

## ORIGINAL ARTICLE

# SEROLOGICAL SURVEY OF RESPIRATORY SYNCYTIAL VIRUS (RSV) AMONG CHILDREN IN ILORIN NORTH-CENTRAL, NIGERIA.

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## ABSTRACT

**Background:** Respiratory Syncytial Virus (RSV) infection is a common worldwide problem. Childhood mortality and morbidity due to RSV is increasing. This study was aimed at determining the sero-prevalence rate of RSV IgG antibody and also to investigate some known risk factors for acquisition of RSV among children presenting with various forms of respiratory tract infections in Ilorin, Nigeria.

**Methods:** A total of 600 samples were collected from children (whose parent or guardian has consented) and analysed for anti RSV IgG antibody using ELISA. Information regarding socio-economic status and other demographic variables were recorded using well structured questionnaire.

**Results:** A seroprevalence of 75% (450/600) was detected in the subjects. The seroprevalence among male was 72.70% and female 77.54% ( $P = 0.171$ ). The age group 6-10 yrs had the highest prevalence of 80% while 0-5 yrs had 73.94%, with a highly significant association ( $P = 0.0001$ ).

**Conclusion:** This study showed high prevalence of RSV, with the highest prevalence in aged 6-10 years which suggests that, RSV is common among school-aged children. Thus the importance of RSV as an agent of respiratory tract infection in children is identified. Preventive measures should be adhered to in the control of the infection, as antiviral drugs are not readily available.

**KEYWORDS:** Respiratory Syncytial Virus, Children, Mortality, Infection.

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## INTRODUCTION

Respiratory infections have been reported to have significant impact on health worldwide and the majority of these infections are caused by viruses. In some cases complications are observed in patients who develop a secondary bacterial infection, children are the most afflicted with acute otitis media, sinusitis, or pneumonia. In very young or older individuals or individuals with chronic medical conditions, viral respiratory infections may induce a severe illness.<sup>1</sup> Viruses that have been implicated in most acute respiratory infections are caused by

Rhinovirus, Respiratory Syncytial Viruses, Enteroviruses, Influenza A and B viruses, Parainfluenza viruses, Adenovirus and Respiratory Coronaviruses.<sup>2</sup> These infections are more common and severe among children and infants, although the elderly patients are also severely afflicted.<sup>3</sup>

Respiratory syncytial virus (RSV) is an enveloped virus with a single stranded positive sense RNA genome belonging to the family Paramyxoviridae.<sup>4</sup> It was first isolated in 1959, from a chimpanzee and was subsequently shown to be of human origin and the cause of serious paediatric respiratory tract

disease.<sup>4</sup> Respiratory syncytial virus (RSV) infection is considered to be one of the most common infections in children, causing significant morbidity and mortality.<sup>5</sup> It is highly contagious, being shed in respiratory secretions for several days, sometimes for weeks and easily spread by direct contact and droplets from the nose. Infection and outbreaks of RSV in related institutions are common occurrence in institutions such as day care settings where the attack rate may be up to 100%.<sup>6</sup> Approximately two-third of infants become infected with RSV during their first year of life and re infections occur throughout life.<sup>5</sup> RSV accounts for about 50% of all cases of pneumonia and up to 90% of all reported cases of bronchiolitis during infancy in some places.<sup>7</sup>

Disease severity with RSV is very variable, for instance of 50% infected infants above one year of age only 3% are hospitalised. Out of RSV hospitalised children only 10% require mechanical ventilation.<sup>8, 9</sup> Low birth weight, young age of less than 6 months, congenital heart disease, and Immunodeficiency/immune suppression are some of the risk factors for infection with RSV.<sup>10</sup> It has also been documented that seasonal change can contribute to increased incidence of RSV infections in temperate regions although in tropical countries there is no specific seasonal pattern in RSV incidence.<sup>11</sup>

Significant information on the epidemiology of the virus in the literature is based on studies from the developed countries as RSV infection had been well reported in industrialized and developing countries. This has made it possible in most parts of the developed world to be able to predict impending epidemics and the burdens as well as severity of RSV. Hence it is possible to plan effective preventive and control measures ahead of epidemics. On the other hand, in the developing countries, particularly in Africa, most information is derived from reports and data from hospital-based studies.<sup>6</sup>

A study has revealed the presence of RSV in children with severe RTI in South west Nigeria.<sup>12</sup> Faneyeet al. in 2014 have also evaluated RSV infection in children younger than 5 years attending a state hospital in Ilorin and reported a prevalence rate of 85.7%. There is still however, an insufficient information on the level of RSV infection in our various hospitals and Medical institutions in Kwara state. This necessitates the conduction this study which was aimed to determine the prevalence of previous RSV infection and associated risk factors for infection in children between  $\leq 1 - 10$  years presenting with various RTI attending a tertiary institution Hospital as well as some state hospitals within Ilorin Northern, Nigeria.

