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Serologic evidence of dengue and chikungunya among patients with acute febrile illness in Ghana, 2016 – 2018

Deborah Pratt [a], Hayashi Takaya [b], Abigail Akua Abankwa [a], Yaw Awuku-Larbi [a], Stephen Nyarko [a], Esinam E Agbosu [c], Magdalene Ofori [a], Stella Bour [a], Dennis Laryea [d], Franklin Asiedu-Bekoe [c], Toshihiko Suzuki [b], Shoji Yamaoka [b], Joseph Humphrey Kofi Bonney [a,*]

[a] Noguchi Memorial Institute for Medical Research, College of Health Sciences, University of Ghana, Legon, Ghana
[b] Tokyo Medical and Dental University, Tokyo, Japan
[c] Kirby Institute, University of South Wales, Sydney, Australia
[d] Public Health Division, Ghana Health Service, Ministry of Health, Accra, Ghana

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ABSTRACT

Objective: This study aimed to describe the exposure levels to Dengue and Chikungunya viruses among individuals presenting with febrile illnesses in Ghana between January 2016 to June 2018.
Methods: The study was conducted in health facilities in seven selected regions in Ghana; namely, Ashanti, Greater Accra, Northern, Upper West, Volta, and Western regions. Patients who met the case definition were enrolled in the study. A total of 1105 blood samples were collected from patients from 2016 to 2018 and serological analysis of Dengue and Chikungunya viruses were performed with ELISA IgM and IgG commercial kits (Abcam, Cambridge, UK).
Results: Analysed results indicated that Dengue and Chikungunya viruses showed seropositivity of 62.0 % and 40.0 % respectively. All processed samples tested negative for Dengue and Chikungunya using the Polymerase Chain Reaction (PCR) assay. Greater Accra and Ashanti regions recorded the highest positivity for Chikungunya and Dengue fever viruses respectively.
Conclusion: Though no detection of Dengue and Chikungunya using molecular tools, the seropositivity suggests the need for an established surveillance for arboviruses to monitor transmission of these pathogens for epidemic preparedness and response.

1. Background

Dengue Virus (DENV) and Chikungunya Virus (CHIKV), the causative agents of Dengue and Chikungunya fever respectively, are single-stranded, positive-sense ribonucleic acid (RNA) viruses. These viruses belong to a group of viruses referred to as arboviruses which are maintained and transmitted by arthropods. The number of DENV cases reported by the World Health Organization increased from 505,430 to 4.2 million over the last two decades [1]. CHIKV, previously confined to Africa and Asia, has since 2004 spread rapidly to over 60 countries including some in Europe and the Americas [2]. The illnesses caused by both DENV and CHIKV, especially in the initial stages of infection, are characterized by sudden onset of fever, malaise, headache, sore throat, abdominal pain, vomiting and bloody diarrhoea, and skin rash [3]. Even though most arboviral infections are mild and self-limiting, they can manifest in severe forms such as life-threatening Dengue haemorrhagic fever and Dengue shock syndrome, and chronic arthropathy in the case of Chikungunya [4]. Clinical evaluation alone is unreliable for a diagnosis because the symptoms overlap with other arboviral infections such as Zika virus (ZIKV) and with nonviral febrile illness. Appropriate laboratory tests are used based on the clinical information, with molecular techniques like Polymerase Chain Reaction (PCR) remaining the most specific diagnostic approach [5]. In Ghana, patients presenting with febrile illness are usually screened for malaria, typhoid, and yellow fever, without investigating for other possible causative viruses. This, in part, could be attributed to the lack of diagnostic capacity, at the treating facilities. Although outbreaks of dengue have not been reported in Ghana, some studies have demonstrated serological evidence of markers to Dengue virus (DENV) infection [6]. Globally, an annual estimate of 50 to 100 million cases of dengue fever in endemic areas has

* Corresponding author at: Noguchi Memorial Institute for Medical Research, College of Health Sciences, University of Ghana, Legon, Ghana.
E-mail address: KBonney@noguchi.ug.edu.gh (J.H.K. Bonney).

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dominated and has accounted for about 250,000 to 500,000 cases of dengue haemorrhagic fever which has led to 20,000 deaths and 264 disabilities [7].

A recent study among blood donors found 43.6 % exposure to dengue virus [8] while another undertaken in suspected yellow fever cases found a seroprevalence of 29.7 % [9]. A hospital-based cross-sectional study detected antibodies to CHIKV in 27.7 % of participants [10].

