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SEROPREVALENCE OF DENGUE VIRUS INFECTIONS IN GHANA

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ABSTRACT

Background: **Dengue virus (DENV) causes febrile illness that may be misdiagnosed with other infectious diseases. This may contribute to the possibility of missing out DENV infections. Recent reports show seroepidemiologic evidence of DENV infections in Ghana, but the frequency of infection and the geographic dissemination are unknown.**

Objectives: **To examine the seroprevalence, the geographical pattern and the age distribution of DENV infections in Ghana.**

Design: **A cross sectional epidemiological study with sera (N = 417) from all 10 regions in Ghana. The sera were obtained during the national surveillance of suspected outbreak of yellow fever (YF) in 2014. The Panbio Dengue IgG Indirect ELISA kit was used for the detection of IgG antibodies.**

Results: **The seroprevalence of dengue IgG was 29.7% among the suspected YF patients in Ghana. Seroprevalence of DENV IgG increased with age. It was 10.4% in the 0-9 year olds, 26.1% in children and adolescents from 10-19 years, 43.1% in the group of 20-29 year old young adults and 57.0% in the age group ≥ 30 years. The seroprevalence ranged from 18.2% in the Western region to 57.9% in the Upper East. The Upper East and the Volta region had a significantly higher seroprevalence than the overall seroprevalence in Ghana (p = 0.0094).**

Conclusion: **The study shows low to moderate levels of dengue virus infection in Ghana and demonstrates that infections occur in all age group and differ between regions. Awareness of DENV infection should be created in the country in cases of undifferentiated fever.**

INTRODUCTION

Dengue fever and severe dengue are outcomes of infections caused by four types of dengue virus (DENV). All four types of dengue virus are known to be transmitted by the *Aedes* mosquitoes. There has been an increase in the number of DENV infections across the world in the last few decades (1) but the role and pattern of dengue virus in Africa has been underestimated (2). The main reason for this challenge is the difficulty to distinguish between the symptoms of initial dengue infection and other infections like measles, gastroenteritis, viral hepatitis, malaria and some bacterial infections.

In Ghana and some other sub-Saharan African countries where malaria is known to be endemic, research has shown that malaria has been over-diagnosed with some proportions of clinically diagnosed malaria turning out to be dengue virus infections (3–5). In 2008, the first dengue virus infection was reported in a Finnish person with fever and history of travel to Ghana. The laboratory confirmation showed the presence of dengue IgM and DENV-2 was isolated (6). Prior to this, no local case of dengue virus transmission and infection has been demonstrated in Ghana. However, in the last decade, there have been a few studies in Ghana also indicating DENV infection in children, adults and HIV/HBV co-infected adults. In 2015, a study conducted among 218 febrile ill children clinically diagnosed with malaria from Accra (in the Greater Accra Region of Ghana), Kintampo (Brong-Ahafo Region in Ghana) and Navrongo (Upper East Region in Ghana) showed a DENV IgG seroprevalence of 21.6% collectively (7). In 2018, a study conducted among 236 HIV-infected individuals reported a dengue prevalence of 87.2% (8). Another 2018 study, among 360 yellow fever (YF) suspect individuals across all regions showed a dengue IgG seroprevalence of 3.6% (9).

From these studies, there is a discrepancy in the reported seroprevalence of dengue virus in Ghana. Therefore, the goal of this study was to further examine the seroprevalence, the geographical pattern and the age distribution of dengue virus infections in Ghana in a cross-sectional study. For the examination, a convenience sample of 417 sera obtained in 2014 from patients with clinical suspicion of YF was investigated.

METHODS

Study site: Ghana is located at latitudes 4° and 11° N of the equator and longitudes 1° E and 3° W and on the West African coast. This country shares borders with Togo to the east, Burkina Faso to the north and northwest, Côte d'Ivoire on the west and the Gulf of Guinea to the south stretching about 560 kilometres across the coastline. Since the life cycle of the mosquitoes that transmit DENVs depend on environmental factors, the ecology of the region is of importance. Based on ecological zones, the country can be divided into three main zones namely the low (sandy coastal plains, with several rivers and streams), the middle and western parts of the country (characterised by a heavy canopy of semideciduous rainforests, with many streams and rivers) and the northern savannah. Ghana has a tropical climate with temperatures and rainfall patterns that vary according to distance from the coast and elevation. The eastern coastal area is relatively dry, the south western corner is hot and humid, and the north of the country is hot and dry. There are two distinct rainy seasons in the southern and middle parts of the country - from April to June and September to November. The north, however, characterised by one rainfall season that begins in May, peaks in August, and lasts until September. The harmattan, a

dry dusty desert wind, blows from the northeast and covers much of the country between December and March, lowering the humidity and visibility, and also creates very warm days and cool nights in the north. In the south, the effects of the harmattan are felt mainly in January (10). Ghana's population was estimated at 27 million in 2014 and there are 10 regions in Ghana: Western, Central, Greater Accra, Volta, Eastern, Ashanti, Brong Ahafo, Northern, Upper East, and Upper West. The regions have been subdivided administratively into 216 districts.

