Original Article

Serological Evidence of *Zika virus* Circulation with Dengue and Chikungunya Infections in Sri Lanka from 2017

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Abstract

Introduction: Arbovirus diseases remain a public health threat in Sri Lanka. Dengue is endemic and two outbreaks of chikungunya infections have been reported. There is limited data on Zika virus (ZIKV) infections in Sri Lanka, and this could be due to a lack of comprehensive ZIKV surveillance. Our aim was to determine the presence of antibodies to dengue, chikungunya, and Zika infections in adults from a suburban population in Sri Lanka. **Methods:** A total of 149 healthy adult volunteers over 18 years of age (mean age: 43±14 years, males – 43%), with no prior diagnosed arboviral infections and no history of overseas travel, participated in the study. ELISA and neutralization assays were carried out to detect past dengue, chikungunya, or Zika infections. **Results:** A total of 94.6% (141/149) of the participants demonstrated dengue IgG antibodies, 37.5% (56/149) were positive for chikungunya IgG, and 5.3% (8/149) were positive for anti-ZIKV IgG antibodies. Neutralization assays confirmed ZIKV-specific antibodies in 6.7% (10/149), when 40/149 of the participating population were tested. **Conclusion:** This clearly demonstrated past ZIKV infections in this population. In addition, this study indicates that >90% of individuals had asymptomatic dengue but no serious symptoms. These results provide a cross-sectional view on the DENV, ZIKV, and CHIKV epidemic status and demonstrate a need for the implementation of enhanced surveillance and more effective measures against the spread of these arbovirus diseases.

Keywords: Chikungunya, dengue, enzyme-linked immunosorbent assay, IgG, neutralization assays, Zika

Introduction

The emergence and re-emergence of viral diseases that are transmitted by vectors have raised global concerns about the reasons for emergence, threats to health, burden to the economy, and the possibility of prevention and control. Globally, arboviral infections constitute a significant public health burden. Three of the most rapidly spreading arboviruses in the world are dengue, Zika, and chikungunya.[1,2] In recent decades, Sri Lanka has emerged as a dengue hyperendemic country within the region, with co-circulation of the four dengue virus serotypes.[3,4] The cyclical dominance of subtypes contributes to a pattern of major outbreaks.[5,6] The consequences can be observed in the rising incidence of dengue cases and dengue-related deaths over the last decade. The largest reported epidemic of dengue was seen in 2017, with a total number of 186,101 cases being notified with 440 deaths, which coincided with the reemergence of DENV-2.[4,7]

Although fewer cases were reported in 2018 (51,659 cases) compared to 2017, there was again another surge in 2019, with 105,049 cases reported.[7,8] Dengue, chikungunya, and *Zika virus* (ZIKV) have similar epidemiology, transmission cycles in urban environments, and clinical symptoms at onset although their subsequent sequelae varied markedly. These viruses have attracted much interest in recent years due to their increasing incidences, expanding geographical ranges, possible effects caused by co-circulation, unpredictable health threats, and burden to the economy.[9]

