

## ORIGINAL ARTICLE

### Dengue virus exposure among blood donors in Ghana

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Dengue is an urban arbovirus whose aetiologic agent is the flavivirus with four distinct antigen serotypes (DENV-1, DENV-2, DENV-3 and DENV-4) that is transmitted to humans through the bite of the mosquito *Aedes aegypti*. Ghana is endemic for *Aedes aegypti* mosquitoes and probably dengue viruses. Due to limited data on dengue virus exposure among Ghanaians, we surveyed 188 healthy adult blood donors for the presence of IgG and IgM antibodies to the four serotypes of dengue. Five milliliters of peripheral blood from the blood donors were collected in plain tubes. Serum was then obtained and ELISA tests were employed to detect both dengue virus total antibodies and IgM. The samples were further tested for dengue virus RNA using RT-PCR. Dengue virus IgG was positive for 43.6% of all the 188 blood donor samples tested but all donors were negative for anti-dengue IgM antibody and dengue virus RNA. The rate of dengue virus total antibody exposure did not differ statistically between urban and rural districts. This study shows for the first time that some regions of Ghana are hyperendemic for dengue virus infection but suggests blood for transfusion is invariably dengue virus free. This report has provided a baseline data that will inform wider discussions about the impact of this dengue fever and also guide policy makers to develop effective and affordable early warning and outbreak response systems for Ghana.

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#### INTRODUCTION

Dengue fever, dengue haemorrhagic fever and dengue shock syndrome are dangerous and debilitating diseases caused by dengue virus (serotypes 1-4). The World Health Organization (WHO) estimates about 2.5 billion people live in regions potentially at risk of dengue infection. About 100 million cases of dengue fever and more than 500,000 cases of dengue hemorrhagic fever occur on yearly basis while approximately 25,000 die from dengue related clinical manifestations (WHO, 2012).

Primary dengue infection has similar clinical picture

as malaria. In sub-Saharan Africa, health resource constraints result in the majority of febrile illnesses being presumptively treated as malaria, although growing evidence suggests that in some cases, malaria may only be responsible for a minority of illnesses.

Dengue virus belongs to the family *Flaviviridae* and is transmitted by mosquitoes (*Aedes*). Flaviviruses are small, lipid-enveloped, positive-stranded RNA viruses (Gubler, 2002). Dengue virus infection in humans is often inapparent (Endy *et al.*, 2011; Simmons *et al.*, 2012) but can lead to a wide range of clinical manifestations, from mild fever to potentially fatal dengue shock syndrome (WHO, 2009). The viremic phase of dengue infection lasts 4–8 days (Gubler *et al.*, 1981) and precedes the onset of symptoms in persons with clinical disease but most

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infections are subclinical (Burke *et al.*, 1988). Plasma viral RNA levels range from  $10^6$  to  $10^9$  copies/mL, and blood collected during this phase may be infective when transfused into susceptible hosts (Sudiro *et al.*, 2001). Dengue has been reported in many African countries and the average mortality rate is about 5%. Although dengue infection or outbreak has not been confirmed in Ghana, it has been detected in Côte d'Ivoire and Burkina Faso, which share common borders with Ghana (WHO, 1995).

## MATERIALS AND METHODS

### The study sites

A cross-sectional seroprevalence study was conducted at the blood transfusion centres at three different sites (hospitals) namely Agogo Presbyterian Hospital (Agogo), Techiman Holy Family Hospital (Techiman) and the Komfo Anokye Teaching Hospital (KATH) in Kumasi between February 2013 and December 2015. The Agogo Presbyterian Hospital is a referral hospital for the Asante Akim North, Central and South Districts of Ashanti Region of Ghana whereas the Techiman Holy Family Hospital is the main referral hospital within the Techiman district of the Brong Ahafo Region of Ghana. The Komfo Anokye Teaching Hospital (KATH) is located in the city of Kumasi. KATH is Ghana's second tertiary hospital.

### Ethical approval

Prior to the study, ethics approval was sought from the KNUST School of Medical Science/KATH Committee on Human Research Publications and Ethics (CHRPE). In all, 188 prospective blood donors (age range: 17-58 years) were enrolled. The participation of the respondents was voluntary and informed consent was obtained from each of them.

### Sample collection and storage

All subjects who visited the laboratories of the above stated blood transfusion centres to donate blood were approached and examined with the following inclusion criteria for the study: age between 16 to 60 years, least weight of 50 kg, haemoglobin level of at least 12.5 g/dl, blood pressure <140/90 mmHg and pulse < 100 BPM. All individuals who

passed the above stated inclusion criteria and were considered physically fit to donate blood were enrolled into the study. All those who did not meet these criteria were excluded from the study.

