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SEROPREVALENCE OF HUMAN *PARAINFLUENZA VIRUS* TYPE 2 INFECTION AMONG CHILDREN (1-5YEARS) IN ZARIA, KADUNA STATE, NIGERIA

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ABSTRACT
Serological survey was carried out to determine the level of Human Parainfluenza virus type 2 (HPIV-2) IgG antibodies in children aged 1-5 years. Blood samples were collected from 379 children who met the selection criteria in selected hospitals in Zaria. Serum IgG antibody level for Human Parainfluenza virus type 2 was measured using commercial ELISA Kits obtained from VIRION-SERION ELISA Classic GmbH Germany. Of the total number of 379 samples, 176 (46.4%) were seropositive for parainfluenza virus type 2 antibody. There is statistical significance between age and seropositivity. Highest seropositivity (69.0%) was seen in the sera of those in age group 4-5 yearss and lowest seropositivity (23.2%) in age group 0 – 1 year (X^2 = 38.734; P.value <0.05). There was male predominance [91 (47.9%)] over females [85 (45%)] but the difference was not statistically significant (X^2 = 0.325; P>0.05). There was no association between the presence of cough and catarrh/running nose with seropositivity (P >0.05). The results also showed that there was no association between the presence of sickle cell disease and parental smoking with seropositivity (P >0.05). Preventive programs against HPIV – 2 infection should be promoted especially in younger children and research why reinfection occurs even in the presence of neutralizing antibodies should be undertaken.

Keyword: Seroprevalence, Human Parainfuenza virus type 2, infection, respiratory tract infection, ELISA.

INTRODUCTION

The World Health Organisation estimates that acute respiratory infections (ARIs) are responsible every year world wide for the deaths of 4 million children under 5 years. Paramyxoviruses are one of the major respiratory pathogens in this age group (Jawetz *et al.*, 2007).

Parainfluenza viruses (PIV) 1 through 4 together with Respiratory syncytial virus are the most frequent viruses isolated from children with upper and lower respiratory tract infections (Raija and Timo 1994; Juan *et al.*, 1998). *Parainfluenza* viruses are responsible for 30-40% of all ARIs in children. These conditions include common cold, croup, bronchiolitis and pneumonia (Laurichesse *et al.,* 1999). *Parainfluenza* viruses are also recognized respiratory pathogens in adults. Adults infected with PIVs tend to have a more variable and less distinctive clinical findings than children and the viral cause of the infection is often unsuspected (Hall, 2001).

Parainfluenza viruses are typical members of the family Paramyxoviridae. They are a large rapidly growing group of viruses that cause significant human and veterinary diseases. These RNA viruses are pleomorphic enveloped particles that are 150-300 nm in diameter, and their genome are organized on a single negative-sense strand of RNA (Raija and Timo, 1994; Henrickson, 2003). The majority of their structural and biological characteristics are similar, but they each have adapted to infect humans at different ages and cause different diseases (Henrickson, 2003). The viral genome of Paramyxoviruses is linear, negative sense, single stranded, non segmented RNA, about 15 kilo bases (kb) in size. The genome is not segmented and this negates any opportunity for frequent genetic reassortment, resulting in the fact that all members of the Paramyxovirus group are antigenically stable (Jawetz *et al., 2007).*

Human parainfluenza viruses (HPIVs) are common community-acquired respiratory pathogens without ethnic, socio-economic, gender, age or geographical boundaries. Many factors have been found that predispose individuals to these infections, including malnutrition, over crowding, vitamin A deficiency, lack of breast feeding and environmental smoke or toxins (Laurichesse *et al.*, 1999). Human parainfluenza serotype 2 (HPIV-2) belongs to the order Mononegavirales, family Paramyxoviridae, subfamily Paramyxovirinae and genus Rubulavirus (Jawetz *et al.,* 2007). The Human Parainfluenza virus type 2 (HPIV-2) has V protein which plays important role in inhibiting the host interferon response and promoting virus growth (Machiko *et al.,* 2008). Human Parainfluenza virus type 2 spreads from respiratory secretions through close contact with infected persons or contact with contaminated surfaces or objects. Infection can occur when infectious material contacts mucous membranes of the eyes, mouth or nose, and possibly through the inhalation of droplets generated by a sneeze or cough (Collins *et al.,* 1995).

The most distinctive disease condition that develops following infection with HPIV is croup (laryngotracheobronchitis).This croup occurs commonly in children age 1-2 years and is more frequent in boys. HPIV-2 has been found to cause outbreaks of croup and it has also been reported to have caused >60% of croup cases in an individual community (Marx *et al.*, 1997). Serologic surveys have shown that 75% of children aged 5 years have antibodies to HPIV-2. HPIV-2 causes annual or biennial fall out breaks (Glezen *et al.*, 1997). In Milwaukee, it was found that white children had a much higher incidence than black children (Henrickson *et al.*, 1994).