Study design and samples for study: This is an epidemiological cross-sectional study and sera (N = 417) from all 10 regions in Ghana were examined. These sera were obtained during a national surveillance of suspected outbreak of YF from January to December in 2014. Blood of about 3-5 ml were drawn by a trained specialist in the disease control unit of the district hospital from patients with fever (> 38 ^{o}C) and jaundice within 14 days. Blood sample was separated into serum at the district hospital laboratory and was transported in a cold box to the National Public Health and Reference Laboratory (NPHRL), Ghana Health Service, Ministry of Health, Korle-bu, Accra. At the NPHRL, serum was divided into three aliquots (0.3-0.7 ml) and one of the aliquots was used for the YF IgM test. Sera were then stored at -80 ^{o}C at the NPHRL. Serologic analysis showed anti-YF IgM responses in two of the sera (11). Approval for the study was obtained from the Ministry of Health Ethical and Protocol Review Committee, Accra, Ghana.

Dengue virus antibody ELISA: The Panbio Dengue IgG Indirect ELISA™ kit was used for the detection of IgG antibodies against dengue virus. The kit was ready-for-use with each of all microwells coated with dengue antigen serotypes 1 to 4 by the manufacturer. All laboratory procedures for this study were carried out at Institute for Virology, University Clinics and Medical Faculty, University of Leipzig, Leipzig, Germany. The sera were thawed to room temperature and diluted to a concentration of 1:100 using the sample diluent according to manufacturer's protocol. Negative and positive control as well as the calibrator were diluted with the sample diluent provided by the manufacturer in the ratio of 1:100. Diluted sera, controls and calibrator (100 µl) were pipetted into the respective microwells. The microwells were then covered and incubated for 30 minutes at 37 ^{o}C. The microwell plate was removed from the incubator and washed six times with washing buffer (phosphate buffered saline, pH 7.2-7.6, with Tween 20 and 0.1% Proclin™). Thereafter, 100 µl of horseradish peroxidase (HRP)-conjugated goat anti-human IgG was added to each well. The microwells were then covered and incubated for 30 minutes at 37 ^{o}C. The microwell plate was removed from the incubator and washed six times with washing buffer. TMB chromogen (100 µl, a mixture of 3,3',5,5'–tetramethylbenzidine and hydrogen peroxide in a citric-acid citrate buffer, pH 3.5-5.8) was added to each well. A blue colour was allowed to develop after which 100 µl of stop solution (1 M phosphoric acid) was added to each well. The absorbance was read at a wavelength of 450 nm with reference filter of 620 nm.

Data analysis and statistical calculations: The Panbio Dengue IgG Indirect ELISA optical density results were interpreted based on the Panbio index (negative < 0.9, equivocal 0.9 – 1.1, and positive > 1.1) as described by the manufacturer's instruction. Positive result means the presence of detectable IgG antibodies which indicates evidence of past dengue infection by any of the four serotypes or a combination of serotypes. Negative result means no evidence of a past DENV infection. Equivocal results suggest sample should be re-tested with the same kit or another reliable kit. All demographic data were entered and analysed in Microsoft

Excel 2010. Age distribution in different regions was compared with the "Comparison of means" tool, the proportion of males and females was compared with the "Comparison of proportions" calculator and proportions of seroprevalence in different regions were compared using the N-1 Chi-squared test of the statistical internet program medcalc (www.medcalc.org/calc/). P < 0.05 was considered significant. The seroprevalence and 95% confidence intervals (CI) were calculated using Excel software and the medcalc tool "Diagnostic test evaluation calculator".

