132] The second study in Delhi found RSV in 17% of under-5s. [239] A larger hospital-based study of nearly 1500 under-5s in Pakistan using the same techniques found RSV in 33% of cases over two years. **[131]** In more recent studies of childhood ALRI in Asia, PCR has been used to identify RSV. With the increased sensitivity of this technique it would be expected that RSV might be detected in a higher proportion of cases. However in Nepal, a large study of over two thousand cases of NSP and SP in children under three years of age, RSV was only found in 15% of cases. It is possible that this low prevalence is related to the high proportion of NSP cases included (94%). In another study of three hundred under-5s with ALRI in India, 43% of the cases included were diagnosed with SP and VSP and the prevalence of RSV was higher, in 20% of cases. [211] Other studies using PCR in Asia that have focused only on children hospitalised with pneumonia have found the proportion of RSV-positive cases to be higher still. Two such studies have been carried out in Vietnam.

The first study of three hundred children over a period of three years identified RSV in 24% of children and the second study of nearly six hundred patients identified RSV in 22% of children over a fourteen-month period. [136, 137] Overall the results from these studies are comparable to this current study where half of the cases were diagnosed with NSP and RSV was detected in 22% of cases.

Given the higher prevalence of RSV in the studies of hospitalised children described above, it is not surprising that the prevalence of RSV-positive cases in this current study increased with severity. RSV was identified in 17% of NSP, 21% of SP and 31% of VSP. Likewise, the study in Nepal using PCR, identified RSV twice as often in children with SP compared to those with NSP (27% v. 15%). [18] Similar results were observed in a community-based study in rural India that followed infants until three years of age, using IF they found RSV in 7% of NSP and 21% of SP. [154] However, a two-year study that used serology and IF to look for RSV in both ambulatory and hospitalised children with ALRI in Thailand found no difference (21% vs. 20%). [268]

In this current study RSV-associated ALRI was more common in younger children; infants accounted for 62% of RSV infections but only 40% of viral-negative cases (P value = 0.05). In the hospital-based study of both ambulatory and hospitalised children in Pakistan, 55% of RSV cases were infants compared to 40% of cases where no virus was detected. [131] In a similar study in India, a significantly higher proportion of RSV-associated cases were found to be infants (76%) compared to virus-negative cases (59%). [211] The study in Vietnam by Do et al. did not find the same association but this may be due to the small proportion of infants included in the study overall, which is reflected in the lower proportions of infants

in both RSV-positive cases (16%) and viral-negative cases (21%). The association between younger age and an increased RSV prevalence has also been reported in Africa. [17]

The detection of RSV in nearly a quarter of childhood pneumonia cases in this current study demonstrates the significant role RSV plays in this setting. The observed association with increased severity and younger age is similar to findings from many other aetiological studies in Asia. It is true that due to the high sensitivity of WHO clinical diagnosis for childhood pneumonia, some cases included may actually be bronchiolitis but overall it is clear that a safe and effective vaccination against RSV in this population could substantially reduce infant and child morbidity from ALRIs. The predominance of RSV in the infant population is well known and this current study confirms that like other populations, for an RSV vaccination to be successful in this region it must be given in infancy. Such a vaccination against RSV has been the focus of research for many years and despite early setbacks, recent vaccine candidates are proving more promising. [171-175] Once a safe and effective vaccine becomes available this population would benefit greatly from its implementation.

INFLUENZA

Influenza was not a common viral pathogen in childhood pneumonia in this current study; InfA was only detected in 2.9% of pneumonia cases, whilst influenza B was not detected at all. Similar results have been reported in the hospital-based studies of ambulatory and hospitalised under-5s in Thailand, India and Pakistan. In Thailand, using serology and IF, InfA and InfB were found in 3% and 1% of cases respectively over a two-year period. [268] The two-year study in India used PCR to detect influenza but still only found InfA in 3%

of cases and detected no cases of InfB. [211] Similarly, the hospital-based study in Pakistan by Ghafoor et al., of under-5s with ALRI, detected influenza in only 1% of cases overall. [131] Other studies in Asia have however observed a slightly higher prevalence of influenza in cases of childhood ALRI. In the hospital-based study of Nepalese children under three years old that used PCR to detect influenza, InfA was found in 7% of ALRI cases and InfB was found in 4% of ALRI cases. [18] A cohort study in India that followed children until three years of age identified InfA in 11% of cases of NSP but 0% of SP cases using IF. [154] The same study only found InfB in 1% of patients. The higher prevalence of influenza in these two studies may be due to their longer duration, which increased the chance of observing influenza epidemics. The study in Nepal observed epidemics of influenza in two out of the three years, whilst the study in India observed one large epidemic of influenza over a 42-month period. [18, 154] These two studies did include many more ambulatory cases compared to this current study and the other hospital-based studies above, however the increased prevalence of influenza is unlikely to be related to this, since in the two studies of hospitalised children in Vietnam the proportions of influenza-associated ALRIs were higher. [136, 137] In these studies of hospitalised children, Do et al. and Yoshida et al. detected InfA in 9% and 15% and InfB in 8% and 0.2% respectively. The study by Do et al. was over three years and observed a high prevalence of influenza all-year round. Although the study by Yoshida et al. only ran for fourteen months, it included a large epidemic of InfA in the first few months of the study. It is therefore more likely that the differences in influenza prevalence are related to the inclusion of influenza epidemics and not the severity of the disease. This current study only ran for twelve months and no large epidemics were observed during this period. It is possible that over a longer period, the presence of influenza epidemics may increase the overall proportion of influenza-associated childhood pneumonia in this population.

