Surveillance for Respiratory Viruses Among Patients Hospitalized with Pneumonia in Sarawak, Malaysia

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Steve Taylor

Thesis submitted in partial fulfillment of
the requirements for the degree of
Master of Science in the Duke Global Health Institute
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ABSTRACT

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Abstract

Introduction: Pneumonia, despite its stereotype as a routine disease, remains the leading cause of morbidity and mortality among children under five worldwide, responsible for nearly 16% of all childhood deaths(1). With an imprecise definition and multiple etiologies, diagnosis and treatment of the disease is difficult when based solely on clinical and symptomatic manifestations(2). This study was conducted as a subset of an ongoing year-long study aimed to determine the viral etiology of and risk factors for pneumonia among 600 patients admitted to Sibu and Kapit Hospitals in Sarawak, Malaysia. Specifically, this sub-study examined molecular diagnostics for two common respiratory pathogens, which often infect children seen at these hospitals and which lacked any such diagnostic capability. We sought to determine the prevalence of respiratory syncytial virus (RSV) subtypes A and B and parainfluenza virus (PIV) types 1- 4. The study describes demographic, viral and behavioral risk factors for these admissions. Additionally, the study aimed to assess viral transmission in the air in hospital wards.

Methods: To determine the viral etiology of pneumonia cases, this cross-sectional study enrolled 129 patients over the age of one month, who had been diagnosed and hospitalized with pneumonia at Sibu or Kapit Hospital in Sarawak, Malaysia between June 15 and July 27, 2017. Nasopharyngeal (NP) swabs were collected and analyzed

using real-time reverse transcription polymerase chain reaction (rRT-PCR) at Sibu Hospital's Clinical Research Centre laboratory. A multivariable model was used to assess risk factors for the presence of different respiratory viruses.

Results: Of 129 specimens collected, 40 samples tested positive for RSV-A (31.01%), two were positive for RSV B (01.55%), one was positive for PIV-3 (0.78%) and one was positive for PIV-4 (0.78%). No samples were positive for PIV-1 or PIV-2. The prevalence of RSV-A was 46% (23/50) at Kapit Hospital and 21.52% at Sibu Hospital (17/79). In Sibu Hospital's pediatric wards, one bioaerosol tested positive for adenovirus and two tested as suspect-positives for adenovirus. One (1) bioaerosol sample from an adult ward at Sibu Hospital tested as a suspect-positive for RSV-A. A multivariable analysis found risk factors of age (>1 year and 1-5 years vs > 5 years) and location of hospitalization (Kapit vs Sibu) potentially important predictors of RSV-A molecular detection.

Conclusions: During this brief demonstration study, we found a high prevalence of RSV-A among pneumonia patients admitted to the two hospitals. Having routine diagnostic capability for these viruses, particularly RSV, could provide prevalence data for estimates of how many hospitalizations might be averted by vaccines.

Dedication

This thesis is dedicated to my family, without whom this opportunity would not have been possible. A special thank you to Bubby for his unwavering confidence in me. You have been and continue to be an inspiration to me.

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Foreword

Having four years of international global health work experience in rural Benin and urban Bangladesh, I drafted this pilot research study with the guidance of Dr. Gray at Duke University to study the prevalence of two respiratory pathogens known to cause pneumonia among both pediatric and elderly populations. With advancements in RSV and PIV treatment and vaccine technology on the horizon, increased surveillance and diagnostic capacities are necessary to inform our understanding of the epidemiology of these respiratory viruses.

1. Introduction

Pneumonia, an infection of the pulmonary parenchyma caused by various pathogens, has remained among the top three leading causes of the global burden of disease since 1990. According to *The Lancet Infectious Diseases* 2015 publication on the Global Burden of Disease (GBD), lower respiratory tract infections are the fifth leading cause of death overall and second leading cause of death among children under the age of five(3). In 2015, an estimated 2.7 million deaths (all ages) were attributable to lower respiratory infections (LRIs)(4).

Over 95% of these pneumonia cases occur in low- and middle-income countries, and the disease disproportionately effects children under the age of five and adults above the age of 60. In 2015, there were an estimated 704,000 pneumonia deaths in children under 5 years of age(3, 5). Approximately 20% of deaths in post-neonates and children ages 28-364 days were due to lower respiratory tract infections (LRTIs)(6).

The GBD Injuries and Risk Factors Study also found that in 2015, LRIs caused an estimated 1.27 million deaths in elderly people (aged >70 years)(3). In this older population, disability-adjusted life years (DALYs) were found to have increased approximately 18.9% between 2005 and 2015, with an even greater increase (25%) in low socio-demographic index countries.

Pneumonias can be classified by severity, type of acquisition (i.e. community- versus hospital-acquired), or by the pathogen or organism that causes infection. Etiologic

causes of pneumonia include bacteria, most frequently *Streptococcus pneumoniae*, viruses, mycoplasma, fungi, parasites and environmental factors such as inhalation of particulate matter and gases(7). Pneumonia is not always caused by a single pathogen; coinfection contributes to the difficulty in diagnosing and treating pneumonia. A 2015 systematic review and meta-analysis of the predictive value of clinical features of pneumonia in children found poor diagnostic performance of WHO-approved clinical signs of pneumonia, and suggested that new point-of-care diagnostics for pneumonia would improve the accuracy of the clinical diagnosis(2). Improved clinical diagnosis of pneumonia could help clinicians in reducing patient exposure to antibiotics in cases of viral pneumonia, which constitute approximately 66% of childhood cases of pneumonia and 25% of all adult cases of pneumonia in the United States(8, 9).

1.1 Respiratory Viruses

In November 2012, international stakeholders joined the World Health Organization and UNICEF in developing a global action plan to address respiratory viruses. The agenda of the Battle against Respiratory Viruses (BRaVe) initiative is to identify gaps in knowledge and increase research efforts in the epidemiology and surveillance of respiratory viruses. While international surveillance for influenza viruses is routinely conducted for the purposes of developing seasonal vaccines, surveillance for non-influenza respiratory viruses is less well established(10). Respiratory syncytial virus

(RSV), and parainfluenza virus (PIV), are two such non-influenza respiratory viruses that were analyzed in this pilot study.

1.1.1 Human Respiratory Syncytial Virus

Respiratory syncytial virus is a single-stranded negative-sense RNA virus in the genus *Pneumovirus* in the Paramyxoviridae family. Human RSV has two antigenic subgroups, A and B, which can co-circulate but typically differ by season and region(11). While humans are currently the only known host for human RSV, the virus was first found and isolated in 1956 from chimpanzees before being isolated from pediatric inpatients and outpatients in 1958-1959(12). Based on a prospective cohort study of 125 infants in the US from 1975-1980 called the Houston Family Study, it is widely believed that virtually all children are infected with RSV by age 2 (11, 13). The virus infects epithelial cells within the nasopharynx and generally causes upper respiratory tract infections (URIs) that progress to lower respiratory tract infections (LRIs), pneumonia, bronchiolitis and tracheobronchitis. Symptoms include fever, severe cough, tachypnea and wheezing. A 2017 systematic review and meta-analysis of RSV hospitalization and mortality found a global hospitalization estimate of 19.19 per 1,000 children less than 1 year of age and a global case-fatality estimate of 6.60 per 1,000 children less than 1 year of age(14). Among children under the age of five, an estimated 36,363 deaths were caused by RSV in 2015(3).

