

A seroepidemiological study of respiratory syncytial virus infection in the Littoral Region of Cameroon

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Abstract

Background

Respiratory syncytial virus (RSV) is responsible for about 300 000 deaths in young children per year, and 99% of these occur in low-income countries. This study aimed to assess the burden of RSV infection among children less than two years with acute respiratory infections (ARI) in the littoral region of Cameroon.

Methods

We carried out a cross-sectional study in seven health facilities in the littoral region of Cameroon. Venous blood was collected using serum separation tubes from eligible children who visited these healthcare facilities with acute respiratory infections. ELISA determined the seroprevalence of anti-IgM RSV. Factors associated with RSV infection were ascertained using logistic regression.

Results

Out of 100 study participants, the overall RSV-associated ARI seroprevalence was 33% (95%CI:23.6-42.3). Factors significantly associated with RSV acquisition were age below six months ($p=0.000$) and mixed feeding ($p=0.015$).

Conclusions

The RSV burden is high among children less than two years with ARI in the littoral region of Cameroon. There is a need for an effective public health RSV surveillance system with standard laboratory techniques and equipment to better understand the RSV disease age-specific incidence, seasonality, and RSV burden among patients in the communities in Cameroon.

Introduction

Global estimates indicate that RSV may cause about 300 000 deaths in young children per year, and 99% of these occur in low-income countries [1]. The respiratory syncytial virus has been established as a leading cause of acute lower respiratory illness (ALRI) in infants and children living in all regions of the world [2, 3]. In 2015, the global disease burden estimated the overall RSV-ALRI mortality could be as high as 118200 (uncertainty range [UR] 94 000–149 400), which would constitute 8.2% of the estimated 1.4 million deaths classified as sepsis or pneumonia [4]. Hall et al. in 2009 found a significant RSV disease burden in neonates, with estimates of 40 episodes per 1000 neonates per year (95% CI 2.5–635.7) [5]. Resource-limited countries have more than twice the incidence of severe disease seen in developed countries [6–8]. The need for precise epidemiologic data regarding RSV as a worldwide pathogen has been growing steadily as novel RSV therapeutics are reaching the final stages of development [9, 10].

By two years of age, almost all children will have been infected with RSV, and approximately 50% infected twice. Re-infection with RSV can occur throughout life and is often symptomatic. Despite an increasing number of epidemiological studies of RSV-associated lower respiratory tract infections (LRTI) published in developing countries, there is still a need to establish a public health RSV surveillance system to improve the incidence estimates and to determine national and regional variations in RSV disease burden in countries [11]. Among children aged less than five years, the incidence of RSV-associated LRTI per 1000 child-years was 34 in Indonesia, and 94 in Nigeria and the incidence of severe RSV-associated LRTI per 1000 child-years was 5 in Mozambique, 10 in Indonesia, and 9 in South Africa [12].

A systematic review study identified 20 studies that investigated 18 risk factors for RSV-associated ALRI in children younger than five years old. Among them, eight risk factors were significantly associated with RSV-associated ALRI, namely prematurity, low birth weight, being male, having siblings, maternal smoking, history of atopy, no breastfeeding, and crowding [13].

A study in Cameroon showed that RSV circulated in the beginning of the dry season from October to December at 5.7% in outpatients with influenza-like illness visiting influenza surveillance centers in 2009. Another study recently showed that RSV was the second most common respiratory virus (13.3%) after human adenovirus in children hospitalized in Yaoundé, Cameroon [14, 15]. The unicentric study did not find a significant age-specific RSV prevalence, which might have underestimated the overall detection rate for selected viruses. The enrolled cases may not be representative of the entire population of children in Cameroon as these studies were based in urban settings. More studies should be expanded in rural or semi-urban regions to provide a better understanding of the epidemiology and spectrum of illness caused by respiratory viruses, especially RSV in Cameroon [16]. Therefore, we sought to assess the burden of RSV infection among children less than two years in a semi-urban setting of the littoral region of Cameroon.

Methods

Study Area

Cameroon, a country in Central Africa, has an area of 475,650 km². According to the 3rd National Population and Housing Census, the estimated population of Cameroon in 2015 was at about 22,179,707 inhabitants. The portion of the population aged below 25 represents 64.2%. Accessibility to public health facilities is more evident for the wealthiest segments of the people, like those living in urban areas (52%). The health sector is structured into primary, secondary, and tertiary levels [14].

