International Journal of Biomedical and Health Sciences
Vol. 7, No. 3, September 30, 2011
Printed in Nigeria

0794-4748/2011 $5.00 + 0.00

http://www.asopah.org

IJBHS 2011013/7305

Seroprevalence of human *Parainfluenza virus* type 3 infection among children 1-5 years in Zaria, Kaduna State, Nigeria

L. D. Rogo[1]*, A. A. Ahmad[4], H. W. Idris[2], A. M. Aliyu[3], J. Sale[4] and A. Muhammad[4]

[1]Department of Microbiology, Faculty of Science, Ahmadu Bello University, Zaria, Nigeria
[2]Department of Pediatrics, Faculty of Medicine, Ahmadu Bello University Teaching Hospital, Zaria, Nigeria
[3]Department of Applied Sciences, College of Science & Technology, P.M.B.2021 Kaduna, Polytechnic, Kaduna, Nigeria
[4]College of Agriculture and Animal Science, Ahmadu Bello University, Mando Road, Kaduna,Nigeria

(Received January 24, 2011; Accepted March 3, 2011)

ABSTRACT: Human *Parainfuenza virus* type 3 (HPIV-3), the causative agent of upper and lower respiratory tract infection can cause severe lower respiratory tract infection in immunocompetent individuals as well as immunocompromised patients. The aim of this study was to assess the seroprevalence of HPIV-3 IgG antibody in children 1-5 years in Zaria. Blood samples were obtained from 379 children whose parents consented. The sera obtained were analyzed using HPIV-3 IgG SERION ELISA classic GmbH Germany to determine the HPIV-3 IgG level. Information about the children's demographic factors and other variables were obtained from their caregivers using a designed questionnaire. Of the 379 serum samples analyzed 323 were positive giving overall seroprevalence for HPIV-3 as 85.2%. Prevalence of HPIV-3 antibodies was 43.0% in males and 42.2% in females. Seropositivity was found to increase with age, cough and parental smoking/ toxin ($P< 0.05$). On the other hand there is no association between the HPIV-3 infection with catarrh/running nose and sickle cell disease. Children aged 1year had the lowest prevalence of 7.4%, while those aged between 1-2 years had the highest prevalence of 26.1%. In view of the high prevalence rate of HPIV-3 infection in this study, it is suggested that further epidemiological studies should be conducted to establish the exact role of HPIV-3 in respiratory tract infections among children in Nigeria.

Keywords: Seroprevalence, Human Parainfuenza virus type 3, Respiratory tract infection, ELISA.

Introduction

Parainfluenza viruses (PIVs) are responsible for 30-40% of all acute respiratory tract infections in infants and children worldwide (1). These conditions include common cold, croup, bronchitis, and pneumonia. It contributes to community acquired respiratory tract infections of variable severity in adults (1,2,3).

*Author to whom correspondence should be addressed.
E-Mail- lawaldahirurogo@yahoo.com

Parainfluenza viruses are pleomorphic viruses whose envelope is derived from the host cell they last infected (4, 5, 6). These viruses are 150-300nm in diameter and posses a single-stranded, non-segmented, negative-sense RNA genome with nucleoproteins P and L, and a lipid bilayer covered with glycoprotein spikes which surround a helical nucleocapsid that measures 12-17nm in diameter (7). These glycoproteins are hemagglutinin–neuraminidase (HN) and fusion (F) proteins, which play a role in the pathogenesis of the disease (8). The viruses are included in the order *Mononegavirales*, the family *Paramyxoviridae* and the subfamily *Paramyxovirinae*. They belong to 2 different genera: HPIV-1 and HPIV-3 belong to the Respirovirus genus, and HPIV-2 and HPIV-4a and 4b belong to the Rubulavirus genus (6, 8).