## METHODS

This cross-sectional study was carried out in selected hospitals located within Ilorin metropolis.

The hospitals were; Children's Specialist Hospital, Kwara State General Hospital, Ilorin and University of Ilorin Teaching Hospital (UIITH). The study was approved by the Ethical Review Board of the University of Ilorin Teaching Hospital. Approval was also given by the study site Institution, written or oral informed consent was taken from the wards of the children before enrolment into the study. Two hundred (200) samples were collected in each hospital making a total of 600 children who were consecutively recruited between January 2015 and December 2016. A well-structured questionnaire was administered and patient's demographic data were collected. Blood samples were collected in labelled sterile plain bottles and allowed to clot and the sera were separated by centrifugation at room temperature. Sera storage was at  $-20^{\circ}\text{C}$  until enough samples were collected for assay.

The various clinics were visited after obtaining ethical approval from Kwara State Ministry of Health and Ethical review committee of UIITH. The study participants are children aged  $\leq 1 - 10$  years who were enrolled by simple consecutive sampling as they presented at the various hospitals. At enrolment, all caregiver's who volunteered to participate were interviewed and demographic data obtained. Blood samples were collected only from children whose caregivers signed an informed consent. Blood specimen of 5 ml venous blood were collected from all enrolled children by the help of a trained nurse and phlebotomist in dry plain specimen bottles and centrifuged at 1500 revolution per minute (1500 rpm/min) for five minutes and the sera were collected into clean and dry plain specimen bottles using clean and dry Pasteur pipettes. The sera were stored at  $-20^{\circ}\text{C}$  until the assay time.

This was achieved by the use of Enzyme-Linked Immunosorbent Assay (ELISA) kit from R-Biopharm AG, 62927 Darmstadt Germany. This is a glycoprotein G-based enzyme-linked immunosorbent assay technique and test result was qualitative. All specimen and kit reagents were allowed to assume room temperature ( $25^{\circ}\text{C}$ ) and gently mixed. The laboratory procedure was performed according to the manufacturer's instruction. The optical density (OD) values were read at 405nm, using Sigma Diagnostic EIA multi well reader II. The kit has 99% specificity and 100% sensitivity.

Data generated was analyzed using SPSS version 21

computer software, statistical associations were done using, chi square test for parametric variables and statistical significance was ascribed based on a p-value of less than 0.05 ( $P < 0.05$ ).

## RESULTS

This study recorded a prevalence of 75% of prevalence of RSV infection out of 600 (450/600) examined samples. Three hundred and fifteen (52.5%) males were tested and 229 (72.70%) were observed to be RSV seropositive, while 221 (77.54%) females were RSV seropositive out of the 285 (47.5%) that were tested. Although there was a slight male predominance but was not statistically significant ( $P$  value = 0.171). The mean age of the study subject was 2.8 years, age group 6-10 years had the highest seroprevalence of 84/105 (80%), followed by age group 0-5 years with seroprevalence of 366/495 (73.94%). It was discovered that there was a significant statistical association between the ages of the patients and RSV ( $P$  value = 0.0001), with children between the ages of 6 and 10 years having the highest prevalence rate (**Table 1**). Those in secondary school had the highest prevalence (88%) and a

statistical association was recorded ( $P$  value = 0.000). Similarly, test of association of mothers' level of education with the infection was carried out and a significant statistical association ( $\chi^2 = 31.170$ ,  $P = 0.000$ ) was observed, children of mothers with primary education had the highest rate of infection followed closely by those with informal education; those with secondary level of education, their children had 78.06% infection rate and the least (61.95%) was observed amongst children of parents with post secondary level of education (**Table 1**).

**Table 2** presents the result of the assessment of the association of some risk factors and the distribution of RSV IgG among the tested children. A significant statistical association was observed between proximity of residence to a livestock settlement and untarred road, day care attendance, birth type, family member recently diagnosed of RTI and parental smoking and seropositivity of RSV in the subjects ( $P < 0.05$ ). However, no statistical association was observed between being exclusively breast fed, number of people sleeping in a room and type of cooking done in the house and RSV infection ( $P > 0.05$ ).

