Seasonality and Prevalence of Respiratory Syncytial Virus in Kinshasa, Democratic Republic of Congo

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A Master's Thesis
Submitted to the Department of Global Health Security,
Division of Global Health Security Detection Program
and the Graduate School Public Health of Yonsei University
in partial fulfillment of the
requirements for the degree of
Master of Public Health

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December 2019

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December 2019

ACKNOWLEDGEMENTS

First and foremost, i thank my God almighty for his everyday grace, blessings and for giving me strength, courage and intelligence to cross this milestone.

Thereafter, i would like to extend my most sincere gratitude to my thesis supervisor Prof Tai-Soon Yong of the environmental medical biology, institute of tropical medicine at Yonsei University as well as my advisers Professor Myungken Lee and Professor Myeong Heon Shin for their guidance and support in writing this thesis.

I would also like to particularly thank Prof Muyembe Tamfum J.J., Prof. Steve Ahuka, Dr Edith Nkwembe, Dr Hugo Kavunga of the National Influenza laboratory, virology department of the National Institute of Biomedical Research, Kinshasa, DR Congo for letting me use the facility and data for this research thesis as well as their unending advises, assistance and support throughout the process of researching and writing this thesis.

I would like to thank and acknowledge Dr Grace Mufwaya for her unvarying assistance during the data collection process and would like to thank my colleagues in Congo and in Korea for their inputs during the researching and writing process.

I must express my very profound gratitude to my parents Don Bruno Kapandji and Marie-Marthe Lukula, to my brothers Dadou, Patrick and Ladis Kapandji and to my sister Francine Kapandji for providing me with unfailing love, support and continuous

encouragement throughout my years of study and through the process of researching and writing this thesis therefore I dedicate my thesis to them.

To the Korean Government and KOICA, i say thank you for giving me the privilege to learn in Korea and to Prof Joshua Sir, i express my gratitude for single-handedly choosing me to participate in the Master's program.

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LIST OF ABBREVIATIONS

ALRTI: Acute Lower Respiratory Tract Infection

CDC: Centers for Disease Control and Prevention

CT: Cycle Threshold

DNA: Deoxyribonucleic acid

DRC: Democratic Republic of Congo

ILI: Influenza-like illness

INRB: National Institute of Biomedical Research

LRTI: Lower Respiratory Tract Infection

NIRL: National Influenza Reference Laboratory

PCR: Polymerase Chain Reaction

RNA: Ribonucleic acid

rRt-PCR: Reverse Transcriptase Polymerase Chain Reaction

RSV: Respiratory Syncytial Virus

SARI: Severe Acute Respiratory Infection

URTI: Upper Respiratory Tract Infection

UTM: Universal Transport Media

WHO: World Health Organisation

ABSTRACT

Seasonality and Prevalence of Respiratory Syncytial Virus in Kinshasa, Democratic Republic of Congo.

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(Directed by Professor Tai-Soon Yong)

The Democratic Republic of Congo (DRC) with its tropical climate in the past was thought not to house many respiratory viruses, with time and after many outbreaks began researches and surveillance on respiratory viruses circulating in the country. The first was influenza sentinel surveillance which showed that around 10% of suspected samples received were positive for influenza and 90% were negative, therefore a research was needed to find which other virus was present and causing infection in the remaining 90%. This study aimed to screen Respiratory Syncytial Virus (RSV) among those remaining samples in Kinshasa in order to determine the proportion and seasonal factors influencing the virus.

The samples used were nasopharyngeal swabs collected from different Influenza surveillance sites in Kinshasa: Kalembelembe, Boyambi, Kinshasa General Hospital, Kingasani Hospital Centre and RVA clinic during the period of January to September

2016. 169 Samples were randomly selected for the research and were chosen regardless of the patient's age, sex, geographic group and symptoms. Molecular analysis was done to determine if the samples were RSV positive or negative at the National Reference Laboratory (NRL) in Kinshasa using real time reverse transcription-polymerase chain reaction (rRT-PCR).

13.6% were positive for RSV. The prevalence was higher in female 65.2% than in male 34.8%, over 95% of RSV infection occurred during the rainy season and among the positive cases 60.9% were from hospitalized patients with severe acute respiratory infection and 39.1% were from influenza-like illness or infection. The largest proportion (78.3%) of RSV positives was found in children under the age of 2 years.

This study showed that RSV is found in Kinshasa, DRC at most during the rainy season and tend to fade away during the dry season. Children are the most affected especially those younger than 2 years and RSV was more prevalent in female than in male.

Key words: **Prevalence, seasonality, Respiratory Syncytial Virus, Kinshasa, Democratic Republic of Congo**

CHAPTER I

INTRODUCTION

1.1. Background

Respiratory Syncytial Virus (RSV) in many countries has shown to be responsible for many respiratory tract infections in children (Troeger, et al, 2018). The viral pathogen causes both upper respiratory tract infection (URTI) and lower respiratory tract infection (LRTI) in children and in adults. The infection in adults is usually mild and less severe than in children, unless the adult has an immunocompromised health issue or/and cardiovascular problems (Kelsey, et al, 2018). It is the most common cause of pneumonia and bronchiolitis in children (Dearden, et al, 2018). In children, RSV is the most common viral cause of respiratory tract infection especially in children younger than 5 years old and it is the viral leading cause of children hospital visits, hospitalization and death resulting from to respiratory tract infections (Scheltema, et al, 2017).

The average hospital length for RSV infection is 3 days in uncomplicated cases and increases with the severity of the infection causing a challenge for hospital services as it requires a lot both in human resources and in provision of medicines and care especially during RSV seasonal peak. But it also a financial burden for the families that have to stay longer at the hospital or often visit the hospital due to RSV infections (Shi, et al, 2017; Madhi, et al, 2003).