Human Parainfleunza virus serotype 3 (HPIV-3), is a member of the *Respirovirus* genus, of the *Paramyxoviridae* family. The virus is one of the most virulent among the group, frequently causing bronchiolitis and pneumonia during the first months of life (9). It is a common community–acquired respiratory pathogen without ethnic, socioeconomic, gender, age or geographic boundaries (10). It is an airborne pathogen that infects human lung epithelial cells from the apical (laminal) plasma membrane domain (3, 11, 12). Viral transmission could also occur via direct inoculation of contagious secretions from the hands or via large particle aerosols into the eyes and nose (13). Prolonged survival of HPIV-3 on skin, cloth and other objects emphasizes the importance of fomites in nosocomial spread. The main pathological features include airway inflammation, necrosis and sloughing of respiratory epithelium, edema, excessive mucus production, and interstitial infiltration of the lungs. These inflammatory features cause swelling of the vocal cords, larynx, trachea, airway inflow and subsequent stridor, which is characteristic of croup (10). The predisposing factors found to predispose individuals to these infections, including malnutrition, overcrowding, vitamin A deficiency, lack of breast feeding, and environmental smoke or toxins (13).

Human Parainfluenza virus type 3 has worldwide distribution. Mortality associated with HPIV-3 is unusual in developed countries and occurs almost exclusively in young infants, people who are immunocompromised and the elderly. In developing countries however, children in the preschool age were found to be more at risk for HPIV-3 associated death (10). The present study was therefore conducted with the aim of determining the seroprevalence of Human Parainfluenza virus type 3 IgG antibody in children 12-60 months attending the out patients clinics of the ABUTH Zaria, Kaduna State.

Materials and Methods

Study Area

The study area covers Zaria metropolis comprising Zaria and Sabon Gari Local Governments areas located 84km from the Kaduna State capital. Zaria Local Government has a population of about 369,800 inhabitants (14). It is located on longitude and latitude $9^{0.}$ It is an urban set up bounded on the east by Soba Local Government, on the west by Giwa Local Government, on the north by Sabon Gari Local Government, and on the south by Igabi Local Government.

Sabon Gari Local Government is an urban set up with a population of 350,000 inhabitants (14). It is along longitude 8^{0} and $9^{0,}$ latitude 10^{0} and $11^{0.}$ It is bounded by Soba Local Government on the east, Giwa Local Government on the west, Makarfi Local Government on the north, and Zaria Local Government on the south.

Sample Collection and Processing

Hospitals were visited after obtaining ethical approval from ABUTH. Children 1-5 years of age were all included in the study. The samples were collected only from children whose parent/guardian gave a written informed consent. A dry sterile plastic syringe (2ml) with 23SWG (standard wire gauges) needle attached to was used for blood sample collection. Blood was collected by applying soft tubing tourniquet to the arm of the patient to enable the veins seen and felt. The punctured site was cleaned using methylated spirit and allowed to air dried. The needle was inserted to the selected straight vein with the bevel of the needle directed upward in the line of the vein. Steadily the plunger of the syringe was withdrawn until 2ml of blood was obtained. The tourniquet was released and the needle was removed from the punctured vein. Pressure was applied to the punctured site to secure homeostasis. The needle was removed from the syringe

and the blood was transferred to a clean dried plain specimen bottle and labeled. The used syringes and needles were disposed appropriately.

The blood samples were centrifuged at 1500 revolution per minute for five minutes (1500 rpm/min for 5min) and the sera collected into a clean and dry plain specimen bottles using clean and dry Pasteur pipettes and stored at -20^0c until needed for analysis (15). All tests were carried out in the Department of Microbiology, Ahmadu Bello University Zaria. Using Enzyme Linked Immunosorbent Assay (HPIV-3 IgG SERION ELISA *classic*, INSTITUT VIRION/SERION, GmbH Germany), samples were assayed for specific IgG antibody against the HPIV-3. Manufacture's instructions were duly followed. The optical density (OD) values were read at 405nm, using Sigma Diagnostic EIA Multi well Reader II.

