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Distribution of antibodies against Coxsackie B viruses, arboviruses and *Toxoplasma gondii* among patients with endomyocardial fibrosis (EMF) compared with normal subjects from EMF endemic and non-endemic zones of Nigeria

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Summary

The sera of eight endomyocardial fibrosis (EMF) subjects, 11 siblings of one of them and 16 normal children matched with the EMF patients for age, sex and socio-economic status from Ogunmakin and Shao/Oloru communities (eight each), situated in EMF-endemic and non-endemic areas of Nigeria respectively, were examined for the presence of antibodies against Coxsackie viruses B1–6, 16 arboviruses and *Toxoplasma gondii*. Sera from 36 other randomly selected normal children from Ogunmakin and 26 other randomly selected children from Shao/Oloru were also tested for the presence of antibodies against *Toxoplasma gondii* and the 16 arboviruses. None of the eight EMF subjects nor the 11 siblings of one of them had antibodies against any of the Coxsackie viruses B1–6 in their sera. Two of the 16 matched control subjects, one from each community, had positive antibodies, at equivocal titres against Coxsackie B1 (Ogunmakin) and B4 (Shao/Oloru). There was no significant difference in the distribution of antibody titres to the arboviruses between the EMF patients and matched controls. Normal children from the Shao/Oloru community had higher percentage antibody reactions and higher titres to the arboviruses compared with the children from Ogunmakin.

All the eight EMF patients had high antibody titres against *Toxoplasma gondii*. Seven (87.5%) of the matched controls from Ogunmakin were sero-positive for *Toxoplasma gondii* compared with three (37.5%) of the matched controls from Shao/Oloru. Of the 36 normal children from Ogunmakin, 32 (88.9%) were sero-positive compared with 11 (42.3%) of the 26 normal children from Shao/Oloru. Four (36.4%) of the 11 siblings of one of the EMF patients had weak sero-positivity.

It is therefore concluded that further studies are needed to clarify the role, if any, of *Toxoplasma gondii* in EMF.

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Résumé

Afin de déterminer la présence d'anticorps aux virus B1–6 Coxsackie, à 16 arbovirus et au *Toxoplasma gondii*, on a examiné le sérum de huit malades souffrant de dégénérescence fibreuse endo-myocardiaque, de 11 frères et sœurs de l'un d'entre eux et de 16 enfants en bonne santé dont l'âge, le sexe et le statut socioéconomique correspondaient à ceux des malades susmentionnés et pris dans les communes d'Ogunmakin et de Shao/Oloru (huit dans chacune), situées au Nigéria respectivement dans des zones où cette dégénérescence fibreuse endomyocardiaque existe à l'état endémique et dans des zones où elle ne l'est pas. Le sérum de 36 autres enfants d'Ogunmakin, en bonne santé et choisis au hasard, et celui de 26 autres enfants de Shao/Oloru pris au hasard, ont aussi été examinés pour y déceler la présence d'anticorps au *Toxoplasma gondii* et aux 16 arbovirus. Aucun des huit malades souffrant de dégénérescence fibreuse endomyocardiaque, ni les 11 frères et sœurs de l'un

d'entre eux, n'avaient d'anticorps contre aucun des virus B1–6 Coxsackie dans leur sérum. Deux des 16 sujets appariés pour le contrôle, un pour chaque commune, présentaient des anticorps positifs en proportions incertaines contre le Coxsackie B1 (à Ogunmakin) et B4 (à Shao/Oloru). Il n'y avait pas de différence significative dans la répartition des proportions d'anticorps aux arbovirus entre les malades atteints de dégénérescence fibreuse endomyocardiaque et les individus correspondants utilisés pour le contrôle. Les enfants en bonne santé de la commune de Shao/Oloru avaient un pourcentage plus élevé de réactions en anticorps et des proportions plus élevées aux arbovirus, comparés aux enfants d'Ogunmakin.

