

# Enteroviruses as a possible cause of hypertension, dilated cardiomyopathy (DCM) and hypertensive heart failure (HHF) in South western Nigeria

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## Abstract

**Background:** Human enteroviruses have long been associated with various diseases of man resulting into a wide range of acute symptoms involving the cardiac and skeletal muscles, central nervous system, pancreas, skin and mucous membranes.

**Objective:** To assess the role of enteroviruses in the etiology of hypertension, DCM and HHF.

**Methods:** We obtained stool specimens from 70 subjects comprising 65 patients and 5 controls and isolation was carried out on RD, L20B, HEp-2C and Vero cell lines and identified by neutralization with standard antisera (RIVM). Thirty-six enteroviruses were isolated and identified to be Coxsackieviruses-B5, A9, Echoviruses 1, 6, 7, 9, 11, 12, 22, 30 and Poliovirus type 1 and 3.

**Results:** Three most frequently occurring enterovirus serotypes which constitute 60.0% of the 30 NPEV typed and 50.0% of all the isolates were Echoviruses, Coxsackie-B5-virus and Coxsackievirus-A9. Echoviruses constituted 50.0% of all the serotypes while Coxsackieviruses-B5 and A9 accounts for the 27.8 % and 5.6% respectively. Enteroviral isolation rate was higher in age groups 51 years and above. The percentage of study subjects who had Coxsackie-B5-viruses and echoviruses was significantly ( $P < 0.05$ ) higher in cases of hypertension, HHF and DCM than in control subjects. Coxackie-B5-virus, Echovirus-6 and Echovirus-11 were found in both study locations.

**Conclusion:** The findings of this study showed that Enteroviruses may likely be involved in the etiology of hypertension, DCM and HHF. Further studies would therefore be necessary for the prevention and control of these diseases.

**Keywords:** Etiology, coxsackieviruses, Echoviruses, dilated cardiomyopathy (DCM), Enteroviruses, hypertension, hypertensive heart failure (HHF).

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## Introduction

Human enteroviruses have long been associated with various diseases of man resulting into a wide range of acute symptoms involving the cardiac and skeletal muscles, central nervous system, pancreas, skin and mucous membranes<sup>1-2</sup>. Their roles in chronic diseases such as the dilated cardiomyopathy, insulin –

dependent diabetes mellitus, motor neurone disease, chronic fatigue syndrome and postpoliomyelitis, syndrome have also been documented<sup>3-8</sup>. Worldwide, an estimated one billion infections occur each year<sup>9</sup>.

Enteroviruses (EVs), particularly the Coxsackie B viruses, are cardiotropic and are important causes of myopericarditis in children and adults<sup>10,11</sup> and are associated with the pathogenesis of dilated cardiomyopathy<sup>12-13</sup>. Recent polymerase chain reaction (PCR) studies in human myocardium have provided evidence that enteroviral infection was detectable in acute and chronic myocarditis, as well as, in end-stage idiopathic dilated cardiomyopathy (IDCM)<sup>10,13</sup>. Some, but not all, studies suggested that positive PCR for virus may be an unfavourable predictor, and it has been proposed that additional prospective data are needed<sup>14</sup>. In a prospective study, Heymans<sup>15</sup> assessed aetiopathogenesis in myocarditis,

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including a positive PCR for virus and serum as viral and autoimmune markers.

Previous studies by Mahrholdt *et al.*<sup>16</sup>; Heymans *et al.*<sup>17</sup>; Heymans<sup>15</sup>; Maekawa *et al.*<sup>18</sup> and Caforio *et al.*<sup>19</sup> have also shown that many viruses are capable of damaging the heart during their replication which often causes damage to the heart cells in 25-50% of all cases of acute heart failure. Pauschinger *et al.*<sup>20</sup> found 24 of 94 patients with IDCM leading to heart failure to have either adenoviral or enteroviral polymerase chain reaction positivity from clinical heart samples.