Virtually all children by the age of 3 years will demonstrate serologic evidence of infection. Immunity to HPIV-2 develops, later increasing more rapidly during the second and third years of life (Henrickson, 2003). The aim of this study was to determine the prevalence of Human *Parainfluenza* virus type 2 among children aged 1 – 5 years attending the out-patient clinics of Ahmadu Bello University Teaching Hospital (ABUTH), Zaria, Kaduna State.

MATERIALS AND METHODS

Area of Study

The study area comprised 3 out patient clinics located in Zaria and Sabon Gari Local Government Areas of Kaduna State. Zaria Local Government has a population of about 975,153 inhabitants (NPC, 2006), it is located on longitude 8^0 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 636, 549 inhabitants (NPC, 2006). 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. The clinics included the General Out Patients Department of ABUTH Shika, Institute of Child Health Banzazzau, and Primary Health Clinic Sabon Gari Zaria.

Sample Collection and Processing

The various clinics were visited after obtaining ethical approval from ABUTH. Children aged 1 – 5 years were recruited in the study. Blood samples were collected only from children whose parents/guardians signed an informed consent. Sterile syringe (2m1) with 23SWG needle attached to the syringe was used for blood collection. Samples were collected aseptically by venous puncture from each. The samples were collected into 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 -20^0C until the assay time (Cheesbrough, 2003*)*. The serum assay for HPIV-2 was carried out in the Department of Microbiology, Ahmadu Bello University Zaria. Using enzyme linked immunosorbent Assay (HPIV-2 IgG VIRION-SERION ELISA Classic, GmbH Germany), samples were assayed for specific IgG antibody against the HPIV-2 virus. Manufacturer's instructions were duly followed. The optical density (OD) values were read at 405nm, using Sigma Diagnostic EIA multi well reader II. The kit has 98.6% specificity and 100% sensitivity.

RESULTS

Among the 379 children between 1-5 years tested for the presence of HPIV-2 IgG antibodies, 56 (14.8%) were aged 0-1year , 117 (30.9%) were aged 13-24 months, 79 (20.8%) were aged 25-36 months, 54 (14.2%) were aged 37-48 Months, while 73 (19.3%) were aged 49-60 months. Of the 379 samples tested, 176 (46.6%) were positive (Table 1).

Table 1 shows the seroprevalence of HPIV-2 IgG antibody among the studied population with respect to age. The seroprevalence was highest (69.0%) in age group 49-60 months and lowest in age group 0 – 12 months (23.2%). The result shows increased seropositivity with age [X^2 = 38.734, (P< 0.05)].

Table 2 shows that 203 (53.6%) of the children had no detectable HPIV-2 IgG with a serum antibody titre to HPIV-2 less than 90lU/ml. A total number of 110 (29%) had a maximum titre value of 891-1090lU/ml a relatively low level of HPIV-2 antibody was found in 33(8.7%) with a titre value of 90-290lU/ml, 15 (4.0%) with a titre of 291-490lU/ml, 8 (2.1%) with a titre value of 491-690lU/ml and 10(2.6%) with a titre value of 691-890lU/ml.

Table 1: Distribution of human *Parainfluenza* virus type 2 IgG antibody according to age group

Age group (Months)	No. of Children screened	No. of sero-positive	% sero-positive per group	% sero-positive per total
0 - 12	56	13	23.2	3.4
13 -24	117	43	36.8	11.3
25 -36	78	35	44.9	9.2
37- 48	54	34	63.0	9.0
49- 60	74	51	69.0	13.5
Total	**379**	**176**		**46.4**

X^2 = 38.734, P=0.000 at 95% CI

Table 2: Profile of human *Parainfluenza* virus type 2 antibody titre according to age group (Titres IU/ml)

Age group (Months)	No of subjects Screened	<90	90-290	291-490	491-690	691-890	891-1090
0 – 12	56	43	5	0	0	0	7
13-24	117	73	12	5	2	2	24
25-36	79	45	4	2	3	3	21
37-48	54	20	4	3	0	3	24
49-60	73	22	8	5	3	2	34
Total	**379**	**203**	**33**	**15**	**8**	**10**	**110**

Table 3: shows the results of the total 379 children tested, 190 (50.1%) were males while 189 (49.9%) were females. Sero-positivity was observed in 91 (47.9%) males and 85 (45%) in females. This shows a slight male predominance over females, which was not statistically significant (X^2 = 0.325; P>0.05).

Table 3: Distribution of human *Parainfluenza* virus type 2 antibody according to sex

Sex	Number Screened	Number Sero-positive	% Sero-positive per group	% sero-positive per total
Males	190	91	47.9	24.0
Females	189	85	45.0	22.4
Total	**379**	**176**		**46.4**

X^2 =0.325, P value=0.320

Of the total number of 379 children tested, 268 (70.7%) had symptoms of catarrh/running nose and 111 (29.3%) had no symptoms of catarrh/running nose. Sero-positivity was observed in 122 (45.5%) of those with catarrh/running nose and 54 (48.6%) of those without catarrh/running nose. This is not statistically significant (X^2 = O.308; P>0.05).