These reports, coupled with the lack of extensive diagnostic capacity in most health facilities, indicate the need for a nation-wide surveillance to identify the overall level of prevalence of DENV and CHIKV infection. Thus, we sought to establish a surveillance and differential diagnostic system for Dengue and Chikungunya viruses and other endemic arboviruses and to identify and characterize these viruses that may be in circulation in selected health facilities in Ghana. This study aimed to describe the exposure levels to Dengue and Chikungunya viruses among individuals presenting with febrile illnesses.

2. Methods

2.1. Case definitions and sample collection

A retrospective cross-sectional study was conducted for Dengue and Chikungunya at 6 hospitals located across the country from 2016 to 2018 (Fig. 1). These health facilities included 1 regional, 1 municipal, 3 district and 1 health centre. The facilities were selected based on previously reported suspected cases and confirmed multiple large recent and historical outbreaks of yellow fever (an arboviral infection) [11,12] cases within the catchment areas of the facilities.

Suspected Dengue cases were defined as having an acute febrile illness with two or more of the following manifestations: headache, retro-orbital pain, myalgia, arthralgia, rash, haemorrhage, chest pains; or occurrence of an illness at the same location and time as other confirmed cases of Dengue fever [4,13].

Suspected Chikungunya cases were defined as having acute onset of fever, >38.5 °C, and severe arthralgia or arthritis that is not explained by other medical conditions [4,14].

Patients attending the outpatient department at the selected health facilities meeting the case definitions for dengue and chikungunya and have ruled out Malaria were enrolled into the study. Five millilitres of whole blood were collected from enrolled participants and centrifuged to obtain serum/plasma for serological assays. We administered structured questionnaires to collect demographic and clinical data from consented participants.

The serum/plasma which are temporarily kept after collection from participants, in liquid nitrogen carriers provided at each study site was transported from the health facility under same ultra-low temperatures conditions each month to the laboratory at Noguchi Memorial Institute for Medical Research where the serological assays were conducted. At the testing laboratory long-term storage of the serum/plasma was in ultra-low freezers at −80 °C or below.