Les huit malades sans exception avaient des proportions plus élevées d'anticorps au *Toxoplasma gondii*. Sept des sujets appariés pour le contrôle originaires d'Ogunmakin (soit 87.5%) étaient séro-positifs pour le *Toxoplasma gondii* par comparaison avec trois sujets appariés pour le contrôle originaires de Shao/Oloru (soit 37.5%). Sur les 36 enfants en bonne santé d'Ogunmakin, 32 (soit 88.9%) étaient séropositifs, par comparaison avec 11 des 26 enfants en bonne santé de Shao/Oloru (soit 42.3%). Quatre des 11 frères et sœurs de l'un des malades atteint de dégénérescence fibreuse endomyocardiaque (soit 36.4%) présentaient une séro-positivité faible.

On en conclut donc que la dégénérescence fibreuse endo-myocardiaque pourrait être causée par une infection par le *Toxoplasma gondii*.

Introduction

Endomyocardial fibrosis (EMF) occurs sporadically in many parts of the world but is endemic in West and East Africa, Brazil and South India [1]. It is the fourth most common cause of cardiac disease in adult Nigerians [2] and it accounts for 22% of heart failure in Nigerian children [3].

Several factors have been considered in its aetiology but the most plausible at present seems to be its association with degranulated eosinophils and high circulating eosinophil granule basic proteins in the cases seen in Europe, and elevated eosinophil counts in those that occur in the tropics. Studies by Olsen and Spry [4], Spry *et al.* [5] and Nakayama *et al.* [6], for example, have shown that the sporadic cases of EMF seen in Europe occur as a result of endomyocardial damage by toxic eosinophil granule basic proteins within the heart. A significant number of degranulated eosinophils are, in such cases, often present in the tissues and the peripheral blood. On the other hand, such an association has been difficult to demonstrate in EMF patients seen in the tropics [7]. A few reports have shown some link between EMF and high eosinophil counts in Nigeria [8–11] while others have disputed it. For example, Urhogide and Falase [12] found no difference between the eosinophil counts of Nigerians with EMF and matched controls. Degranulated eosinophils were absent from the blood of the two groups of subjects they studied and from the bone marrow of those with EMF. The levels of circulating eosinophil granule basic protein (ECP/EPX) in both groups were not significantly different. Similar findings have previously been reported from India and Brazil [7]. For these reasons, attention is being focused on other aetiological factors.

EMF has a distinct climatic restriction which is most evident in areas of the world like Nigeria, Brazil and India, where the disease is endemic. In Nigeria, EMF occurs only in the southern rainforest part of the country and is rare in the northern part, which has a savannah type of vegetation. This climatic restriction suggests to us that there may be an aetiological link between EMF and infective agents which are confined to the tropical rainforest belt of the country. Filariasis has been the prime suspect [11] but no concrete proof has been provided. Moreover, filariasis is not confined to the tropical rain forest of Nigeria [13].

In our search for the aetiology of the tropical form of this disease, we recently looked for the presence of eosinophilia and degranulated eosinophils in normal children living in the rain forest (EMF endemic) and savannah (EMF non-endemic) areas of the country to determine whether differences of relevance to the aetiopathogenesis of EMF could be detected. None was found. The sera of some of these children were therefore examined and differences looked for in the distribution of antibodies against other infective agents — enteroviruses, arboviruses and *Toxoplasma gondii* — in the two communities. The results

obtained in these communities were compared with those of eight proven cases of EMF and 11 siblings of one of them.

Subjects and methods

Communities studied

The two rural communities studied were Ogunmakin village together with 11 other surrounding villages and Shao/Oloru villages. Ogunmakin and its surrounding villages are all within an area of 2 km radius and are situated in the rainforest vegetation belt of Nigeria, about 20 km south-west of Ibadan. The villages have no electricity or pipe-borne water supply and are served by one primary and one secondary school. The location, demographic and climatic features of the area are shown in Table 1 and Fig. 1 [14].