In children, less than five years in Cameroon, low respiratory infections, malaria, diarrheal diseases, and nutritional deficiencies are the leading causes of morbidity and mortality. In children aged two months to five years, malaria (21%), diarrhea (17%), pneumonia (17%), and HIV/AIDS (7%) are the leading causes of this mortality. Essential family practices and interventions with a high impact on the child's health (vaccination, exclusive breastfeeding, etc.) are not, however, sufficiently implemented to reverse the figures, as mentioned above [14]. In 2011, there were 189 health districts, 4034 health facilities with the public (72%), and private (28%). These health facilities serve the general population. Most of the services are still out-of-pocket and considered expensive by the public. The high cost of healthcare services in private health facilities encourage users to resort to informal care or home care [14].

This study was conducted in seven health facilities: the regional reference hospital of Nkongsamba, Bare sub-district health center, Eboumbeng sub-district health center, Eboumbeng integrated health center, Bonangoh integrated health center, Nlongko'o sub-district health center, and Bare integrated health center. Regional Hospital of Nkongsamba represents a secondary hospital with a catchment area of about 320 000 inhabitants. The rest of the study sites are part of the primary healthcare level. The study sites are representative of the healthcare system in Cameroon. We selected the littoral region at random out of the four areas in Cameroon, which has a surveillance system of influenza and other respiratory diseases. Thus, the region possesses a reasonable research capacity in monitoring respiratory infections. The littoral region was then selected at random out of the four for this study. The littoral region comprises of 26 health districts and 212 health facilities.

Study design, Target population, and Sampling

This multicentric cross-sectional study was conducted within six months, from March to September 2018. The target population was children less than two years who visited the study sites with acute respiratory infections (ARI). The sampling procedure was as follows: firstly, we randomly selected the littoral region among four areas that comprise sentinel sites for influenza-like illnesses based on regional health retrospective data. And, secondly, we chose two health districts (Melong and Nkongsamba) among 21 health districts of the region. Thirdly, we selected at random the seven study sites from the list of 45 health facilities in the two health districts respecting the selection criteria of being situated at most 5 km from the reference laboratory and possess a well-functioning refrigerator (Fig. 1). The regional hospital of Nkongsamba is also the health district hospital that serves as the reference hospital for all the other study sites in the rural communities. The sample size (n) calculated using the single proportion formula was 100, where population proportion(p) = 0.50,

confidence level of 95% and desired effect size (d) = 0.10 to obtain a sample that is enough to ensure precision with a 4% to adjust for nonresponse rate. Since the prevalence for outpatient and admitted patients with RSV infection is unknown, we assumed $p = 0.50$ [21].

The stratified sampling proportional allocation strategy did the allocation of the sample size to the study sites. The size of each stratum was based on the number of pediatric ARI cases of the year 2017. In this study, each study site was considered as a stratum (Fig. 1).

Here: Figure 1. Study site selection and sample size allocation

Inclusion and exclusion criteria

A case of ARI was defined as illness fulfilling age-specific clinical inclusion criteria with onset within seven days in a child aged less than two years. Acute respiratory infection was defined as an illness presenting with one or more of the following symptoms: fever, cough, earache, nasal congestion, rhinorrhea, sore throat, vomiting after coughing, wheezing, and labored, rapid, or shallow breathing. We excluded children who had respiratory symptoms lasting more than 14 days because RSV infection may have been acquired in the health facility during the perinatal period, who had neutropenia from chemotherapy, had been hospitalized elsewhere within four days or were newborns who had been hospitalized since birth.

Laboratory assessments

The criteria for selection of recruitment and collection sites included were: at most 5 km from testing site, and a well-functioning refrigerator. Venous blood of 2-ml was collected from all enrolled patients centrifuged and plasma transferred into 1-ml cryotubes containing virus transport medium. Plasma was stored at 4–8 °C at the collection site for a maximum of 48 hours. Samples were then transported, maintaining the cold chain using the triple packaging system to the testing site and stored at -80 °C pending testing.

RSV Immunoglobulin M (IgM) ELISA testing procedure: A 96-well plate was precoated with Respiratory syncytial virus antigens to bind cognate antibodies. Controls or test samples were added to the wells and incubated. Following washing, a horseradish peroxidase (HRP) labeled anti-Human IgM conjugate was added to the wells, which binds to the immobilized Respiratory syncytial virus-specific antibodies. 3,3',5,5'-Tetramethylbenzidine (TMB) was then catalyzed by the HRP to produce a blue substrate that changes to yellow after adding an acidic stop solution. The yellow coloration is directly proportional to the amount of Respiratory syncytial virus IgM sample captured in plate.