Though symptoms are more severe in children, RSV has been implicated as a cause of LRI among hospitalized adult patients (15). In one US-based study, RSV was one of the four most common pathogens identified out of adults admitted to hospitals for community-acquired pneumonia between 1990-1992(16). More recently, RSV has been found to have a disease burden similar to that of non-pandemic Influenza A within elderly adult populations(17).

While therapy is largely supportive, there are currently more than 54 vaccines and 46 antiviral drug products for RSV being developed and assessed in various phases of clinical trials, several with favorable preliminary results(18-21). Promising developments in immune prophylaxis with targeted monoclonal antibodies (mAbs) such as Palivizumab are available for high-risk infants and children(22). Other mAbs being developed include MEDI8897, which is currently in a Phase IIb clinical trial (23). Among adult patients, the new antiviral ALS-008176 has demonstrated the ability to reduce the viral load of RSV (24). The growing number of RSV vaccines and mAb technologies in the past several years adds to the increased need for RSV surveillance and epidemiologic research.

1.1.2 Human Parainfluenza Viruses

Parainfluenza viruses are also single-stranded, negative-sense RNA viruses in the Paramyxoviridae family. There are four primary types of human PIVs, PIV1-4, with two

subtypes of type 4 (4a and 4b). PIV-5 has been detected in both animals and humans, however its ability to naturally infect humans and cause disease is still controversial (25). While PIV-3 is most commonly associated with pneumonia, all four types are common causes of LRIs, particularly among children and infants. PIV-1 and PIV-2 are more commonly associated with croup in children >6 months of age, while PIV-3, which clinically mimics RSV, is most closely associated with pneumonia and bronchiolitis. PIV-4 typically causes mild URIs in adults and children >5 years, though it is associated with more severe illness in infants with underlying conditions (26). A 2017 study of respiratory viruses in children across eight countries in tropical and southern hemisphere countries found an equal prevalence (9.7%) of parainfluenza viruses and RSV among children experiencing influenza-like-illnesses(27).

Currently there are no antivirals with demonstrated efficacy for PIV, though supportive therapies exist and pneumococcal vaccines have been associated with reduced incidence of PIV-associated pneumonia(28). Evaluations of vaccines for PIV-3 have been ongoing since the early 1990s (29). Today there are 13 ongoing vaccine trials for human PIV-3 in the US and one in the EU(10).

1.1.3 Animal Reservoirs for Non-human RSV and PIV

It is well known that in the past two decades, 60% of the emerging and re-emerging infectious diseases that have impacted human populations have been zoonotic in origin,

and that of these zoonotic infectious diseases, an estimated 72% originate from wildlife, as distinct from non-wildlife (i.e. domesticated animals) (30, 31). In particular, novel respiratory viruses such as SARS, avian influenza viruses, swine influenza viruses, enteroviruses, and adenoviruses have caused significant outbreaks in recent years (32, 33). Furthermore, Southeast Asia has been identified as a hotspot for emerging infectious diseases, due to the tropical climate and anthropogenic factors that have led to increased contact between humans, wildlife and the ecosystem.

As non-human RSV and PIV viruses have been found in animal reservoirs, including cattle, goat and sheep populations, there may be reason to establish surveillance for cross-over of similar respiratory viruses between animals and humans(34, 35). Bovine RSV (bRSV) was isolated from cows in the 1970s and continues to infect cattle herds worldwide despite widespread use of multivalent vaccines in calves(34). While distinct, bRSV is closely related to human RSV and has demonstrated similar histopathologic manifestations(36). Bovine parainfluenza virus type 3 (BPIV-3) and bRSV have both been associated with bovine respiratory disease complex (34, 37, 38). Seroprevalence of ovine-RSV has been detected in cattle, suggesting transmission between ruminants occurs(39). Despite circulation of bovine-PIV and ovine-, bovine- and caprine-RSV within animal populations, as far as we know, there is no evidence of zoonotic transfer of either animal-reservoired RSV or animal-reservoired PIV to humans. While this study

did not survey for these non-human strains of RSV and PIV, we did ask enrolled patients questions about their history of contact with animals, including cattle and goats.

1.2 Pneumonia in Sarawak

A meta-analysis of the global burden of childhood pneumonia found the greatest proportion of severe pneumonia cases occurred in southeast Asia (39%)(1). Given the burden of disease in southeast Asia, however, few recent studies have been conducted in Malaysia and the surrounding tropical territories, to assess the burden and epidemiology of lower respiratory tract infections.

One retrospective study of respiratory virus infections in hospitalized children ≤5 years of age in Kuala Lumpur, Malaysia, conducted between 1982-2008 found a total of 2,708 laboratory-confirmed positive cases of respiratory viruses out of a total 10,269 samples(40). Of the positive cases, 70.6% were positive for RSV (18.61% overall prevalence), 13.2% tested positive for PIV (3.48% overall prevalence), 11% tested positive for influenza and 5.2% tested positive for adenoviruses. The study found RSV had the most pronounced seasonality of the viruses, peaking between September and December.

A second retrospective study of RSV in children hospitalized with bronchiolitis and pneumonia between 1982-1997 in Malaysia found a prevalence of 18.4% for RSV, with seasonal variations correlated with the number of days of rain in a month, peaking in

November, December and January (41). Neither of these two studies sub-typed RSV or PIV.

Sibu Hospital in Sarawak has seen increasing pneumonia admissions with 1,611 admissions in 2013, 1,607 admissions in 2014, and 1,903 admissions in 2015. Kapit Hospital in Sarawak also sees a relatively large number of pneumonia admissions (approximately 300 admissions per year) considering the markedly smaller population it serves. Current treatment for pneumonia cases in these two hospitals includes oxygen therapy and increased fluid intake. Antivirals such as ribavirin are rarely provided for patients and are only prescribed for particularly severe and clinically suspected cases.

Several clinical trials for orally administered antivirals are also underway in Malaysia, including a trial on ALX0171, an RSV therapy administered via inhalation, in infants and young children at Sibu Hospital(42). Prior to this pilot study and the introduction of rRT-PCR laboratory techniques to Sibu Hospital, the research team sometimes relied on the Quidel QuickVue dipstick immunoassay test, a rapid antigen detection test (RADT), to identify RSV-positive patients. To date, 10 children have been enrolled in the trial. Study staff ensured that none of the children enrolled in the clinical trial were included in our study.

1.3 Rationale and Study Aims

1.3.1 Gaps in Non-Influenza Respiratory Viruses Surveillance and Epidemiology

Historically, advocacy and policy for combatting pneumonia have lagged behind other infectious diseases such as TB and malaria, though networks such as the global health network for childhood pneumonia have recently started to champion the issue(43). Pneumonia receives less funding than other major infectious diseases, and studies have demonstrated that pneumonia, despite the greater disease burden, has historically received less media attention than AIDS, TB and malaria(44).