**Table 1: Prevalence of anti-RSV infection among children in relation to socio-demographic variables.**

Variables	Numbers Tested	Numbers Positive	$\chi^2$ (p value)
Gender			
Male	315	229 (72.70)	1.874 (0.171)
Female	285	221 (77.54)	
Age Group (Years)			
0-5	495	366 (73.94)	26.56 (0.0001)*
6-10	105	148 (80)	
Educational Level			
Pre School	151	91 (60.26)	25.909 (0.000)*
Nursery	333	260 (78.08)	
Primary	91	77 (84.61)	
Secondary	25	22 (88)	
Mother's Educational Level			
Primary	100	86 (86.0)	31.170 (0.000)
Secondary	196	153 (78.06)	
Post secondary	205	127 (61.95)	
Informal Education	99	84 (84.5)	
<b>Total</b>	<b>315</b>	<b>150</b>	

$\chi^2$ = Chi square

P value = level of significance

Significant association exists at  $p < 0.05$

\*Statistical significance

**Table 2:** Table showing association between some risk factors and RSV infection in children.

Risk factor	Number tested	Number (%)	Positive	x2 (p value)
Birth type				
Vaginal delivery	471	344 (73.04)		
Caesarean section	129	106 (82.17)		4.506 (0.034*)
Exclusive breast feeding				
Yes	429	316 (73.66)		
No	171	132 (77.19)		0.613 (0.434)
Attendance of day care				
Yes	171	102 (59.65)		
No	429	348 (81.11)		30.058 (0.000)*
Proximity of residence to livestock settlement				
Yes	317	250 (78.86)		
No	283	200 (70.76)		60.62 (0.012)*
Number of people sleeping in a room				
1-2	160	112 (70.00)		
3-4	279	215 (77.06)		
5-6	112	89 (79.46)		
Above 6	49	34 (69.39)		4.788 (0.188)
Type of cooking done in the house				
Gas	69	54 (78.26)		
Stove	219	169 (77.17)		
Coal pot	70	55 (78.57)		
Fire wood	17	10 (73.30)		
A combination of two of gas, stove, coal pot, firewood	221	162 (73.30)		3.654 (0.455)
Parental smoking				
Yes	107	97 (90.65)		
No	453	353 (77.92)		17.044 (0.000)*
History of RTI				
Yes	114	91 (79.82)		
No	486	359 (73.87)		1.927 (0.165)
Proximity of residence to untarred road				
Very close	193	130 (67.36)		
Averagely close	217	182 (83.87)		
Not close	190	138 (72.63)		15.688 (0.000)*

Family member recently diagnosed of RTI			
Yes	277	180 (64.98)	
No	323	270 (83.59)	26.56 (0.000)*

P value = level of significance

Significant association exists at  $p < 0.05$

\*Statistical significance

**Table 3: Relationship between Symptoms of respiratory tract infection and RSV Infection.**

Parameter	Number tested	Number positive (%)	$\chi^2$ (p value)
Presence of catarrh			
Yes	478	350 (73.22)	
No	122	100 (81.97)	11.477 (0.003)*
Persistence sneezing			
Yes	232	128 (55.17)	
No	368	322 (87.5)	93.67 (0.000)*
Presence of cough			
Yes	497	359 (72.23)	
No	103	91 (88.35)	12.623 (0.000)*
Sore throat			
Yes	86	71 (82.56)	
No	495	370 (74.75)	
Don't know	19	9 (47.37)	15.688 (0.000)*
Fever			
Yes	381	293 (76.90)	
No	279	157 (71.69)	3.04 (0.218)
<b>Total</b>	<b>600</b>	<b>450</b>	

P value = level of significance

Significant association exists at  $P < 0.05$

\*Statistical significance

## DISCUSSION

The seroprevalence of RSV infection is high in most geographic regions of the world and is among the commonest cause of severe respiratory tract infection in infants and children with 76% of children being re-infected by the second year of life.<sup>13</sup> Previous studies conducted in Africa on RSV infection have shown that there is a significant and high seroprevalence of IgG antibodies in children population.<sup>14</sup> In this present study, 600 samples were tested for RSV IgG antibody and seroprevalence recorded was 75.0% (450/600). This differ with the study of Faneyeet *al.*, in Ilorin which reported a prevalence of 85.7% which was slightly higher than what was observed in this study.<sup>3</sup> This slight decrease in prevalence could be due to the fact that the study was conducted only in children less than 5 years of age, however, this study population cut across children up to 10 years of age. Our result hereby also shows that there is a high level of RSV circulation and transmission among inhabitants of Ilorin.