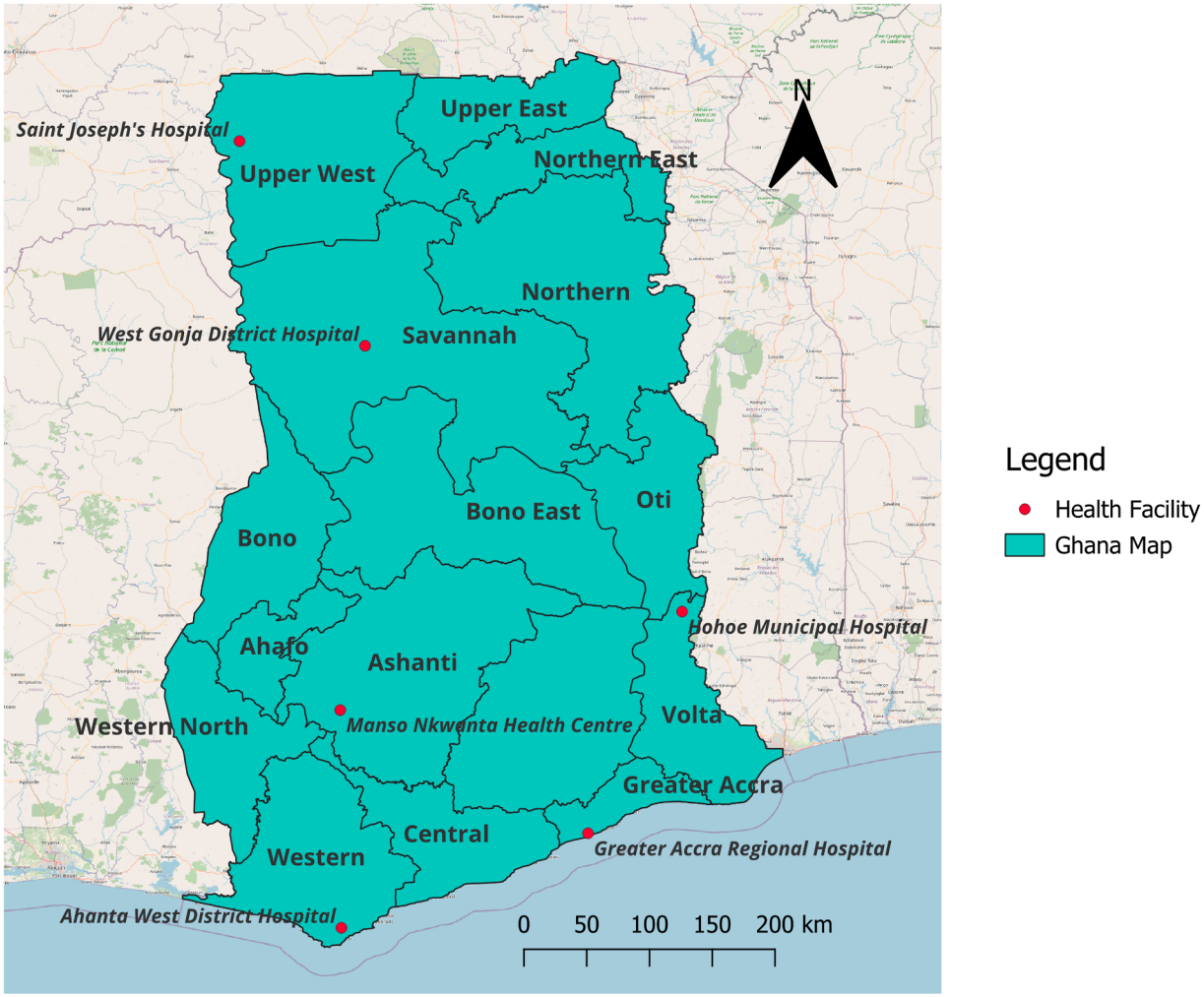

Fig. 1. Showing the distribution of selected healthcare facilities across the country where blood samples were collected from patients meeting the case definition.

2.2. Laboratory testing

2.2.1. Serology

Serum samples were tested for specific Dengue and Chikungunya antibodies (IgG-IgM combined antibody) using anti-dengue and anti-chikungunya IgM-IgG combined ELISA (Enzyme Linked Immunosorbent Assay) commercial kits (Abcam, Cambridge, UK). The testing procedures were done according to the manufacturer's recommendations.

2.2.2. Molecular testing

Reverse transcription-polymerase chain reaction (RT-PCR) was performed for the detection of Dengue and Chikungunya. Following the manufacturer's protocol, nucleic acid was first extracted and purified from serum/plasma with the QIAamp viral ribonucleic acid (RNA) mini kit (QIAGEN, Hilden Germany).

The nucleic acid was subjected to the US Centres for Disease Control and Prevention (CDC) Trioplex real-time RT-PCR (https://www.cdc.gov/zika/pdfs/trioplex-real-time-rt-pcr-assay-instructions-for-use.pdf) assay for the diagnosis of Dengue, Chikungunya and Zika viruses using specific primers and probes designed by the US CDC.

2.3. Ethical considerations

For the participation of the arbovirus surveillance, a signed informed consent was sought from patients before patient details and samples were collected. The study received approval from the Noguchi Memorial Institute for Medical Research's Institutional Review Board (NMIMR-IRB) under protocol number NMIMR-IRB CPN /20–21 revd.2023.

Table 1
Demographic characteristics of individuals enrolled into Dengue, chikungunya, and coinfection seropositivity by age group and sex.

Characteristics	Overall (N)	Dengue			Chikungunya			Coinfections		
		n	% (n/N)	p-value	n	% (n/N)	p-value	n	% (n/N)	p-value
Total	**1105**	**681**	**0.62**		**438**	**0.4**		**291**	**0.26**	
Age group, in years										
≤5	24	12	0.5	<0.001	3	0.13	<0.001	2	0.08	<0.001
5–17	125	54	0.43		17	0.14		10	0.08	
18–39	569	341	0.6		247	0.43		158	0.28	
40–60	251	174	0.69		122	0.49		87	0.35	
≥60	67	50	0.75		34	0.51		23	0.34	
No response	69	50	0.72		15	0.22		11	0.16	
Sex										
Male	387	256	0.66	0.022	158	0.41	0.65	111	0.29	0.222
Female	693	409	0.59		272	0.39		175	0.25	
No response	25	16	0.64		8	0.32		5	0.2	
SYMPTOMS										
Jaundice										
Absent	793	484	61	0.095	308	38.8	0.165	202	25.5	0.118
Present	128	88	68.8		58	45.3		41	32	
Diarrhoea										
Absent	568	326	57.4	0.01	237	41.7	0.399	148	26.1	0.611
Present	455	297	65.3		178	39.12		125	27.5	
Extreme weakness after rehydration										
Absent	858	512	59.7	0.086	351	40.9	0.22	226	26.3	0.83
Present	149	100	67.1		53	35.6		38	25.5	
Nausea										
Absent	382	233	61	0.999	132	34.6	0.003	89	23.3	0.054
Present	646	394	61		285	44.1		186	28.8	
Vomiting										
Absent	534	317	59.4	0.255	226	42.3	0.223	142	26.6	0.92
Present	495	311	62.8		191	38.6		133	26.9	
Loss of appetite										
Absent	104	65	62.5	0.752	37	35.6	0.273	25	24	0.503
Present	926	564	60.9		381	41.1		251	27.1	
Muscle pain										
Absent	171	102	59.7	0.643	70	40.9	0.95	50	29.2	0.473
Present	858	528	61.5		349	40.7		228	26.6	
Joint pain										
Absent	166	103	62.1	0.777	67	40.4	0.95	50	30.1	0.306
Present	864	526	60.9		351	40.6		227	26.3	
Conjunctivitis										
Absent	901	540	59.9	0.048	364	40.4	0.274	235	26.1	0.9
Present	71	51	71.8		24	33.8		19	26.8	
Chest pain										
Absent	636	364	57.2	0.001	252	39.6	0.45	161	25.3	0.182
Present	388	262	67.5		163	42		113	29.1	
Rapid respiration										
Absent	720	408	56.7	<0.001	308	42.8	0.031	190	26.4	0.623
Present	283	205	72.4		100	35.3		79	27.9	
Recent loss of hearing										
Absent	893	552	61.81	0.955	352	39.4	0.507	237	26.5	0.206
Present	16	10	62.5		5	31.3		2	12.5	
Bleeding										
Absent	924	567	61.4	0.244	361	39.1	0.002	239	25.9	0.04
Present	89	49	55.1		50	56.2		32	36	