Cardiomyopathy been reported to be associated with viruses like coxsackieviruses, adenovirus, and echovirus. Enteroviruses and adenoviruses have been considered the most common pathogens of viral cardiomyopathy, but parvovirus B19 (PVB19) is increasingly found in endomyocardial biopsies of patients with acute myocarditis or IDCM<sup>16,21</sup>. Recent biopsy series in patients with DCM have revealed that long-term persistence of cardiotropic viruses triggers heart failure at long term: >70% of patients with DCM carry a cardiotropic virus in the heart as reported by Tschope *et al.*<sup>22</sup> and Kuhl *et al.*<sup>23</sup>.

Isolation and identification of enteroviruses is important to establish an etiologic/epidemiologic link among cases during an outbreak and to recognize serotype-specific cases, such as dilated cardiomyopathy and hypertensive heart failures. Confirmation of a suspected enterovirus infection is important for management of patient, prognosis, reducing hospitalization, preventing outbreaks, and excluding other infectious diseases, and therefore prevents unprescribed antibiotic usage. In addition, rapid confirmatory diagnosis of enteroviral infections could facilitate use of the novel antiviral drugs, such as pleconaril, targeted at the *Picornaviridae*<sup>24-26</sup>. However, the impact of the endemic enteroviral infection on the cardiovascular system in Africans has received scanty attention by researchers. There is, however, dearth of literatures on the precise role or contributions of enteroviruses in the pathogenesis of DCM and HHF in Nigeria. Thus, this study attempted to isolate and identify enteroviruses as a possible cause of hypertension, DCM and HHF in South Western Nigeria.

## Methods

### Study Area

All of the patients used for this study came from Ibadan city in Oyo state, one of the largest city in Africa and

its immediate environs and few were from Abeokuta and its environs both in South- Western, Nigeria.

### Study Population

Informed consent was obtained from each patient before investigations were carried out. A total of seventy (70) subjects (comprising 65 patients and 5 controls) were selected for studies based on M-mode echocardiography (ages 4-83 years; mean age: 49.7 years). The 65 study subjects comprised 48 (73.8%) males and 17(26.2%) females (Table 3) while the control groups comprised 4(80.0%) males and 1(20.0%) female (Table 4). The study subjects were so confirmed of the clinical condition by the Consultant Cardiologists. The control subjects were also so confirmed free of the clinical conditions. Demographic and clinical information of the subjects were obtained by chart abstraction and recorded on a prepared data collection form. Other relevant information such as socio-economic status, occupation, history of smoking and alcohol intakes and parity (females only) were obtained using a Performa specially designed for this purpose.

### Sample collection

Stool samples were collected aseptically from the subjects (65 patients and 5 controls) and the laboratory investigation such as viral isolation and identification carried out according to standard laboratory procedures of WHO manual<sup>28</sup>. All specimens were collected aseptically and transported in cold chain. The 70 samples from 65 patients and 5 controls were analyzed anonymously, as required by ethical rules<sup>27</sup>.

### Specimens preparation for virus isolation

All stool samples were treated with chloroform before inoculation into cell culture<sup>28</sup>.

### Cell lines and tissue culture media

Green monkey kidney (Vero), human epidermoid carcinoma (HEp-2) and human rhabdomyosarcoma (RD), and genetically engineered mouse cell (L20B) lines were used in this study<sup>28</sup>. With adherence to aseptic procedures and WHO<sup>28</sup> protocols, cells seeded into test tubes and at specified cell concentrations. Cell lines were cultured in Eagle's minimum essential medium with Earle's salts (Gibco, Invitrogen, Paisley, UK/ Gibco, BRL, Grand Island, N.Y.) supplemented with 5% /10% fetal bovine serum, 2 mM Lglutamine, 26.8 mM NaHCO<sub>3</sub>, 100 U of penicillin per ml, and 100g of streptomycin per ml and amphotericin B [0.25g/ml]). All tissue culture media and reagents were composed and prepared according to the working

standard method described by WHO<sup>28</sup> for the virologic investigation of enteroviruses.

### Virus isolation

Virus isolation and *in vitro* propagation was carried out using Vero, HEp-2, RD and L20B and all processed stool samples were inoculated onto monolayers of these four different cell lines and incubated in the stationary sloped position at 37°C. The cultured tubes were observed daily, using a standard/inverted microscope, for appearance of cytopathic effect (CPE) characteristic for enterovirus for the first 7 days; day 10 and finally at 14 days post-inoculation as described by WHO<sup>28</sup>.