RESULTS

Study subjects and regional distribution of samples: A total number of 417 sera were tested. A greater proportion of the sera tested was from males (n = 241, 57.8%) than from females (n = 176, 42.2%). Also, the majority of sera were from children and adolescents below 20 years (63.3%) (Table 1). The highest proportion of sera was from the Brong Ahafo region (n = 197, 47.2% of total sera) whereas few sera were from Greater Accra region (n = 2, 0.5% of all sera) and the Northern region (n = 9, 2.2%). Between 19 and 44 sera were from the other regions. The age of the patients from which the sera were obtained ranged from 5 months to 79 years, with a median age of 14 years and an average age of 18.6 years. The mean age of the patients from individual regions did not significantly deviate from the mean age of all patients (Table 2).

Table 1

Sex and age distribution of patients

CHARACTERISTICS		
Sex	**Number of sera**	**% of total**
Male	241	57.8[1]
Female	176	42.2[1]
Total	417	100
Age (years)	**Number of sera**	**% of total**
0-9	154	36.9
10-19	111	26.6
20-29	58	13.9
≥30	93	22.3
Missing age	1	0.2

[1] *The difference in the proportion of males and females was statistically significant (p = 0.0017)*

Table 2

Distribution of patients in the different regions

Region	**Number of sera**	**% of total**	**Median age**	**Mean age ± standard deviation**	**p-value[1]**
Ashanti	44	10.6	18.0	20.8 ± 16.8	0.38
Brong Ahafo	197	47.2	14.0	17.7 ± 15.0	0.57
Central	20	4.8	14.5	16.6 ± 14.5	0.60
Eastern	38	9.1	16.5	21.3 ± 17.6	0.31
Greater Accra	2	0.5	-	-	-
Northern	9	2.2	12.0	20.7 ± 23.2	0.68
Upper East	19	4.6	11.0	21.8 ± 24.1	0.15
Upper West	22	5.3	8.5	15.8 ± 18.4	0.45

Volta	33	7.9	16.0	20.3 ± 17.0	0.55
Western	33	7.9	13.0	16.5 ± 13.1	0.48
All regions	417	100	14	18.6 ± 16.3	-

[1]*P-values for the difference of the mean age of patient in the regions compared with the overall mean age.*

Seroprevalence of DENV in different age groups and geographical locations: From the 417 sera that were tested, 124 sera were DENV IgG positive, making an average seroprevalence of 29.7% among these patients in Ghana (95% confidence interval (CI): 25.3-34.3%). The seroprevalence was 29.5% (n = 71 sera) among males and 30.1% (n = 53 sera) among females. The difference is not statistically significant (p = 0.8948). Seroprevalence of DENV IgG increased with age. It was 10.4% in the 0-9 year olds, 26.1% in children and adolescents from 10-19 years, 43.1% in the group of 20-29 year old young adults and 57.0% in the age group ≥ 30 years (Figure 1).