Worldwide RSV has been and remains a major public health problem as it poses a high burden on health system both in developed countries and in developing countries. In the 2005 report of the global overview of RSV burden, it appeared that RSV was the most viral pathogen causing severe acute lower respiratory infection as it was associated with 22% of severe acute LRTI. Around 55 000 to 199 000 of deaths that occurred in children younger than 5 years old in 2005 were caused by RSV (Nair, et al, 2010). In the year 2015, the new global report and estimates on RSV burden showed that RSV caused 31.1 million acute lower respiratory tract infection (ALRTI) and from those infections 3.2 million resulted in hospitalization with 59 600 deaths in children younger than 5 years. The report also showed that number of deaths caused by RSV ARLI moved from 28% in 2005 to 5% in 2015 (Shi, et al, 2017). Nonetheless RSV still is a major burden worldwide especially in developing countries and to date continues to weight on people's health and health system around the world.

Overall 60 to 70% of all children in the world have been infected by RSV by the age of 2 years and 2-3% of those resulted in hospitalization (Stein, et al, 2016). Children that have been infected by RSV within their early life have shown to develop recurrent wheezing and asthma during childhood (Obando, et al, 2018).

The diagnosis of RSV is mostly based on clinical symptoms. Among those symptoms are runny nose, fever, nasal congestion, cough wheezing, chest congestion, difficult breathing. Besides, the clinical symptoms based diagnosis, other diagnostic

method are used such as viral culture, rapid antigen detection tests and reverse-transcriptase polymerase chain reaction (Griffiths, et al, 2017).

Currently there is no official effective cure for RSV; the treatment is based on supportive care by treating the symptoms or giving oxygen therapy in severe cases making it difficult for broken health system and facilities that do not have the proper resources (Ferolla, et al, 2019). There is no vaccine for RSV at the moment but candidate vaccines are being studied (Lee, et al, 2017). Palivizumab is given as a preventive measure for RSV high risk people such as premature babies, people with cardiopulmonary and cardiovascular diseases as well as immunocompromised individuals, but the process is highly costly and not easily accessible (Dearden, et al, 2018). Nevertheless, many new strategies are being studied to find a cure to RSV.

The more there are researches to find cures against RSV, the more there is a need for accurate data regarding RSV epidemiology worldwide but there is still a lack of knowledge in many areas of RSV, lack of data especially in high burden countries like developing countries and this allows RSV to remain a big problem (WHO, 2016). Lack of data on RSV makes the viral pathogen a major public health problem (Sanicas, et al, 2016) because there is a high need for more epidemiological studies and established RSV surveillance especially in developing countries in order to perfect RSV prevention, diagnostic measures and treatment. Therefore, data on the timing and prevalence of the virus should be available in every country to provide a good knowledge on the viral pathogen and infection. With that being said, more researches should be directed toward

describing the epidemiology in countries that doesn't have data on RSV (Obando, et al, 2018). This research study on seasonality and prevalence of RSV in Kinshasa, DRC which is the first ever laboratory confirmed RSV study done in Kinshasa the capital city and province of the Democratic Republic of Congo (DRC) may contribute in resolving the issue of data unavailability on RSV incidence in Kinshasa, DRC. As it is important to know the pattern of RSV to efficiently treat it, this first study may provide some of the necessary information needed in order to have a picture and representation of the circulation of RSV in Kinshasa, DRC.

1.2. Literature review

In public health, RSV infection is an important burden as it is globally epidemic and is associated with high mortality and morbidity in developed and developing countries (Abraha, et al, 2015). RSV causes seasonal epidemics every year all over the world, making children and adults sick, but mostly children and causes severe infections in children younger than 5 years old. RSV has a very high mortality and morbidity rate in young children. By the age of 2 years old every child has been infected with RSV. Literature comes from researches and studies done on RSV seasonality, prevalence, RSV surveillance, burden and global estimates. Not all countries have RSV surveillance hence the lack of data in many countries; some countries have inserted RSV within their influenza surveillance and surveillance of other respiratory viruses (Obando, et al, 2018).

Ali, 2012 stated that RSV mostly causes infection in children younger than 2 years of age.

In the literatures, incidence of RSV infections appeared to be lower in Asian countries than in African countries and 70% RSV infection, death and burden was found in developing countries in children 1 month to 5 years. In Africa, most LRTI were found to be caused by RSV (Sricharoenchai, et al, 2016). In a study done in 2013 in African countries, it was found that the largest percentage of death from pneumonia within a period of 3 years was caused by RSV; for example, in Kenya whereby the prevalence for RSV positive was 12.5% (Ahmed, et al, 2012). RSV has shown to affect people from all ages, sex, cultural background, socioeconomic status and demographics. Some studies have shown that RSV could infect the same person more than once as reinfection was observed each year with rate between 6% and 83% which goes to show that an initial infection from RSV does not provide immunity for upcoming infections (Tregoning, et al, 2010)

The risk factors that increases the chance of RSV infection are temperatures and seasonal factors, age, sex in some studies, prematurity, low weight at birth, having siblings, overcrowded houses, working with children, compromised immune system, cardiopulmonary diseases and cardiovascular diseases (Kelsey, et al, 2018; Stein, et al, 2016). Rahman, et al, 2014 found no significant association between RSV incidence and gender, race, specimen and symptoms but a significant association with age group of a p value of $p < 0.001$

In age, it has already been said that RSV causes a high under 5 year old mortality and causes high number of hospitalization and hospital visits, but also does affect older children and adults although it causes milder infections and wears off quickly, it may in favourable circumstances cause more serious infections (Falsey, et al, 2000). There isn't much literature and studies on infection of RSV in adults, therefore the pathophysiology is not well known, but the few studies done show that it is most commonly found in immunocompromised adults and suggest that the smaller airways in children and smaller maternal immunity are the reasons why children are the most affected (Loubet, et al, 2016).