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The ZIKV cases have been sporadically reported in many countries in the world.[10,11] The World Health Organization (WHO) reported that 87 countries of the world had evidence of ZIKV infection until July 2019.[10,12] While Southeast Asia has been known as a ZIKV endemic region for more than 60 years, large ZIKV epidemics have only been reported recently. Although the virus was first isolated in Asia from mosquitoes in Malaysia in 1966, the first human cases were only reported in 1977 in Central Java, Indonesia.[13] According to the WHO, till December 2018, around 159 cases of ZIKV infections have been identified in India, the neighboring country of Sri Lanka.[14] All these cases were detected based on reverse transcription–polymerase chain reaction, of which 63 were found in pregnant women. The Zika infection was reported in four regions of India, i.e. Tamil Nadu, Rajasthan, Gujarat, and Shastri Nagar area of Jaipur.[15] A recent study was conducted on 462 samples collected from patients showing dengue symptoms and asymptomatic patients from 2004 to 2017 in Myanmar; however, ZIKV infection was confirmed in 4.9% of patients showing the dengue symptoms and 8.6% in asymptomatic persons at Mandalay and Yangon, respectively.[16] The most recent study showed long-term and widespread ZIKV infection since 2006 in Thailand.[17] A total of 3089 samples were analyzed from 1717 symptomatic patients from January 2016 to December 2017 among 76 Thai provinces,[17] and 368 cases were confirmed as ZIKV infection in 29 provinces in Thailand.[17] In 2016, a total of 455 cases were confirmed in Singapore[18] and, in Thailand, 386 cases were reported in 29 out of 76 provinces from 2015 to 2017.[17] During this period, cases of ZIKV infection were also reported in other Southeast Asia nations including Malaysia[19] and Myanmar.[16,20] In 2016, 3 tourists were confirmed to have ZIKV infection after visiting Vietnam.[21] As of June 2019, a total of 265 cases have been reported in Vietnam, most of which occurred in Ho Chi Minh City.[22,23] In addition, in 2016, a case of Zika-associated microcephaly was reported in the Central Highlands of Vietnam and 5 family members and 2 neighbors were confirmed positive for ZIKV infection, further investigations showed 1.1% of the community had ZIKV-specific antibodies.[23] Despite the endemicity for dengue and the high density of mosquito vectors, the number of cases of ZIKV infection in Vietnam remains substantially lower than the number of cases of dengue. Vietnam lies within the tropical zone where *Aedes aegypti* mosquitoes are endemic. In Vietnam, the number of ZIKV infections peaked at 219 in 2016, and has subsequently decreased, with only one reported case in 2019.[23] While neighboring countries have reported ZIKV outbreaks in recent years, there are limited data available on the extent of ZIKV infection in local populations in Sri Lanka. In addition, it has been hypothesized that dengue hyperendemicity may lead to cross-reactive immunity toward ZIKV, thus limiting the size of ZIKV epidemics in South Asia.[24] However, there were limited seroprevalence data to support this hypothesis. Cross-reactivity between ZIKV and DENV antibodies has led to difficulties in the interpretation in some studies.[24] Annual reported dengue cases vary considerably in Sri Lanka. The largest reported epidemic of dengue was seen in 2017, with a total number of 186,101 notified cases with 440 deaths. Although fewer cases were reported in 2018 (51,659 cases) compared to 2017, there was again another surge in 2019, with 105,049 cases reported. In 2021, very few cases (35,054) were reported.[7] Dengue seroprevalence remains high, with >90% of the adult population (>44 years) being seropositive in Sri Lanka.[25] Recent studies have suggested that while DENV is cross-reactive with ZIKV, the level of cross-neutralization and hence disease protection is limited.[26] It is surprising that Zika is not reported in Sri Lanka although dengue, chikungunya, and ZIKV are widely reported in both South and Southeast Asia and these three viruses share the same mosquito vector *Aedes aegypti* and *Aedes albopictus*.[27] The objective of this study was to determine the seroprevalence of ZIKV antibodies among the population in Sri Lanka during and after the 2016 Zika epidemic using ZIKV neutralizing assays to elucidate the extent of the ZIKV spread, compared to DENV and CHIKV in the local population of a low-income area in a suburb of Colombo, Sri Lanka.

Methods

The study community was a low-middle income, densely populated suburban community of 146 families in Ratmalana, in the Colombo district [Figure 1]. Overall, garbage disposal and drainage system in the area was observed to be inadequate, and mosquitoes were found to be breeding in stagnant water that had accumulated in discarded containers (e.g. tires, pots, and nonbiodegradable plastic containers) (unpublished data). Healthy volunteers (149) between 18 and 80 years from the above suburban community were included in the study and samples were collected in 2017. The sample size was calculated according to the formula used for cross-sectional studies.[28]

The sample size was calculated using the formula given below.

$$n = \frac{z^2\, p(1-p)}{d^2}$$

P = prevalence of measure under study; since dengue IgG-positive samples would be identified first, the reported rate of IgG positive was taken as $P = 0.682$[25] and d = acceptable level of precision of the measure under study was taken as 7.5%, we assumed that z = 1.96 for 95% confidence interval. A questionnaire was used to obtain information on gender, age, occupation, and location of their residence.