Analyses of results of the 2015 GBD LRI models demonstrate the need for increased efforts in preventing pneumonia deaths(5). To achieve progress, researchers must first address several gaps in the epidemiology of non-influenza respiratory viruses that are associated with LRIs, to include both RSV and PIV. Recent publications have described these gaps in surveillance, recognizing the barrier of timely diagnostic testing and specifically exploring the importance of non-influenza respiratory virus research for the development of therapeutics (10, 18). An analysis of the gaps in RSV surveillance and epidemiology cites the need to integrate RSV laboratory detection with epidemiology, calling for the increased use of rRT-PCR to enhance the sensitivity of RSV detection and to allow detection of other respiratory viruses(18). Contributing to the international surveillance of the estimated burden of RSV across all age groups, not just the under-5

population, would contribute to both local-level clinicians' understanding of the epidemiology of RSV but also the international-level effort for future planning for clinical treatment and prevention of RSV.

1.3.2 Study Aims for Respiratory Viral Surveillance

This cross-sectional pilot study was a sub-investigation of a year-long study sponsored by the Navy Medical Research Centre, Asia, Vysnova Partners, conducted in partnership with Duke University and Sibu Hospital Clinical Research Centre. The study, entitled Cross-sectional Surveillance for Novel Respiratory Virus Infections among Patients Hospitalized with Pneumonia in Sarawak, Malaysia, is conducting ongoing surveillance for respiratory viruses, seeking to determine the prevalence of specific respiratory viruses among pediatric and adult pneumonia admissions at Sibu and Kapit Hospitals. The year-long study will conclude in May, 2018, and analysis of results will include the results of data collected for the sub-study. In addition to RSV, the full panel of viruses being analyzed includes influenza A, B, C & D viruses, adenoviruses, enteroviruses and coronaviruses.

This sub-study was conducted between June and August of 2017, relying on specimens collected from enrollments only in the months of June and July to determine the prevalence of RSV and PIV. Specifically, we hypothesized that we would detect

human respiratory syncytial virus and human parainfluenza virus in approximately 15-20% and 5-15% of patients, respectively.

In addition to investigating these two respiratory viruses, the sub-study also aimed to assess viral presence in the air of hospital wards with the objective of informing hospitals of evidence that respiratory viruses may be transmitted through the air. For the purposes of this thesis, the analysis of the bioaerosol specimens collected included an analysis for influenza A, B, C & D viruses, adenoviruses, enteroviruses and coronaviruses.

To conduct the study, our team collaborated with the Sibu Hospital Clinical Research Centre to set-up a Biosafety Level-2 (BSL2) laboratory space. The Duke One Health team furnished the laboratory space with the BioRad real-time PCR machine. Our introduction of rRT-PCR has likely increased the sensitivity of respiratory virus detection over the use of RADTs and permits the timely co-detection of other respiratory pathogens as well. Following RNA extraction, the molecular assays for RSV and PIV can be conducted in under two hours in order to quickly provide diagnostic results to clinicians.

2. Methods

2.1 Setting

The study was conducted between June and August, 2017 in Sibu and Kapit Hospitals in the state of Sarawak, Malaysia, on the northwest side of the island of Borneo. The region of Sarawak is tropical and has an equatorial climate with high humidity and temperatures ranging from 78F°-90F° (23-32C°). June through August is considered Sarawak's dry season, when the region sees the least amount of rainfall; however, rain showers occurred regularly and in June alone, Sibu saw approximately 250 mm of rainfall(45). Approximately 70% of Sarawak is forested with large concentrations of animals, including an estimated 45 species of bats and over 600 species of birds(46, 47). It is one of the world's largest producers of palm oil, however, deforestation in Sarawak has been rampant, with over 1,700 square kilometers logged in 2015 alone(48). Anthropogenic deforestation has been strongly associated with the emergence of infectious diseases in recent years, to include the emergence of Nipah virus in Malaysia in 1998, following slash-and-burn deforestation and encroachment into wildlife habitats(49).

According to the most recent census, the population in the Sibu Division was approximately 300,000 in 2010, and the population in the Kapit Division was just under 113,000. The population in the Sarawak state is approximately 2.5 million. Sibu Hospital

is a referral hospital for central Sarawak, serving approximately 10 other hospitals and a population of 615,922 as of 2010. It is a major specialist hospital with approximately 750 beds. Kapit is located 75 miles south-east (120 kilometers) inland from Sibu, a three-hour boat ride up the Rejang River. Considerably smaller than Sibu, Kapit is home to a population of 60,000 inhabitants. Kapit Hospital is a government hospital with approximately 134 beds that serves as the referral hospital for several smaller towns along the river.

2.2 Participants

For this study, we adapted inclusion and exclusion criteria from two U.S., large and comprehensive, community-based pneumonia studies published in New England Journal of Medicine in 2015 (Appendix A)(8, 9). Participants of all ages, with the exception of infants under 30 days, that had been admitted to Sibu or Kapit Hospitals were recruited after the attending physician had made a clinical diagnosis of pneumonia. Immunocompetent patients who had been hospitalized within <7 days (children) or <28 days (adults) and immunosuppressed patients who had been hospitalized within <90 days (all ages), were excluded from the study, as were patients who had recently undergone a tracheotomy or had pre-existing conditions such as cystic fibrosis or cancer with neutropenia.

The major ethnic groups in Sarawak are Iban, Malay, Chinese, Melanau, Bidayuh, and Orang Ulu. As of 2010, approximately 28% of the population in Sibu was Iban and 82% of the population in Kapit was Iban. Based on the recommendations of the Sibu and Kapit Hospital collaborators, study materials, to include the Patient Information Sheet, Consent Form, and Questionnaire, were made available in English, Malay and Mandarin. Per the Malaysian Medical Research and Ethics Committee (MREC) regulations, assent forms were required for all children between the ages of 7 and 18.

The year-long study aims to enroll a total of 600 patients; therefore, for the months of June and July, the expected sample size was 100. Due to an unexpectedly high number of pneumonia admissions in Kapit Hospital during the month of June, however, a total of 129 patients were enrolled in the study between June and July, with 79 from Sibu Hospital and 50 from Kapit Hospital. These 129 subjects were sampled out of a total 567 patients that were admitted to Sibu and Kapit Hospitals with pneumonia between June and July 2017.

Table 1: Total pneumonia cases by hospital, June and July, 2017.

Ward		June*		July		Total	
		All Admissions	Enrolled Admissions	All Admissions	Enrolled Admissions	All Admissions	Enrolled Admissions
Sibu	Pediatric	103	16	83	39	186	55
	Adult (Female)	67	5	58	5	125	10
	Adult (Male)	61	4	59	10	120	14
	Sibu Total	**231**	**25**	**200**	**54**	**431**	**79**
Kapit	Pediatric	43	26	29	14	72	40
	Adult (Female)	33	5	20	1	53	6
	Adult (Male)	7	0	4	0	11	0
	Kapit Total	**83**	**31**	**53**	**19‡**	**136**	**50‡**
Total		**314**	**56**	**253**	**73**	**567**	**129**

* All admissions for the month of June and enrollments from June 15-30; ‡ 4 ages missing from Kapit enrollments in July

Sample size calculation was based on the year-long study of respiratory viruses and the primary objective of determining the viral etiology of pneumonia for the two hospitals. The precision for which the resultant prevalence statistics are determined was estimated by calculating the 95% confidence interval around a binomial (cases detected/patients studied). While our pilot study had no Sarawak baseline prevalence data from which to calculate sample size estimates, we considered other similar studies to develop working prevalence figures, presuming that the most prevalent pathogens we will detect among the subjects will be influenza A. If we use the true prevalence (TP) figure of 30% for influenza-A, a sample size of 600 subjects would allow our study to estimate a prevalence within 3.8% of the TP.