Five millilitres pre-donation venous blood specimens were collected from each prospective blood donor under strict aseptic conditions for screening. After the routine screening of the blood donors' serum samples for HIV, HBsAg, VDRL, Hepatitis C virus, the remaining serum samples of all the study participants were stored at -20 °C. The frozen plasma samples were later transported to the virus research laboratory of the Department of Clinical Microbiology of the School of Medical Sciences of Kwame Nkrumah University of Science and Technology where this study was performed.

### Testing of sera for DENV-specific IgM/IgG antibodies

The samples were allowed to thaw at room temperature and then tested for dengue virus specific antibodies (IgM/IgG) using AccuDiag™ Dengue IgM/IgG ELISA kit (Diagnostic Automation/Cortez Diagnostic, Inc. Calabasas, California 91302, USA) following the manufacturer's instructions. The microwells are coated with purified dengue virus antigen from Vero cell cultured type 1-4 dengue. In the presence of human sera any antibody present will bind to the wells; results are obtained following the completion of the test procedure. Donors were considered positive against dengue virus when the ELISA result was defined as having an index value greater than 1.1. Both the sensitivity and specificity of the kit were 100% according to the manufacturer.

### Testing of sera for DENV-specific IgM antibodies

Dengue virus (DENV) Detect™ IgM capture ELISA (InBios, USA) was used in testing for the presence of IgM antibodies in human serum to Dengue derived recombinant antigens (DENRA) following the manufacturer's instruction. Prior to the test, the components of the kits were pre-warmed to room temperature. A 1: 100 dilutions of the samples and the controls (positive and negative controls) were

made using the DENV Sample Dilution Buffer. The optical densities of the wells were measured at 450 nm using iMark Microplate Reader (Bio-Rad, USA). The Immune Status Ratios (ISR) of the negative and the positive controls which were calculated as the ratio between the averaged optical density (OD) of dengue recombinant antigen (DENRA) to average OD of normal cell antigen (NCA) were used in the determining the validity of the assay. The ISR of the unknown samples were also calculated as the ratio between the averaged OD of DENRA to average OD of NCA. An unknown sample with  $ISR \leq 1.65$  was considered as negative for Dengue IgM. An unknown sample with  $1.65 < ISR < 2.84$  was considered as equivocal meaning the test must be repeated in duplicate and the average ISR evaluated. Samples that remained equivocal after repeated testing were considered as negative for Dengue IgM. Samples with  $ISR \geq 2.84$  were considered as positive for Dengue IgM antibodies.

#### **Real-time PCR testing for Dengue Viruses**

The RNA of all samples that were IgG and IgM positive were extracted using Qiagen Viral RNA Minikit (Qiagen, Germany). Real-time PCR was performed on the extracts to identify dengue virus: DENV1, DENV2, DENV3, and DENV4. The reactions were carried out in a 25  $\mu$ l reaction mixture containing 5  $\mu$ l of RNA template, 2x Reaction Mix containing

3.2mM MgSO<sub>4</sub>, 1  $\mu$ l of 1 mg/ml Bovine Serum Albumin, 50 mM MgSO<sub>4</sub>, 0.5  $\mu$ l forward primer, 1  $\mu$ l of reverse primer, 0.5  $\mu$ l of probe and 1  $\mu$ l of superscript III platinum-Taq Enzyme Mix (Invitrogen). Thermocycling parameters were as follows: reverse transcription at 45 °C for 30 min, denaturation at 95 °C for 5 min, followed by 45 cycles at 95 °C for 3 seconds and annealing at 57 °C for 35 seconds. The sequences of probes and primers are described by Drosten *et al.*, 2002 (Drosten *et al.*, 2002).

#### **Data analysis**

All data were entered using microsoft excel spreadsheet. A simple proportion of dengue positive vs negative were assessed by means of Students *t*-test.

#### **RESULTS**

Of the total 188 blood serum samples tested 82 (43.6%) were positive for dengue virus IgG whereas 106 (56.4%) tested negative. Also, all 188 blood serum samples tested negative for anti-dengue virus IgM and the dengue virus RNA.

The overall prevalence rate of dengue infection was thus 43.6%. However, the prevalence for Agogo, Techiman and Kumasi were 37.8%, 37.8% and 24.4% respectively (Table 1). The mean age of the entire study population was 28.8 years (age range:

**TABLE 1. Sociodemographic of 188 healthy adult blood donor stratified by three study sites in Ghana: Kumasi, Agogo and Techiman**