In this current study, the median age of children with InfA detected in their NPA was 15.7 months (IQR 3 to 36 months). This was the highest median age of all viral pathogens screened for. Results published from other studies in Asia have found the median age to be slightly higher than this. A study in infants and children under fifteen years old hospitalised with clinical pneumonia in Bangkok, found the median age of influenza-positive cases to be 27 months. [183] Similarly a community-based study of influenza of under-5s with respiratory symptoms in Bangladesh found a median age of 24 months in over 300 children who were influenza positive (60% InfA and 40% InfB). [141] The study in Vietnam of children hospitalised with ALRI found the median age of children in whom InfA was detected to be 19 months. The small number of cases detected in this current study may not have been representative of this population.

HUMAN METAPNEUMOVIRUS

Over twelve months, this current study identified human metapneumovirus in 6% of cases of childhood pneumonia. Due to its recent discovery only a few studies have been published that have screened for hMPV in childhood pneumonia. Similar proportions to this current study have been found in all previously mentioned studies in Asia that have sought to identify this virus using PCR.

The two-year study of ambulatory and hospitalised under-5s in India found hMPV in 4% of pneumonia cases. [211] Similarly, in Nepal, hMPV was detected in 4% of children under three years old with pneumonia. [18] Whilst in Vietnam, two studies detected hMPV in 5% and 7% of children admitted to hospital with ALRI. [136, 137] In other developing countries, hospital-based studies using PCR have also observed similar results. In Mexico, Kenya and

South Africa, hMPV was found in 6%, 4% and 6% respectively. [17, 202, 205] This current study together with these studies above, show that when screened for hMPV is detected consistently in children with ALRI in the developing world.

The age distribution of hMPV has however been found to be more variable. In this current study, the age distribution of hMPV was similar to that observed for RSV. The median age of hMPV-positive cases in this current study was 8 months (IQR 6 to 25 months) and a high proportion (64%) of these cases were infants. This however contrasts to previous studies that have described the age distribution of hMPV infections in Asia. In Vietnam, the study of under-5s by Do et al. did not publish a median age, but did find all twenty-one children with hMPV-associated ALRI were older than 12 months. [136] The study by Yoshida et al, found a median age of 17 months in cases where hMPV was detected, however the overall median age was higher (16 months). [137]

This current study has established the prevalence of hMPV in children with ALRI in this population to be similar that of previously published studies, although the age distribution was younger. Further research of this new virus is essential to clarify the age group at highest risk of hMPV-associated ALRI to guide the development of any future interventions. This current study only included eight cases of hMPV-associated ALRI and such a small sample may not be representative of the local population. For the same reasons, data from this study are insufficient to compare the clinical and radiographical characteristics of hMPV to other viral pathogens. It was however observed that all but one child with hMPV had a radiographic diagnosis of OI. In addition, none of the cases of hMPV had a diagnosis of VSP. The clinical and epidemiological characteristics of this

recently discovered virus both in this setting and other resource-poor settings need further investigation.

ADENOVIRUS

ADV was the most frequently identified virus in this current study and was detected in 24.0% of all cases. This is much higher than all previous studies in Asia that have used both traditional techniques and molecular diagnostics. A two-year hospital-based study in Pakistan of over 1400 children with ALRI found ADV in 3% of outpatients and 1% of in-patients, and ADV was found in all age groups up to five years old including neonates. [131] In Thailand, the two-year hospital based study of both ambulatory and hospitalised under-5s that used serology and IF to detect viral pathogens, identified ADV in 7% of ALRI cases. [268] In India, the three-year cohort study of children up to three years of age found ADV in less than 1% of cases of ALRI. [154] More recently two studies in Vietnam have used PCR to detect ADV. In both studies, ADV was found in only 5% of hospitalised ALRI cases. [136, 137] Even though Do et al. included children up to fifteen years old, all the ADV isolates came from under-5s. [136] In the majority of studies in developing countries in other regions of the world, ADV has also been detected in similarly low proportions. Tupasi et al. in a two-year cohort study of under-5s in the Philippines found ADV in 3.5% of children with ALRI using IF. [134] In Kenya, a study that used PCR to detect viral pathogens found ADV in 3.8% of children hospitalised with pneumonia. [17] In Argentina, two studies of under-5s both identified ADV in 3% of cases of ALRI. [241, 271] In Chile, the burden of ADV in children under two-years old hospitalised with ALRI was slightly higher. [229] Over eight years, ADV was identified in 13% of children using IF, second only to RSV (26%). [229] Only one recent study in Mozambique has detected such a high burden of ADV in

children with pneumonia as this current study did. [138] This one-year study of under-5s hospitalised with SP used PCR to detect ADV in 21% of 807 children. [138] In the developed world, aetiological studies have also observed a low prevalence of ADV in childhood ALRIs compared to this current study. Studies in Spain, Finland, USA and Taiwan have detected ADV in 3% to 12% of children. [235, 236, 240, 245]