Laboratory diagnostics

The blood was collected into plain vacutainer tubes by trained preintern medical officers and was allowed to clot at room temperature, and then transported to the laboratory for storage and testing. A commercially available IgG antibody detection assay Panbio DENV IgG indirect enzyme-linked immunosorbent assay (ELISA) (Alere, Australia) (serological specificity 100%, serological sensitivity [secondary] 97.9%, serological sensitivity [endemic] 62%), CHIKV IgG capture ELISA (Novatec, Germany; specificity 100.0%, sensitivity

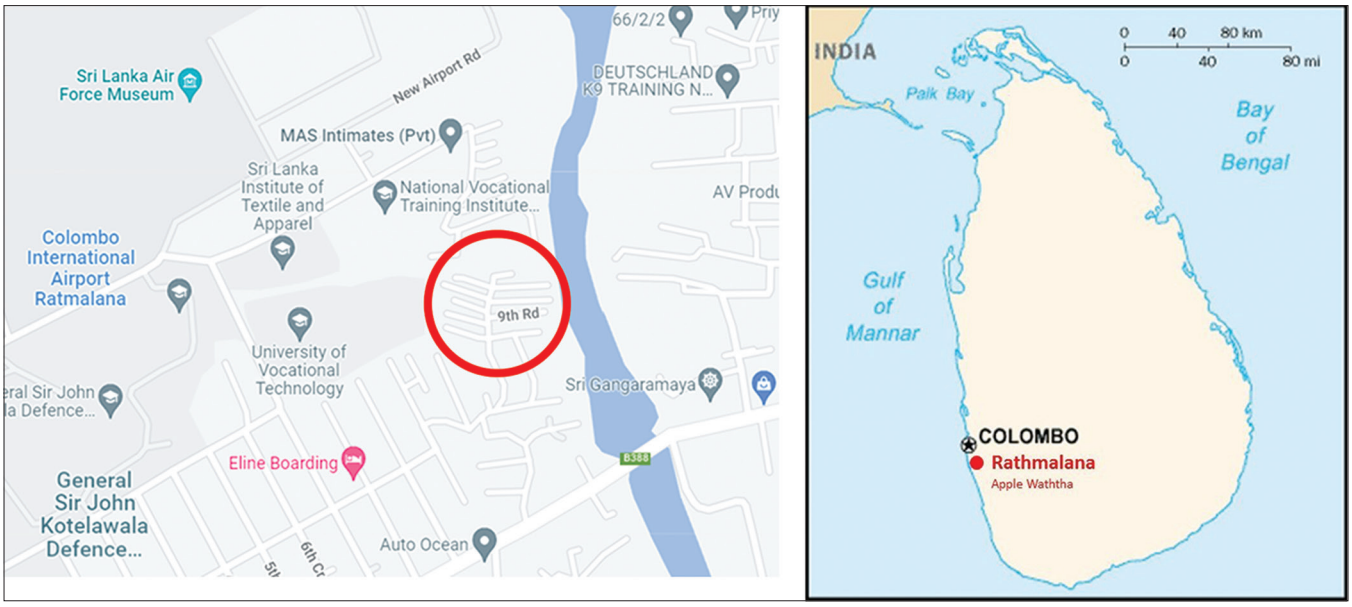

Figure 1: Location of the study population mapped in Sri Lanka. Right side map shows the Sri Lanka location in the world map and left side map shows where the sample collection site in Ratmalana Colombo, Sri Lanka, circled in red color. The study community was a low-middle income, densely populated suburban community of 146 families in Ratmalana, in the Colombo district

98.68%), and human anti-ZIKV IgG ELISA (R and D Systems, USA) were used to detect antibodies to DENV, CHIKV, and ZIKV. The manufacturer's instructions were followed for each kit.

Social demographic data obtained from 149 volunteered participants from study community were analyzed by using Microsoft Excel. Univariate analyses of potential socioeconomic and demographic risk factors for DENV, ZIKV, and CHIKV were analyzed using Microsoft Excel.