### Virus identification

Poilo and NPEVs isolated in this study were identified using specific polyclonal antisera as described by the WHO<sup>28</sup> standard protocols for identifications of polio and non-polio enteroviruses.

### Antisera

Pooled horse antisera against the most frequently isolated ECHO and Coxsackieviruses were provided by the National Institute of Public Health and the Environment (RIVM), Bilthoven, The Netherlands. Interpretation of the results was done with the assistance of a list of the neutralization patterns of individual viruses<sup>28</sup>.

### Identification by Neutralization Assay

Identification of virus serotypes was performed by neutralization test using type-specific antisera (National

Institute of Public Health and the Environment, The Netherlands) as described by WHO<sup>28</sup>.

### Virus titration

Virus titre was determined by measuring TCID<sub>50</sub> of each virus isolate in RD cells, as previously described<sup>28</sup>.

## Results

### Virus isolations

A total of 36 isolates belonging to 10 clearly defined enterovirus serotypes were recovered from samples collected from the subjects confirmed to be DCM and HHF. One of the 36 isolates was recovered from the control group. The cell line of the positive samples showed cellular degeneration as cytopathic effect (CPE) which was very rapid, becoming 4<sup>+</sup> in 1-3 days with all cells detached from the glass base. CPE for the remaining enteroviruses generally requires 4-7 days or longer and often accompanied by individual small, round, and sometimes refractile cells or by a degenerative appearance across the monolayer, and might not progress to 4<sup>+</sup> or general cell lysis. CPE for the remaining were not specific for enteroviruses and were not tested further.

### Virus typing and identification

Table 1 shows the frequency of enteroviruses among the various studied groups. The 10 serotypes were identified as Coxsackie-B5-viruses [10 (27.8%)], echovirus 6 and 11 [4 (11.1%) each], echovirus 7, 9, 12, and 30 [2 (5.6%) each], Coxsackievirus A9 [2(5.6%)], poliovirus (PV) type 1 [2(5.6%)], PV3 [2(5.6%)] and mixture of PV1 and PV3 [2(5.6%)], and echovirus 1 and 22 [1 (2.8%) each] as shown in table 1.

**Table 1: Frequency of Occurrence of Enteroviral Isolates**

Enteroviral Isolates	No. (%)	Subjects (%)		Locations (%)	
		Study Groups	Control Groups	FMC,	UCH,
Coxsackie-B5-virus	10 (27.8)	+	-	+	+
Echovirus 7	2 (5.6)	+	-	-	+
Echovirus 11	4 (11.1)	+	-	+	+
Echovirus 9	2 (5.6)	+	-	-	+
Echovirus 6	4 (11.1)	+	-	+	+
Coxsackievirus-A9	2 (5.6)	+	-	+	+
Echovirus 1	1 (2.8)	+	-	+	-
Echovirus 22	1 (2.8)	+	-	+	-
Echovirus 12	2 (5.6)	+	-	+	+
Echovirus 30	2 (5.6)	+	-	-	+
Poliovirus 1 (PV1)	2 (5.6)	+	-	+	+
Poliovirus 3 (PV3)	2 (5.6)	+	-	+	+
PV1+PV3 Mixtures	2 (5.6)	+	+	+	+
<b>Total</b>	<b>36 (100.0)</b>	<b>35(97.2)</b>	<b>1(2.8)</b>	<b>7(19.4)</b>	<b>29(80.6)</b>

### Distribution of Enterovirus Isolates in relation to the group studied

Table 2 shows the distribution of enterovirus isolates in relation to patient groups studied. It showed that enteroviral infection was most prevalent among study subjects with DCM [26(63.4%)], followed by hypertensives [8(40.0%)], and HHF [1(25.0%)]. However, one (20.0%) of the controls was positive for enterovirus.

### Distribution of Enterovirus Isolates in relation to Location of study

Table 3 shows distribution of enterovirus isolates in relation to locations of study. It showed that enteroviral infection was more prevalent among subjects at FMC, Abeokuta [6(60.0%)] than their counterparts at UCH, Ibadan [29(52.7%)]. It also showed that 1(50.0%) of the two controls from Abeokuta was positive for enterovirus (table 3).