In this current study, ADV was the viral pathogen with the highest rate of co-detection and in 15% of adenoviral cases at least one other pathogen was detected. The most common pathogen to be detected alongside ADV was RSV. Similar observations have been made in both Chile and Mozambique. [138, 229] In Chile, ADV was detected together with another pathogen in one-third of cases, whilst in Mozambique co-detection of an additional pathogen occurred in 41% of ADV-positive cases. Furthermore, in this current study, ADV was most prevalent in children with NSP and its prevalence decreased with increasing severity. This current study did not include healthy controls and therefore it is not possible to compare the prevalence of ADV detection between those with clinical pneumonia and asymptomatic children. This will however be important to establish in the future if the role of ADV detection in cases of childhood pneumonia is to be understood. In a study that was carried out in Kenya, PCR has already been used to detect viral pathogens in both cases of ALRI and URTI. In this study ADV was detected in <1% of well children, 8% of mild URTI patients and 4% of LRTI patients. [17]

Uncertainty still surrounds the clinical significance of ADV detection in nasopharyngeal specimens in children with ALRI, particularly when identified using PCR. It is thought that ADV nucleic acid may persist in the respiratory tract after infection. ADV has been isolated from nasopharyngeal specimens of asymptomatic healthy children; Brandt et al.

found ADV in 6.5% of patients with respiratory disease but also in 3.0% of asymptomatic controls; [231] Edwards et al. also isolated ADV from 0.6% of well children. [218] A recent study using PCR found repeated ADV-positive nasopharyngeal specimens in children with URTIs. [244] The adenoviruses detected in repeated symptomatic URTIs in the same patient were of both the same serotype and different serotypes and in some cases were detected in children for more than six months. This prolonged presence of ADV in nasopharyngeal specimens and the relationship to both URTIS and ALRIs needs further clarification.

Together the above observations suggest that ADV might not actually play a significant role in the pathogenesis of childhood pneumonia. However, the high prevalence of ADV in this population means that further studies are urgently needed to understand its pathogenic contribution to childhood pneumonia. Should future studies find ADV to play a role in the pathogenesis of childhood pneumonia, its high prevalence in this community would make ADV a key target for future interventions.

SEASONALITY

This current study was not designed to evaluate the seasonality of individual pathogens or their relationship to environmental factors. Apart from the small number of cases, the main limitation was the short duration of only twelve months. This short observation period may not represent a typical seasonal pattern and does not allow the year-to-year occurrence to be compared. However, the results from the twelve months that were observed showed clearly that the largest peak of childhood pneumonia corresponded closely to that of RSV. RSV was present for 5 months of the year beginning after the rainy season and was completely absent during the rainy season. A birth cohort study in rural India found a

similar relationship to the rainy season. [154] However, in other studies in Asia, different patterns have been observed. In another birth cohort study in rural Bangladesh, almost the opposite pattern was reported. [272] Here, half of the RSV cases were detected during the rainy season and RSV was infrequently detected during the winter months. In Bangkok, a two-year study of ambulatory and hospitalised cases of ALRI in children, observed a peak of ALRI corresponding to an increase in RSV activity in both years of the study. [268] Like this current study, the first RSV epidemic occurred at the end of the rainy season. However in the second year, the peak of RSV activity occurred during the rainy season. A similar variation in RSV activity was reported in a three-year hospital-based study in urban Nepal. [18] Here RSV epidemics were observed both in the winter months and during the rainy season. Two recent studies of Vietnamese children hospitalised with ALRI, found RSV activity to be greatest during the rainy season. [136, 137]

The low prevalence of the other individual pathogens in this current study and the short duration of the study period mean it is difficult to draw any firm conclusions about their seasonality in this setting. The results suggest that hMPV activity increased after the monsoon. However, like RSV, previous Asian studies have found varied seasonal patterns. [18, 136, 211] To better understand the seasonality of all these viral pathogens in this region, aetiological surveillance would ideally continue. However in this and many other resource-limited settings such continual surveillance is not sustainable. Therefore a larger study of several years duration is needed if the understanding of viral seasonality in this region is to be improved. A longer period of study would also increase the prospect of observing epidemics of viral infections, in particular influenza.

RADIOLOGY

One of the strengths of this study was the availability of corresponding radiographical data to the aetiological data. In fact a chest radiograph was performed in over 95% of the children. In this study, radiographic pneumonia was defined according to the WHO standardised guidelines for interpretation. [79] No other studies of viral aetiology in childhood pneumonia have yet employed these standardised radiographic diagnoses. However their use in this current study will permit comparison with any future studies of viral aetiology that utilise the same method. Together such studies may help establish the relationship between the aetiological and radiographical diagnoses.

The difficulty in determining the aetiology based on radiographic appearances was clearly illustrated in this current study where viral pathogens were detected in similar proportions of children with PEP and OI (51.6% v. 49.1%). In addition, PEP was detected in similar proportions of viral positive and viral negative children (39.5% vs. 36.1%), as was OI. This supports previous suggestions that radiographic appearances cannot be used to determine the aetiology of paediatric pneumonia. [70, 248]

As a single pathogen, RSV had a tendency to be associated with radiographic changes and was detected in over twice the proportion of patients with radiographic changes (PEP and OI) compared to 'nil' (23.5% v. 10.3%). Furthermore, the proportion of RSV positive isolates was also higher in children with PEP compared to OI (27.4% v. 19.3%).