We selected the 8 Zika IgG-positive and 32 other Zika IgG-negative samples (40 / 149) to be further screened for the presence of cross-neutralizing antibodies to ZIKV using a Plaque-reduction neutralization test (PRNT). The assay was performed as described by Nguyen *et al*., 2020.[23] Serum samples were first heat-inactivated at 56°C for 30 min. In the first PRNT screening for ZIKV antibodies, the serum was diluted in 2-folds using EMEM (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) containing 2% fetal bovine serum (FBS) (1:20–1:10240). The PRNT was performed in replicates of 2 for ZIKV (PRVABC59 strain). At each dilution, 50 µL of serum sample was mixed with 50 µL virus at 100 PFU/mL. The virus–immune complex mixture was then incubated at 37°C for 1 h. A total of 50 µL of virus–immune complex mixture was then added onto Vero cell monolayers in 12 well plates (Corning Costar, Sigma-Aldrich, St. Louis, Missouri, USA) and incubated at 37°C in 5% CO_2 for 1 h. After incubation, overlay medium (2 mL of EMEM/1% methylcellulose [Wako Pure Chemical Industries Ltd, Osaka, Japan], in 2% FBS) was added into each well. The plates were incubated at 37°C in 5% CO_2 for 4–6 days until visible plaque formation. Cells were then fixed using 4% paraformaldehyde phosphate buffer solution (Wako) for 1 h at room temperature and then stained with 1.25% crystal violet (Wako). The plaques were then counted by naked eye. The neutralization titers, $PRNT_{50}$, were defined as the highest serum dilution which reduced the number of plaques by 50%.[23]

Results

Demographic and socioeconomic background characteristics

A total of 149 participants (mean age: 43 ± 14 years), 64 (43%) males and 85 (57%) females, participated in the study. Their age range was between 18 and 77 years. The highest number of participants was from the age group of 28–37 years, which represented 23.4% of the study population. Most of the participants (70 participants representing 46.9% of the study population) had studied up to the secondary level in school. There were 59 (39.5%) homemakers. Among observations in their households, outdoor uncovered water tanks 13 (9%), general cleanliness 89 (60%), open large drains 44 (30%), and indoor potted plants 19 (13%). There were 108 (72%) participants using bed nets, 43 (29%) were using mosquito coils, 13 (9%) were using insect sprays, and 12 (8%) were using other alternatives as mosquito control measures.

There were 26 (17%) participants who had not attended any public gatherings within the last 6 months or any outdoor activities whereas 42 (28%) were going to school to pick up their children. There were 87 (58%) participants going to temples/mosques/churches and 45 (30%) were going to entertainment places and for other festivities regularly.

Zika virus, DENV, and CHIKV seropositivity in the study population

Of the 149 samples tested, 141 (94.6%) were positive for anti-DENV IgG [Table 1], which is a high proportion to be positive

for DENV-IgG, and this reflects the dengue hyperendemicity in the region. Previous studies have found that by age 12 in the Colombo district, over 70% of child-age population would have been exposed to DENV.[29] Thus, our results confirm those of previous studies and reflect the dengue situation in Sri Lanka. There were 56 (37.5%) positives for anti-CHIKV IgG antibodies and 8 (5.3%) positives for anti-ZIKV antibodies found in the study population. There are no associations among positivity according to gender or ethnicity. Out of eight IgG-positive persons for Zika, three were positive for all three viruses ZIKV, DENV, and CHIKV whereas five were positive for DENV and ZIKV viruses. There were no cases positive for ZIKV only. Since all the ZIKV positives were positive for dengue as well, this suggests that these eight positive cases may have cross-reactive antibodies to DENV IgG antibodies. We emphasize that the manufacturer claims that the anti-ZIKV IgG kit has a special reagent to minimize cross-reactivity against cross-reactive antibodies found in dengue-infected patients.[30]

The comparison between IgG positives of dengue, chikungunya, and Zika and selected socioeconomic demographic data is shown in Supplementary Material Table 1.