The distribution of RSV according to age group indicates that age group 6-10 yrs had the highest seroprevalence of 80% ( $P = 0.000$ ). This finding showed that age had a significant association with RSV infection, and this is in disagreement with earlier submissions of researchers that children aged less than 5 years have higher prevalence of RSV infection.<sup>3</sup> It is noteworthy here that most of earlier research centres on children less than 5 years of age, however, from the observations from this study it can be seen that children older than 5 years are also at higher risk of RSV infection. This high prevalence among 6- 10 years age group could be as a result of re infection, since there is no permanent immunity to the infection those antibodies modify the disease, as such reinfection usually present simply as non febrile upper respiratory infection.

Selected risk factors that have been previously reported by several workers for acquisition of RSV infection were analysed, including; gender, educational level, socioeconomic class, attendance of day care, educational level, birth type and birth weight, parental smoking, proximity to livestock settlement, family member with symptoms of respiratory tract infection, being exclusively breastfed during infancy<sup>15</sup>. From the data generated, gender was found not to have a statistical significant association in relation to RSV infection  $P$ - Value (0.171). Although more males were recruited for this study presenting with RT, the percentage seropositivity was 72.70% which was lower to what was

observed in the female (77.54%), this result is in disagreement with various other reports indicating that the RSV infection rate is higher in male owing to the anatomical nature of male trachea which has been suggested to be shorter and narrower than that of the females and was substantiated in the Canadian PICNIC study.<sup>16</sup> Results on educational level and RSV shows that, children in secondary school had the highest prevalence (88%) and  $P$  value 0.000, this observation is again also expected as it is a reflection of the age of the subjects. The test of association of attendance of day care with the infection showed there was a significant association ( $P = 0.000$ ).

Educational level of the mother shows a statistical association ( $P$  value = 0.000). The seropositivity and prevalence was high in children whose mothers have an informal education or primary education. Exclusive breast feeding of enrolled children in relation to RSV IgG positive children was also investigated but no statistically significant relationship  $P$ -value (0.434) was found. This is in agreement with the report of Faneyeet *al.* in 2014 who also reported a non statistical association between the duration of breast feeding and presence of RSV IgG antibody. However, this is not in agreement to past reports that indicated breastfeeding for > 2months as a significant protective factor against RSV infection although breastfeeding alone is not sufficient to protect against Primary RSV infection in neonates and young infants.<sup>10, 17, 18</sup>

Proximity of the subjects' residence to a live stock settlement and untarred road were statistically significant (0.0012 and 0.000 respectively). This indicates that respiratory viruses could be transmitted via aerosol from dust particles.

There was no statistical association with symptoms of catarrh ( $P$  value= 0.061). Seropositivity was observed to be 73.22% of those with the symptoms and 83.33% of those without the symptoms of catarrh/runny nose. This result is akin to the work of Sale *et al.* whose findings showed that respiratory symptom is not statistically significant to a viral respiratory tract infection. In the cough distribution of RSV antibodies, seropositivity was observed to be 72.23% for those with cough and 83.35% for those without cough. This was also found to be statistically significant.<sup>19</sup>

Children of parents that smoke had higher infection with RSV compared to those of non-smoking parents and this was found to be significantly associated with the infection ( $P = 0.000$ ). History of



the subjects having family member who was recently diagnosed of respiratory tract symptoms shows that there was a statistical association ( $P = 0.000$ ), this is indicative of the fact that RSV could easily be transmitted from infected patients to others.

### CONCLUSION AND RECOMMENDATIONS

Observation from the study highlights the importance of RSV as an agent of respiratory tract infections in children. Age, Parental occupation, duration of breastfeeding and presence of respiratory symptoms are important demographic and risk factors of RSV Infection in children. This study suggests the need for rapid, easy and less expensive methods of diagnosis for clinical management and availability of vaccines. A better understanding of RSV pathogenesis will aid the design and evaluation of live attenuated RSV vaccines and therapeutic drugs. As antiviral drugs are not readily available, preventive measures such as proper hygienic practices and parental supervision should be adhered to in the control of the infection.

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