Shao and Oloru villages also have no electricity or pipe-borne water supply and are situated in the dry woodland savannah belt of the northern part of Kwara State of Nigeria. Oloru is about 20 km north of Ilorin (the capital of Kwara State of Nigeria) and serves as common market-place to the surrounding villages. It has one primary and one secondary school. Shao is about 5 km south-west of Oloru and is also served by one secondary and two primary schools. The location, demographic and climatic features of the area are also shown in Table 1 and Fig. 1.

Subjects

A total of 485 students of primary and secondary schools in Ogunmakin area were screened. Of these, 422 (216 males and 206 females) were found to have no clinically detectable illness and were used for our community survey on degranulated eosinophils. The rest were excluded because of either haemoglobinopathy (two subjects) or lack of co-operation (six). Four hundred and fifty students of primary and secondary schools in the Shao/Oloru area were also screened and 426 of them (214 males and 212 females), free from any clinically detectable disease, were used for our study. The rest were excluded on the basis of cardiovascular illness such as rheumatic valvular disease (four), haemoglobinopathy (three), or lack of co-operation (17).

Serological tests

Assays for arbovirus, and *T. gondii* antibodies were performed on sera from 36 normal subjects from Ogunmakin and 26 others from

Table 1. Demographic and climatic features of Ogunmakin and Shao/Oloru area

	Ogunmakin	Shao/Oloru
Population		
1963 national census	3379	3031
1985 projection	5675	4836
Dominant occupation	Farming	Farming
Dominant ethnic group	Yorubas	Yorubas
Social amenities		
Electricity supply	None	None
Sources of drinking	Ponds, brooks, shallow wells	Ponds, brooks, shallow wells
Number of villages	12	4
Mean monthly temperature (°C)		
Minimum	10.9 ± 2.4	20.9 ± 1.8
Maximum	32.2 ± 2.8	32.4 ± 2.3
Mean monthly rainfall (mm)	174 ± 122	100.6 ± 76
Mean monthly humidity (%)		
1100 h	82.3 ± 5.7	76.3 ± 3.6
1500 h	71.4 ± 9.2	52.8 ± 15.1

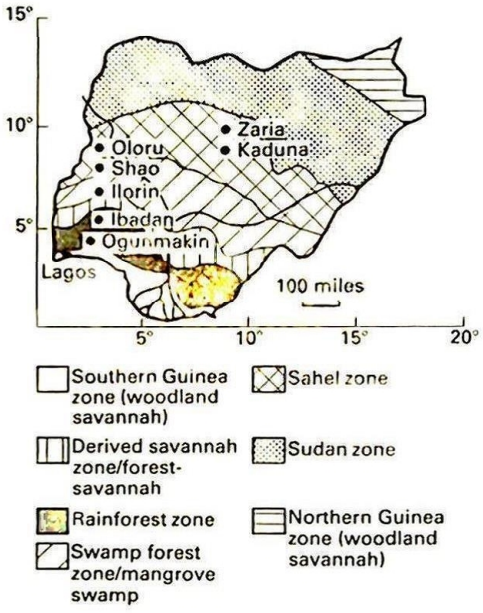

Fig. 1. Map of Nigeria showing vegetation and study sites.

Shao/Oloru randomly selected from the whole batch of 848 children. In addition, sera from eight EMF subjects confirmed on angiography, 11 siblings of one of them living with him at Ikire, a town situated 30 km south-east of Ibadan, and 16 other normal children from Ogunmakin and Shao/Oloru communities (eight from each community) matched with the EMF subjects for age, sex and socio-economic status were also tested for the presence of antibodies against arbovirus, *T. gondii* and Coxsackie B virus. Haemagglutination inhibition tests were carried out against 16 arbovirus antigens (chikungunya, sindbis, yellow fever, dengue 1, dengue 2, wesselsbron, west Nile, banzi, spondweni, tick-borne encephalitis, sandfly fever, tahyna, bunyamwera, ilesha, bwamba, germiston) using standard techniques [15,16]. Titres of 1:160 or higher were regarded as recent infections.

Serological tests for Coxsackie viruses B1–6 were performed with the IgM ELISA antibody technique [17] and titres reported as positive, negative or equivocal.

