ISSN (Print): 2476-8316
ISSN (Online): 2635-3490
Dutse Journal of Pure and Applied Sciences (DUJOPAS), *Vol. 5 No. 2b December 2019*

Serological Detection of Enteroviruses (EV71) From HIV Infected Children in Kaduna, Nigeria

Egboche, F.O., *Abraham, O. J., Ijah, U.J.J.
[1]Department of Microbiology,
Kaduna State University,
Kaduna State, Nigeria.

[2]Department of Science Laboratory Technology,
Federal Polytechnic Idah, Kogi state, Nigeria.

[3]Department of Microbiology,
Federal University of Technology,
Minna, Niger State, Nigeria.
Email: josephoyiguh@yahoo.com

Abstract
Enteroviruses are single-stranded, non-enveloped positive sense RNA viruses with small diameter of about 28-30nm, in the family Picornaviridae. They replicate in the human alimentary tract, throat, blood, urine, Cerebro Spinal Fluid, stool, fluid and lower intestine. They are however predominated by polioviruses as the major causes of paralytic disease in children and usually transmitted by person- to -person by direct contact with the viruses that are shed from the gastrointestinal tract or upper respiratory tract. This study determined the seroprevalence and molecular detection of Enteroviruses 71 in HIV infected children (0 to 5 years) using gold based immunochromatographic assay (GICA).
A total of 100 blood samples were aseptically collected from confirmed HIV infected children admitted in General hospital Kaduna, Nigeria. Rapid confirmatory test kits were used to detect Enteroviruses 71 (EV71)-IgM antibody for Enteroviruses 71 specific in the HIV infected children. Out of the 100 samples screened 5(5%) samples was positive of EV71 specific IgM antibodies captured by immobilized EV71 formed an antibody-antigen complex on the test line. However, no paralytic disease was recorded in the five positive cases, but there was a severity of hand foot & mouth disease (HFMD), fever, sore throat, headache, swollen gland, aseptic meningitis, acute respiratory illness, jaundice and diarrhea which was common indicator of EV71 infection. The study revealed that Enteroviruses 71 infection is circulating among children in Kaduna State, and this needs proper assessment of its magnitude and impact in order not to result to epidemic situation

Keywords: Children, Enteroviruses 71, Gastro-intestinal tract, IgM-antibodies, Picomaviridae

INTRODUCTION
Enteroviruses are small diameter (28-30nm), single-stranded, non-enveloped positive sense RNA viruses from the family picornaviridae (Xu *et al*., 2010). They are transient inhabitants of the human alimentary tract, throat, blood, urine, CSF, stool, fluid and lower intestine (Huang *et al*., 2012). The picornaviridae family contained six genera: Enterovirus (Enteroviruses), Rhinovirus (rhinoviruses), Hepatovirus (Hepatitis A virus), Parechovirus (parechoviruses), Aphthovirus (foot and mouth diseases), and Cadioviruses (cardioviruses) (Zhao *et al*., 2011).