Procedure

The procedure according to manufacturer's instruction were used to carry out the assay. Sufficient amount of microtitre wells for the standards, controls; samples as well as substrate blank were prepared in duplicate. About 10µl each of diluted samples and ready-to-use standards and controls respectively were pipetted into the wells. The first well was left empty as blank, mixed by gentle shaking and incubated for one hour at 37ºC. The wells were emptied after incubation and washing solution was added into the wells. This procedure was repeated three times. The left over of the washing solution was afterwards removed by gentle tapping of the microtitre plate on a clean tissue paper. Approximately 100µl each of ready-to-use conjugate reagent was added into the wells. The substrate blank was left empty. The plate was incubated at 37ºC for 30 minutes, the same procedure for washing was followed, and 100µl each of the ready-to-use substrate was pipetted into the wells. This time the substrate was pipetted into the blank, incubated at 37ºC for 30 minutes. 100µl of stop solution was finally pipetted into the wells to terminate the substrate reaction. The plate was thoroughly mixed and the bottom wiped, the reading of the absorption at 405nm was made using ELISA reader machine (SIGMA DIAGNOSTIC).

Results

Of the 379 samples tested for HPIV-3 IgG antibody, 323 were positive giving an overall seroprevalence of 85.2% (Table 1). It also shows the seroprevalence of HPIV-3 IgG antibody among the studied population with respect to age. Of the total samples tested, 56 (14.8%) were aged 0-12 months, 117 (30.9%) were aged 13-24 months, 78 (20.6%) were aged 25-36 months, 54 (14.2%) were aged 37-48 months and 74 (19.5%) were aged 49-60 months. The seroprevalence was highest (26.1%) in age group 13-24 months and lowest in age group 0-12 months (7.4%). The result shows increased seropositivity with age. $X^2 = 71.69$, $P < 0.05$.

Fifty six (14.78%) of the children had no detectable HPIV-3 IgG antibodies with a titer less than 65 IU/ml while 67 (17.78%) had a maximum antibodies level of 866-1065IU/ml, a level of 666-865 was found in 26 (6.86%), 466-665 IU/ml level was found in 38 (10.03%), level of 266-465 IU/ml was found in 50 (13.19%) and a relatively low level of HPIV-3 antibodies was found in 142 (37.47%) with a titer of 66-265IU/ml as shown in (Table 2). The protection rate against HPIV-3 seems to be lower in children ages 1 year or less.

Table 1: Distribution of HPIV-3 Antibody by Age

Age (months)	Number of Children Tested	Number of Seropositive	% Seropositive in Each Age Group	% seropositive for Overall Total
0-12	56	28	50.5	7.4
13-24	117	99	84.6	26.1
25-34	78	75	96.2	19.8
35-46	54	50	92.6	13.2
47-60	74	71	95.9	18.7
Total	379	323		85.2

X^2 =71.69, P value =0.000 at 95% CI

Table 2: Profiles of HPIV-3 Antibody Titers According to Age.

Age (months)	No. of subjects	Titres (IU/ml)					
		<65	66-265	266-456	466-665	666-865	866-1065
0 – 12	56	28	20	1	0	1	6
13 – 24	117	18	45	20	10	6	18
25 – 36	78	3	28	13	11	5	18
37 – 48	54	4	21	3	6	7	13
49 – 60	74	3	28	13	11	7	12
Total	379	56	142	50	38	26	67

Keys: IU = International Unit, <65 IU/ml IgG Antibody = Negative; ≥65 IU/ml IgGAntibody =Positive

Among the 379 children tested, 190 (50.1%) were males and 189 (49.9%) were females. Seropositivity was observed in 163 (43.0%) males and 160 (42.2%) of the females (Table 3). This shows that there was slight male predominance over females, which was not statistically significant, (P value= 0.343). X^2 =0.09.

Of the total 379 children tested, 268 (70.7%) had symptoms of catarrh/running nose while 111 (29.3%) had no such symptoms. Seropositivity was observed in 229 (60.4%) of those with catarrh/running nose while 94 (24.8%) of those without these symptoms were seropositive (Table 4). This is however not statistically significant (X^2 = 0.097, P value = 0.434).