2.3 Procedures

2.3.1 Patient Enrollment and Nasopharyngeal Sampling

Due to MREC regulations and language barriers, all physical contact and verbal interactions with patients required the presence of a licensed Medical Officer (MO). Five (5) MOs from Sibu Hospital and seven MOs from Kapit Hospital were trained on the procedures for consenting, enrolling and collecting specimens from subjects. Study team members provided packets of study-related instruments and information for MOs, to include standardized operating procedures (SOPs) for the enrollment process and NP swab collection and a list of inclusion and exclusion criteria. MOs were observed the first several enrollments to ensure SOPs were adhered to. Due to our dependency upon the availability of MOs for enrollment, this study used convenience sampling.

After determining patient eligibility, an MO approached the subject, explaining the study and allowing the patient or patient's guardian sufficient time to read through the Patient Information Sheet and Consent Form. MOs frequently showed parents or guardians the FLOQSwab that would be used to perform the NP swab, but stressed that abstaining from the study would not detract from patient care. No compensation was offered for participation in the study and during the consenting process the MOs explicitly stated that the patient would not receive the results of the study.

If the subject was willing, the MO obtained consent and administered a brief questionnaire, further explaining, reading or translating questions when necessary. The MO then sat the patient comfortably, tilting his or her head back 70 degrees, slowly inserting the flexible, sterile NP swab into the subject's nostril to a depth approximately equal to half of the distance between the nostril and the outer opening of the ear. The swab was rotated several times for three seconds, before being removed and immediately placed into a BD Universal Viral Transport tube containing 3 ml of sterile viral transport medium (VTM). The consent form, questionnaire and VTM tube containing the NP swab were labelled with the same study number before the specimen was stored in the ward refrigerator (1-3^{o} C). A study team member collected any specimens from the refrigerator and moved them to the -80^{o}C freezer within 24 hours. In Kapit where there was no -80^{o}C freezer, specimens were moved for a -30^{o}C freezer, where they remained for a maximum of four days before a team member from Sibu came to collect and transport them in a cooler with ice packs to the Sibu laboratory -80^{o}C freezer.

2.3.2 Bioaerosol Sample Collection

To assess aerosolization of the study viruses in the hospital wards, a two-stage bioaerosol sampler that was designed by and was on loan from the National Institute for Occupational Safety and Health (NIOSH) was mounted at a height of approximately 1.5

meters on a tripod in patient ward areas for a documented period of time between one to two hours. Bioaerosol collection was done eight times in Sibu Hospital and twice at Kapit Hospital. Sample collection was conducted using SKC Personal Air Sampling Pumps. which vacuumed air through an inlet into the two-stage bioaerosol sampler, where particulate matter 4 μm and larger was collected on the sides of a 15ml conical tube (the first stage), particles a 1 to 4 μm were captured in a 1.5 ml conical tube (the second stage) or particles <1 μm were captured by a filter in a cassette atop the sampler. Air flow through the conical tube creates centrifugal force that throws particulate matter onto the sides of the tubes where they remained until processed with a solution of phosphate buffered saline (PBS) with 1% bovine serum albumin (BSA) to maintain viral viability. All bioaerosol specimens were processed within 24 hours of sample collection and were stored in the -80^{o}C freezer.

2.3.3 Ethical Review Board

All study procedures were approved by the ethical review boards at Malaysia National Medical Research Registry MREC, Duke University, Duke-NUS (National University of Singapore) Medical School, and the Navy Medical Research Centre, Asia.

2.4 Measures: RNA Extraction and rRT-PCR

All specimens were stored at -80°C until RNA extraction was performed using QIAmp Cador Pathogen Mini Kit (cat. 54106). Extractions were conducted in a BSL-2 biosafety cabinet using a 10% Distel disinfectant solution as an approved tuberculocidal. Extractions (100µl) were then stored in cryogenic vials in a -80°C freezer until ready for rRT-PCR. Primers and probes were identified and validated following a literature review of van de Pol et al. (2007)(50). Cycling conditions for the six of the singleplex assays for RSV-A, RSV-B, PIV 1, 2, 3 and 4 were identical: a 30-minute denaturing stage at 50°C followed by two minutes of the annealing stage at 95°C, before 40 repetitions of the extending stage, alternating between 95°C for 15 seconds and 60°C for 30 seconds. rRT-PCR was conducted on a BioRad CFx96 C1000 Touch Thermal Cycler Real-Time system.

Table 2: Primers and probes used to conduct rRT-PCR for the six viruses.

RSV-A	RSVAs Primer	5′- AGATCAACTTCTGTCATCCAGCAA-3′
	RSVAas Primer	5′- TTCTGCACATCATAATTAGGAGTATCAAT-3′
	RSVA FAM Probe	5′- FAM-CACCATCCAACGGAGCACAGGAGAT-TAMRA-3′
RSV-B	RSVBs Primer	5′- AAGATGCAAATCATAAATTCACAGGA-3′
	RSVBas Primer	5′-TGATATCCAGCATCTTTAAGTATCTTTATAGTG-3′
	RSVB FAM Probe	5′-FAM-TTCCCTTCCTAACCTGGACATAGCATATAACATACCT-TAMRA-3′
PIV-1	PIV1s Primer	5′- TGATTTAAACCCGGTAATTTCTCAT -3′
	PIV1as Primer	5′- CCTTGTTCCTGCAGCTATTACAGA-3′
	PIV1 FAM Probe	5′- FAM-ACGACAACAGGAAATC-BHQ-3′
PIV-2	PIV2s Primer	5′- AGGACTATGAAAACCATTTACCTAAGTGA-3′
	PIV2as Primer	5′- AAGCAAGTCTCAGTTCAGCTAGATCA-3′
	PIV2FAM Probe	

		5′- FAM-ATCAATCGCAAAAGCTGTTCAGTCACTGCTATAC-TAMRA-3′
PIV-3	PIV3s Primer PIV3as Primer PIV3FAM Probe	5′- TGA TGA AAG ATC AGA TTA TGC ATA TC -3 5′- CCG GGA CAC CCA GTT GTG -3′ 5′- FAM-TGG ACC AGG GAT ATA CTA CAA AGG CAA AAT AAT ATT TCT C-TAMRA-3′
PIV-4	PIV4s Primer PIV4as Primer PIV4FAM Probe	5′- CAAAYGATCCACAGCAAAGATTC -3′ 5′- ATGTGGCCTGTAAGGAAAGCA -3′ 5′- FAM-GTATCATCATCTGCCAAATCGGCAATTAAACA-TAMRA -3′

Adapted from: van de Pol et al. (2007)(50).