Data collection and analysis

Nurses and laboratory technicians of each study health facility were trained to collect clinical data using a pilot-tested questionnaire and blood samples and transported to the research lab respecting the SOP of sample collection, transportation, and storage. Raw data and laboratory results were recorded and stored in a central database (Microsoft Excel). The statistical analysis was performed using Stata, version 11.0 (StataCorp, College Station, TX). The primary outcome RSV prevalence was determined by proportion of RSV positive children (95% exact confidence interval (CI) for proportion), calculated by dividing the total number of RSV positive children by the total number of children tested for RSV. Means and standard deviations were calculated for continuous variables. The Student t-test was used for continuous variables and the Pearson Chi-square test for categorical variables. A stepwise logistic regression analysis was used to analyze RSV-associated factors. A p-value of < 0.05 was considered statistically significant.

Results

In total, 100 eligible children were enrolled, with (65/100) 65% of cases from the hospitals. The male-to-female ratio was 0.9:1, with an average age of 10.6 months (SD = 6.11), and those below six months formed 22%. More than half of the children were diagnosed clinically for malaria 55 (55.6%) and 71 (82.6%) on mixed feeding (breast milk and bottle-feeding).

Few had underlying conditions like the previous wheezing (9.1%), chronic lung disease 8 (8.1%), low birth weight 9 (10.3%), and 19 (19%) were born premature (Table 1).

The most frequent clinical signs/symptoms were fever, cough, wheezing, difficulty breathing, vomiting, and inability to drink or breastfeed. A severe lower respiratory infection like bronchiolitis (30.0%) and pneumonia (16.7%) were clinically diagnosed among RSV-positive patients by the clinicians or attending physicians.

Table 1

Characteristics of children under two years with RSV-associated Infection

Variables	no. (%) RSV +	N (%) RSV -	p-value
Health facility (N)			
Primary health center	13 (39.4)	22 (32.8)	
Hospitals	20 (60.6)	45 (67.2)	
Total	33 (100.0)	67 (100.0)	
Gender (N)			
Male	10 (33.3)	31 (52.5)	
Female	20 (66.7)	28 (47.5)	
Total	30 (100.0)	59 (100.0)	
Age (months)			0.000
0–5	16 (48.5)	06 (09.0)	
6–11	10 (30.3)	25 (37.3)	
12–23	07 (21.2)	36 (53.7)	
Total	33 (100.0)	67 (100.0)	
Underlying condition / illness			
Malaria	14 (42.4)	41 (62.1)	
Chronic lung disease	01 (12.5)	07 (10.6)	
Previous wheezing	05 (15.2)	04 (06.1)	
Type of feeding			0.015
Exclusive breastfeeding	09 (30.0)	04 (07.1)	
Exclusive bottle-feeding	01 (03.3)	01 (01.8)	
Mixed breast and bottle	20 (66.7)	51 (91.1)	
Total	30 (100.0)	56 (100.0)	
Birth weight (kg)			
Low Birthweight \leq 2.5	03 (10.7)	06 (10.2)	
Normal weight $>$ 2.5–4.0	25 (89.3)	53 (89.8)	
Total	28 (100.0)	59 (100.0)	
Child immunization status			
Vaccinated	27 (84.4)	61 (95.3)	
Partially vaccinated	04 (12.5)	03 (04.7)	
Unvaccinated	01 (03.1)	-	
Total	32 (100.0)	64 (100.0)	

Variables	no. (%) RSV +	N (%) RSV -	p-value
Maternal education			
Uneducated	01 (03.13)	02 (03.13)	
Primary	09 (28.13)	12 (18.75)	
Secondary	17 (53.13)	36 (56.25)	
University	05 (15.63)	14 (21.88)	
Total	32 (100.0)	64 (100.0)	

Table 1 shows 20 (66.7%) children under mixed feeding were RSV positive ($p = 0.015$). Of the 100 samples, the RSV-associated ARI seroprevalence was 33.0% (95%CI:23.6–42.3) among children less than two years at the seven study sites in the littoral region of Cameroon. Almost half of the children below six months were RSV positive, and the prevalence decreased as the ages of the children increased ($p = 0.000$) (Fig. 2).