Table 4: Distribution of human *Parainfluenza* virus type 2 antibody based on the symptom of catarrh/running nose

Catarrh/Running Nose	Number Screened	Number Sero-positive	% Sero-positive per group	% Sero-positive per total
presence	268	122	45.5	32.4
Absence	111	54	48.6	14.2
Total	**379**	**176**		**46.4**

X^2 0.308, P value=0.329

Table 5: shows the results of the total number of children tested (379), 266 (70.2%) had cough while 113 (29.8%) had no cough. Sero-positivity was observed in 116 (43.6%) of the HPIV-2 antibody but was not statistically significant (X^2 =2.871; P>0.05).

Table 5: Distribution of human *Parainfluenza* virus type 2 antibody according to presence of cough

Cough	Number Screened	Number Sero-positive	% Sero-positive per group	% Sero-positive per total
presence	266	116	43.6	30.6
Absence	113	60	53.0	15.8
Total	**379**	**176**		**46.4**

X^2 = 2.871, P value=0.057

Table 6: it shows that out of the 379 children tested 9 (2.4%) have sickle cell disease while 370 (97.6%) have no sickle cell disease. Seropositivity was observed in 2 (22.2%) but is not statistically significant (X^2 = 2.173; P>0.05).

Table 6: Prevalence of *Parainfluenza* virus type 2 IgG antibody in children with sickle cell disease

Sickle cell disease	Number Screened	Number Sero-positive	% Sero-positive per group	% Sero-positive per total
Presence	9	2	22.2	0.5
Absence	370	174	47.0	45.9
Total	**379**	**176**		**46.4**

X^2 =2.173, P value=0.127

Table 7 shows the results of the total number of children tested (379), 46 (12.1%) had parents who smoke cigarettes while 333 (87.9%) had no history of parental smoking. Seropositivity was observed in 26(56.5%) of the children of those who smoke but was not statistically significant (X^2 = 2.140; P >0.05).

Table 7: Prevalence of human *Parainfluenza* virus type 2 IgG antibody in children whose parents smoke

Parental smoking	Number Screened	Number Sero-positive	% Sero-positive per group	% Sero-positive per total
presence	46	26	56.5	6.8
Absence	333	150	45.0	39.6
Total	**379**	**176**		**46.4**

X^2 = 2.140, P value=0.096

DISCUSSION

In this study, IgG antibody was measured to determine the proportion of sero-positivity and the level of the antibody with respect to age, gender, presence of respiratory symptoms, sickle cell disease, and parental smoking. Human *Parainfluenza* virus type 2 overall seroprevalence of 46.4% was found in this study for HPIV-2 IgG antibodies. This is in contrast to the report of Glezen and Denny (1997*)* which showed that 75% of children aged 5 years had antibodies to HPIV- 2. The study also revealed that there is increase in sero-positivity with age. This could be as a result of reinfection of older children that occurs in the presence of antibodies elicited by an earlier infection. Since there is no permanent immunity to the infection those antibodies modify the disease, as such reinfection usually present simply as nonfebrile upper respiratory infection. This conforms to the findings of (Jawetz *et al., 2007)*.

The profile of HPIV-2 antibody titre indicated that 110(29%) had maximum level of antibody titre ranging between 891 and 1090 IU/ml. This could be as a result of reinfection.

In the sex distribution of HPIV-2 antibodies, seropositivity was also observed to be high in males (47.9%) than in females (45.0%). This also agrees with the work of Henrickson (2003), although the result was not statistically significantly (X^2 = 0.325; P>0.05). Sero-positivity was observed to be 45.5% of those with the symptoms and 48.6% of those without the symptoms of catarrh*/*running nose, although the result was not statistically significant (X^2 = 0.308; P>0.05), this could be as a result of other microbial agents rather than the virus.

In the cough distribution of HPIV-2 antibodies, seropositivity was observed to be 43.6% for those with cough and 53% for those without cough. This was not also statistically significant, but could also be as a result of other microbial agents responsible for the cough.

The results revealed that of the 9 (2.4%) subjects tested to have sickle cell disease, seropositivity was observed in 2 (22.2%) but was not statistically significant (X^2 =2.173; P>0.05). This could be due to limited number sickle cell disease individual involved in the present study. The results also showed that 46 (12.1%) had been exposed to parental smoking. Twenty six (56.5%) were sero-positive of HPIV-2 antibody but was not statistically significant. This is in contrast with the findings of Laurichesses *et al* (1999) who identified environmental smoke as a predisposing factor to the infection in England and Wales.

CONCLUSION AND RECOMMENDATIONS

Careful observation from the study showed the importance of HPIV-2 as an agent of acute respiratory tract infections in children. This suggests the need for rapid, easy and less expensive methods of diagnosis for clinical management and availability of vaccines.

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