When it comes to gender many studies suggest that RSV can be more prevalent in one gender than the other, but those results are most of the time not statistically significant and usually biased especially when the number of sample is not taken equally in both gender (Salimi, et al, 2015; Rahman, et al, 2014). In Biko Hospital study the findings showed that RSV incidence was greater in male gender children than in female but they did not count it as a risk factor (Dearden, et al, 2018). Another study had the same findings but also found that more male coming from overcrowded houses were positive for RSV and more female that were not breastfed were positive for RSV which makes breastfeeding a protective factor from RSV (Ferolla, et al, 2019).

RSV seasonality varies globally and differs from one country to another. Countries that have an RSV surveillance system show more variation in RSV season and countries with no RSV surveillance show less variation in RSV season due to lack of data.

Preventive measures could be considered useless due to the major seasonal variation that may occasionally occur within countries; therefore a strong surveillance system is recommended (Obando, et al, 2018). Past Studies revealed that in temperate climate regions and countries like Europe and North America RSV followed the decrease in the temperature meaning RSV was more prevalent during the cold season or winter; However, in tropical and sub-tropical climate regions that have a lot of rain, RSV was mostly found during the rainy wet season, which is the result expected for Kinshasa DRC as it is found in the tropical climate region. In countries near the Equator like Mozambique and Malaysia, which have a heavily rainy humid season; RSV tend to go up to 10 months (Robertson, et al, 2004; Khor, et al, 2012). Countries like Brazil, Australia and USA which cover large territories and have more than one climatic region showed different intercountry RSV seasonality (Freitas, et al, 2016; Australia Gov, CDC); Since DRC also covers a very large territory and comprises more than one climatic region, same results are expected.

Paynter, et al, 2014 found a direct association between environmental factors and presence of RSV and for a seasonal RSV outbreak to occur. Because of the way the environment influences viral transmission, environment was stated to play a big role in the maintenance of RSV seasonality by influencing viral transmission during a specific period of time. Literature showed that RSV throughout the year occurs in seasons depending on the climate of regions, hemisphere and equatorial proximities (Ferolla, et al, 2013).

Currently there is no data available on RSV in Kinshasa, DRC and as mentioned above data availability is important for any given public health burden disease or infection such as RSV infection. Therefore a research study on RSV in Kinshasa, DRC was needed to fill the gap in information and to provide data. This could be done by firstly finding the proportion of RSV in Kinshasa, DRC then look at the seasonal factors and influences on RSV incidence in Kinshasa, DRC. Another reason why this study was conducted was to find which other respiratory virus besides Influenza was circulating and infecting people as the above question was raised after the first influenza research and sentinel surveillance in DRC revealed that out of all the samples screened for Influenza virus in Kinshasa only 15% were positive for Influenza virus in Kinshasa, and 9% country-wise; with that came the need to conduct researches on the remaining negative samples to find if other respiratory viruses could be present. Due to RSV public health burden and importance worldwide, it was only fair to firstly screen those remaining negative samples for the presence of RSV in the national influenza reference laboratory (NIRL) (Kavunga, et al, 2018; Nkwembe, et al, 2017; Muyembe, et al, 2012).

There are many callings in literatures of respiratory tract infections. In this study the terms used are influenza-like illness (ILI) and severe acute respiratory infection (SARI) taking notes that all SARI patients are hospitalized or in patients. Both terms are from the world health organization (WHO).

1.3. Study Goal

This study aimed to investigate RSV incidence in Kinshasa, DRC in order to find and state the proportion of RSV occurrence as well as the seasonal factors influencing Respiratory Syncytial Virus in Kinshasa DRC.

1.4. Study Objectives

1) Determine the prevalence of respiratory Syncytial virus circulating in Kinshasa DRC.
2) Determine the seasonal factors influencing respiratory Syncytial virus prevalence in Kinshasa DRC.

1.5. Study Question

What is the prevalence and seasonal factors influencing RSV in Kinshasa province of the Democratic Republic of Congo?

1.6. Hypothesis

1) Respiratory Syncytial Virus presence in Kinshasa, DRC could be affected by socio-demographic characteristics such as gender, age and also severity of infection and hospitalization.

2) Respiratory Syncytial Virus incidence could be different according to the rainy season and the dry season in Kinshasa province of the Democratic Republic of Congo.

CHAPTER II

METHODOLOGY

2.1 Study Design

A cross sectional study is a study that analyses data or representation of a population at a specific point of time whereby both the exposure and the outcome are measured at the same time. An Analytical cross sectional study is a study that analyses or determines the health outcome from an exposure and data from exposure and health outcome are selected and analyzed to compare the results and determine the prevalence of a health condition (Setia, 2016).

This research study design is analytical quantitative cross sectional study to determine the prevalence as well as the seasonality of RSV in Kinshasa, DRC by finding the proportion of RSV found in nasopharyngeal swabs from samples collected from January to September 2016 in Kinshasa, DRC and analyzed in the NIRL of the national institute of biomedical research (INRB) in Kinshasa during the year 2017 using secondary data.

2.2. Target population

The population used for this study was taken from the in and out patients of the hospitals that were part of the influenza sentinel surveillance project. The sentinel sites

chosen were both public or private clinics and hospitals in Kinshasa, DRC: RVA clinic, Boyambi Clinic, Kalembelembe Pediatric Hospital, Kinshasa General Reference Hospital and Kingasani Hospital Center (See Table 2.1 for characteristics of sentinel sites). The chosen sites demographically represented the population of Kinshasa as they received patients from all parts of the province. The criteria used for choosing the 5 sentinel sites were a higher accessibility, higher affordability for all patients including free and low cost services, higher number of qualified personnel, attendance of 50% on urban area and 30% on rural area, have good storage and a cold chain which is crucial when dealing with respiratory viruses and have the ability to easily ship samples to the laboratory. The medical staffs at the sentinel sites were trained to recognize patients that met the case definition for the study and were trained to efficiently collect, store, pack and transport the samples.