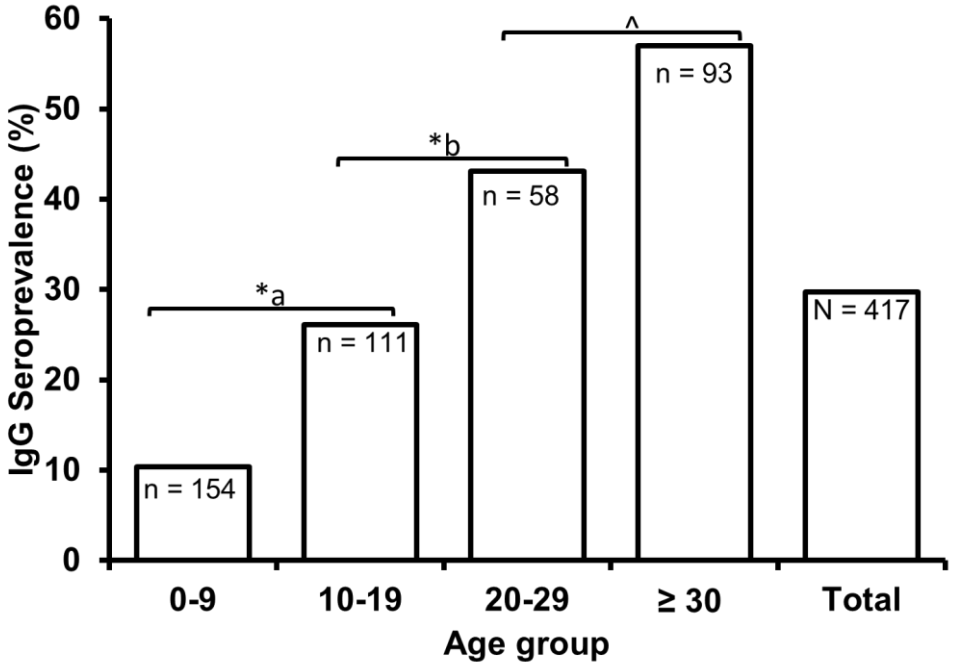

Figure 1: Age distribution of DENV IgG in Ghana. The DENV IgG seroprevalence in the different age groups was compared using the N-1 Chi-squared test (www.medcalc.org/calc/). "n" indicates the number of sera from the age group tested and "N" indicates overall number of samples tested. An asterisk (*) indicates significantly higher seroprevalence (p < 0.05). Note: *[a]p = 0.008, *[b]p = 0.0248, ^p = 0.0975.

In the different regions, the seroprevalence of DENV IgG ranged from 18.2% in the Western region to 57.9% in the Upper East region (Figure 2). The Upper East and the Volta regions had a significantly higher seroprevalence than the overall seroprevalence in Ghana (p = 0.0094). These two regions also recorded a significantly higher seroprevalence than the Central (p = 0.0164 and p = 0.0244 respectively), Western (p = 0.0036 and p = 0.0048 respectively), Ashanti (p = 0.0037 and p = 0.0047 respectively) and Brong Ahafo (p = 0.0022 and p = 0.0018 respectively) regions (Table 3).

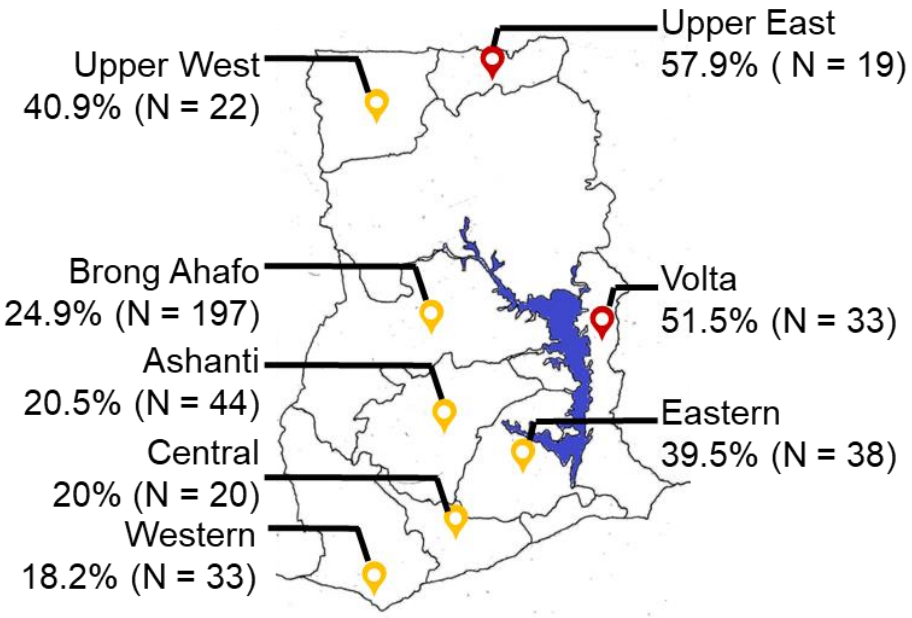

Figure 2: ***Regional DENV IgG seroprevalence in Ghana*****. Total of 417 sera were tested using Panbio Dengue IgG Indirect ELISA. The overall average DENV IgG seroprevalence was 29.7%. N indicates the total number of sera from each region. The ORANGE locations indicate regions of seroprevalence ranging from 18.2% - 40.9%. The RED locations indicate regions with seroprevalence greater than 50%.**

Table 3

Comparison of proportion of IgG seroprevalence in the different regions

Region	Average	Ashanti	Brong Ahafo	Central	Eastern	Upper East	Upper West	Volta	Western
Average		0.2005[a]	0.2174	0.3522	0.2096	0.0094*	0.2656	0.0094*	0.1609
Ashanti			0.5381	0.9636	0.0610	0.0037*	0.0818	0.0047*	0.8023
Brong Ahafo				0.6279	0.0648	0.0022*	0.1076	0.0018*	0.4049
Central					0.1360	0.0164*	0.1482	0.0244*	0.8723
Eastern						0.1925	0.9158	0.3142	0.0515
Upper East							0.2834	0.6589	0.0036*
Upper West								0.4447	0.0665
Volta									0.0048*
Western									