*Author for Correspondence

The Enteroviruses of human origin include: The polioviruses, type 1 – type 3; Coxsackieviruses of group A, type 1- 24 (there is no type 23 which is the same as echoviruses 9); Coxsackieviruses of group B, type 1-6; echoviruses, type (1-33 (no types 10, 22, 23, or 28); enterovirus type 68-71; enteroviruses 72; Hepatitis A virus has been provisionally classified as a serotype (Baba *et al.,* 2012).The virion of Enteroviruses consists of a capsid shell of 60 subunits, each of four proteins (VP1-VP4) arranged with icosahedral symmetry around a genome made up of a single strand of positive-sense RNA (Wang *et al.,* 2012). Epidemiology of enteroviruses is predominated by polioviruses as the major causes of paralytic disease in children (Chang *et al.,* 2004). Poliomyelitis has three epidemiologic phases of endemic, epidemic, and vaccine era. Enteroviruses occur in all age groups, but children are usually more susceptible than adults because of the acquired immunity of the adult population. Aseptic meningitis is the common reported illness due to enteroviruses infection but notable clinical manifestation of certain types particularly coxsakie group A involve the skin and mucous membrane and respiratory tract (WHO, 2015). Gastrointestinal disturbance has been reported in some echoviruses infection and cardiac disease is particularly associated with group B coxsakie virus infections (Stikas *et al.,* 1986). Modes of transmission of Enteroviruses are usually transmitted by person to person by direct contact with the viruses that are shed from the gastrointestinal tract or upper respiratory tract. However in some patients, especially children, these infections may cause serious disease that may produce lifelong problems and infrequently, may cause death (Nathanson, 2015). Some Enteroviruses can be transmitted indirectly when uninfected person come in contact with food or fluids contaminated by secretions (faeces, oral secretion or droplets) from infected individuals. The viruses are capable of surviving on surfaces like tables and door handles for several days (Adeniji and Faneye, 2014). Signs and symptoms of Enteroviruses have an average incubation period of about 3-10 days. Symptoms when present in uncomplicated infections, last about a week, individual can shed infectious viruses even if they have no symptoms or during the incubation period and or after the symptom stops (Oyero and Adu, 2010). Infected Infant may develop one or more of the following symptoms; Common cold (nasal discharge, cough, mild fever, mild malaise), Hypoxia; (low oxygen in the blood, shortness of breath, wheezing, rapid breathing, skin coloration change rapid heart rate), Aseptic meningitis ; (most common among the HIV infected children), Photophobia (avoidance of light due to discomfort), Myopericarditis, Intermittent chest pain, Herpangina; (small flat sores on the oral mucosa), Hand, foot and mouth disease (HFMD); small nodules and blisters that are tender and appear gray which occur on the hand, feet and in the oral cavity , Encephalitis ; symptoms range from lethargy and drowsiness to seizures and coma, Paralysis; (infrequent in both polio and non-polio infection) flaccid paralysis that is often asymmetric with proximal extremity muscles affected(Rao *et al.,* 2013). The peak seasons for the disease are late spring to summer, although infections occur with high incidence throughout the year in tropical areas. The patients usually have a high fever above 38°C, unlike HFMD caused by other enterovirus serotypes such as coxsackievirus A16 (Lee, 2016).

Laboratory Diagnosis of Enteroviruses varies; the virus may be recovered or isolated from throat swab, blood, stool, CSF, fluid and lower intestine (Adeniji and Faleye, 2010). The diagnosis range from; electron microscopy, PCR, complement fixation, anti-body fluorescence, neutralization test and heamagglutination can be used to identify the virus in tissue or secretion (WHO, 2015).Treatment and control of enteroviruses (polio) Management is a supportive Management and addresses symptoms. No antiviral medications are currently approved for the treatment of enterovirus infections, effective medical care, surgical care, consultation, diet and activity is highly supported (Ooi *et al.,* 2011). Control at the other hand is by maintaining high standard of hygiene and sanitation (Chan *et al.,* 2011).

Enteroviruses71 among HIV infected children are more prevalent in lower socioeconomic population and younger children are the most important transmitter (Adeniji and Faleye, 2014). EV71 occasionally involves the central nervous system (CNS) and can cause serious and potentially fatal neurological complications, thus, has emerged as a major concern among pediatric infectious diseases, particularly in Africa (Tapparel *et al.,* 2013).

Data and medical information of Enteroviruses 71 among HIV infection children are not accurately available in Northern part of Nigeria and most of the infections caused by Enteroviruses, especially in HIV infected children cannot be clinically distinguished from other diseases caused by other viruses or bacteria (WHO, 2015). This study determined the seroprevalence and molecular characterization of Enterovirus 71 in HIV infected children in Kaduna, Kaduna State, Nigeria.

MATERIALS AND METHODS

Ethical consideration

Ethical permit was obtained from Kaduna State Ministry of Health, and written informed consents were obtained from all parents of participating infants.

Study Area

This study was carried out in the Dept of Microbiology, Kaduna State University, Kaduna. Kaduna is located in North-Western Nigeria, and is the capital of Kaduna State, Nigeria. It has an estimated population of 6,113,503 people (2006 census). The state covers a total Land area of 46,053 km^2 and located on Latitude 10°20′N and Longitude 7°45′E.