Table 2.1 Characteristics and description of 5 Sentinel Sites for sample collection

Sentinel Site	N° of Beds	Outpatient per months	Inpatient per months	N° of Doctor	N° of Nurse
Kingasani Hospital Centre	139	1250	170	8	53
General Reference Hospital of Kinshasa	2000	1820	789	403	1411
Boyambi Clinic	41	1061	16	8	23
Kalembe lembe Pediatric hospital	142	693	299	31	106
RVA Clinic	23	1020	35	3 + 5*	37

*Visiting doctor.

2.3. Case definition

The case definition was patient that presented with influenza-like illness (ILI) or showed signs of Severe Acute Respiratory Infection (SARI). ILI patient's symptoms were sudden onset of fever with a temperature of ≥38°C accompanied by cough or sore throat and the SARI patients were all hospitalized patients with or without fever accompanied by cough and difficulty in breathing plus one or more of the following symptoms: vomiting, unconsciousness, lethargy, convulsion and shortness of breath within the past 7 days (Ortiz, et al, 2009).

The specimens collected from patients with the patient's permission and acknowledgement of the research process were nasopharyngeal swabs that were obtained by having the patient's head tilted back at 70˚ angle then inserting a swab at the back of the throat or nose for a few seconds then take it out, the swab was then put in a cryovial tube containing 3ml of universal transport medium (UTM) and was kept at a temperature of 4-8˚C within the sentinel sites before it could be shipped to the laboratory for analysis. Later on the nasopharyngeal swabs were sent to the national reference laboratory in Kinshasa DRC the national institute of biomedical research where they would be analyzed.

2.4. Laboratory analysis

The samples came to the INRB and were analyzed in the NIRL located inside the INRB. Upon reception, the samples were first registered and given a reference number, then divided into 3 aliquots by transferring 1ml of samples respectively in 3 different eppendorf tubes, the tubes were then kept frozen at -80˚C before being analyzed for detection of RSV in the laboratory. The laboratory analysis done for detection of RSV was firstly extraction of nucleic acid material and secondly the identification of the virus by Polymerase Chain Reaction (PCR). Respiratory virus nucleic acid material is RNA, not DNA, therefore it was needed to do a reverse transcription PCR from RNA to DNA and the reference laboratory in Kinshasa INRB is to date the only laboratory in the Democratic Republic of Congo capable of detecting respiratory tract viruses using real-time reverse-transcription polymerase chain reaction assay (rRt-PCR assay).

2.4.1. RNA extraction

The extraction of the viral RNA was done using QIAamp Viral mini kit (Qiagen) following the Qiagen kit protocol. The kit contained:

- Quick-Start Protocol
- QIAamp mini spin columns
- Carrier RNA (Poly A)
- Buffer AVL
- QIAGEN® Proteinase K
- Buffer AW1 (concentrate)
- Buffer AW2 (concentrate)
- Buffer AVE

The viral RNA extraction or purification was done by following the Qiagen kit protocol following steps: lyses of the cellular material, precipitation of the nucleic acid material, adsorption and purification of the nucleic acid material.

2.4.2. Amplification

The purified RNA was amplified using the ABI 7500 Fast Ambion Applied Biosystems AgPath One-Step real-time reverse-transcription polymerase chain reaction assay (rRt-PCR assay) to screen for the presence of Respiratory Syncytial virus (RSV).

As stated before the NIRL in INRB to date is the only laboratory in DRC that is capable to perform Respiratory virus diagnostic using rRt-PCR assay and has met the standards of the External Quality Assessment Program (EQAP) administered by the WHO. Table 2.2 provides a description of the rRT-PCR machine set up including the different stages, number of cycles, temperature and time.

Table 2.2. Amplification Information

Stages	**Cycle**	**Temperature**	**Time**
Denaturation	1	45 °C	10 minutes
Annealing	1	95 °C	10 minutes
Elongation	45	95 °C	15 seconds
		55 °C	1 minutes

2.5. Outcome and Statistical Analysis

The outcome of this study was laboratory confirmed presence of RSV in the nasopharyngeal swabs of the study participants representing the population of Kinshasa in the Democratic Republic of Congo. The Statistical analysis was performed using the SPSS 25. A 95% confidence interval (CI) odds ratio was done to determine the association between the outcome and exposure. The statistical significance for this study was defined as $p < 0.05$.

CHAPTER III

RESULTS

3.1. Findings

Out of the 169 samples that were analyzed, 23 cases (13.6%) of the nasopharyngeal swabs were positive for respiratory syncytial virus and 146 samples were negative for RSV. Out of the 23 RSV positive samples, 8 (34.8%) positives samples were from male patients and 15 (65.2%) were female patients, 22 (95.7%) of RSV incidence occurred during the rainy season and 1 (4.3%) was found during the dry season. 18 (72.3%) out of the 23 samples positive for RSV were children between the age of zero and two and the denominator for age was 0-2 years following literatures and results from RSV researches around the world. According to the severity of the RSV infection, 60.9% of RSV positives were from hospitalized patient with severe acute respiratory infection and the 39.1% remaining were from patients suffering from influenza-like illness.

3.2. RSV Prevalence by age

In this study, 18 out of 23 RSV positive samples were from children between ages zero to two which was the denominator age for the study, as age increased RSV incidence decreased. One RSV positive sample was from a 6 years old patient, 2 RSV positives samples came from an 11 years old patient and a 25 years old patient and the remaining 2

RSV positive samples were from patients older than 40 years. Table 3.1 shows the average age for RSV incidence in Kinshasa, DRC including the standard deviation and the range.