2.4. Data analysis

The study used surveillance data collected between 2016 and 2018 from six (6) health facilities across selected regions in Ghana. The number of samples tested and the percentage of samples that tested positive for dengue and chikungunya for each year were mapped out by months to describe the monthly distribution of Dengue and Chikungunya. Categorical variables were summarized as frequencies and percentages. Binary logistic regression was used to estimate univariate odds ratios (OR) with a 95 % confidence interval (CI) to examine the demographic and symptoms associated with seropositivity of Dengue, Chikungunya and coinfection. P-values of <0.05 were considered statistically significant. All analyses were carried out in STATA v16 and Microsoft Excel.

3. Results

3.1. Demographic characteristics

A total of 1105 suspected cases for either Dengue or Chikungunya were recruited for the study between 2016 and 2018. Of these 62 % (681/1105) were seropositive for dengue, 40 % (438/1105) seropositive for Chikungunya and 26 % (291/1105) had seropositivity for both Dengue and Chikungunya (Table 1). Majority of the individuals were within the 18–39 years age group (Table 1). Dengue, chikungunya as well as seropositivity for both infections varied significantly by age group. Dengue (75 %, 50/67) and Chikungunya (51 %, 34/67) seropositivity rate was highest among individuals aged 60 years and above (Table 1). However, seropositivity rate for both Dengue and Chikungunya coinfections were most recorded among individuals (34 %, 87/251) aged 40 to 60 years.

Although dengue seropositivity significantly varied by sex (p-value = 0.022), there was no association between seropositivity of chikungunya (p-value = 0.650) as well as coinfections (p-value = 0.222) and sex. More females were enrolled than males (Table 1). Dengue seropositivity was more commonly detected among males than in females (Table 1).

All the PCR results were negative for Dengue and Chikungunya.

Fig. 2 shows the distribution of participants seropositive for Dengue and Chikungunya across the regions where the study was conducted. Participants from Greater Accra presented with the highest Dengue seropositivity whiles the highest Chikungunya seropositivity was recorded amongst individuals from the Ashanti region. Dengue seropositivity was not recorded among participants from the upper west region.

3.1.1. Trend in positivity for dengue and chikungunya over the 3-year surveillance period

Dengue and Chikungunya positivity were observed throughout the surveillance period. Positivity for both Chikungunya and Dengue did not follow a clear pattern over the 3-year period. Over the period, Dengue