Here: Figure 2. The proportion of RSV infection by the age of the children less than two years with ARI

Univariate analyses identified the factors associated with respiratory syncytial virus among children less than two years (Table 2). Only the type of feeding ($p = 0.015$) and age ($p = 0.000$) were significant. Logistic regression analysis revealed that children below six months were highly less likely to acquire an RSV infection than those above six months [OR = 0.10 (95%CI: 0.03–0.30)]. Also, children on exclusive breastfeeding were highly less likely to acquire an RSV infection than those on mixed feeding [OR = 0.17 (95%CI: 0.04–0.64)]. These relationships were both strongly significant. No lethal RSV infection was observed. The clinical presentation of the RSV-positive children was not significantly different from children with RSV-negative acute respiratory infections. RSV-associated ARI patients were inappropriately prescribed antibiotics (91.3%).

Table 2
Odds ratios of factors associated with patients with RSV infection

Variables	Prevalence of RSV infections	
	n (%)	OR (95%CI)
Health facility		
Primary healthcare center	13 (39.39)	1.32 (0.56–3.12)
Hospital	20 (60.61)	1
Sex		
Male	10 (33.33)	2.21 (0.88–5.52)
Female	20 (66.67)	1
Age (months)		
< 6	16 (48.48)	0.10 (0.03–0.30)
≥ 6	17 (51.52)	1
Underlying condition / illness		
Chronic lung disease	01 (12.50)	0.26 (0.03–2.23)
Previous wheezing	05 (15.15)	2.76 (0.69–11.09)
Feeding type		
Exclusive breastfeeding	09 (30.00)	0.17 (0.05–0.61)
Mixed feeding	21 (70.00)	1
Low birth weight	03 (10.71)	0,94 (0.21–4.08)
Prematurity	06 (16.13)	0.72 (0.23–2.23)
Maternal education		
Primary or no schooling	10 (31.25)	0.61 (0.24–1.57)
Above Primary	22 (68.75)	1
Household smoking	04 (12.50)	0.48 (0.14–1.60)
Indoor air pollution	22 (68.75)	2.20 (0.90–5.35)

Discussion

This is the first multicenter study to better understand the burden of RSV-associated ARI in Cameroon. The prevalence of 33% recorded is similar to studies from Turkey, Iran, Brazil, and Egypt (17). Our result was superior to that of a previous study showing a rate of detection of RSV at 5.7% of patients with influenza-like illness visiting influenza surveillance centers in 2009 in the central region of Cameroon. One possible reason was that we included all ambulatory or hospitalized cases different from the previous study that included only outpatients made up of children and adults. Another probable cause could be that this study was conducted in the late phase of the rainy season and maybe a decreasing period of the RSV season in Cameroon [18]. Another study showed that RSV was the second most common respiratory virus (13.3%) among hospitalized children ≤ 15 years with severe acute respiratory infections (SARI) after human adenovirus in Yaoundé, Cameroon [16].

This study showed a significant RSV disease burden among children below six months which is in-line with other researchers [19] and suggest the necessity of passive protection against RSV infection at birth, either through maternal immunization or administration of a birth dose of an extended half-life mAbs at birth and the importance of developing vaccines for active infant immunization to provide durable protection against RSV disease.

Though the male-female ratio of RSV-positive patients was 1:2, gender was not a significantly associated factor. This result is similar to a prevalence study in Brazil [24]. Nevertheless, some studies in the literature have revealed being male as a risk factor to acquire RSV infection [13, 19].

More than two-thirds of the children on mixed feeding were RSV positive, a significantly higher proportion than those on exclusive breastfeeding. This finding showed that patients on mixed feeding were more likely to acquire RSV than those on exclusive breastfeeding, indicating children on exclusive breastfeeding were well protected and probably with stronger immune systems against a viral infection like RSV. Many epidemiological studies show that breastfeeding can reduce the frequency, severity, and mortality of respiratory disease in infants [20]. According to the literature, breast milk contains a series of components with immunomodulating properties, which represent a benefit for the infant. Its antimicrobial, anti-inflammatory and immunomodulatory agents are multifunctional and act synergistically.