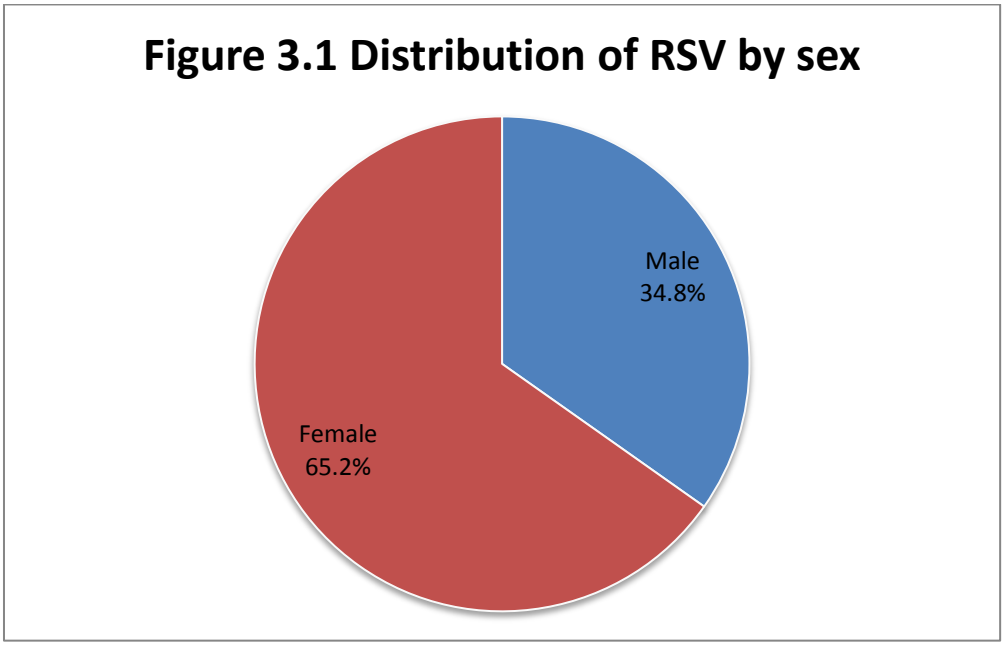
Table 3.1 RSV distribution by age

RSV Outcome	**Age**			
	Mean	STD	Lowest*	Oldest
RSV Positive sample	6.3	13.2	< 12 months	46
RSV Negative sample	18.3	21.5	< 12 months	71

* Lowest age: sample from patient younger than 12 months.

3.3 RSV Outcome by Gender

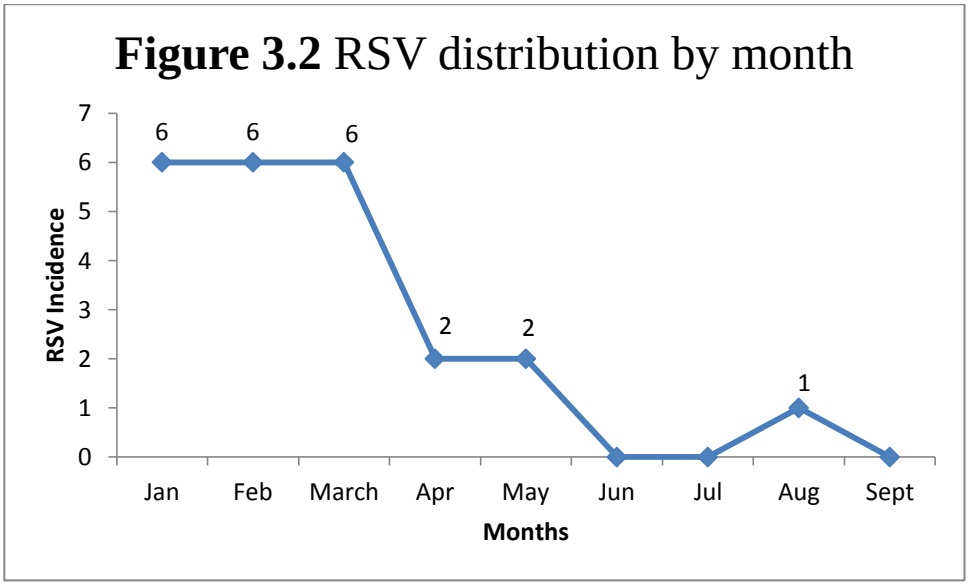
When it comes to gender, out of the 79 male and 90 female that participated in this study RSV was more prevalent in female than in men as seen in figure 3.1 and the statistical analysis shows that the association between gender and the presence of RSV was not statistically significant therefore stating that RSV incidence is not affected by gender. (Table 3.2)



3.4 RSV Seasonality

RSV positive samples in this study were unevenly distributed throughout the year between the two seasons found in Kinshasa, DRC which are the dry season that goes from June to September and the rainy season that goes from October to May in Kinshasa from the findings in this study. In Figure 3.2 which describes the distribution of RSV in the months of January to September 2016, it shows that January, February and March had the most occurrence of RSV with six RSV positive samples found respectively, followed by the months of April and May which had two RSV positive samples and lastly the month of August where only one RSV positive sample was found. During the dry season

which is considered the cold season in DR Congo meaning June to September there were little to no positive sample during those months.