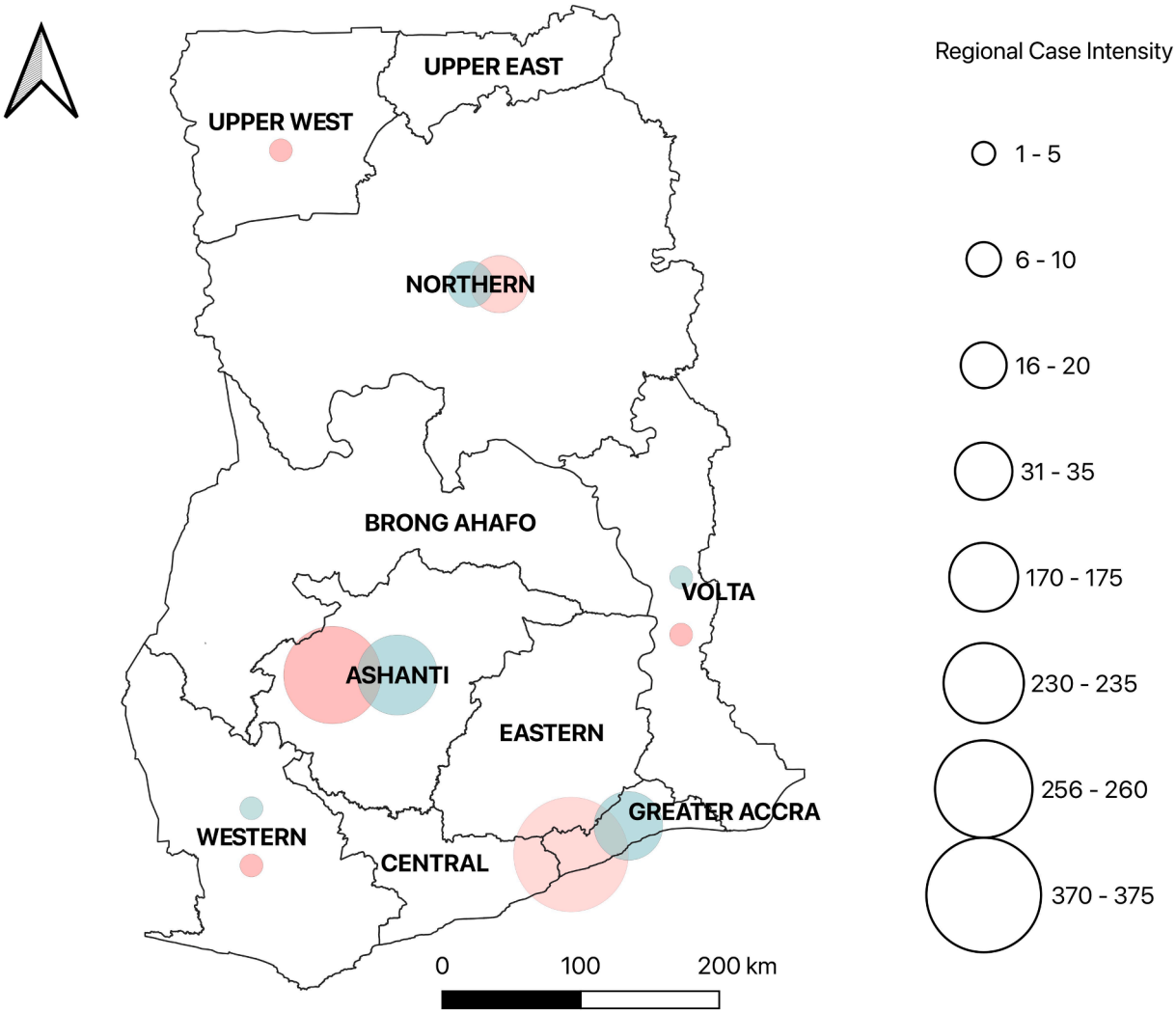

Fig. 2. Illustrates the regional intensity of Chikungunya and Dengue seropositive.

seropositivity was more commonly detected than chikungunya seropositivity. Dengue seropositivity was highest in May 2017 whiles chikungunya seropositivity was highest in June 2016. Fig. 3 shows the trend in percentage positivity for chikungunya and dengue over the 3-year period.

Fever was more commonly detected among patients who were seropositive for Dengue, Chikungunya, and coinfection. Recent hearing loss was least commonly detected among patients who were seropositive for Dengue, Chikungunya and coinfection.

3.1.2. Association of dengue, chikungunya and coinfection seropositivity by covariates

Table 2 shows the factors associated with the seropositivity of Dengue, Chikungunya, and Chikungunya-Dengue coinfections. Age was associated with Dengue, Chikungunya, and coinfections. Sex was only associated with dengue (Table 2. Individuals aged ≤60 years were more likely to be associated with dengue (OR = 2.0, 95 % CI 1.11–3.5) as compared with individuals aged 18–39 years. Females were less likely to associated with dengue seropositivity as compared with males (OR = 0.74, 95 % CI 0.57–0.96).