** Numbers in this table represent p-values obtained by comparing the proportions using N-1 Chi-squared test. Significantly higher DENV IgG seroprevalence between regions was marked with an asterisk considering p-values < 0.05 as significant.*

DISCUSSION

This study and other similar studies in Ghana have shown fingerprints of DENV infection although the country has not recorded DENV outbreaks yet (7–9,12). From this study, the overall seroprevalence of DENV IgG in patients medically managed for suspicion of yellow fever was 29.7%. The seroprevalence among males (29.5%) and females (30.1%) was similar. The DENV IgG seroprevalence increased with age from 10.4% for children below 10 years to 57% for adults above 30 years. The low seroprevalence among the under 10 year old indicates that dengue fever is not a frequent childhood disease. The increasing seroprevalence of DENV IgG in the age groups was expected because the time of exposure to mosquito bites in an individual's life increases with age.

Serum samples were from patients suspected of yellow fever. Since symptoms and signs of severe dengue and yellow fever overlap, the serum sample may be enriched for DENV infections. If this is the case, the true seroprevalence of dengue in the general population is lower than the 29.7% found.

The dengue IgG prevalence value is based on the assumption that the sensitivity and specificity of the ELISA are 100%. This is unlikely. In a recent comparison study of two dengue IgG ELISA kits with sera from Sudan, the Panbio test had a sensitivity of 91.1% and a specificity of 79.4% compared with a neutralization assay (13). Prevalence calculations with these values lead to a DENV IgG frequency of 12.9% (95% CI: 9.8%-16.5%). Based on these calculations, the dengue IgG prevalence in the serum sample is between approximately 12% and 30%.

The limited number of sera from individual regions indicates that differences between the regions should be regarded with caution. Few samples were from the Greater Accra region (n=2) as well as the Northern region (n=9). This makes it difficult to generalise the results obtained from these areas. However, some samples from these regions were DENV IgG positive. This indicates that the population in these areas has been exposed to dengue virus. Other studies have shown DENV infection in Greater Accra region, as well (7,12). The highest dengue seroprevalence was found in the Upper East in the north-east of the country and the Volta region in the east. The two regions showed a DENV IgG seroprevalence of more than 50%. Two previous studies examined Breteaux and container indices and the man-biting frequency of Aedes mosquitoes in the northern regions and on the University of Ghana campus in Accra. It was found that the indices and the man-biting frequency were ten times higher in the northern region compared with the study site in Accra (14,15). This suggests that regional variations of infestation with Aedes mosquitoes may account for differing seroprevalence in the country.

The DENV seroprevalence in the study is lower than the seroprevalence seen in many Southeast Asian countries such as Malaysia (55%), Singapore (59%), Thailand (79%) and Indonesia where more than 80% of children of 10 years and older were infected at least once. In Africa, DENV seroprevalence studies showed a frequency of 12.5% in Kenya, 67% in central and eastern Sudan and 4.1% in northern and north western provinces in Zambia. In Burkina Faso, a study conducted between 2003 and 2004 revealed a seroprevalence of approximately 30% (16). This is similar to the seroprevalence reported in this study. Hence, the DENV seroprevalence in Africa is relatively low compared to what has been shown in Southeast Asian countries.

The dengue seroprevalence data in Ghana obtained indicate that the majority of the population is susceptible to DENV infection.

Dengue fever outbreaks have not yet been observed in Ghana. However, outbreaks have recently been reported from the neighbouring countries including Burkina Faso in 2013 and 2016 (17,18) and Cote d'Ivoire in 2018 and 2019 (19,20). Two DENV nucleotide sequences found recently in patients in Ghana were closely related to a DENV-2 strain isolated in the 2016 outbreak in Burkina Faso. This suggests that the virus circulates between Ghana and Burkina Faso (18). From the perspective of public health, the DENV reports from Ghana and data from neighbouring countries mean that DENV surveillance should be increased and the serotypes of DENVs in circulation determined. From a clinical standpoint, the findings imply that there should be awareness of dengue fever in Ghana as a possible diagnosis in cases of undifferentiated fever in patients of all age groups.

CONCLUSION

In summary, the study confirms that dengue virus infections occur in Ghana and shows that infections occur in all age groups. This and previous studies about the seroprevalence in Ghana show varying results and further studies should be pursued in future for better understanding of DENV in Ghana. Considering the high Aedes larvae and pupae indices and man-biting frequency in parts of the country, there may be DENV outbreaks in Ghana in the future similar to those that took place in recent years in the neighbouring countries.

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