All NP swabs and bioaerosol samples were tested for RSV-A, RSV-B, PIV 1, 2, 3 and 4. In addition to RSV and PIV, bioaerosol samples were tested for influenza A, B, C, D, adenovirus, enterovirus and coronavirus. As validation via culture or rRT-PCR of cycle threshold (Ct) value cut-offs was not feasible in the CRC laboratory, Ct value cut-offs were determined based on a literature review to be similar to those used for influenza: Ct values <38 were positive; Ct values 38-40 were considered suspect; and Ct values >40 were considered negative(50-53). A positive control and a no-template control were included in each run to ensure that the reaction was successfully set up and that there was no contamination.

All pre-existing health conditions were self-reported by the subject or their guardian during the questionnaire, then confirmed by the MO through the patient's medical records. Criteria for previously diagnosed medical conditions were defined by the Malaysia Clinical Practice Guidelines (4th Edition).

2.5 Analysis

Survey data and rRT-PCR results were entered into REDCap version 7.0 and verified by two study team members. Data were imported into STATA version 15.0 (StataCorp, College Station, TX) and frequencies for dichotomous and categorical variables were tabulated against positive RSV and PIV samples. Given the low prevalence of positive RSV B, PIV-3 and -4, and given that no samples were positive for PIV-1 or PIV-2, these outcomes were not included in the final analysis.

To assess potential risk factors, an initial bivariate screening was conducted to assess the association between the covariates of interest and the outcome variable of positive detection of RSV-A by rRT-PCR. For the purpose of this analysis, the three suspect-positive RSV-A samples, which were run a second time for validation and revealed consistent amplification curves, were considered positive.

Logistic regression was used to assess the following covariates as possible risk factors for the detection of RSV-A: age category, gender, location (hospital), week of enrollment, ethnicity, pre-existing medical conditions, current treatments or medications, and history of exposure to animals. Pearson's chi-squared test was used for categorical variables. Those predictors with a bivariate test statistic p-value ≤ 0.1 were then included in a stepwise, backward elimination logistic regression model. Predictors with a p-value <0.05 were retained in a final model to calculate adjusted odds ratios.

3. Results

3.1 rRT-PCR detection of RSV and PIV in NP swabs

From June 15 to July 27, 2017, we enrolled 129 patients who met the inclusion criteria. Forty (40) samples were positive for RSV-A (31.01%), two were positive for RSV-B (01.55%), one was positive for PIV-3 (0.78%) and one was positive for PIV-4 (0.78%). No samples were positive for PIV-1 or PIV-2.

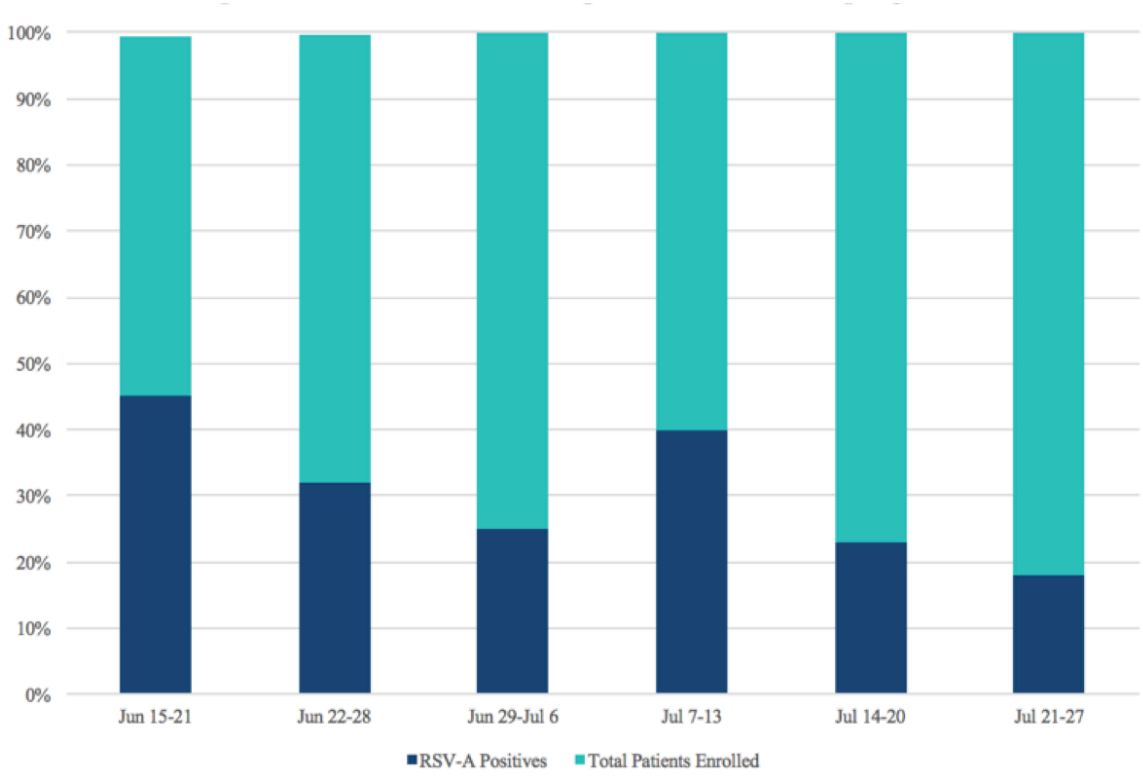

Figure 1: Proportions of RSV-A positive enrollments by week at Sibu and Kapit Hospitals.

Eighty-five (85) of the 129 patients were children under the age of 5 (65.89%); ten of the patients were aged 6-18 (7.75%) and 30 were adults over the age of 18 (23.26%). The median age was 1.84 years (22 months). Of the enrolled patients, 73 were male and 56 were female (56.59% of the participants enrolled were males). Only one pregnant patient was enrolled.

Table 3: Characteristics of enrolled subjects

Characteristics	All Total N (%)	Sibu N (%)	Kapit N (%)
Total	129 (100)	79 (61.24)	50 (38.76)
Sex			
Female	56 (43.41)	36 (45.56)	20 (40.00)
Male	73 (56.59)	43 (54.43)	30 (60.00)
Age Groups*			
<1	32 (24.81)	18 (22.78)	14 (28.00)
1-5	53 (41.09)	31 (39.24)	22 (44.00)
6-18	10 (07.75)	6 (07.59)	4 (08.00)
>18	30 (23.26)	24 (30.38)	6 (12.00)
missing	4 (03.10)	0 (0.00)	4 (08.00)
Household Size			
≤2	16 (12.40)	9 (11.39)	7 (14.00)
3-5	48 (37.21)	29 (36.71)	19 (38.00)
6-10	52 (40.31)	30 (37.98)	22 (44.00)
>10	13 (10.08)	11 (13.92)	2 (04.00)
Ethnicity			
Iban	90 (69.77)	46 (58.23)	44 (88.00)
Malay	10 (7.75)	8 (10.13)	2 (04.00)
Chinese	7 (5.43)	7 (08.86)	0 (0.00)
Bidayuh	1 (0.78)	0	1 (02.00)
Melanau	13 (10.08)	13 (16.45)	0 (0.00)
Other'	8 (6.20)	5 (06.33)	3 (06.00)
Pre-existing Conditions			
Hypertension	15 (11.63)	11 (23.92)	4 (08.00)
Diabetes mellitus	5 (3.88)	3 (03.80)	2 (04.00)
Heart disease	5 (3.88)	5 (06.33)	0 (0.00)
COPD	10 (7.75)	9 (11.39)	1 (02.00)
Asthma	5 (3.88)	4 (05.06)	1 (02.00)