Toxoplasma antibody titres were assayed in all the sera by the cytoplasm-modifying dye test [18] and the indirect later agglutination test [19]. Titres of 31 units and above with the dye test and 1:256 or above with the latex agglutination test were taken as positive.

Statistical tests

Analysis of variance with Yate corrections was performed to compare the statistical significance of the differences in antibody titres in the various groups. The values were compared to *F* distribution tables. The tests of significance of the differences in the positivity rates of the viral and toxoplasma antibodies were carried out using chi-squared tests. The results were considered significant at the 95% confidence level. The confidence intervals of the means were expressed as mean ± standard error of the mean.

Results

The age and sex distribution of the various groups of subjects studied are shown in Table 2.

Coxsackie viruses B1–6

None of the eight EMF subjects or the 11 siblings of one of them had antibody against any of the Coxsackie viruses in their sera. Two of the 16 matched control subjects, one from each community, however, had positive antibody, at equivocal titres, against Coxsackie B1 (Ogunmakin subject) and B4 (Shao/Oloru subject).

Arboviruses

The results are shown in Tables 3–6. Antibody titres to at least one of the arboviruses were detected in 80% of all the subjects. Although none of the EMF patients or the matched control subjects had antibody to sandfly, bunyamwera, germiston, sindbis and bwamba viruses, high antibody titres to wesselsbron, tick-borne encephalitis, dengue 1 and 2, banzi, yellow fever, west Nile and spondweni viruses were detected in the EMF subjects, siblings of one of them, and the control subjects from the two communities. None of the EMF subjects or

Table 2. Characteristics of the subjects included in the study

	Total number	Male/female ratio	Mean age (yr)	Age range (yr)
EMF subjects	8	7:1	20.3 ± 5.6	15–30
Siblings of one of the EMF subjects	11	7:4	12.4 ± 6.5	3.5–26
Control subjects from Ogunmakin	8	7:1	20.3 ± 5.6	15–30
Control subjects from Shao/Oloru	8	7:1	20.3 ± 5.6	15–30
Other Ogunmakin subjects	36	7:1	20.4 ± 5.6	15–30
Other Shao/Oloru subjects	26	7:1	20.4 ± 5.5	15–30

Table 3. The percentage positive sera for arboviral antibodies in the EMF subjects and the control subjects (actual numbers are given in parentheses)

Viruses	EMF subjects (n = 8)	Ogunmakin subjects (n = 8)	Shao/Oloru subjects (n = 8)	Siblings of one EMF (n = 11)
Alphaviruses				
Chikungunya	12.5 (1)	25 (2)	12.5 (1)	9.1 (1)
Sindbis	0	0	0	0
Flaviviruses				
Yellow fever	62.5 (5)	50 (4)	62.5 (5)	72.7 (8)
Dengue 1	75 (6)	50 (4)	62.5 (5)	100 (11)
Dengue 2	75 (6)	62.5 (5)	62.5 (5)	100 (11)
West Nile	50 (4)	50 (4)	62.5 (5)	90.9 (10)
Banzi	62.5 (5)	50 (4)	50 (4)	72.7 (8)
Wesselsbron	50 (4)	62.5 (5)	62.5 (5)	72.7 (8)
Spondweni	62.5 (5)	62.5 (5)	62.5 (5)	81.8 (9)
Tick-borne encephalitis	75 (6)	50 (4)	62.5 (5)	81 8 (9)
Bunyaviruses and bunyavirus-like				
Sandfly	0	0	0	0
Bunyamwera	0	0	0	0
Bwamba	0	0	0	0
Germiston	0	0	0	0
Tahyna	0	25 (2)	0	0
Ilesha	0	12.5 (1)	12.5 (1)	0

Table 4. Logarithmic mean of haemagglutination inhibition antibody titres to arboviruses in the EMF subjects and the matched control subjects