Sample collection

Demographic information was collected from 100 confirmed HIV- infected children age 0-15 years old on in-patient and out-patient admission at the General Hospital Kaduna using questionnaires. About 2 ml venous blood collected from each of them to obtain serum for analysis of HIV status.

Screening Procedure for Enteroviruses 71

Samples confirmed for HIV infection were screened for Enteroviruses 71 (EV71)-IgM antibody. Enteroviruses 71 (EV71) was detected from the plasma of HIV positive infant (1-15) years with EV71- IgM GICA kit (Bioneovan.com Beijing, China) according to the manufacturer's instructions. A 10µl of serum samples was pipetted into the sample wells of the EV71- IgM GICA cassette and the results were read within 15-20 minutes.

RESULT

Age Distribution of EV71 in HIV infected children in Kaduna State

Five blood samples out of the 100 samples screened were positive for EV71 specific IgM antibodies giving a seroprevalence of 5% (5/100) (Table 1). EV71 antibodies were detected in one sample from age group 0-3 years giving a seroprevalence rate of 6.3% (1/16), and 4 samples were positive in the age group 4-6 years with a seroprevalence rate of 13.3% (4/30), while the remaining age groups were negative (Table 1).

Table 1. **Age Distribution of EV71 in HIV infected children in Kaduna, Kaduna State**

Age (Years)	No screened	No positive	Percentage (+ve)
0-3	16	1	6.3
4-6	30	4	13.3
7-9	16	0	0.0
10-12	21	0	0.0
13-15	17	0	0.0
Total	100	5	5.0

Sex Distribution of EV71 among HIV Infected Children in Kaduna, Nigeria

Of the forty two male samples screened, two were positive with a seroprevalence of 4.8% (2/42), while three samples from females were positive with a seroprevalence of 5.1% (3/58) (Table 2).

Table 2: **Sex Distribution of EV71 among HIV Infected Children in Kaduna, Nigeria**

Sex	No screened	No Positive	% positive
M	42	2	4.8
F	58	3	5.1
Total	100	5	5.0

Distribution of EV 71 among Symptomatic HIV infected children in Kaduna, Nigeria

Results showed that 5 out of the 9 children with symptoms HFMD were positive for EV 71, while 5 of the 14 children with jaundice had antibody to EV 71 (Table 3). Also the 5 children who were positive for EV 71 had symptoms of diarrhoea, fever and cough, while 4 had blisters and sore throat (Table 3). One had symptoms of aseptic meningitis (Table 3).

Table 3: Distribution of EV 71 among Symptomatic HIV infected children in Kaduna

Symptoms	No screened	No +ve for EV71	% prevalence
HFMD	09	05	55.6
Jaundice	14	05	35.7
Diarrhoea	42	05	11.9
Blister	06	04	66.7
Cough	09	05	55.6
Fever	13	05	38.5
Sore throat	05	04	80.0
Aseptic meningitis	02	01	50.0
Total	100	19	19.0

Key: HFMD = Hand, foot and mouth disease.

DISCUSSION

Human enterovirus 71 (EV-A71), genus Enterovirus, and family Picornaviridae is a serious public health threat because it can cause large outbreaks of hand, foot and mouth disease (HFMD), and has also been associated with severe and some-times fatal neurologic complications that affect mostly infants and children and that range from aseptic meningitis and encephalitis to poliomyelitis-like acute flaccid paralysis (WHO, 2015). Co-infection with other infectious diseases and other factors such as malnutrition, vitamin deficiency, delay in hospital admission combined with a lack of specific intensive care equipment (such as extrac

orporeal membrane oxygenation) or intravenous immunoglobulins and host genetics are of factors responsible for the severity of EV-71 infections (Li *et al*., 2013).