By symptoms, diarrhoea, chest pain, conjunctivitis and rapid respiration were found to be significantly associated with dengue seropositivity (Table 2). Nausea, rapid respiration and bleeding were found to be significantly associated with Chikungunya seropositivity whiles bleeding was significantly associated with seropositivity of both Chikungunya and Dengue.

4. Discussion

The study was conducted in selected health facilities across the country to establish an algorithm for molecular and serological testing of suspected cases of Dengue and Chikungunya and further characterize circulating viral strains. Out of 1105 patients, 681 were sero-positive for anti-Dengue fever virus and 424 for anti-Chikungunya fever virus total antibodies, giving a positivity rate of 62 % and 40 % for Dengue and Chikungunya respectively. Nucleic acid for both viruses were not detected by any of the molecular amplification methods used.

The early and proper diagnosis of Dengue fever virus and Chikungunya virus infections is a hurdle yet to be overcome. P~~re~~sentation of signs and symptoms associated with Dengue and Chikungunya are like other commonly known diseases such as malaria [15]. Thus, m~~o~~st often than not, patients who show these similar symptoms are misdiagnosed. These occurrences or situations may likely affect the true state and prevalence of Dengue and Chikungunya diseases in Ghana. So, there was the need to conduct this study from the years 2016 to 2018 in selected regions of Ghana to better understand the prevalence of these diseases and possible ways of prevention in case of any outbreak.

This study was conducted in 6 of 10 regions across the country for three years. Each of these regions has its own distinct characteristics and may differ in altitude and the two season patterns in the country [16]. Again, Human and the environmental characteristics may drive the dynamics of vector-borne diseases [16]. Thus, transmission of theses fevers may lead to the prevalence of dengue and chikungunya or both at the same time [17]. Climatic factors affect vector distribution that may affect transmission of Dengue and Chikungunya, and this poses a threat to public health [16]. Out of the 6 regions, the prevalence of Chikungunya and Dengue seropositive was high in Greater Accra and Ashanti regions respectively. This could be attributed to the overpopulation in these areas because of rural urban migration, that might have led to high increased in the storage of water in barrels due to the fear of water shortage. Aedes mosquito which is the host of these viruses prefers to breed in clean stagnant waters such as those stored in these barrels [18]. Also, from a study conducted in 2021 showed that the spatial distribution of aedes breeding sites is in close proximity to humans [19]. This therefore leads to a direct proportionality rate thus, where the human population is high, it is highly possible that the populates may have been exposed to these fevers [20]. Western, Volta and Upper West regions, known for their moderate levels of rainfall, recorded low Chikungunya prevalence rates as shown in Fig. 1. Volta and the Western North~~,~~, however, recorded the lowest prevalence for dengue fever virus. No or Dengue seropositive were recorded in the Upper West. Out of 1105 participants, 681 tested positive for dengue fever and 424