Table 3: Distribution of HPIV-3 Antibody by Sex

Sex	Number of children tested	Number seropositive	% seropositive
Male	190	163	43.0
Female	189	160	42.2
Total	379	323	85.2

X^2= 0.09, P value= 0.343

Table 4: Distribution of HPIV-3 Antibody by Symptoms of Catarrh/Running Nose

Catarrh /R. Nose	No. tested	Seropositive	% seropositivity
Presence	268	229	60.4
Absence	111	94	24.8
Total	379	323	85.2

X^2 =0.036, P value =0.481

Out of 379 children tested 266 (70.2%) were having symptoms of cough while 113 (29.8%) were without symptom of cough. Seropositivity was observed in 219 (57.8%) of those with symptom of cough and 104 (27.4%) of those without symptom of cough (Table 5). This was found to be statistically significant with a P value =0.009

Table 6 shows the seroprevalence of HPIV-3 IgG antibody among sickle cell children tested in the study. Of the total number of 379 children tested, 9 (2.37%) were with sickle cell disease and 370 (97.6%) were those that have no sickle cell disease. Seven (7) of those with sickle cell disease (1.85%) were observed seropositive. Although this was not statistically significant with P value =0.394. X^2 =0.406.

Table 5: Distribution of HPIV-3 Antibody by Symptom of Cough

Cough	No. tested	Seropositive	% seropositive
Presence	266	219	57.8
Absence	113	104	27.4
Total	379	323	85.2

X^2 =5.931 P value =0.009

Table 6: Distribution of HPIV-3 Antibody by Sickle Cell Disease

Sickle cell	No. tested	No. Seropositive	% Seropositive
Presence	9	7	1.85
Absence	370	316	83.4
Total	379	323	85.2

X^2 =0.406 P value =0.394

Of the total 379 children tested in the study 46 (12.1%) were those with history of parental smoking and 333 (87.9%) were those without parental smoking. Seropositively was observed in 45, (11.9%) of these with parental smoking and 278 (73.4%) in those without parental smoking (Table 7). It was found to be statistically significant with P value = 0.004. X^2 =6.602.

Table 7: Distribution of HPIV-3 Antibody According to History of Parental Smoking

Parental smoking	No. tested	Seropositive	% seropositive
Presence	46	45	11.9
Absence	333	278	73.4
Total	379	323	85.2

X^2 =6.602 P value =0.004

Discussion

Human *Parainfluenza virus* type 3 is a medically important respiratory pathogen with no predilection for any race and second only to Respiratory syncytial virus as a major cause of lower respiratory tract illness in infants and young children (16). They are responsible for 30 - 40% of all acute respiratory tract infections in infants and children worldwide (1). Although re infection in healthy older children and adults are typically less severe, serious lower respiratory tract illness caused by HPIV-3 has been reported among immunocompromised individuals (17).

The present study found 323 (85.2%) of the subjects to be seropositive for HPIV-3 IgG antibodies. This is slightly above the finding of Mariana et *al* (18) who demonstrated 83.76% prevalence in Brazilian children. This is not supprising because the two studies were carried out around the same season (rainy season). It may also be attributable to the comparable living standard of the two study areas. It contrasts with the finding of Palern *et al* (19) who reported (18.6%) prevalence in Cuban children even though the study was conducted around the same season of the year. The possible reason may be the differences in the living condition between Cuba and the present study area. The presence study showed that a significant number of children remain unprotected against HPIV-3 in Zaria.

The study also revealed an increase in seropositivity with age. This finding conforms to those of Laurichesse *et al* (20), Hohenthal *et al* (21) and Arden *et al* (22) who demonstrated that HPIV-3 seropositivity increase with age. This shows that children of older ages developed higher antibodies that protect them from severe form of subsequent infection with HPIV-3 which could be due to booster effect on the immunity of older children resulting from recurrent infection.

In assessing the sex distribution of HPIV-3 antibodies, seropositivity was observed to be slightly higher in males (43.0%) than in females (42.2%). This also agrees with the finding of Spring (23) although the result was not statistically significant (P> 0.05). This could be due to the fact that HPIV-3 has no predilection for sex.

In assessing the symptom of catarrh/running nose distribution of HPIV-3 antibodies, seropositivity was observed to be higher (60.4%) in those with the symptoms which agrees with the work of Mariana *et al* (18) which may due to the present of active infection, although the result was not statistically significant (P > 0.05).