In this study, a prevalence of 5% was obtained from the 100 infants' samples studied. In a study of non – polio-enterovirus (NPEV) reported by Fernandez-Garcia *et al*. (2016), the results obtained from some countries in West Africa except Cape Verde 0%(0/5 specimens), were higher, for instance, Gambia 12.5%(8/64), Guinea-Bissau 16.3%(8/49), Guinea 11.8%(42/355), Mauritania 18.5%(20/108), Niger 15.9%(95/596), and Senegal 14.9%(63/423) than the 5% obtained in this study in Nigeria. In a previous study carried out in Ibadan, Nigeria among children ages 1 -10 years (Stanway *et al*., 2015), a higher prevalence rate of 15 (25%) of the sixty (60) stool samples screened by RT-snPCR was reported for the enterovirus VP1 gene. The differences in detection protocols might yield different results based on the sensitivity. Other methods of detection includes direct detection of enterovirus genome from the clinical sample, cell-culture based algorithm, particularly a combination of RD and L20b cell lines, as recommended by World Health Organization (WHO), and gene detection RT-snPCR (Stanway *et al*., 2015). The samples for laboratory investigation should be selected according to the disease manifestations, either throat, rectal, and ulcer swabs, and samples of serum, urine, CSF, and fluid from vesicles. The sensitivity, specificity, and usefulness of findings vary according to the sample (Chang *et al*., 2016). In particular, virus detection in samples from sterile sites, such as vesicular fluid, CSF, serum, urine, or those gathered at autopsy, is more reliable than that in samples from non-sterile sites, such as the throat or rectum, where the presence of the virus might merely indicate coincidental carriage. The gold standard for diagnosis of enterovirus infection is virus isolation. Several human and non-human primate cell lines can be used, including rhabdomyosarcoma, which is most efficient, human lung fibroblast cells, and African green monkey kidney cells. In rhabdomyosarcoma cells, a characteristic cytopathic effect is observed typically 7–10 days after inoculation, and the virus is identified by neutralization tests in intersecting pools of type-specific antisera, EV71-specific antisera, or by an indirect immune-fluorescence assay with EV71-specific monoclonal antibodies (Chang *et al*., 2016).

The virus was detected in the age groups 1 – 6 years in this study (Table 1). This is in agreement with the report of WHO (2015) and Lee (2016), that EV71 infections affect mostly infants and children. Humoral immunity with neutralizing antibodies is crucial in protecting against EV71 and CA16 infections, with seroepidemiology providing information of great importance to assure population immunity (Li *et al*., 2013).The implication of EV 71 in this study population and in this area is that there might be a build-up of a large population of susceptible children every few years sufficient to sustain transmission. The virus has the polymerase of a +ssRNA virus, an RNA-dependent RNA polymerase (RdRP) with low fidelity, having as many as 10^{-3} to 10^{-5} mutations per nucleotide copied in each replication cycle, and this could result in misincorporation of 1–2 new nucleotides. There are exchanges of genomic domains between poliovirus and other co-circulating enterovirus species which affect the plasticity of enteroviral genome. This has been to exist between circulating vaccine-derived polioviruses (cVDPV) and non-polio enteroviruses of species C (EV-Cs)(Fernandez-Garcia *et al*., 2016).

Gender distribution of EV 71 infection in this study showed no significant difference ($p<0.05$) between males with seroprevalence of 4.8% (2 /42) and females with a seroprevalence of 5.1% (3/58) (Table 2). This result does not agree with the 29.73% (11/37) and 17.39% (4/23) seroprevalence obtained for male and female respectively (Xu *et al*., 2010) in a similar studies carried out among apparently healthy school aged children in Ibadan, Southwestern Nigeria. Mamdary and Poh (2018), in a similar study in Thai population reported that there was no

significant difference in male-female ratio of EV 71 infection. Also, Ooi *et al.* (2010), in a similar study among children in Cambodia observed that, gender was not a risk factor of EV 71 infection. However, Li *et al.* (2013), in a similar epidemiology studies in Guangdong reported that boys were more susceptible to enterovirus than girls, since they may have more physical activities which may diminish immunity due to stress, thus favouring the spread of HFMD.