Viruses	EMF subjects (n = 8)	Ogunmakin control subjects (n = 8)	Shao/Oloru control subjects (n = 8)	Siblings of one EMF subjects (n = 11)
Alphaviruses				
Chikungunya	2.11	2.31	1.78	1.30
Sindbis	0	0	0	0
Flaviviruses				
Yellow fever	20.65	10 05	20.04	13.76
Dengue 1	35.78	24 59	30.92	28.45
Dengue 2	25.25	23 13	23.81	36.07
West Nile	11.62	11.96	23.99	20.90
Banzi	11.94	7.10	12.65	9.42
Wesselsbron	9.76	10 32	23.81	14.64
Spondweni	15.47	30.08	28.40	19.23
Tick-borne encephalitis	19.44	6.50	14.15	9.50
Bunyaviruses and bunyavirus-like				
Sandfly	0	0	0	0
Bunyamwera	0	0	0	0
Bwamba	0	0	0	0
Germiston	0	0	0	0
Tahyna	0	1.30	0	0
Ilesha	0	1.30	0	0

the siblings had positive titres against ilesha and tahyna viruses. One each from the matched control subjects in the two communities had low positive titres against ilesha virus and only one from the eight Ogunmakin control subjects had positive antibody titres to tahyna virus. One of the EMF subjects and one each from the matched control subjects in the two communities were sero-positive against chikungunya virus.

In the general population, a significantly greater number of children from Shao/Oloru had antibody reactions compared with children from Ogunmakin villages. Children from Shao/Oloru also had higher antibody titres. Seven (19.4%) of the 36 Ogunmakin subjects were sero-negative to all the arboviruses, while only one (3.8%) of the 26 Shao/Oloru subjects was sero-negative to all the arboviruses.

Toxoplasma antibodies (Tables 7–9)

All the eight EMF patients were sero-positive for toxoplasma antibodies by both the Sabin-Feldman dye and indirect latex-agglutination tests. All the titres were high, with the dye test showing titres ranging from 125 to 1000 units and the indirect latex-agglutination test showing titres ranging from 1/1024 to 1/4000. Similarly, seven (87.5%) of the eight control subjects from Ogunmakin showed positive antibody titres. Two of these had low positive titres of 62 units on the dye test but high titres on the indirect latex-agglutination test. The remaining five had moderately high titres on both the dye and latex tests. By comparison, only three (37.5%) of the eight Shao/Oloru subjects had positive toxoplasma antibody titres. The titres in these three were strongly positive at 250 units

Table 5. The percentage positive sera for arboviral antibodies in the normal subjects from Ogunmakin, Shao and Oloru (actual numbers are given in parentheses)

Viruses	Ogunmakin subjects (n = 36)	Shao/Oloru subjects (n = 26)	P-values
Alphaviruses			
Chikungunya	33.3 (12)	26.9 (7)	> 0.05
Sindbis	0	7.7 (2)	> 0.1
Flaviviruses			
Yellow fever	27.8 (10)	69.2 (18)	< 0.05
Dengue 1	38.9 (14)	80.8 (21)	< 0.05
Dengue 2	30.6 (11)	65.4 (17)	< 0.05
West Nile	33.3 (12)	69.2 (18)	< 0.05
Banzi	19.4 (7)	76.2 (20)	< 0.05
Wesselsbron	33.3 (12)	69.2 (18)	> 0.05
Spondweni	44.4 (16)	73.1 (19)	< 0.05
Tick-borne encephalitis	33.3 (12)	80.8 (21)	< 0.05
Bunyaviruses and bunyavirus-like			
Sandfly	0	0	0
Bunyamwera	0	15.4 (4)	> 0.05
Bwamba	25 (9)	34.6 (9)	> 0.1
Germiston	2.8 (1)	15.5 (4)	> 0.1
Tahyna	30.6 (11)	38.5 (10)	> 0.5
Ilesha	5.6 (2)	11.5 (3)	> 0.5

and above, by dye test. The remaining five subjects had negative titres. The percentage positivity of the sera of the EMF subjects and those of Ogunmakin control subjects, for toxoplasma antibodies, were similar. Those of the Shao/Oloru subjects, were, however, significantly lower ($P < 0.05$). Of the 36 other normal subjects from Ogunmakin, 32 (88.9%) were sero-positive for toxoplasma. This was against 11 (42.3%) of 26 normal subjects from Shao/Oloru. The difference between these two rates was statistically significant ($P < 0.05$).