**Table 2: Distribution of Enteroviruses in relation to the groups studied**

Study Groups	No. Tested (%)	No. Positive (%)	Control Groups	No. Tested (%)	No. Positive (%)
Normotensives	0(0.0)	0(0.0)	Normotensives	5(100.0)	1(20.0)
Hypertensives	20(30.8)	8(40.0)	Hypertensives	0(0.0)	0(0.0)
Hypertensive Heart failure	4(6.2)	1(25.0)	Hypertensive Heart failure	0(0.0)	0(0.0)
Dilated Cardiomyopathy	41(63.1)	26(63.4)	Dilated Cardiomyopathy	0(0.0)	0(0.0)
<b>Total</b>	<b>65(100.0)</b>	<b>35 (53.8)</b>	<b>Total</b>	<b>5(100.0)</b>	<b>1 (20.0)</b>

**Table 3: Distribution of Enterovirus isolates in relation to locations studied**

Gender	No. Tested (%)	No. Positive (%)
<b>Study groups</b>		
UCH,	55(84.6)	29(52.7)
FMC,	10(15.4)	6(60.0)
<b>Total</b>	<b>65(100.0)</b>	<b>35(53.8)</b>
<b>Control groups</b>		
UCH,	3(82.9)	0(0.0)
FMC,	2(17.1)	1(50.0)
<b>Total</b>	<b>5(100.0)</b>	<b>1(20.0)</b>

### Distribution of Enterovirus Isolates in relation to various risk factors and demographics of subjects

Table 4 shows the distribution of enterovirus isolates in relation to various risk factors and demographics of study subjects. It showed that enteroviral infection was more prevalent among ages 51 years and above [19(54.3%)] compared to their counterparts in ages below 51 years [16(53.3%)]. However, this difference was not statistically significant ( $p > 0.05$ ). The distribution of enteroviruses in relation to sex is shown in table 4. It showed that enteroviral infection was more prevalent among males [27(56.3%)] compared to their female counterparts [8(47.1%)]. The results showed that the enterovirus isolates occurred frequently among the low-income class subjects (73.5%) and high-income class subjects (60.0%) compared to their counterparts in the 4 member-middle class (19.0%). There was a significant

difference in the rate of enteroviral infection between subjects in the high and the low socio-economic groups ( $p < 0.05$ ).

Enteroviral isolates were more prevalent among farmers (100.0%). This was followed by businessmen and unemployed subjects (66.7% each), traders (61.0%), civil servants (60.0%), students (57.1%), clergy, custom officer and pensioners (50.0% each). Enteroviral isolates were least prevalent among professionals (25.0%) while no enterovirus isolates was obtained from the teachers studied (table 4).

From table 4, it showed that enteroviral infection was highest among subjects with history of heart failure (63.4%), hypertension (56.1%), DCM (61.0%), alcoholics (73.3%), smoking (75.0%), and multiparous females (66.7%) compared to their

counterparts without such history. Females with no history of pregnancy (nulliparous) had the least enteroviral infection rate (12.5%). There was a significant association ( $p < 0.05$ ) between the rate of enterovirus infection and clinical history of subjects (table 4). Statistically, enterovirus isolation rate was significantly ( $p < 0.05$ ) higher in those with hypertension, DCM and HHF than those in the control group (table 4).

Table 4 shows the distribution of enterovirus isolates in relation to various risk factors and demographics of control subjects. It showed that enteroviral infection was only found among male control subject age 51 years and above [1(25.0%)]. The control results also showed that the enterovirus isolates occurred only among the low-income class and a farmer (table 4). Statistically, enterovirus isolation rate was significantly ( $p < 0.05$ ) higher in the study group than the control group (table 4).