Like other Enteroviruses, EV71 is most frequently recognized among the younger children most especially the HIV infected and immune-compromised children and can cause a broad spectrum of symptoms even death. In the five positive cases (5%) recorded in this study out of the 100 samples screened, there was severity of hand foot and mouth disease (HFMD), fever, sore throat, headache, swollen gland, aseptic meningitis, acute respiratory illness, jaundice and diarrhoea. HFMD is typically a benign self-limiting illness observed among young children and infants. Outbreaks are often associated with day care centres, nurseries and primary schools (Sabanathan *et al.*, 2014). Vesicular lesions usually occur on the palms of the hands and soles of the feet, but all parts of the limbs, including groins and buttocks may be affected. Oral ulceration is usually present, and there is clinical overlap with herpangina, an illness characterized by high fever, sore throat and oral ulceration that also affects young children. The transmission of EVs is most efficient in crowded settings, and therefore during outbreaks most countries in the region have adopted social distancing measures, such as closures of childcare facilities and schools and cancellation of public functions involving children (Lee *et al.*, 2016; Duong *et al.*, 2017).

CONCLUSION

The prevalence of Enterovirus 71 in HIV infected infants attending the public health facilities in Kaduna metropolis was established. Symptoms exhibited by infected infants are typical symptoms of EV71 infection. Molecular serotyping may give more insight into the subspecies of the virus present.

REFERENCES

Adeniji, J.A. and Faleye, T.O. (2014). "Impact of cell lines included in enterovirus isolation protocol on perception of nonpolio enterovirus species C diversity," *Journal of Virological Methods*. **207**:.238–247.

Baba, M.M., Oderinde, B.S., Patrick, P.Z. and Jarmai, M.M. (2012). "Sabin and wild polioviruses from apparently healthy primary school children in northeastern Nigeria," *Journal of Medical Virology*. **84**(2): 358–364,

Chan, Y.F., Wee, K.L., Chiam, C.W., Khor, C.S., Chan. S.Y. et al. (2011). Comparative genetic analysis of VP4, VP1 and 3D gene regions of enterovirus 71 and Coxsackieviruses A16 circulating in Malaysia between 1997- 2008. *Tropical Biomedicine* **29**(3): 451- 466.

Baba, M.M., Oderinde, B.S., Patrick, P.Z. and Jarmai, M.M. (2012). "Sabin and wild polioviruses from apparently healthy primary school children in northeastern Nigeria." *Journal of Medical Virology*, **84**(2): 358–364.

Chan, Y.F., Wee, K.L., Chiam, C.W., Khor, C.S., Chan, S.Y. et al. (2011). Comparative genetic analysis of VP4, VP1 and 3D gene regions of enterovirus 71 and Coxsackieviruses A16 circulating in Malaysia between 1997- 2008. *Tropical Biomedicine*, **29**(3): 451- 466.

Chang, L., Hsia, S.H., Wu, C.T., Huang, Y.C., Lin, K.L. et al. (2004). Outcome of enterovirus 71 infections with or without stage-based management: 1998 to 2014. *Pediatric Infect Dis J.* **23**: 327-331.

Chang, P-C., Chen, S-C. and Chen, K-T. (2016): The Current Status of the Disease Caused by Enterovirus 71 Infections: Epidemiology, Pathogenesis, Molecular Epidemiology, and

Vaccine Development. *International Journal ofEnvironmental Researchaand Public Health.* **3**(2): 234-243.

Duong, V., Mey, C., Eloit, M., Zhu, H., Danet, L. et al. (2016): Molecular epidemiology of human enterovirus 71 at the origin of an epidemic of fatal hand, foot and mouth disease cases in Cambodia. *Emerging Microbes & Infections*. 5, e104; doi:10.1038/emi.2016.101.

Fernandez-Garcia, M. D., Kebe, O., Fall, A. D., Dia, H., Diop, O.M. et al. (2016): Enterovirus A71 Genogroups C and E in Children with Acute Flaccid Paralysis, West Africa. Emerging Infectious Diseases. **22**(4): 753 – 755.

Huang, C., Liu, C., Chang, Y., Chen, C., Wang, S. andYeh, T. (2012). Neurologic complications in children with enterovirus 71 infection. *N Engl J. Med*. **341**: 936-942.

Lee, K. Y. (2016): Enterovirus 71 infection and neurological complications. Korean J Pediatr. **59**(10):395-401.