The titres in the 11 siblings of the EMF subjects were positive in four (36.4%) and negative in the rest. All the positive titres were weak, with two being 125 units on the dye test and the others 62 and 31 units, respectively. It should be noted, however, that these siblings, as a group, had a lower mean age than the EMF subjects or the control subjects from the two communities.

Discussion

The occurrence of EMF in restricted geographical zones in the tropics [1] and its occasional coexistence with dilated cardiomyopathy (DCM) [20,21] and myocarditis [22] are some of the compelling indicators for an infective aetiology for EMF. Coxsackie B virus infection has been found to be one of the aetiological factors of DCM in Nigerians [23] and, because EMF occasionally coexists with DCM, Coxsackie virus B infection may also be reasonably suspected in the pathogenesis of EMF. The present study, however, in which the EMF subjects and the normal children in the age group at risk of EMF and living in the EMF-endemic area did not have significant antibody to any of the Coxsackie B viruses, does not support this consideration. The general lack of sero-positive antibody to these viruses in all the EMF subjects and the normal subjects would

Table 6. Logarithmic mean of haemagglutination inhibition antibody titres to arboviruses in the normal subjects from Ogunmakin and Shao/Oloru

Viruses	Ogunmakin subjects (n = 36)	Shao/Oloru subjects (n = 26)	P-values
Alphaviruses			
Chikungunya	2.11	3.11	> 0.05
Sindbis	0	1.49	> 0.05
Flaviviruses			
Yellow fever	1.98	12.8	< 0.05
Dengue 1	2.29	24.08	< 0.05
Dengue 2	1.8	16.15	< 0.05
West Nile	2.38	12.23	< 0.05
Banzi	1.34	16.86	< 0.05
Wesselsbron	2.11	11.68	< 0.05
Spondweni	2.78	13.61	< 0.05
Tick-borne encephalitis	2.5	20.29	< 0.05
Bunyaviruses and bunyavirus-like			
Sandfly	0	0	> 0.05
Bunyamwera	0	1.74	> 0.05
Bwamba	3.28	4.94	> 0.05
Germiston	1.10	1.74	> 0.05
Tahyna	3.2	4.79	> 0.05
Ilesha	1.13	1.49	> 0.05

Table 7. Toxoplasma antibody titres (units) using the dye test in the EMF subjects and normal controls

EMF subjects (n = 8)	Ogunmakin children (n = 8)	Shao/Oloru children (n = 8)	Siblings of one of the EMF subjects (n = 11)
500	62	2000	7
125	7	7	7
500	62	0	7
250	500	1000	125
1000	125	250	7
500	125	7	7
200	250	7	0
500	250	7	0
			62
			31
			125

Table 8. Toxoplasma latex-agglutination antibody titres in the EMF subjects and normal controls

EMF subjects (n = 8)	Ogunmakin children (n = 8)	Shao/Oloru children (n = 8)	Siblings of one of the EMF subjects (n = 11)
1/4000	1/1024	1/4000	1/16
1/1024	1/16	1/16	1/16
1/2000	1/512	1/64	1/16
1/2000	1/2000	1/2000	1/4000
1/1024	1/1024	1/2000	1/16
1/4000	1/4000	1/16	1/16
1/1024	1/4000	1/16	1/32
1/4000	1/2000	1/16	0
			1/512
			1/512
			1/512

Table 9. Percentage positive sera for toxoplasma antibodies in the normal subjects from Ogunmakin and Shao/Oloru

	Ogunmakin (n = 26)	Shao/Oloru (n = 26)	P-values
Sero-positive sera	88.9	42.3	< 0.05
Sero-negative sera	11.1	57.7	< 0.05

suggest that infections from these enteroviruses are less prevalent in this age group compared with adults from the same population who had been shown to have significant antibody titres to the viruses [23]. No evidence, therefore, exists in this study to implicate Coxsackie B virus infections in the aetiology of EMF.