Ninety-one percent of the RSV-positive children were inappropriately prescribed antimicrobial drugs (antibiotics). Antibiotic treatment was not appropriate for patients with RSV infection. This finding was similar to that of a study in Saudi Arabia that demonstrated a high prevalence of antibiotic misuse ranging from 42–92%, especially in children [21]. The reasons for antibiotic misuse are complex, and several contributing factors are evidently associated with the overuse of antibiotics in both the patient's (or parents of children) level and doctor's level. These factors include cultural factors, behavioral characteristics, socioeconomic status, and level of education [21]. Antimicrobial resistance is a global public health challenge, which has been accelerated by the overuse of antibiotics worldwide. Antibiotic overprescribing is a particular problem in primary care, where viruses cause most infections. General practitioners issue about 90% of all antibiotic prescriptions and respiratory tract infections are the leading reason for prescribing. Multifaceted interventions to reduce overuse of antibiotics are effective and better than single initiatives. Interventions should encompass the enforcement of the policy of prohibiting the over-the-counter sale of medicines, the use of antimicrobial stewardship programmes, the active participation of clinicians in audits, the utilization of valid rapid point-of-care tests, the promotion of delayed antibiotic prescribing strategies, the enhancement of communication skills with patients and the performance of more pragmatic studies [22]. There is a need for a global strategic effort to develop a portfolio of vaccines that target AMR [21].

Malaria infection is suspected in all patients with fever. Malaria may result in fever and raised respiratory rate; therefore, it could be a confounding infection mimicking ALRI in countries where malaria is endemic. Increasing awareness is essential for healthcare workers who should provide adequate diagnosis and treatment of both acute respiratory infections and malaria.

Infants with underlying medical conditions, prematurity, low birth weight, poor parental education, household smoking, and exposure to indoor air pollution have been revealed as risk factors to acquire RSV infections. Still, none of the elements were shown as significant associated factors in our study [23, 24].

Further large cohort and interventional studies in LMIC settings are needed to elucidate these risk factors, as these studies are best suited for this purpose. Parents and caregivers who do not have adequate knowledge concerning preventive measures put infants at higher risk. The infant's home environment is also essential. Infants from low-income families tend to be more at risk, perhaps in part due to lack of access to medical care and in part, to lack of maternal education. One of the most substantial environmental risk factors for infant respiratory infection is exposure to passive tobacco smoke [24]. A case-control study of 53 infants with bronchiolitis found that any exposure to passive smoke was the strongest predictor ($p = 0.004$) [24]. The study sites of our research are located in the rural and semi-rural settings in the littoral regions of Cameroon, where most of the families use solid fuel like firewood or charcoal for cooking. Exposure to household air

pollution is preventable. Still, resources are limited in low-income populations with competing for health priorities: with high-quality evidence of the accurate scale of the problem and cost-effectiveness of interventions, resources to reduce the global burden of disease can be effectively allocated.

Among the RSV-positive children, the most frequent clinical symptoms/signs were fever, cough, wheezing, difficulty breathing, vomiting, and inability to drink or breastfeed. This is the typical symptomatology of acute respiratory infection; RSV is one of the etiologies indicating, therefore, that the healthcare system in settings in Cameroon and other LMICs should put in place diagnostic possibilities in their health facilities to guide management and infection control. This also shows that emphasis should be placed on prevention. An RSV vaccine will play a vital role in reducing the infant mortality that is highest in low- and middle-income countries.

A third of the RSV-positive children were clinically diagnosed to have bronchiolitis, and one-sixth had pneumonia by the clinicians or attending physicians. This indicates that RSV is an established cause for acute lower respiratory infections in children. In settings where viral etiologies are not systematically checked and resources not available, the critical solution to save lives is through preventive measures like vaccines and monoclonal antibodies.

The strength of our study lies in the uniqueness of conducting such a seroprevalence study in the rural and semi-rural communities in Cameroon faced with several human resources, infrastructural and logistical bottlenecks. The study sites were mostly first-line primary and secondary healthcare facilities with little experience in health care research. This study was integrated into their routine health care activities and has undoubtedly built a clinical research capacity that needs to be further strengthened in such settings.

A limitation of our study was that we relied on a single laboratory test for detecting RSV antigen, the ELISA test. This is likely to have led to underestimates or overestimates of the disease burden. The use of multiple methods, including polymerase chain reaction, in addition to the ELISA test, may have been the best option. However, in the field of community settings, the ELISA test was the only practical, affordable, and feasible choice. Compared to virus isolation, using the ELISA test to detect RSV has been shown to have a sensitivity of 94% and a specificity of 97%. There is also the possibility of higher false positives as compared to the standard test RT-PCR. Still, we limited this by using IgM RSV ELISA, which has a life span of just 30 days in the immune system and also included only children with acute respiratory infections with an early onset of not more than seven days. However, the evidence from this study documents a substantial disease burden associated with RSV in the littoral region of Cameroon.