Characteristic	Total population	IgM/IgG positive	IgM/IgG Negative	P-value
	N = 188	N = 82	N = 106	
<b>Gender</b>				
Male	170 (90.4)	79 (96.3)	91 (85.8)	0.024
Female	18 (9.6)	3 (3.7)	15 (14.2)	
<b>Age group</b>				
17-19	29 (15.4)	12 (14.6)	17 (16)	
20-29	88 (46.8)	43 (52.4)	45 (42.5)	0.484
30-39	45(23.9)	19 (23.2)	26(24.5)	0.943
40-49	18(9.6)	6(7.3)	12 (11.3)	0.582
50-58	8(4.3)	2 (2.4)	6 (5.7)	0.404
<b>Study site</b>				
Agogo	64 (34.0)	31 (37.8)	33 (31.1)	
Techiman	62 (33.0)	31 (37.8)	31 (29.2)	0.861
Kumasi	62 (33.0)	20 (24.4)	42 (39.6)	0.066

17 to 58 years) whereas that of Agogo, Techiman and Kumasi were 29.9, 30.5 and 26.7 years respectively.

The predominant males (90.4%) among the study population had a higher dengue seroprevalence of 96.3%, compared with 3.7%, observed among the minority females (9.6%), (Table 1). Majority of the study donors who tested positive for dengue antibodies fell into the 20-29 age range. Donors within the oldest group ( $\geq 50$  years) not only were a minority but also had the lowest dengue seroprevalence as shown in Table 1.

## DISCUSSION

To the best of our knowledge this is the first study that provides evidence on circulation of dengue virus antibodies among blood donors in the Ashanti and Brong Ahafo regions of Ghana where outbreaks of dengue fever have never been documented. The absence of anti-dengue virus IgM in the current study suggests the blood donors were not actively infected with dengue virus. The absence of anti-dengue virus IgM antibodies could also be attributed to the stringent pre-blood donation assessments to ensure that only healthy and qualified blood donors actually donate blood in Ghanaian hospitals.

The current study found dengue virus exposure of 43.6% among blood donors in all three-study sites. Although Kumasi is a city or an urban centre there was no statistical difference in the virus exposure compared to both Agogo and Techiman, which are comparatively smaller towns. Urban dwellers are normally less exposed to mosquitoes compared to people living in smaller districts but recent reports show that a major driver contributing to the spread of dengue virus to urban centres around the world is modern transportation and travel to endemic areas (Wilder-Smith and Schwartz, 2005; Gubler, 2006; Wilder-Smith and Gubler, 2008; Gubler, 2011).

The results of the current study are in line with a similar study in Tanzania that showed a high dengue virus seroprevalence of 50.6% (Vairo *et al.*, 2014) and another study in Nigeria that reported 45% for DENV-2 infection (Fagbami *et al.*, 1977). Also, den-

gue virus seroprevalence rate in the adult population in Singapore was 45% (Wilder-Smith *et al.*, 2004) in line with findings from the current study. However, the results from the current study are in sharp contrast to a report by Pascal Richard and colleagues who studied the seroprevalence of anti-dengue virus IgG among 783 adult blood donors from the French Caribbean islands of Guadeloupe and Martinique and reported that 90.7% of the samples tested positive for dengue antibodies (Azou *et al.*, 2015).

Geographically Ghana falls within the dengue endemic countries (Bhatt *et al.*, 2013) but there are no official records on dengue virus outbreak or diagnosed infections yet. Appawu *et al.*, reported 56% of households in each study site as positive for *Aedes* larvae in a survey to estimate larval indices and man-vector contacts of potential vectors of viral haemorrhagic fevers such as dengue and yellow fever (Appawu *et al.*, 2006). The high population size of *Aedes* in some Ghanaian households could be responsible for the high seroprevalence rate of dengue reported in this study.

Age group 20 – 39 was the most exposed to dengue virus infection consistent with a report by Vairo's group that showed majority of the blood donors exposed to dengue were aged 26 – 35 (Vairo *et al.*, 2014). Although the exact age at which each blood donor was infected cannot be determined the results suggest prolonged exposure to the infection was a risk factor. The few limitations to this study were: firstly, the sample size was less representative of the general population and could have affected the results, either by underestimating or overestimating the inferred prevalence. Secondly, the vast majority of the study participants were males. As expected, males dominate the blood donor population worldwide and particularly in Africa where some socio-cultural and religious beliefs preclude females from donating blood. Thirdly, the plaque reduction neutralisation test (PRNT) should have been used as the confirmatory test for anti-DENV IgG-positive samples to determine antibody and serotype specificity (Roehrig *et al.*, 2008). PRNT was not available in Ghana at the time of

this study.

## CONCLUSION

Taken together, the current study suggests blood for transfusion in Ghana are dengue virus free although many Ghanaians are exposed to dengue virus infections. It is anticipated that these findings would provide a starting point for a wider discussion about the impact of dengue infections and will help to guide policy makers develop effective and affordable early warning and outbreak response systems for Ghana.

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## COMPETING INTERESTS

The authors declare that they have no competing interests.

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