This study also showed that seropositivity is higher in those children with symptom of cough (57.8%) than in those without the symptom (27.4%), which was found to be statistically significant (P< 0.05). This conforms to the work of Subhash and Thomas, (24)2007) who also showed statistical significant association between IgG seropositivity of HPIV-3 and respiratory tract infection symptom of cough. It could be due to an immune response by the host to clear the airways blockages cause by caused by inflammatory reaction due to the HPIV-3 infection.

It was observed from this study that out of 2.37% of the patients with sickle cell, 1.85% were seropositive subjects but this was not statistically significant ($P > 0.05$). This is in contrast with the work of Welliver *et al* (3) who reported association of HPIV-3 infection and sickle cell disease. However the lack of statistical significance could be due to fact that the number of sickle cell children in the study population is very small.

In this study, it was observed that children whose parents smoke have increased seropositivity of HPIV-3 infection ($P < 0.05$) which conforms with the work of Laura *et al* (13) who reported environmental smoke or toxin to be one of the predisposing factors to HPIV-3 infection. It may be as a result of the ability of the smoke/toxin to erode the mucosa surface epithelia providing easy assess to the cell.

The profile of HPIV-3 antibody titers indicate that only 67 (17.78%) showed very high titers ranging between 866 – 1065 IU/Ml. It is clear that any detectable HPIV-3 IgG antibody is an indication of immunity. This high IgG level may be due to the booster effect as a result of continuous re-infection with the HPIV-3. Some children with low protective HPIV-3 such as those with titer less than 65 IU/Ml which form (14.78%) of the studied population may stand the risk of severe HPIV-3 infection. Higher number of children from the population with IgG antibody titers between 866 – 1065 IU/Ml was observed in age groups 1 – 2 and 2 – 3 respectively, which suggest previous exposure of the individuals to HPIV-3 infection or acute infection.

Conclusion and Recommendations

The present study has revealed the importance of HPIV-3 as an etiologic agent of Respiratory tract infection in Zaria children. The study emphasizes the need to rapidly diagnose viral respiratory infections for clinical management of infected children. It also provide epidemiological data that may be useful in control effort and vaccine trials, mostly in developing countries where less information regarding respiratory virus is available.

In view of the high prevalence rate of HPIV-3 Seropositivity, it is recommended that attention should be given to this virus for proper management of patients with respiratory tract infection especially in children with symptoms of cough, within the age of 1-2 years and children who have history of exposure to environmental smoke/toxin from the parent.

References

1. Santanu B, Mausumi B and Amiya KB. Role of Nucleolin in *Human Parainfluenza virus* type 3 infections of human lung epithelial cells. *Journal of Virology* 2004;78 (15):8146-8148.
2. Glezen WP, Frank AL, Taber LH and Kasel JA. *Parainfluenza virus* type 3: seasonality and risk of infection and reinfection in young children. *Journal of Infectious Disease* 1984; 150: 851-857.
3. Welliver RC, Wong DT, Sun M and McCorthy N. *Parainfluenza virus* bronchiolitis: epidemiology and pathology. *American Journal of Disease in Children,* 1986;140: 34-40.
4. Collins PL, Chanock RM and Mcintosh K. *Parainfluenza* viruses 1996;1205-1241. In B.N. Fields, P.M. Howley, and D. M. Knife (ed.), Fields Virology. Lippincott-Raven, Philadelphia, Pa
5. Puppe JH, Niesters GM and Groen JW Weighg JA, Aron G, Groundahl B and Schmitt H. Evaluation of a multiplex reverse transcriptase PCR ELISA for the detection of nine respiratory tract pathogens. *Journal of Clinical Virology* 2004;30 (3): 165-174.
6. Porotto M, Murrell M, Greengard O, Doctor L and Moscona A. Influence of the *Human Parainfluenza virus* 3 attachment proteins neuraminidase activity on its capacity to activate the fusion protein. *Journal of Virology* 2005; 79:238-2392.
7. Jose CA, Maria PP, Maria LG, Nieves C, Dean DE and Juan EE. Detection and identification of *Human Parainfluenza viruses*, 1, 2, 3, and 4 in clinical samples of pediatrics patients by multiplex Reverse Transcriptions PCR. *Journal of Clinical Microbiology* 2000; 38 (3):1191-1195.
8. Jawetz, Melnick, and Adelberg's. Medical Microbiology. In: Geo, F. B., Karen, C. C., Janet, S. B. and Stephen, A. M. 23rd Int. edition 2004; 550-569 McGraw Hill publisher.
9. Michael AH and Amiya KB An infectious clone of *Human Parainfluenza virus type* 3. *Journal of Virology* 1997; 71 (6): 4272-4277.
10. Gulen F, Cicek CZ, Kurugol Z, Demir E, Zeyrek D, Ozdemir R, Tanac R and Karatos T. *Parainfluenza virus* type 3 outbreaks in Izmir children, Turkey. *Tropical Doctors* 2007;37 (4): 252-4.