Almost all (141 out of 149 or 94.6%) participants were positive for DENV IgG antibodies whereas 56 of them were also positive for CHIKV IgG antibodies [Table 1]. There were five participants positive for both DENV and ZIKV IgG antibodies. Three participants were positive for all three types of IgG antibodies against all three viruses and 81 participants tested positive for DENV IgG antibodies only. None of the participants had IgG antibodies against ZIKV only whereas one participant had IgG antibodies against CHIKV only. Furthermore, none of the participants had IgG antibodies against ZIKV and CHIKV only without IgG antibodies against DENV. Another interesting finding is that seven participants did not have IgG antibodies against all three viruses [Figures 2 and 3].

ZIKV IgG antibodies are mostly present among older age groups while they were lacking in younger age groups (37 years or less).

Neutralizing antibody levels to *Zika virus*

Of the 149 samples, 40 samples were selected randomly as well as to include all Zika IgG-positive samples to detect the presence of neutralizing antibodies to ZIKV. Of these 40 samples, 32/40 were positive for dengue and 15/40 were positive by IgG ELISA for chikungunya. Of the 8 ZIKV IgG-positive samples, 6 demonstrated neutralizing antibodies to ZIKV (PRNT50 = 1:40–1:320). Of the eight samples, one has exhibited ZIKV antibody titers that were >8-fold higher (PRNT50 ZIKV =1:160–1:1280) [Table 2]. Among the 32 IgG-negative samples, 4 showed neutralizing antibodies to ZIKV (PRNT50 ZIKV =1:160–1:320).

Further, we have calculated a sensitivity and specificity of the ZIKV IgG ELISA compared to ZIKV PRNT as a gold standard. There are three cutoff values for predicting the positive, negative, and equivocal ranges in the ELISA kit used. There are three cut-off values for predicting the positive (>0.200), negative (<0.100), and equivocal ranges (in between 0.100 and 0.200 values) in the ELISA kit used. To determine the positive and negative predictive values [Table 3], for the equivocal range data, the cutoff point to classify as DENV positive was above 0.150 and negative cutoff point was below 0.140. We calculated a sensitivity of 60%, a specificity of 93.3%, a

Table 1: DENV immunoglobulin G, CHIK immunoglobulin G, and Zika immunoglobulin G results

	Dengue IgG Panbio	Chikungunya IgG Novatec	Zika IgG R and D
Positive	141	56	8
Percentage	94.6	37.5	5.3

IgG: Immunoglobulin G

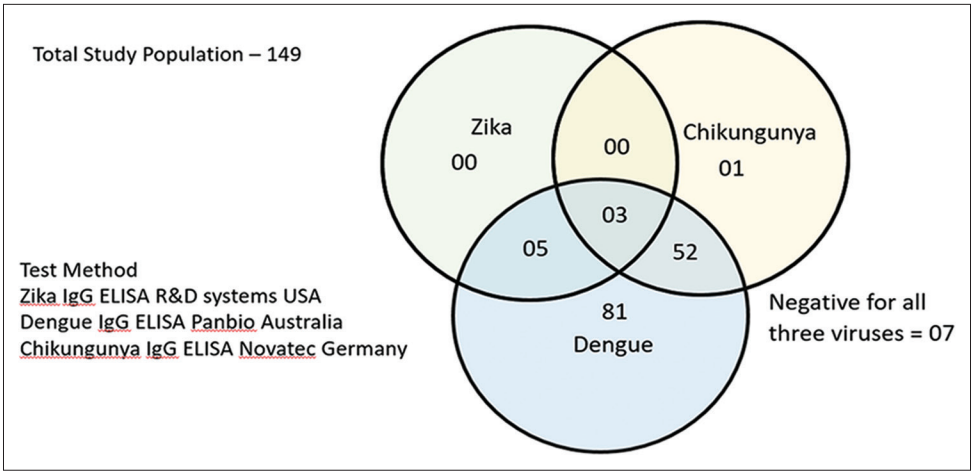

Figure 2: Number of participants positive for each of three viruses in IgG ELISA in ZIKA, DENV, and CHIKV. ELISA: Enzyme-linked immunosorbent assay. This figure summarizes the infections which share the antibodies in common for all three infections. Total participants were 149. DENV =141 positive, CHIKV =56, DENV + ZIKV =05, DENV + ZIKV + CHIKV =03, DENV only =81, ZIKV only =00, CHIKV =01, ZIKV + CHIKV =00, no DENV + no ZIKV + no CHIKV =07.