The detection of viral pathogens in over half of the cases of PEP supports the fundamental role that viral pathogens, particularly RSV, play as either a causative or contributing factor

in the pathogenesis of childhood pneumonia. Until the techniques for diagnosing bacterial aetiology substantially improve, the contribution of bacterial-viral co-infection to this observation cannot be established. However regardless, these results suggest that the prevention of viral infections may be just as vital as the prevention of bacterial infections in the global reduction of childhood pneumonia.

Other studies of the viral aetiology of childhood pneumonia have not used the WHO standardised radiographic diagnoses, but a recent study in Vietnam that used PCR to detect viral pathogens in children hospitalised with ALRI also performed chest radiographs. Like this current study, they reported consolidation in similar proportions of viral-positive and viral-negative cases (28% vs. 28%). [137]

The WHO standardised method of interpretation provides a valuable tool to help establish a clearer picture of the relationship between the aetiology of childhood pneumonia and radiographic appearances. [79] Any further research should therefore utilise the WHO radiographic diagnoses so that data can be compared and integrated. If using this standardised method fails to establish a radiographical-aetiological relationship, the role of radiography in the diagnosis of paediatric pneumonia may become redundant.

LIMITATIONS

Other viral pathogens, thought to play a potential role in childhood pneumonia, were not screened for in this current study. A wider diagnostic panel of viruses, including RV, PIV, hBoV and hCoV, would improve future studies in this region and help establish the

aetiology of childhood pneumonia. In particular, two studies have found rhinovirus to be the most common virus in children hospitalised with ALRI. [137, 138] Along with ADV, the relationship of RV to childhood pneumonia deserves further investigation.

The detection of viral pathogens from NPA samples only implies their role in the development of pneumonia through their inhalation from the nasopharynx into the lung. It is possible that in some cases the virus detected by NPA is not the causative pathogen. However the risks associated with lung aspiration limit its use especially in resource-limited settings such as this particularly in cases of NSP. Therefore NPA remains the most suitable sampling technique. The use of NPAs in this current study means that it is not possible to differentiate between nasopharyngeal carriage and a causative role of the pathogens detected. Whilst NPA remains the technique of choice, future studies should either include asymptomatic controls or undertake longitudinal surveillance of nasopharyngeal pathogens in relation to acute episodes of pneumonia so that the significance of the detection of these viral pathogens in the development of pneumonia can be better understood.

Currently effective methods to establish bacterial aetiology are lacking and only blood cultures were utilised in this study to identify bacteria. This current study was therefore unable to explore the role of bacterial pathogens and viral-bacterial co-infections. It is likely that a greater proportion of children in this current study had a bacterial aetiology than the four cases detected by this method.

CHAPTER 7

CONCLUSION

The majority of studies of childhood pneumonia in the developing world have focused on urban populations. Without performing studies in rural areas, it is impossible to understand how relevant the results from urban studies are to the majority of children in the developing world who live in rural areas. This current study is one of only a few to be carried out in a rural resource-limited setting in South-East Asia, and it therefore provides essential knowledge regarding the epidemiology and aetiology of childhood pneumonia in such a setting.

With training, local staff were able to apply the ARI case-management guidelines effectively both correctly diagnosing and treating over 93% of children. An unexpectedly high proportion of migrant infants and children diagnosed with clinical pneumonia in this rural clinic on the Thai-Myanmar border had SP or VSP. This is much higher than other similar hospital-based studies and could be due to a variety of reasons including cultural, financial and educational reasons. Further research is therefore needed to explore the maternal knowledge of pneumonia, the use of self-treatment and traditional medicine for suspected pneumonia and the caregivers' response to suspected pneumonia in this population. It is possible that community-based health education could reduce the morbidity of childhood pneumonia in this population.

Like other studies, this current study demonstrates the substantial burden of clinical pneumonia in infants, with nearly half of the cases occurring in this age group. It also

demonstrates the susceptibility that infants have to develop severe disease, with nearly two-thirds of SP and VSP cases occurring in this age group, and infants were twice as likely to be diagnosed with SP or VSP than older children. Any future interventions or research in this population therefore need to focus on this vulnerable age group.

Another important observation made by this current study is the substantial number of children with clinical pneumonia that have associated hypoxaemia, and most of these children were only identified using pulse oximetry. Given the lack of suitable clinical predictors and the cost of oxygen therapy itself, the results of this current study support the use of the pulse oximetry in resource-limited settings both to ensure all children with hypoxaemia receive oxygen therapy and to rationalise the use of expensive resources. [273] Therefore there is a need to improve awareness of the high prevalence of hypoxaemia in children with severe pneumonia, and the use of pulse oximetry and oxygen therapy in the case-management of pneumonia needs to be supported in resource-poor settings. Auscultatory wheeze was a common finding in children with clinical pneumonia and its presence was associated with increased severity of symptoms. The IMCI has already adapted its approach to recommend the additional use of salbutamol in these children. [263] It is now important that health workers are trained and encouraged to use salbutamol as its use could reduce the need for hospitalisation in childhood pneumonia.