**Table 4: Distribution of Enterovirus Isolates in relation to Risk factors among the study groups**

Risk Factors	Study Groups		Control Groups	
	No. Tested (%)	No. Positive (%)	No. Tested (%)	No. Positive (%)
<b>Age group (years)</b>				
Below 51	30(44.6)	16(53.3)	1(20.0)	0(0.0)
51 and above	35(55.4)	19(54.3)	4(80.0)	1(25.0)
<b>Gender</b>				
Male	48(73.8)	27(56.3)	4(80.0)	1(25.0)
Female	17(26.2)	8(47.2)	1(20.0)	0(0.0)
<b>Clinical History</b>				
<i>History of Heart Failure</i>				
Yes	41(63.1)	26(63.4)	0(0.0)	0(0.0)
No	24(36.9)	9(37.5)	5(100.0)	1(20.0)
<i>History of Hypertension</i>				
Yes	41(63.1)	23(56.1)	0(0.0)	0(0.0)
No	24(36.9)	12(50.0)	5(100.0)	1(20.0)
<i>History of DCM</i>				
Yes	41(63.1)	25(61.0)	0(0.0)	0(0.0)
No	24(36.9)	10(41.7)	5(100.0)	1(20.0)
<i>History of Alcohol</i>				
Yes	15(23.1)	11(73.3)	0(0.0)	0(0.0)
No	50(76.9)	24(48.0)	5(100.0)	1(20.0)
<i>History of Smoking</i>				
Yes	12(18.5)	9(75.0)	0(0.0)	0(0.0)
No	53(81.5)	26(49.0)	5(100.0)	1(20.0)
<i>Parity (Females; n=17)</i>				
Below 4	4(23.5)	2(50.0)	0(0.0)	0(0.0)
Multiparity (4 and above)	6(35.3)	4(66.7)	0(0.0)	0(0.0)
Nulliparity	7(41.2)	1(14.3)	1(100.0)	0(0.0)
<b>Socio-economic Class</b>				
High Income Class	10(15.4)	6(60.0)	0(0.0)	0(0.0)
Middle Income Class	21(32.3)	4(19.0)	3(60.0)	0(0.0)
Low Income Class	34(52.3)	25(73.5)	2(40.0)	1(50.0)
<b>Occupations</b>				
Students	7(10.8)	4(57.1)	2(40.0)	0(0.0)
Farmers	2(3.1)	2(100.0)	0(0.0)	0(0.0)
Traders	23(35.4)	14(61.0)	2(40.0)	1(50.0)
Civil servants	10(15.4)	6(60.0)	1(20.0)	0(0.0)
Pensioner	2(3.1)	1(50.0)	0(0.0)	0(0.0)
Businessmen	3(4.6)	2(66.7)	0(0.0)	0(0.0)
Teachers	3(4.6)	0(0.0)	0(0.0)	0(0.0)
Custom officer	2(3.1)	1(50.0)	0(0.0)	0(0.0)
Professionals	8(12.3)	2(25.0)	0(0.0)	0(0.0)
Clergy	2(3.1)	1(50.0)	0(0.0)	0(0.0)
Unemployed	3(4.6)	2(66.7)	0(0.0)	0(0.0)
<b>Total</b>	<b>65(100.0)</b>	<b>35(53.8)</b>	<b>5(100.0)</b>	<b>1(20.0)</b>

## Discussion

This study examined stool samples of hospital inpatients and outpatients with hypertension, Hypertensive heart failure (HHF), and dilated cardiomyopathy (DCM). Subjects with normal blood pressure were used as control. The study showed that enterovirus isolation rate was significantly ( $p < 0.05$ ) higher in those with hypertension, DCM and HHF than those in the control group. Among the enteroviruses isolated, echoviruses and Coxsackie-B5-viruses were the most implicated. Enteroviral infection is thought to be the most common cause of HHF and DCM leading to heart failure. Many studies have evaluated the role of enterovirus infections (particularly Coxsackieviruses B4 and B5) as environmental triggers of diseases. Several lines of evidence also suggested that enterovirus infections may be involved in the etiology of the dilated cardiomyopathy (DCM) leading to heart failure and hypertensive heart failures<sup>11-13,29-31</sup>.