3.5. RSV prevalence by case severity SARI and ILI

In this study the severity of infection was stated by whether patients from which the samples were taken had severe acute respiratory infection (SARI) and were hospitalized or had a milder infection which was an influenza-like illness (ILI). RSV findings showed that out of the 75 enrolled SARI and 94 enrolled ILI in this study, RSV was more prevalent in SARI samples and less prevalent in ILI samples. (Figure 3.3)

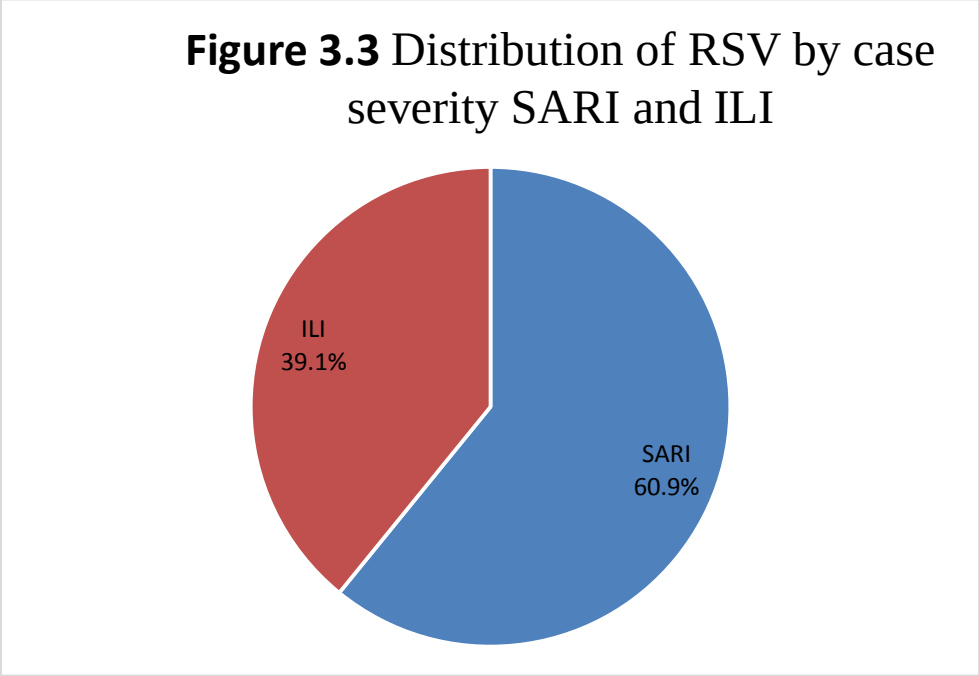


Figure 3.3 Distribution of RSV by case severity SARI and ILI

3.6. Data Analysis

As seen in the statistical analysis test table 3.2 children less than 2 years of age are less likely to be negative for RSV with an odds ratio of 0.149 and a confidence interval of 0.052-0.425 which is statistically significant. People in the dry season are more likely to be negative for RSV with an odds ratio of 23.230 and a confidence interval of 3.053 and 176.960 which is statistically significant. Females are more likely to be negative for RSV with an Odds ratio of 0.563 and a confidence interval of 0.225 and 1.410 which is not statistically significant. Hospitalised patients with severe acute respiratory infection are

more likely to be negative for RSV with an Odds ratio of 0.461 and a confidence interval of 0.188 and 1.134 which is not statistically significant.

3.7. PCR Value

PCR machine value comes from the machine and is called Cycle Threshold (CT), the cycle threshold is the highest amplification cycle from which the virus reaches its threshold, at that moment the nucleic material of the virus can no longer be amplified, it varies between samples and depends on the viral load of the sample, the larger the viral load the lower the CT would be and the lower the viral load the bigger the cycle threshold will be. Whenever there is no amplification, meaning the sample does not contain any virus, the machine would not give out a CT and say the CT is undetected (UD). Table 3.3 shows the CT of all of the 23 RSV positive samples and table 3.4 shows the average CT of all the samples including the controls (positive and negative) that were used during the amplification process.

Table 3.2 RSV Outcome and odds ratio finding

Variables		RSV Outcome		Odds ratio	95% CI	
		Negative	Positive		Lower limit	Upper limit
Age	0-2*(n=69)	51	18	0.149	0.052	0.425
	Other(n=100)	95	5			
Sex	Female(n=90)	75	15	0.563	0.225	1.410
	Male(n=79)	71	8			
Season	Dry(n=76)	75	1	23.23	3.052	176.960
	Rainy(n=93)	71	22			
Severity	SARI(n=75)	61	14	0.461	0.188	1.134
	ILI(n=94)	85	9			

*Reference age for comparison of RSV incidence by age according to general results from other studies.

Table 3.3 Positive sample cycle threshold

Positive Sample Laboratory N°	**PCR Cycle Threshold (CT≤40)**	**Positive Control CT (<35)**	**Negative Control CT (UD)**
16GP0011	18	32	UD
16GP0013	24	32	UD
16GP0022	28.7	32	UD
16GP0025	36.3	32	UD
16GP0051	32	32	UD
16GP0058	27.8	32	UD
16GP0173	18.8	33.1	UD
16GP0185	39.7	33.1	UD
16GP0204	19.9	33.1	UD
16GP0210	26.2	33.1	UD
16GP0339	21.4	33.1	UD
16GP0345	38.3	33.1	UD
16GP0399	33.1	33.1	UD
16GP0435	39.4	33.1	UD
16GP0482	40	33.1	UD
16GP0514	17	33.1	UD
16GP0519	38	33.1	UD
16GP0522	26	33.1	UD
16GP0523	40	33.1	UD
16GP0529	37.8	33.1	UD
16GP0740	31.7	33.4	UD
16GP0745	22.1	33.4	UD
16GP1308	37.3	33.4	UD

Table 3.4 PCR value in RSV-positive samples

Samples	**Mean**	**STD**
RSV negative	UD*	UD*
RSV positive	30.2	8.1
Positive Control	32.9	0.5