Like other studies of childhood pneumonia that have used the WHO radiographic definitions to interpret paediatric chest radiographs, this current study also found clinical symptoms and signs to be poor predictors of PEP. Using the current WHO clinical diagnosis of childhood pneumonia, only 38% of children had evidence of PEP on radiography, which is comparable to other similar studies. There was however a much

better correlation if a broader spectrum of changes associated with ALRI was considered. In fact nearly three-quarters of children with clinical pneumonia had radiographic evidence of either OI or PEP. This current study did not observe any correlation between the presence of OI or PEP and the detection of viral pathogens. Given the association between PEP and bacterial aetiology observed in the vaccine trials it is possible that the differences in radiographical diagnosis observed in this current study were due to undetected bacterial pathogens. However, until further studies can better clarify the relationship between radiographic changes and aetiology the utility of the clinical diagnosis in this setting cannot be completely understood. If OI truly is a marker of viral infection the clinical diagnosis is in urgent need of reassessment, however if both OI and PEP can be observed in bacterial infection then the current WHO clinical diagnosis will persist as a valuable tool for the case-management of childhood pneumonia in this setting.

The use of molecular methods for the detection of viral pathogens in this current study means that a high sensitivity was achieved in comparison to traditional techniques for the comprehensive set of viruses that were screened for. Using PCR viral pathogens were detected in nearly half of the cases of clinical pneumonia (49%). This is a substantial burden and is comparable to similar studies in Asia. Undoubtedly ADV and RSV were the leading pathogens identified in this current study, detected in 24% and 22% respectively. The role of RSV in childhood pneumonia is well established. The results from this current study confirm that in this population, its prevention through an effective and safe vaccination has the potential to reduce episodes of clinical pneumonia by over one-fifth. Furthermore given the association of RSV detection with increased severity, such a vaccination against RSV may also reduce the need for hospital admission. The young median age of those children with RSV does however mean that for such a vaccination to

be successful it needs to be given during early infancy. Nearly two-thirds of RSV-positive cases were in infants, the same age group that accounted for the majority of cases of clinical pneumonia in this study. An effective vaccination against RSV given in infancy could therefore have a substantial effect on the reduction of morbidity and mortality from childhood pneumonia along the Thai-Myanmar border.

As previously mentioned, the detection of ADV or any viral pathogen from the nasopharynx does not prove the causative role of the pathogen but only suggests that the virus may be responsible for the pneumonia. Therefore further studies of ADV are needed before it's role in childhood pneumonia and therefore the implications of the results from this current study can be fully understood. The low prevalence of InfA, InfB and hMPV in this population suggest that interventions, such as vaccinations, against any of these pathogens would not be expected to have such a dramatic effect on the reduction in childhood pneumonia.

In nearly half of the children with ALRI in this study, no pathogen, either viral or bacterial, was detected. It is possible that some of these cases had an undiagnosed bacterial pathogen or that a viral pathogen that was not screened for accounted for these ALRIs. It is also feasible that in the cases of pneumonia where a viral pathogen was identified, a bacterial co-infection was missed. Once improved microbiological techniques for bacterial diagnosis become available, their combination with viral PCR in the study of childhood pneumonia will allow the relationship of bacterial and viral infections to be better understood. Without effective techniques to diagnose bacterial pneumonia or large vaccine probe studies to assess the burden of bacterial pathogens in this population we must continue to assume, as the WHO originally did, that a large proportion of these children have a bacterial

pneumonia either with or without a viral pathogen. [6] Therefore, despite the high prevalence of viral pathogens in this region, until the contribution of bacterial pathogens, both alone and together with viral pathogens, is better understood, it remains both prudent and appropriate to continue using the highly sensitive IMCI/WHO guidelines to ensure treatment of all potential bacterial childhood pneumonia. [59, 65] However, if pneumococcal and Hib vaccinations are successfully deployed, the role of the WHO clinical diagnosis of pneumonia in this region and in others where viral pathogens are found to be prevalent will need urgent reassessment.

APPENDIX 1 - STANDARDISED PAEDIATRIC CLINICAL QUESTIONNAIRE

Date:
Study Code:

Unwell Infant & Child History & Examination Sheet

Date of Birth (dd/mm/year) Age at Examination (months)

Sex Weight (kg)

Has the child been assessed using the Emergency Protocol?

History

Any other complaint by Mother describe here:

1. Cough **YES/NO**
   If yes duration _____days
2. Fever **YES/NO**
   If yes duration _____days
3. Runny nose **YES/NO**
   If yes duration _____days
4. Noisy breathing **YES/NO**
   Describe _____
5. Earache **YES/NO**
   If yes duration _____days
6. Sore throat **YES/NO**
   If yes duration _____days
7. Vomiting **YES/NO**
   If yes duration _____days
8. Diarrhoea **YES/NO** If yes
   Duration _____days
   Frequency of stools _____/day
   Is there blood in the stool **YES/NO**
9. Rash **YES/NO**
   If yes duration _____days
   Describe the rash _____
10. Any problems with the baby's eyes? **YES/NO**
    Describe _____

11. When did the child last pass urine _____(hours ago)

12. How is the baby being fed (circle)?
*Breast feeding / Bottle Feeding / Mixed feeding / NA / Other give details*_____

13. Does the baby suck well/child drink well? **YES/NO**

14. Has the baby taken any antibiotics or medicine since last seen? **YES/NO**

Episode			
What medicine?			
Started			
For how long (days)?			