11. Chanock RM, Murphy BR and Collins PL. Parainfluenza viruses. In M. D. Knipe and P. M. Howley (ed). Field Virology 4th ed., Vol. 1 Lippincott W. and Wilkins Philadelphia. 132001;41.
12. Lamb, R. A. and KolaKosfsky, D. Paramyxovividae: the virus and their replication. 1305. In D.M. Knipe, and P.M. Howley (ed), Fields virol 4th ed., vol.1 2001.William and Wilkins Lippincott-philadelphia pa.
13. Laura, M.P., Matteo, P., Olga, G. and Anne, M. Fusion Promotion by a Paramyxovirus Heamagglutinin-Neuraminidase protein: PH modulation of Receptor Avidity of Binding Site I and II. *Journal of Virology* 2007; 81 (17): 9152-9161.
14. National Population Commission in Nigeria –NPC 1991. Nigeria Demography and Health Survay.
15. Cheesbrough, M. District Laboratory Practice in tropical countries (Part2).Cambridge University press. 2000. Pp. 248-266.
16. Sigh-Naz, N., Willy, M. and Riggs, N. Outbreak of *Parainfluenza virus* type 3 in a neonatal nursery. *Pediatrics Infectious Disease Journal*, 1990; 9:*31-33*
17. Legoff, J., Guerot, E., Ndjoyi-Mbiguino, A., Matta, M., Si-Mohammed, A., Gutmann, L., Fagon, J. Y. and Balec, L. High prevalence of respiratory viral infection in patients hospitalized in an intensive care unit for acute respiratory Infections as detected by nucleic acid based assays. *Journal of Clinical Microbiology*, 2005; *43:* 455-457.
18. Mariana, M.M.F., Fernanda, E. and Araujo, M. *Parainfluenza virus* infections in tropical city: Clinical and epidemiological aspects. *Brazilian Journal of Infectious Disease*, 2008; 12(3):1413.
19. Palerm, C. L., Gonzalez, M. G., Savon, V. C. E., Valdes, R. O., Gonzalez, B. G., Goyenechea, H. A. J. and Galindo, S. B. Seroepidemiological surveillance of *Human Parainfluenza viruses* (HPIVs) type 2 and 3 in an infantile population selected in Havana City. *Review Cuvana Medicine for Tropics*, 2005;*53 (3):* 169-174.
20. Laurichesse, H., Dedman, D. and Watson, J. M. Epidemiological features of *Parainfluenza virus* infections: laboratory surveillance in England and Wales, 1975-1997. *European Journal of Epidemiology*, 1999;15 (5): 475-484.
21. Hohenthel, U., Nikoskelainen, J. and Vainiopaa, R. *Parainfluenza virus type 3* infections in a hematology unit. *Bone Marrow Transplantation*, 2001; 27(3): 295-300.
22. Arden, K.E., McErlean, P. and Nissen, M.D. Frequent detection of human rhinoviruses, *Paramyxoviruses*, *Coronaviruses*, and *Bocavirus* during acute respiratory tract infections. *Journal of Medical Virology*, 2006; 78 (9): 1232-1240.
23. Spring, H. Professional guide to diseases. Eigth edition, 2005. P2-3. Lippincott William and Wilkins Publisher.
24. Subhash, C.P. and Thomas, J.M. *Parainfluenza virus*. e*Medicine Specialties Infectious Diseases*, 2007; 1:2-10.