Note: *Undetected

CHAPTER IV

DISCUSSION

This analytical quantitative cross-sectional study on RSV prevalence and seasonality took part in five general hospitals and clinics in Kinshasa, Democratic Republic of Congo and aimed at determining the proportion of and seasonal factors influencing Respiratory Syncytial Virus in Kinshasa, capital province of DRC. Two hypotheses were raised; the first hypothesis stipulated that respiratory syncytial virus presence in Kinshasa, DRC could be affected by socio-demographic characteristics such as gender, age and also severity of infection including hospitalization and the second hypothesis stipulated that respiratory syncytial virus incidence could be different according to the rainy season and the dry season in Kinshasa province of the Democratic Republic of Congo. Both Hypotheses in this study were confirmed: RSV was mostly present during the rainy season and RSV was more prevalent in children age 0-2 years, more prevalent in women than in men and was more prevalent in SARI samples than in ILI samples.

4.1. RSV prevalence

In many countries there is a need for data on the presence of respiratory syncytial virus (Griffiths, et al, 2017). This study tackled the prevalence using the variables gender,

age, season and severity of infections. Statistical analysis using SPSS 25 was used to determine the association between the variables and the outcome which was rRt-PCR detection of RSV and also the likelihood of RSV infection. This study showed that among inpatients and outpatients analyzed, 13.6% (N=23) was positive for RSV which was close to the prevalence of RSV findings in other countries such as the following countries in Africa: Kenya 17% (N=2050), South Africa 10.7% (N= 5743), Gambia 8.7% (N= 4799), Ghana 14.1% (N= 128), Mozambique 9.8% (N= 1172) and Burkina Faso 11.9% (N= 209) (Bigogo, et al, 2013; Pretorius, et al, 2016; Weber, et al, 2002; Kwofie, et al, 2012; Fodha, et al, 2004; Ouédraogo, et al, 2014). In Madagascar, in an acute respiratory infection study done over one year the percentage of RSV positive was 11.8% (Hoffmann, et al 2012).

These results can be representative of what is happening in the community of Kinshasa, DRC. Because of the small number of positives, the association between health facilities and RSV incidence was not checked nor taken in consideration.

4.2 Gender

In this study RSV was more prevalent in female than in male and at the same time it showed that females are more likely to be negative for RSV in Kinshasa. Other studies have shown that RSV was more predominant in male than in female whereby male were more affected (Glezen, 1977). The difference in findings from other studies could be due

to the fact that more female than male were part of this study population. Moreover the statistical analysis showed that there was no statistically significant association between gender and the presence of RSV which correlated with many other studies around the world whereby RSV is more prevalent in one gender but the finding is not statistically significant (Salimi, et al, 2015; Rahman, et al, 2014). Many studies also failed to show or find a direct association between gender and RSV infection (Dearden, et al, 2018).

4.3. Age

When it comes to age distribution of RSV, the virus occurrence and prevalence have demonstrated to be the same in all countries; it shows similarity in developed countries as well as developing countries (Glezen, et al, 1981; Glezen & Denny, 1973).

Nolan et al, 2015 found in her study on the prevalence of RSV that RSV prevalence declined as age decreased and that finding was the same in this study as the RSV positive samples were the least in adults and children older than 5 years of age and was more prevalent in children at the age of 2 and/or younger than 2 years. This study on RSV in Kinshasa had 18 positives in children age 0-2, 1 RSV positive in children 5-10, 2 RSV positive in age 10 to 25, and 2 RSV positive in age 40 and above which includes the elderly.

The findings from this study on RSV in Kinshasa is similar to many studies whereby children under the age of 2 were less likely to be negative for RSV and had higher risks and chances of being infected by RSV, a few cases of RSV positives in elderly were found in this study which also goes along with most reviews that also found sporadic cases of RSV infections in elderly. It is safe to say that the findings from this study correlated with many reviews and papers that looked at the association between age and RSV because this study also found a strong association $p< 0.001$ between age and RSV incidence in Kinshasa, DRC.

4.5. RSV by case severity SARI and ILI

Although this study findings showed that RSV was more present in samples of patients hospitalized with severe acute respiratory infection (60.9%) and less present in samples from patients that had influenza-like illness (39.1%) and that SARI patients are less likely to be negative for RSV, the statistical analysis showed that there was no statistically significant association between the severity of the infection and the presence or incidence of RSV.

This finding goes hand in hand with other literatures and studies done to find if there was an association between acute respiratory infection and RSV presence (Singleton, et al, 2010; Ouédraogo, et al, 2014; Al-Ayed, et al., 2014). However, in a study on the prevalence of acute respiratory infection caused by RSV, findings showed

that there was an independent association between RSV and the risk of developing an acute respiratory infection, but this specific study only included children younger than the age of 5 years old, had a larger number of study sample and was done on a shorter period of time (5 months), those differences could have favoured the difference in findings from that study and this study on RSV in Kinshasa (Kabego, et al, 2018).

4.6. Seasonality

Respiratory syncytial virus has shown to have seasonal outbreaks in many countries around the world, in countries with mediterranean and temperate climate, RSV occurs during the relatively cold seasons, and in countries with tropical climates it occurs during the wet rainy season (Weber, et al, 1998). Studies have shown that seasonal RSV occurred in phases where there was a shorter phase of seasonal RSV infection and a longer phase of seasonal RSV infection (Elena, et al, 2005).

The Democratic Republic of Congo is a very large country, second largest in Africa after Algeria and is passed through by the Equator line; the country has a predominantly tropical climate with two rainy season peak that can go up to seven months (World Bank, 2007). Parts of DRC that are located on the north of the country experience wet rainy season from the month of April to the month of November, wherever the regions located on the south of DRC experience rainy season from October to May. The province of Kinshasa from which the samples were collected is located on

the south bank of DRC therefore experiences rainy season from October to May and dry season from June to September. The rainy season is relatively hot, wet and humid whereby the dry season is less hot and a tad cold and is considered the "cold season" with temperatures dropping to 20˚C and 17˚C at its lowest (Burton, 2010).