15. Does the baby have any medical problems such as heart problems, kidney problems or neurological problems? **YES/NO**
    Details
16. Is the baby allergic to any medicines? **YES/NO**
    Details
17. Are any family members unwell at the current time? **YES/NO**
    Details

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Examination

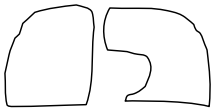
Observations

HR RR Temp O2 saturations Dextrose CRT

1. Is the infant (circle):
   Alert / Quiet / Sleepy / Irritable / Responding only to voice / Responding only to pain / Unresponsive
2. What is the colour of the infant (circle)?
   Pale / Mottled / Cyanosed / Normal / Jaundiced
3. Are there any lymph nodes palpable? **YES/NO**
   If yes where?_____
4. Is the infant dehydrated? **YES/NO**
   If yes (circle) *Mild* *Moderate* *Severe*
5. Is the infant's cry normal? **YES/NO**
   If no describe _____

Cardiovascular Examination

1. Describe the heart sounds
   Normal *Murmur*, describe _____



Respiratory Examination

1. Any of the following present (circle all that apply)?
   Grunting *Stridor* *Chest indrawing*
   Tracheal tug *Nasal flaring* *Head bobbing* *None*

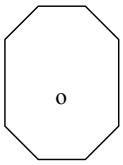
Wheeze: R/L/B/N
Crepitations: R/L/B/N

2. Listen to the chest is it clear? **YES/NO**

If no describe the noise and location on the diagram

Abdominal Examination

1. On palpation of the abdomen is there a mass / liver/ spleen palpable? **YES/NO**
   If yes please draw on the diagram
2. Is the genitalia normal? **YES/NO**
   If no details



Neurological Examination

1. Does the infant have normal muscle tone? **YES/NO**
   Describe if no _____
2. Does the infant have normal posture? **YES/NO**
   Describe if no _____
3. Is the infant moving all limbs normally? **YES/NO**
   Describe if no _____
4. Are the infant's pupils equal and reactive? **YES/NO**
   Describe if no _____
5. Describe the anterior fontanelle (circle)
   Raised *Normal* *Depressed*

Investigations:

ENT Examination

1. Throat **Normal/Abnormal**
   If abnormal describe _____
2. Mouth **Normal/Abnormal**
   If abnormal describe _____
3. Ear **Normal/Abnormal if abnormal which one R/L/Both**
   If abnormal describe _____

Skin

1. Any, rash, pustules, cellulitis or skin abnormality? **YES/NO**
   Describe rash and location_____

Eyes

1. Any abnormality? **YES/NO**
   Describe if yes _____

Outcome *Home* *Home with follow up* *Admit IPD*
Diagnosis
Treatment (include length of treatment)

Form completed by:

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APPENDIX 2 - DEMOGRAPHIC QUESTIONNAIRE

Date:
Study code:
MKT code

Acute Respiratory Infection Questionnaire

PATIENT DETAILS

Mother ANC Code if known

Date of Birth (dd/mm/yyyy) ___ / ___ / _____ Age _____years

Male/Female (circle) Ethnic Group: _____

Name of village: _____ **Thai/Burmese** Side (circle)

PAST MEDICAL HISTORY

1. Previous episodes of **Pneumonia?** **YES/NO**
   If yes, give details: Age: _____ treat in **IPD/OPD** (circle)
   Date: _____ treat in **IPD/OPD** (circle)

2. Previous admissions to **IPD** (In-patient Department) **YES/NO**
   If yes, details : Diagnosis _____ Age: _____ **IPD/OPD**
   Diagnosis _____ Age: _____ **IPD/OPD**

FAMILY ACCOMODATION

1. Number of rooms _____
2. Open fire in hut for cooking (circle)? **YES/NO**
3. What fuel is used on the fire? (circle) **Charcoal / wood / gas / other** _____
4. Number of people living in the house in the last one month _____
5. Any family member's currently unwell (circle)? **YES/NO**