Li, W., Yi, L., Su, J., Lu, J., Ke, C. et al. (2013): Seroprevalence of Human Enterovirus 71 and Coxsackievirus A16 in Guangdong, China, in Pre- and Post-2010 HFMD Epidemic Period. *PLoS ONE.* **8**(12): e80515. doi:10.1371/journal.pone.0080515

Linsuwanon, P., Puenpa, J., Huang, S-W., Wang, Y-F., Mauleekoonphairoj, J. et al. (2014): Epidemiology and seroepidemiology of human enterovirus 71 among Thai populations. *J Biomed Sci*. **21**(1): 16.

Mandary, M. B. and Poh, C.L. (2018): Changes in the EV-A71 Genome through Recombination and Spontaneous Mutations: Impact on Virulence. Viruses. **10**(320); 21pages. doi:10.3390/v10060320

Nathanson, N. and Kew, O.M. (2015). "From emergence to eradication: the epidemiology of poliomyelitis deconstructed," *American Journal of Epidemiology*. **172**(11): 1213- 1229.

Ooi, M.H., Wong, S.C., Lewthwaite, P., Cardosa, M.J. and Solomon, T. (2010): Clinical features, diagnosis, and management of enterovirus 71. *Lancet Neurol*. **9**: 1097–1105.

Ooi, M.H., Wong, S.C., Lewthwaite, P., Cardosa, M.J., Solomon, T. (2011). Clinical features, diagnosis, and management of enterovirus 71. *LancetNeurology*, **9**(11): 1097-1105.

Oyero, O.G. and Adu, F.D. (2010). "Non-polio enteroviruses serotypes circulating in Nigeria," *African Journal of Medicine and Medical Sciences*, **39**(3): 201–208.

Rao, D.C., Ananda, B.M., Raghavendra, A., Dhananjaya, D., Kumar, S., Maiya, P.P. (2013). "Non-polio enteroviruses and their association with acute diarrhea in children in India," *Infection, Genetics and Evolution*, **17**: 153–161.

Sabanathan, S., Tan, L.V., Thwaites, L., Wills, B., Qui, P.T. et al. (2014): Enterovirus 71 related severe hand, foot and mouth disease outbreaks in South-East Asia: current situation and ongoing challenges. *Epidemiol Community Health*. **68**(6): 500 – 502.

Stanway, G., Brown, F., Christian, P., Hovi, T. Hyypiä, T. et al. (2015). Family Picornaviridae. In: VirusTaxonomy: Eighth Report of the InternationalCommittee on Taxonomy of Viruses. (Edited byElsevier Academic Press), pp 757-778, London.

Strikas, R.A., Anderson, L.J. and Parker, R.A. (1986). "Temporal and geographic patterns of isolates of nonpolio enterovirus in the United States, 1970–1983," *Journal of Infectious Diseases*. **153**(2) 346–351.

Tapparel, C., Siegrist, F., Petty, T.J. and Kaiser, L. (2013). "Picornaviruses and enterovirus diversity with associated human diseases," *Infection, Genetics and Evolution*, **14**(1): 282–293.

Wang, Y., Zou, G., Xia, A., Wang, X., Cai, J, et al. (2015). Enterovirus 71 infection in children with hand, foot, and mouth disease in Shanghai, China: epidemiology, clinical feature and diagnosis. *Virology Journal.*;**12**: 83.

World Health Organization (2015), Enterovirus Surveillance Guidelines: Guidelines for Enterovirus Surveillance in Support of the Polio Eradication Initiative, World Health Organization, Geneva, Switzerland.

Xu, F., Yan, Q., Wang, H., Niu, J., Li, L. et al. (2010). Performance of detecting IgM antibodies against Enterovirus 71 for early diagnosis.*PLoSOne* **5**(6): e11388.

Zhao J, Xu J, Chen WW, Li YL, Tang Y. et al. (2011).The early diagnosis value of EV71 IgM class antibodies in the hand, foot, and mouth disease.*Zhonghua Shi Yan He Lin Chuang Bing Du XueZaZhi.* **25**(2): 140- 142.