The presence of high titres of arboviral antibodies in some of the EMF subjects would seem to suggest possible acute arboviral infections in them during the period of the study. However, antibody demonstrated in this study is mainly against the flaviviruses and marked cross-reactions occur within this group. It is therefore difficult to be sure of the particular virus(es) responsible for these high titres. Previous epidemiological work in Nigeria would, however, suggest that these serological reactions might be due to yellow fever or dengue viruses [24,25]. The significance of the viral infections in the EMF patients is uncertain but may indicate possible viral basis of some of the recurrent heart failures which occur commonly in these patients [1].

The uniform occurrence of multiple arboviral antibodies in Ogunmakin and Shao/Oloru, the EMF endemic and non-endemic zones of Nigeria respectively, as shown in this study, suggests that these viral infections are common in both communities. In fact, higher prevalence of sero-positive titres were found in the Shao/Oloru group, the non-EMF endemic area, than in the Ogunmakin subjects. The difference in the prevalence rates of EMF in these different geographical areas of Nigeria cannot, therefore, be attributable to differences in the prevalence rates of these viral infections. Other factors must, therefore, be found to explain these differences.

The presence of antibodies to tahyna and tick-borne encephalitis in up to 30% of the normal children in both communities (Table 5) showed that infections by both arboviruses are common in these zones. These have not been

recognized in previous viral studies in Nigeria [25]. The pattern of distribution of antibody titres, or any of the other viruses screened for in this study, however, does not indicate that any of them may play a direct role in the aetiopathogenesis of EMF. In fact, the percentage positive sera for antibodies to those viruses were significantly higher in the Shao/Oloru subjects who are from the EMF non-endemic region. However, no firm conclusion could be drawn from this study in view of the size of the subjects. Further studies would, therefore, be required to determine whether any of those arboviruses, which have now been shown to be prevalent in the young children in both communities, has any direct aetiological relationship to EMF.

The other infective agent we considered in this study was toxoplasma. Ludlam and Somers [26], in a study of serological reactions to toxoplasma in normal Ugandan subjects and subjects with various heart diseases, had concluded against the possibility of this infection in the aetiology of EMF patients. In the present study, high antibody titres to toxoplasma were found in the Ogunmakin subjects and low titres in the Shao/Oloru subjects, from EMF-endemic and non-endemic areas, respectively. Also, all the eight EMF subjects had high toxoplasma antibody titres similar to those of the Ogunmakin subjects.

It has previously been shown that toxoplasmosis is highly endemic in the southern part of Nigeria [27,28]. The prevalence rates of seropositive antibodies also increase with age. The prevalence found in the EMF subjects and the normal Ogunmakin subjects in this study, however, far exceeded the prevalence found in similar age groups from previous studies.

On the other hand, the prevalence of seropositive toxoplasma in the northern part of Nigeria (where EMF is rare) has been shown to be lower than in the southern part where EMF is endemic [29], and our present findings of very low prevalence rates in the Shao/Oloru subjects, from the EMF non-endemic woodland savannah region, is in support of these previous reports. It is therefore possible that the differential prevalence of EMF in these various parts of Nigeria is due to the different prevalence rates of toxoplasma infection in the two zones. However, it is also possible that toxoplasmosis was simply a coexistent disease among the subjects with EMF used in this study or a fortuitous association. Further studies are needed to clarify this.

In conclusion, we have, in this study, been unable to find a role for enteroviruses and arboviruses in the aetiology of EMF in Nigeria. Since the prevalence of toxoplasma infection in the rainforest region of Nigeria where EMF is found far exceeds that of the savannah part of the country where EMF is rare, and because there is a high sero-positivity against *T. gondii* among all the EMF subjects studied, there is a need for further studies to evaluate whether toxoplasmosis has an aetiological relationship with the tropical forms of EMF or not.

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