If yes details: _____
_____

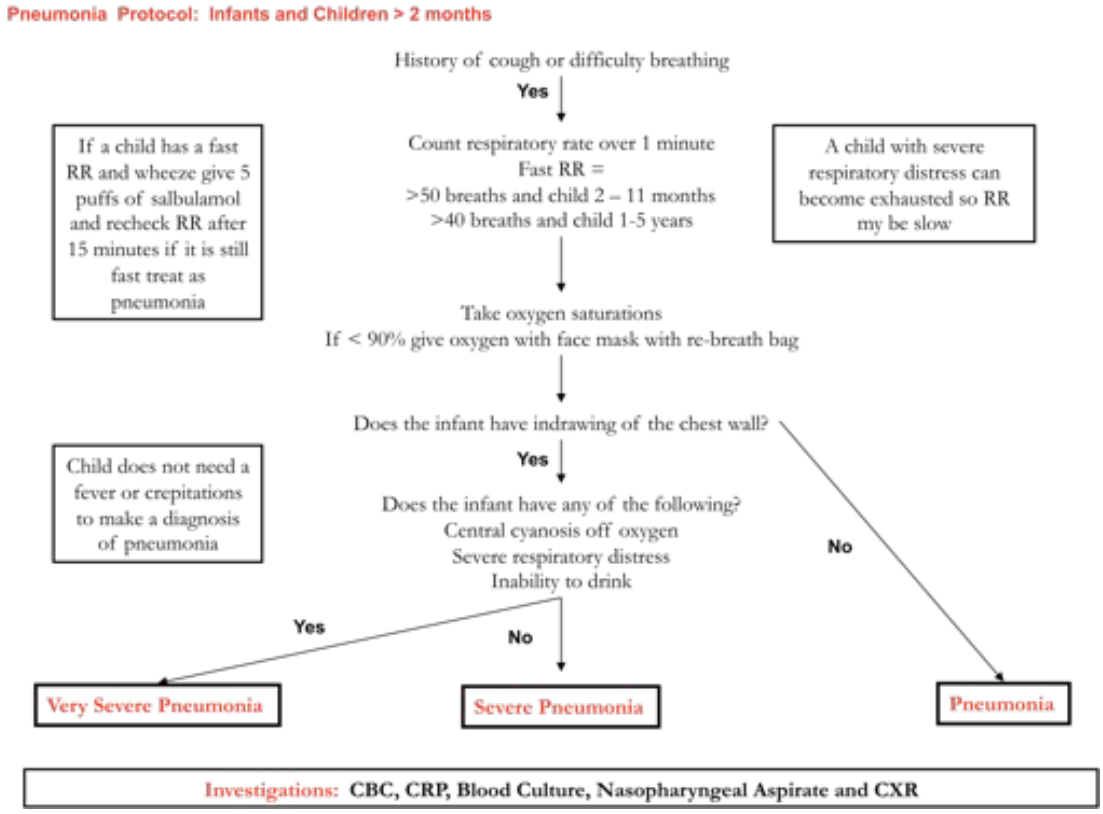
DETAILS OF HOUSEHOLD MEMBERS

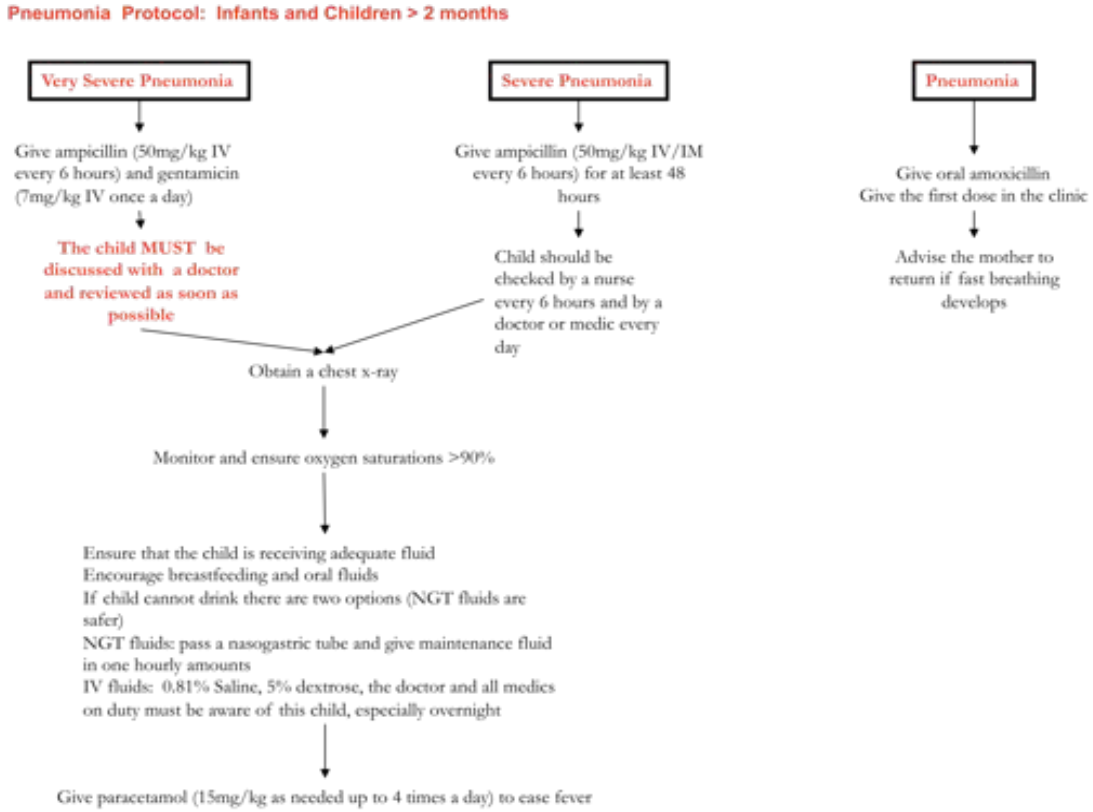
Name	Relationship to infant	DOB or age (years) dd/mm/year	Smoke Y/N	If smoke, what and how many?
	Mother			
	Father			