The pattern of distribution of each of the 10 identified NPEV serotypes differs in each of the 2 location studied with Coxsackie-B5-virus, Coxsackievirus A9, Echoviruses 1, 6, 9, 11, 12, 22 and 30, as well as, Polioviruses present in the 2 locations studied. Echoviruses 7, 9 and 30 were isolated only from patients in Ibadan. The findings of this present study suggest that enteroviruses are endemic in Nigeria and the frequency of occurrence among this patients studied is higher compare to other studies<sup>32-37</sup>. Previous study in Nigeria suggested that Coxsackievirus B infections have been associated with clinical manifestation of DCM and HHF<sup>4</sup>. Muir et al.<sup>30</sup> reported a significantly higher prevalence of enterovirus-specific IgM responses among patients with DCM.

The three most frequently occurring enterovirus serotypes which constitute 60.0% of the 30 NPEV typed and 50.0% of all the isolates were Echoviruses, Coxsackie-B-viruses and Coxsackievirus A9. Coxackie-B5-virus was found in the two study locations in Ibadan and Abeokuta. Echovirus-6 and 11 was also found in both locations. Statistically, enterovirus isolation rate was significantly ( $P < 0.05$ ) higher in the study group than the control group. Occurrence of echovirus-6 was also documented as localized in Spain<sup>38</sup>, while both echovirus-6 and 11 had been implicated in an outbreak of mixed enterovirus infections in Kansas City<sup>39</sup>. In a study by Li et al.<sup>11</sup>, nine of 14 myocardial samples from endemic DCM cases in China were positive for the enteroviral RNA. The isolation of

echovirus 9 and 12 in this study deviates from the findings of Adejokun<sup>36</sup> who reported the absence of these two serotypes in their study.

The isolation of Enteroviruses in this study gives an indication that enteroviral infection may likely have a role in the etiology of the different diseases studied. Our finding is similar to the work of Antia<sup>40</sup> who found Coxsackievirus in the stool of one out of five children with DCM who later developed heart failure. It is also similar to Adejokun<sup>36</sup> who reported Coxsackie-B-virus, Coxackievirus A9, echovirus 6, 7 and 11 in patients who presented with DCM in Ibadan.

The study showed significant association between the enteroviral infection and the socio-economic status of the subjects. The presence of these enteroviruses in the subjects were not surprising, it may have been probably due to over-crowding, poor sewage disposal and poor living standard notably in the two locations of study. The findings of this study also showed significant association ( $P < 0.05$ ) between enteroviral infection and clinical history of subjects. In keeping with others<sup>19,32,41-43</sup>, this study found frequency of enteroviral infections to be higher among subjects with DCM (63.4%) than hypertensives (40.0%) and HHF(25.0%), supporting a causative association in adults. Frequencies of the individual viruses have been highly variable. The differences in the frequency of occurrences of these isolates were also reported by previous studies<sup>14,23,36,43-46</sup>.

Our findings are comparable to that of Ferreira *et al.*<sup>29</sup> and Fujioka *et al.*<sup>45</sup> who isolated enteroviruses; Coxsackie-B-viruses and echovirus-1 from DCM patients. All these previous reports and our present findings support the enteroviral involvement (especially group B and group A Coxsackieviruses) in the aetiology of DCM and HHF<sup>11-13,29-31</sup>. Wojnicz *et al.*<sup>47</sup> reported that 18(40.0%) among patients with left ventricle (LV) dysfunction demonstrated nonreplicative enterovirus. Wojnicz *et al.*<sup>47</sup> also reported that 10(56.0%) out of 18 patients with left ventricle (LV) dysfunction were found to have active viral replication. Why *et al.*<sup>32</sup> reported 34.0% prevalence of enteroviral infection among patients with idiopathic dilated cardiomyopathy (IDCM). A recent cross-sectional study has reported a much higher frequency of positive PCR for one or more viruses in patients with idiopathic LV dysfunction<sup>21</sup> or LV diastolic dysfunction in the absence of myocarditis<sup>22</sup>.

## Conclusion

The higher frequency of echoviruses and Coxsackie-B5-viruses as well as the presence of other enteroviral serotypes in this study suggest the possible association of these viruses in the etiology of HHF and DCM patients as have been previously reported by earlier workers. Attention must be directed toward currently recognized cardiotropic viruses and remain alert to the possibility that other viruses are common cardiac pathogens. Thus, this study further confirms the continued circulation of NPEV among hypertensives, HHF and DCM patients in Nigeria.

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