Cancer	1 (0.78)	1 (01.26)	0 (0.00)
Other§	17 (13.18)	16 (20.25)	1 (02.00)
None	92 (71.32)	48 (60.76)	44 (88.00)
Medical Treatment in last 6 months			
Diabetes medicine	5 (3.88)	3 (03.80)	2 (04.00)
Hypertension medicine	14 (10.85)	11 (13.92)	3 (06.00)
Oral corticosteroid	1 (0.78)	1 (01.26)	0 (0.00)
Inhaled corticosteroid	19 (14.73)	16 (20.25)	3 (06.00)
Cancer medicine	0 (0.00)	0 (0.00)	0 (0.00)
Others‡	7 (5.43)	7 (08.86)	0 (0.00)
None	98 (75.97)	53 (67.09)	45 (90.00)
Animals touched or within 1 meter in the last 30 days			
Pigs	8 (6.20)	6 (07.59)	2 (04.00)
Chickens	26 (20.16)	13 (16.45)	13 (26.00)
Ducks	9 (6.98)	5 (06.33)	4 (08.00)
Other poultry	4 (3.10)	4 (05.06)	0 (0.00)
Horses	0 (0.00)	0 (0.00)	0 (0.00)
Cows	1 (0.78)	1 (01.26)	0 (0.00)
Goats	0 (0.00)	0 (0.00)	0 (0.00)
Other†	47 (36.43)	34 (43.04)	13 (26.00)

COPD: Chronic Obstructive Pulmonary Disease; § dyslipidemia, gout, obstructive sleep apnea, hyperthyroidism, benign prostatic hyperplasia, wheeze, reactive airway disease, preschool wheeze, Bronchiolitis obliterans, iron deficiency anemia; ' Penan, Ulu-Sekapan, Kadazan, Bisaya, Bugis, Dayak, Kenyal; ‡ colchicine, allopurinol, aspirin, gout medication, multi-vitamin, iron supplement, Nafarin-A; † cats, dogs

A bivariate analysis of dichotomous risk factors against the outcome of RSV-A positive found potentially important risk factors (defined as Pearson chi2 $p \leq 0.1$) to include: hospital site; pre-existing conditions of high blood pressure, chronic obstructive pulmonary disease (COPD), other conditions or no conditions; and previous or current treatment with inhaled corticosteroids or other medications. The variables "medical treatment of hypertension medication" and "no medication" were found to be potentially important risk factors, however, they were omitted from the final logistic

regression model due to collinearity with the variables "pre-existing condition of hypertension" and "no pre-existing condition", respectively.

A stepwise, backward elimination logistic regression model was fit using these variables. The final model included age and location of hospitalization. When controlling for age, patients enrolled at Kapit hospital had a higher adjusted odds ratio of testing positive for RSV-A (adjusted OR=2.94, 95% CI 1.23-7.03). When controlling for location, the adjusted odds ratio of RSV-A infection for participants <1 year was 32.65 (95% CI 3.88-274.57), and the adjusted odds ratio of RSV-A infection for participants ages 1-5 years was 13.71 (95% CI 1.70-110.49). Both age covariates were strongly associated with RSV-A infection, though the confidence interval was wide.

Table 4: Risk factors for molecular detection of RSV-A

Risk Factor	Total N	RSVA + (%)	RSVA - (%)	Unadjusted OR (95%CI)	Adjusted OR (95% CI)
Hospital					
Kapit	50	23 (46.00)	27 (54.00)	3.11 (1.43-6.73)	2.94 (1.23-7.03)
Sibu	79	17 (21.52)	62 (78.48)	Ref.	Ref.
Self-reported HBP					
Yes	15	1 (6.67)	14 (93.33)	0.14 (0.02-1.08)	--
No	114	39 (34.21)	75 (65.79)	Ref.	
Self-reported COPD¨					
Yes	10	0 (0.00)	10 (100.00)	0.15 (0.00-0.94)	--
No	119	40 (33.61)	79 (66.39)	Ref.	
Self-reported Other¨ §					
Yes	17	1 (5.88)	16 (94.12)	0.12 (0.00-0.82)	--
No	112	39 (34.82)	73 (65.18)	Ref.	
No self-reported pre-existing conditions					
Yes	92	37 (40.22)	55 (59.78)	7.62 (2.18-26.66)	--
No	37	3 (8.11)	34 (91.89)	Ref.	
Inhaled corticosteroids Treatment					

Yes	19	2 (10.53)	17 (89.47)	0.22 (0.05-1.02)	--
No	110	38 (34.55)	72 (65.45)	Ref.	
Other Treatment¨‡					
Yes	7	0 (0.00)	7 (100.00)	0.22 (0.00-1.51)	--
No	122	40 (32.79)	82 (67.21)	Ref.	
Age Category					
<1	32	18 (56.25)	14 (43.75)	37.29 (4.51-308.24)	32.65 (3.88-274.57)
1-5	53	19 (35.85)	34 (64.15)	16.21 (2.04-128.56)	13.71 (1.70-110.49)
6-17	10	1 (10.00)	9 (90.00)	3.22 (0.18-56.88)	2.57 (0.14-46.80)
>18	30	1 (03.33)	29 (96.67)	Ref.	Ref.

OR: Odds Ratio; HBP: high blood pressure; COPD: Chronic Obstructive Pulmonary Disease; ¨ OR generated using exact logistic regression, median unbiased estimates; § Other conditions: dyslipidemia, gout, OSA, hyperthyroidism, benign prostatic hyperplasia, wheeze, reactive airway disease, preschool wheeze, Bronchiolitis obliterans, iron deficiency anemia; ‡ Other treatments: colchicine, allopurinol, aspirin, gout medication, multi-vitamin, iron supplement, Nafarin-A.

3.2 rRT-PCR detection of respiratory viruses in bioaerosol samples

Between June 29 and July 28, 2017, eight convenience aerosol samples were collected from Sibu Hospital and two convenience aersosol samples were collected from Kapit Hospital. The median duration of the run-time was 74 minutes. When possible, bioaerosol samplers were deployed in the mornings when the wards were busiest, before any patients were discharged for the day. One (1) bioaerosol sample tested suspect positive for RSV-A, one tested positive and two tested suspect-positives for adenovirus.