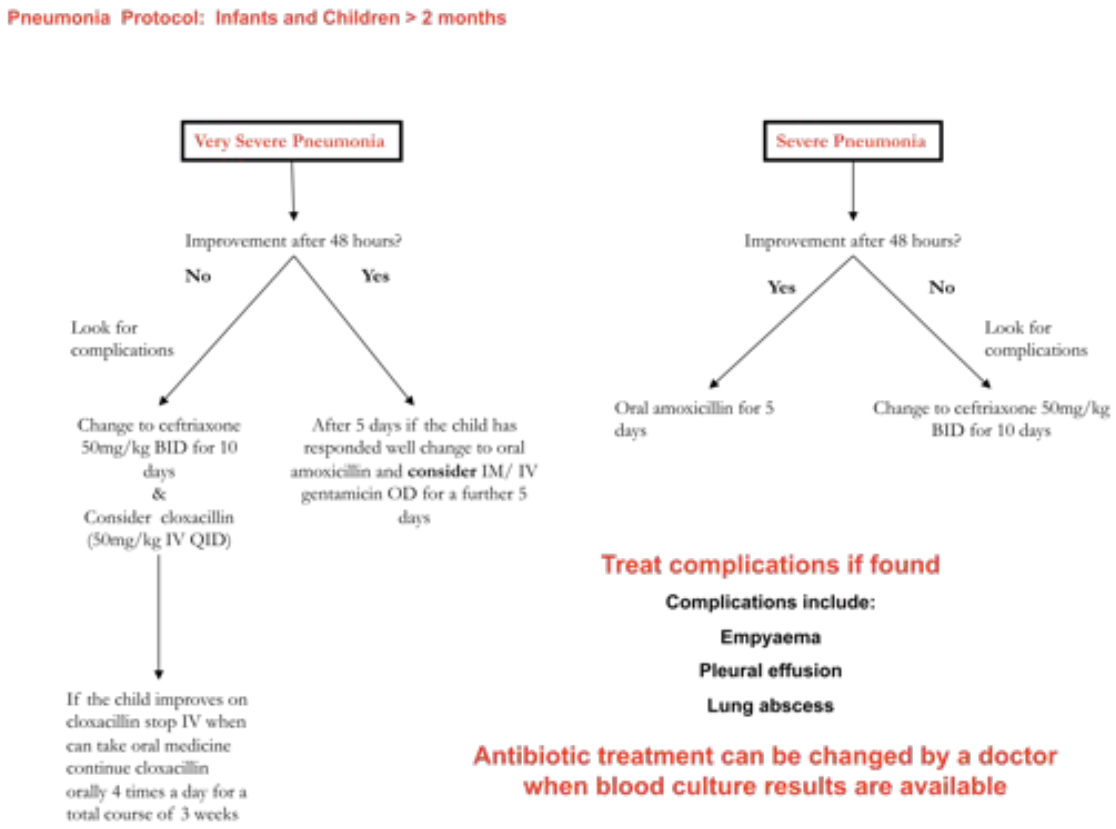
Form filled in by: **Date:**

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APPENDIX 3 - PAEDIATRIC ARI GUIDELINES




Pneumonia Protocol: Infants and Children > 2 months
Very Severe Pneumonia
Give ampicillin (50mg/kg IV every 6 hours) and gentamicin (7mg/kg IV once a day)
The child MUST be discussed with a doctor and reviewed as soon as possible
Severe Pneumonia
Give ampicillin (50mg/kg IV/IM every 6 hours) for at least 48 hours
Child should be checked by a nurse every 6 hours and by a doctor or medic every day
Pneumonia
Give oral amoxicillin
Give the first dose in the clinic
Advise the mother to return if fast breathing develops
Obtain a chest x-ray
Monitor and ensure oxygen saturations >90%
Ensure that the child is receiving adequate fluid
Encourage breastfeeding and oral fluids
If child cannot drink there are two options (NGT fluids are safer)
NGT fluids: pass a nasogastric tube and give maintenance fluid in one hourly amounts
IV fluids: 0.81% Saline, 5% dextrose, the doctor and all medics on duty must be aware of this child, especially overnight
Give paracetamol (15mg/kg as needed up to 4 times a day) to ease fever


Pneumonia Protocol: Infants and Children > 2 months
Very Severe Pneumonia
Improvement after 48 hours?
No
Yes
Look for complications
Change to ceftriaxone 50mg/kg BID for 10 days
&
Consider cloxacillin (50mg/kg IV QID)
After 5 days if the child has responded well change to oral amoxicillin and consider IM/ IV gentamicin OD for a further 5 days
If the child improves on cloxacillin stop IV when can take oral medicine continue cloxacillin orally 4 times a day for a total course of 3 weeks
Severe Pneumonia
Improvement after 48 hours?
Yes
No
Look for complications
Oral amoxicillin for 5 days
Change to ceftriaxone 50mg/kg BID for 10 days
Treat complications if found
Complications include:
Empyaema
Pleural effusion
Lung abscess
Antibiotic treatment can be changed by a doctor when blood culture results are available

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