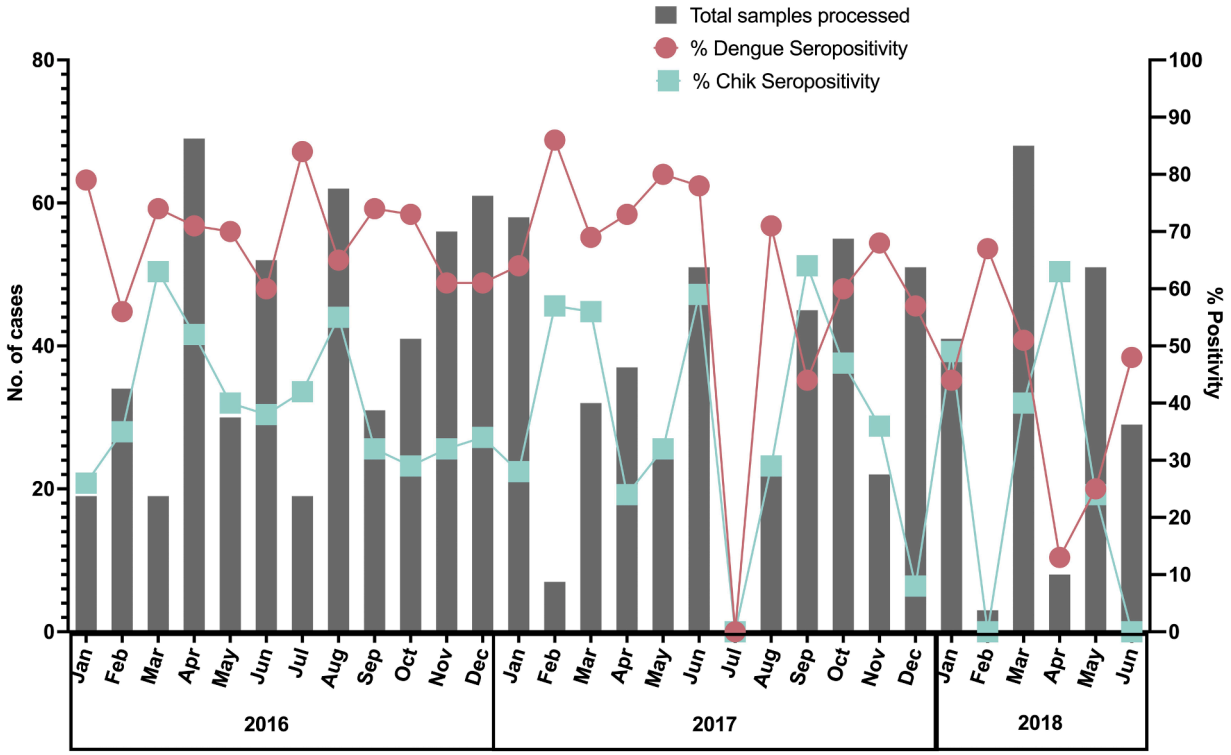

Fig. 3. Shows the trend of chikungunya and dengue % positivity for 30 months.

Table 2
Dengue, Chikungunya, and coinfections by covariates.

Characteristics	Dengue		Chikungunya		Coinfections	
	OR (95 % CI)	p-value	OR (95 % CI)	p-value	OR (95 % CI)	p-value
Demographics						
Age group, in years						
18–39	Reference	<0.001	Reference	<0.001	Reference	<0.001
≤5	0.67 (0.3–1.51)		0.19 (0.05–0.63)		0.24 (0.05–1.02)	
6–17	0.51 (0.34–0.75)		0.21 (0.12–0.35)		0.23 (0.12–0.44)	
40–60	1.51 (1.1–2.07)		1.23 (0.92–1.66)		1.38 (1.003–1.90)	
≥60	2.0 (1.11–3.5)		1.34 (0.81–2.23)		1.36 (0.80–2.33)	
Sex						
Male	Reference	0.021	Reference	0.612	Reference	0.221
Female	0.74 (0.57–0.96)		0.94 (0.73–1.21)		0.8 (0.64–1.11)	
Symptoms*						
Fever	0.69 (0.36–1.31)	0.261	0.61 (0.34–1.1)	0.098	0.68 (0.37–1.27)	0.227
Jaundice	1.4 (0.94–2.1)	0.096	1.3 (0.9–1.9)	0.166	1.38 (0.92–2.07)	0.119
Diarrhoea	1.4 (1.08–1.8)	0.01	0.9 (0.7–1.15)	0.399	1.07 (0.81–1.42)	0.611
Extreme weakness after dehydration	1.38 (0.95–1.99)	0.087	0.8 (0.56–1.15)	0.22	0.96 (0.64–1.43)	0.83
Nausea	1.0 (0.77–1.3)	0.999	1.5 (1.15–1.94)	0.003	1.33 (0.99–1.78)	0.055
Vomiting	1.16 (0.9–1.49)	0.255	0.86 (0.67–1.1)	0.223	1.01 (0.77–1.34)	0.92
Loss of appetite	0.93 (0.62–1.42)	0.752	1.27 (0.83–1.93)	0.274	1.18 (0.73–1.88)	0.503
Muscle pain	1.08 (0.77–1.51)	0.643	0.99 (0.71–1.38)	0.95	0.88 (0.61–1.26)	0.474
Joint pain	0.95 (0.68–1.34)	0.777	1.01 (0.72–1.42)	0.95	0.83 (0.57–1.19)	0.306
Conjunctivitis	1.7 (1.0–2.91)	0.05	0.75 (0.45–1.25)	0.276	1.04 (0.6–1.79)	0.9
Chest pain	1.55 (1.19–2.02)	0.001	1.1 (0.85–1.43)	0.45	1.21 (0.91–1.61)	0.182
Rapid respiration	2.01 (1.49–2.71)	<0.001	0.73 (0.55–0.97)	0.031	1.08 (0.79–1.47)	0.623
Recent loss of hearing	1.03 (0.37–2.86)	0.955	0.7 (0.24–2.03)	0.509	0.4 (0.09–1.75)	0.222
Bleeding**	0.77 (0.5–1.2)	0.245	2.0 (1.29–3.1)	0.002	1.61 (1.02–2.54)	0.041

*The reference for each symptom is compared to the absence of the symptom, **The type of bleeding is defined to be frank and not massive, mostly gum or nostril.

participants tested positive for Chikungunya fever from 1053 participants tested: giving a seropositivity rate of 62 % for Dengue fever and 40 % for Chikungunya fever exposure. This is consistent to the study done in 2022 stating these arboviral diseases are increasing in west Africa which includes Ghana, and it may likely lead to great epidemics soon [16].