The suspect RSV-A sample was detected in the large-particle collection tube of the sampler deployed in the adult female ward in Sibu Hospital. That sampler was deployed on the morning of July 24, 2017, from 9:59am-11:03am. A total of nine NP

swabs were collected on July 24, two of which were positive for RSV. One (1) of the two NP swabs was from Sibu Hospital's pediatric ward, the other was from Kapit's pediatric ward. The one NP swab collected from the adult female ward on July 24 was negative for RSV.

The one positive and two suspect-positive adenovirus samples were all collected in the Sibu Hospital pediatric ward. An adenovirus suspect positive was detected in the medium-particle collection tube of the sampler deployed from 1:42om-2:40pm on July 3. While no NP swabs were collected from the pediatric ward on July 3, one NP swab collected from a pediatric patient on July 4 was also a suspect positive for adenovirus. A sampler deployed on July 27 from 1:28pm-3:15pm detected a suspect adenovirus in the filter of the sampler, the smallest particle size of the two-stage sampler. Two days prior to the collection, on July 25, an NP swab collected from a pediatric patient in this ward tested positive for adenovirus; however, the bioaerosol sampler deployed in the pediatric ward that day was negative for all respiratory viruses. The final bioaerosol sample was collected July 28, 2017 from 8:38-9:46am, with the positive adenovirus detected in the largest particle size of the sampler. No NP swabs were collected that day.

4. Discussion

4.1 Prevalence and risk factors of RSV-A

The study found a remarkably high and sustained prevalence of RSV-A (31.01% overall) among the pneumonia patients admitted to the two hospitals. Given previous studies of respiratory viruses in Malaysia, we expected to see an RSV prevalence between 15-20% (40, 41). The prevalence was particularly high at Kapit Hospital (46.00%), though the prevalence increased over the course of the second and third week in Sibu Hospital (21.52%). Given the high prevalence of RSV-A positives in Kapit the week of June 15-21, it is conceivable our surveillance captured the tail-end of an RSV-A outbreak at Kapit Hospital. As reviews of RSV epidemiology have suggested, we found that while there was some co-circulation of RSV subtypes, one subtype predominated (RSV-A)(11).

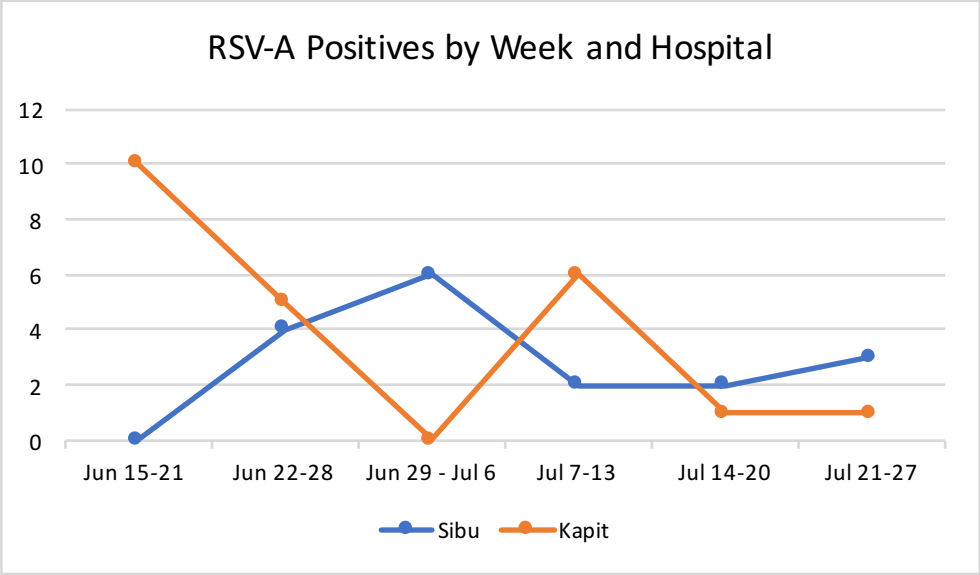

Figure 2: Graph of RSV-A positives by week at Sibu and Kapit Hospitals.

A 2015 meta-analysis of risk factors for RSV associated with LRIs in pediatric patients under the age of five found that males had a statistically significant increased risk for RSV-associated acute LRIs, with an overall meta-estimated odds ratio of 1.32 (95% CI 1.24-1.40) (54). Our study did not find gender to be a potentially significant risk predictor.

Even though we assessed contact with animals as a risk factor for RSV and PIV, we found no association between animal exposure and RSV-A, which perhaps suggests transmission of the virus in this population was via the conventional human-to-human route. There is no evidence to date that RSV is zoonotic, so these findings are in keeping with our expectations of the standard epidemiology of RSV. To our knowledge, RSV has also not been detected in pigs or chickens, the animals that subjects came into contact

with most frequently. Only one enrolled subject had come into contact with cattle, and none came into contact with goats.

The analysis found the two risk factors of having no pre-existing conditions and being on no other treatment or medication to be potentially statistically significant predictors of RSV-A infection. Because we recruited subjects in a hospital setting, it is possible that the association between no pre-existing conditions or medications is representative of the infection for which of the patient was hospitalized. Those subjects who were hospitalized with pneumonia and reported Chronic Obstructive Pulmonary disease (COPD) or use of inhaled corticosteroids may have been more likely to be hospitalized for chronic, pre-existing conditions that contributed to their pneumonia. Conversely, those with no pre-existing conditions or those subjects who were not taking any medications were perhaps more likely to be hospitalized for an acute infection caused by a respiratory virus.

Because COPD and hypertension are conditions predominantly affecting older populations, when conducting the analysis only within age groups of above 18 and under 18, these risk factors were no longer statistically significant.

4.2 Prevalence of RSV-B, PIV 1-4

While the study showed a high prevalence of RSV across the two hospitals, we found an unexpectedly low prevalence of PIV 3 and 4. A prevalence of 0% for PIV types 1 and

2 is in keeping with our understanding that these two virus types are more commonly associated with croup, rather than pneumonia. However, we anticipated a higher prevalence of 5-10% for PIV-3(40).

4.3 Positive and suspect-positives in bioaerosol samples

The one positive and two suspect-positive adenovirus bioaerosol samples are consistent with recent studies that have found a higher prevalence of adenovirus than other respiratory viruses in hospital settings (55). Furthermore, studies have demonstrated the survival of airborne respiratory viruses such as influenza are dependent on the relative humidity of the setting, with drier conditions (20-35% relative humidity [RH]) more ideal than intermediate (50% RH) or humid conditions (80% RH)(56). The sampling conditions in Sibu were therefore less than ideal for particle viability, as the RH in Sibu was consistently between 70-90% in both June and July of 2017 (57). Given that two of the suspect adenoviruses were also detected in the afternoon sampling time frame, it may not be necessary to collect bioaerosol samples during the busiest time of the day.