In a nutshell, RSV burden is high among children less than two years with ARI in the littoral region of Cameroon. Accurate clinical and laboratory diagnosis of RSV infection among these patients with ARI is necessary to reduce the disease burden, large-scale RSV spread, and the misuse of antimicrobial drugs. Further studies are required to better understand antimicrobial drug overuse and abuse, especially in this era of antimicrobial resistance. There is a need for an effective public health RSV surveillance system with standard laboratory techniques and equipment to better understand the RSV disease age-specific incidence, seasonality, and RSV burden among patients in the communities in Cameroon.

Declarations

Ethical approval and consent to participate

The study was approved by the Cameroon Bioethics Initiative Ethics Review and Consultancy Committee (Reference number CBI/429/ERCC/CAMBIN), and permission was sought locally from the authorities of the health facilities and the regional health delegation of Littoral, Cameroon. Verbal and written informed consent were sought from the parents or guardians of study participants, and their information kept confidential.

Consent for publication

Not Applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare no conflict of interest.

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Author's Contributions

Conception and design: HM

Investigation: HM CEB

Project Administration: HM CEB AE

Supervision: HM SY CEB AE

Formal Analysis: HM

Writing – Original Draft Preparation: HM SY CEB AE

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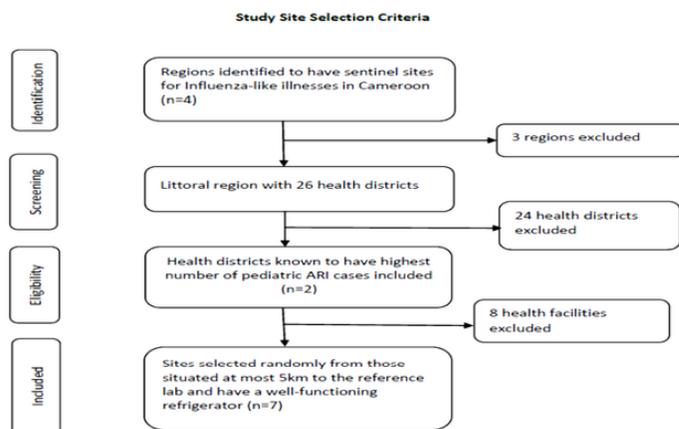
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Figures

Fig1. Study site selection and sample size allocation



Sample Size Allocation

Health facilities	Target Population (≤ 2 years)	Total number of ARI cases (2017) *	Sample size
Bare sub district hospital	2191	194	15
Bare integrated health center	1496	133	10
Eboumbeng sub district hospital	2212	196	15
Eboumbeng integrated health center	1443	128	10
Bonangoh integrated health center	1426	126	10
Nlongko'o sub district hospital	1465	130	10
Nkongsamba reference hospital	4370	387	30
TOTAL	14604	1293	100

*ARI = Acute respiratory infections of children under 15 years

Figure 1

Study site selection and sample size allocation

Fig 2. Proportion of RSV infection by age of the children less than 2 years with ARI

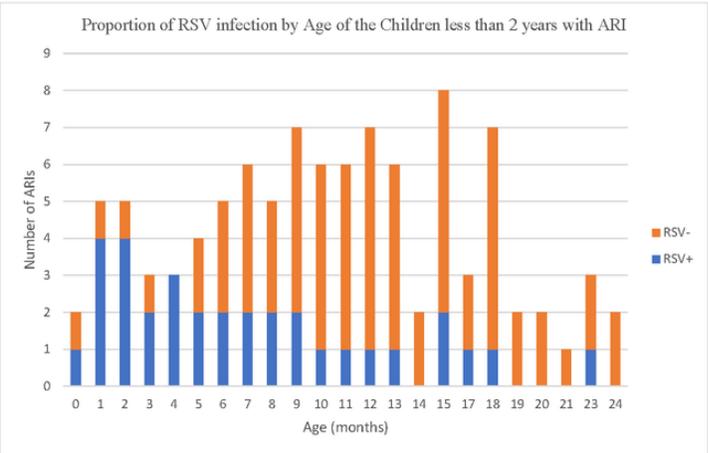


Figure 2

The proportion of RSV infection by the age of the children less than two years with ARI