This study in Kinshasa, DRC showed that RSV occurred predominantly 95.7% during the rainy season, which goes in accordance with most study findings on RSV seasonality for tropical climates. RSV incidence in this study decreased together with the temperature meaning the colder it got, the less RSV incidence occurred and this too was found in studies done in countries with similar climate to that of DRC. The cold or dry season in Kinshasa, DRC is holiday time and for 3 months straight all schools are closed, this can partially explain the decrease in RSV incidence as there might be lesser chances of contamination between children during that time since they do not attend school.

4.7. Study Strengths and limitations

4.7.1 Study Strengths

This study is the first study done in Kinshasa DRC that gives a picture on the proportion of RSV in Kinshasa DRC and the first ever study done that studies the seasonal factors influencing the presence of RSV in the country at a community level based representation. This study used PCR laboratory detection to screen for RSV which

is one of the gold standards for detection of RSV around the world. This study may contribute in the future in implementation of preventive measures in the country, educational campaigns on RSV, increase knowledge on RSV, estimating public health burden on the community and health system due to RSV infections, stating the needs for proper equipment and resources for management of RSV infections and allocation of those resources. The results from this study done on RSV in Kinshasa, DRC correlated with many studies and reviews done and published on RSV globally.

4.7.2 Study Limitations

The study population was randomly chosen but not randomized from the Influenza sentinel sites, so it represents the community of Kinshasa but could be argued to represent the entire population of Kinshasa. The sample used for this study were from the year 2016 which due to funding cessation only ran from January 2016 to September 2016 therefore when it comes to seasonality, there is a limitation due to the missing remaining months of October, November and December.

Seasonality studies are advised to go on for a longer period of time therefore strongly suggesting more studies and more researches to properly represent RSV seasonality in the country. And another point is DRC is the second largest country in Africa after Algeria and is passed through by the equator making the country fall into 2 different climatic regions, however seasonal factors and influences found in this study

only applies to Southern part of DRC where Kinshasa is found and can not apply to the entire country, therefore studies need to be done in both climatic regions of DRC to represent each one accordingly. Furthermore this study on RSV in Kinshasa is limited to the tropical climate region where Kinshasa falls whereby the rainy season goes from October to May and the cold dry season goes from June to September meaning the findings from this study only represent one climate region and not the all territory of DRC.

Lastly, this study did not look into the incidence of the subtypes of RSV which requires more time, funding, resources and equipment.

4.8 Recommendations

Further researches should be done on a much larger population and on a randomized population based study for the prevalence of RSV in the entire country. In addition, a study that covers many years should be done for an accurate and better representation of seasonality of RSV in all climate regions of DRC to represent the entire country. Research on RSV subtypes circulating in the country, association with risk factors as well as defining the risk factors in DRC for RSV should be done to help in the future with the prevention of RSV infections in DRC. A study on the burden of RSV in DRC including financial impact is recommended since burden of disease is important to know in public health and in any given country because it provides estimates on that

disease by providing information about the disease and health information which are respectively important for the prevention and control of any health condition, for resources' allocation, for estimation of intervention's cost when needed and lastly to provide data on RSV burden in DRC which the country currently lacks.

CHAPTER V

CONCLUSION

This analytical quantitative cross-sectional study on the seasonality and prevalence of respiratory syncytial viruses in Kinshasa, DRC aimed to investigate the proportion of RSV and seasonal factors influencing RSV in Kinshasa, DRC at community level. The objectives were to give the prevalence of the virus and give a picture on the seasonality of the virus in Kinshasa which is the capital city of DRC by analyzing nasopharyngeal samples from inpatients and outpatients of 5 sentinel sites in Kinshasa collected from January to September 2016 and analyzed using PCR in the NIRL at the INRB.

After analysis of the samples, interpretation of the results as well as statistical analysis and interpretation, the results obtained from this research study showed that RSV infection occurs at most during the rainy season in Kinshasa, DRC with over 90% of RSV positive sample found in the rainy season and whereby people are less likely to develop an RSV infection during the dry season. A peak in RSV infection was observed between January and March 2016. This research study also revealed that children are the most infected by RSV in Kinshasa, DRC and that the virus was more predominant in children younger than 2 years and the same age range was found to be more likely to suffer from an RSV infection. Female in this study were the most infected by RSV compared to male, whereas male were found to be more likely to develop an RSV infection.

APPENDIX 1 Authorisation letter to use samples and data from the INRB

REPUBLIQUE DEMOCRATIQUE DU CONGO
Ministère de la Santé Publique
Institut National de Recherche Biomédicale

07 OCT 2019

Merveille Kasenga Kapandji
Yonsei University, South Korea
Phone: +821032769008
Email: mckkapandji@gmail.com

Dear Merveille Kasenga Kapandji,

Authorization to use the 2016 samples and data from the National Influenza laboratory at the National Institute of Biomedical Research Kinshasa, Democratic Republic of Congo

This is to confirm that you are approved to use the 2016 samples and data from the National Institute of Biomedical Research Kinshasa, Democratic Republic of Congo for your registered research paper titled: **"Seasonality and Prevalence of Respiratory Syncytial Virus in Kinshasa, Democratic Republic of Congo"**

As an employee of the state and duly approved for your training, the data should be used only for the purpose of laboratory analysis, statistical analysis and reporting, and only for the aforementioned study.

Sincerely,

Signature

Dr Edith Nkwembe Ng.
CNOM 4332
Médecin Biologiste
RDC

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