4.4 Implications for policy and practice

The high prevalence of RSV-A suggests that these hospitals may serve as good sites for further RSV research. Currently, two other randomized, double-blind, placebo-controlled trials in Malaysia are pending for antivirals AK0529 and Lumicitabine. Prior to this pilot study, Sibu Hospital, which is involved in several of these RSV therapy trials, was dependent on rapid diagnostic tests for recruitment of RSV-positive patients into their trials. Sibu Hospital's CRC reported having a difficult time finding RSV-positive pediatric patients to enroll. Given the high prevalence of RSV-A detected at Sibu Hospital between June 15 –July 27, 2017, it seems likely that the RADT being used to recruit patients had lower sensitivity than the rRT-PCR we used. Several studies have demonstrated higher sensitivity for RSV diagnosis using rRT-PCR over rapid antigen tests(58, 59). The molecular diagnostic techniques introduced at this hospital through the pilot study have therefore potentially increased the capacity of the MOs and researchers to identify and enroll patients in future RSV-related trials.

4.5 Implications for further research

This study provides baseline surveillance data to estimate the prevalence of both RSV and PIV within the Sibu and Kapit pneumonia patient populations; however, with emerging treatments for both on the horizon, there are still epidemiologic gaps in

surveillance for the burden of these diseases. While this study contributes to an understanding of the burden of RSV in Sarawak, continued studies are required to assess RSV-related mortality and the disease outcomes of RSV infection. Following future vaccine introduction, further surveillance will be required to assess changes in the burden and adverse events (18).

In addition to assessing disease outcome as severe or non-severe in both the short- and long-term, other covariates such as smoking status or breastfeeding habits for infants, should be assessed. Finally, the use of rRT-PCR for this study will allow us to detect and assess co-infections of other respiratory viruses as they relate to clinical outcomes.

Bioaerosol sampling offers researchers a unique and non-invasive form of respiratory virus surveillance. The detection of adenovirus and suspected detection of RSV-A in bioaerosol samples suggests a further need for aerosol studies of respiratory viruses in hospital settings.

4.6 Study strengths and limitations

This study is unique in its use of a One Health approach to infectious diseases, combined with the innovative use of cutting-edge technology to attempt to capture environmental air samples of viral particulate matter. Strengths of the study include the use of singleplex rRT-PCR to sub-type RSV and PIV. As far as we are aware, this is the

first surveillance done of RSV subtypes A and B and PIV types 1-4. While rRT-PCR is highly sensitive, it is possible that some infections could have been missed. Two different study team members read and interpreted the Ct amplification curves to ensure assay results were agreed-upon.

The study was unable to draw conclusions about causality due to the cross-sectional study design. As this was a pilot study, we also did not have information on the duration or incidence of pneumonia, and could therefore not assess our study for incidence-prevalence bias or duration ratio bias.

Because of logistical constraints and dependency on Medical Officers, the study was unable to employ a random sampling technique. As we were able to collect information on all patients hospitalized with pneumonia in June and July at each hospital, we know that our study captured approximately 37% of all pneumonia pediatric cases but less than 10% of all adult pneumonia cases admitted (9% female and 10.7% male). We relatively under-sampled adult pneumonia cases, but this biased sampling is mitigated as most epidemiological studies are concerned with RSV infection in children. To adjust for this potential bias in subsequent months of surveillance, the study team adopted a stratified random sampling technique across the adult and pediatric wards, enrolling the first two patients to be admitted with pneumonia on three randomly generated days of the week.

Several unexpected limitations arose due to the feasibility of inclusion and exclusion criteria. For instance, while inclusion criteria initially included chest radiography within 48 hours of admission, access to x-ray technology at the hospitals, Kapit Hospital in particular, was not always feasible. Furthermore, a decision was made to exclude known TB-positive patients admitted to the TB wards of the hospital from the first two months of enrollment.

Other limitations include the self-reporting of pre-existing conditions. While MOs verified subjects' reports using their medical records, no clinical tests were conducted at the time of enrollment to verify patient's status, possibly contributing to a misclassification bias.

Despite the study's limitations, as this was a subset of a year-long study, we have the opportunity to further analyze our data, to include further genetic characterization of positive RSV and PIV specimens.

5. Conclusion

In conclusion, this study identified respiratory syncytial virus subtypes A and B and parainfluenza virus types 3 and 4 in nasopharyngeal swabs collected from patients hospitalized with pneumonia in two hospitals in Sarawak, Malaysia. Additional studies are needed to further assess the association between RSV infection and short- and long-term outcomes. Active surveillance of respiratory viruses in Malaysia will help better prepare stakeholders to identify potential outbreaks and monitor for zoonotic crossover of infectious diseases to humans. Baseline data on the prevalence of specific respiratory viruses in the hospitalized pneumonia patient population may help inform medical practitioners and researchers as to the etiology of pneumonia in Sarawak and guide future interventions and vaccine or pharmaceutical trials.

Appendix A

Table 5: Inclusion and exclusion criteria by age.

Inclusion Criteria Children (1 month to 18 years)	Inclusion Criteria Adults (18 years or more)
They were admitted to Sibu or Kapit hospital; Have evidence of acute infection, defined as reported fever or chills, documented fever or hypothermia, or leukocytosis or leukopenia; Have evidence of an acute respiratory illness, defined as new cough or sputum production, chest pain, dyspnea, tachypnea, abnormal lung examination, or respiratory failure; A parent or legal guardian provides written informed consent. In addition to parental consent, signed assent document will be sought from children 7 to 18 years of age. The evidence of illness is consistent with pneumonia as assessed by means of chest radiography within 72 hours before or after admission.	They were admitted to Sibu or Kapit hospital on the basis of a clinical assessment by the treating clinician; Have evidence of acute infection, defined as reported fever or chills, documented fever or hypothermia, leukocytosis or leukopenia, or new altered mental status; Have evidence of an acute respiratory illness, defined as new cough or sputum production, chest pain, dyspnea, tachypnea, abnormal lung examination, or respiratory failure; Have evidence consistent with pneumonia as assessed by means of chest radiography by the clinical team within 48 hours before or after admission.
Exclusion Criteria Children (1 month to 18 years)	**Exclusion Criteria Adults (18 years or more)**
If they had been hospitalized recently (<7 days for immunocompetent children and <90 days for immunosuppressed children) If they had already been enrolled in this study within the previous 28 days If they resided in an extended-care facility If they had an alternative diagnosis of a respiratory disorder If they are newborns who never left the hospital If they have a tracheostomy tube If they have cystic fibrosis or If they have cancer with neutropenia If they have received a solid-organ or hematopoietic stem-cell transplant within the previous 90 days If they have active graft-versus-host disease or bronchiolitis obliterans If they have human immunodeficiency virus infection with a CD4 cell count of less than 200 per cubic millimeter (or a percentage of CD4 cells <14%).	If they had been hospitalized recently (<28 days for immunocompetent patients and <90 days for immunosuppressed patients), If they have already been enrolled in this study within the previous 28 days If they were functionally dependent nursing home residents, If they have a clear alternative diagnosis If they have undergone tracheotomy If they have a percutaneous endoscopic gastrostomy tube If they have cystic fibrosis If they have cancer with neutropenia, If they have received a solid-organ or hematopoietic stem-cell transplant within the previous 90 days, If they have active graft-versus-host disease If they have bronchiolitis obliterans If they have human immunodeficiency virus infection with a CD4 cell count of less than 200 per cubic millimeter.

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