The highest proportion of dengue seropositivity was recorded among individuals aged 18–39 years, thus 54 % with a mean age of 33. This is contrary to the study conducted by Lim et al. showing results for dengue seropositive recording high between the ages of 0 to 9 since they showed clear limitation of adults being underrepresented in their studies [21]. It is consistent with the study conducted by Abe et al. stating that Dengue seropositivity were highly recorded in older with a mean age of 29 years, which is sparingly lower than the mean age obtained for this study, thus 33 years [22]. And the same for a study conducted by Paulson et al. confirming the positivity rate for both Dengue and Chikungunya seropositive higher amongst the age groups of 18–45 years [23]. This was like Chikungunya and coinfection. The lowest proportions for Dengue, Chikungunya, and coinfections were recorded among individuals aged 5 years and younger, constituting 14 %.

Females were more sero-prevalent to Dengue and Chikungunya than males since the ratio of females recruited for the study was more than that of females. Previous studies have reported females are more likely to stay at home longer and thus are at risk of exposure to Chikungunya or Dengue infections than Males [24]. As the mosquitoes that transmit these viruses tend not to travel farther away from households. The seropositivity rate for Dengue and Chikungunya for females is 64.17 % and that for males is 35.83 % [24]

Recent research has shown that patients with coinfection from these two viruses have a clinically severe disease with a high mortality rate when compared to Dengue and Chikungunya virus single infection [25,26]. Studies done showed most of the symptoms we obtained from our study. Most especially that of bleeding, Joint pains and headache [25]. Most of the symptoms in other studies in relation to the coinfections is bleeding. Looking at the symptoms shown by coinfected patients, it confirms the fact that these patients present clinically severe disease. Most of the recent studies showed a low percentage of coinfection, thus between 1 and 23 %, confirming the lower percentage of coinfections obtained from this study [25]. Symptoms showed by the Dengue and Chikungunya fever patients from our study were consistent with studies done by Adam et al. except records on conjunctivitis [27].

Dengue and Chikungunya viruses are less well known by inhabitants of this country [28]. Lack of testing capacity of Dengue and Chikungunya for plasmodium infections will continually persist due to the same signs and symptoms like fever, loss of appetite, headache etc. [28]. This study showed a high seroprevalence for both infections across all age groups except that of children under five, indicates the need to strengthen surveillance system across the country to determine the true burden of the infection and the association with other diseases. Moreover, infections may be at suboptimal level now and there is a likelihood that it may results in endemics and or pandemics.

The limitation of this study is that different serotypes of Dengue and Chikungunya were not tested which we aim to include in future studies.

The ideal way of controlling the high mortality and morbidity rates of Dengue and Chikungunya infections is through continuous surveillance, public education and prevention and control strategies. For effective prevention and control strategies of Dengue and Chikungunya, it is vital to understand the various social, economic, and demographic risk factors that increase the odds of these infections in the population and the adoption of measures addressed to epidemic impact alleviation in most geographical areas.

Ethical approval

The study received approval from the Noguchi Memorial Institute for Medical Research's Institutional Review Board (NMIMR-IRB) under protocol number NMIMR-IRB CPN /20–21 revd.2023. Informed consent was obtained from all participants.

CRediT authorship contribution statement

Deborah Pratt: Writing – review & editing, Writing – original draft,

Investigation, Data curation. **Hayashi Takaya:** Writing – review & editing, Supervision, Investigation. **Abigail Akua Abankwa:** Writing – review & editing, Writing – original draft, Investigation, Data curation. **Yaw Awuku-Larbi:** Writing – review & editing, Validation, Data curation. **Stephen Nyarko:** Writing – review & editing, Writing – original draft, Validation, Data curation. **Esinam E Agbosu:** Writing – review & editing, Investigation. **Magdalene Ofori:** Writing – review & editing, Validation, Investigation, Data curation. **Stella Bour:** Writing – review & editing, Investigation, Data curation. **Dennis Laryea:** Writing – review & editing, Validation, Supervision. **Franklin Asiedu-Bekoe:** Writing – review & editing, Validation, Supervision. **Toshihiko Suzuki:** Validation, Supervision, Funding acquisition. **Shoji Yamaoka:** Supervision, Funding acquisition, Conceptualization. **Joseph Humphrey Kofi Bonney:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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