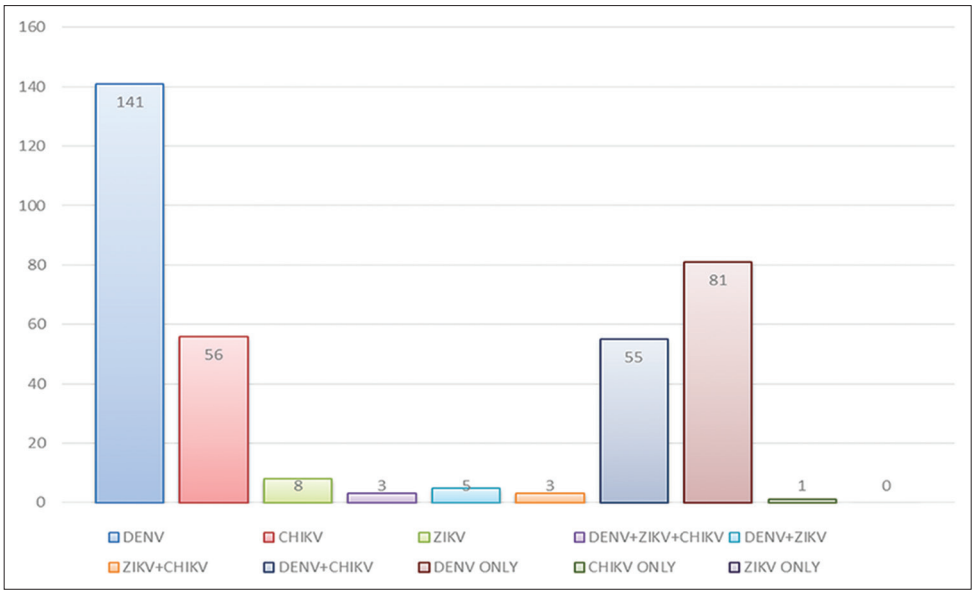

Figure 3: Number of participants positive for each of three viruses in IgG ELISA in ZIKA, DENV, and CHIKV. ELISA: Enzyme-linked immunosorbent assay. This figure shows the positive participants for each of three viruses in IgG ELISA in common

positive predictive value of 75%, and a negative predictive value of 87.5% [Supplementary Material], whereas the kit leaflet gave no values but mentioned it has a high specificity with minimal cross-reactivity with DENV IgG antibodies and high sensitivity.

Optical density values were obtained using the anti-Zika IgG ELISA test (R and D Systems, USA) on a panel (n = 149) of ZIKV. OD values of <0.100 were considered as negative, OD values of > 0.200 were considered as positive and OD values from 0.100 to 0.200 were considered as equivocal. This was determined at a wavelength of 450 nm with a correction wavelength at 540 or 570 nm. PRNT50 cutoff for positive was determined as ≥160.

Discussion

In this study, we determined the ZIKV, CHIKV, and DENV IgG antibody levels among 149 participants. Out of the 149 samples tested, 141 were positive for DENV IgG antibodies, 81/149 were only positive for DENV, and 7/149 were negative for all 3 types. CHIKV IgG antibodies were present in 56/149 patients with 1/149 positive only for CHIKV and ZIKV IgG antibodies present in only 8/149 (5.3%) [Figure 2]. While not being conclusive, the small number of ZIKV antibody positives, being positive among the older population, suggests that ZIKV has not been circulating in Sri Lanka for a long period of time (~37 years ago) (supplemental data) and suggests that the population has not been widely exposed to ZIKV in recent times. However, CHIKV IgG antibodies were prevalent in younger age groups suggesting that CHIKV has been circulating in Sri Lanka in more recent times. This agrees with the known history of CHIKV in Sri Lanka whereby a large epidemic occurred from 2005 to 2007 in Sri Lanka.[31]

However, 10/149 (6.7%) had ZIKV neutralizing antibodies (PRNT =1:160–1:320) with one case exposed to ZIKV infection confirmed using PRNT50 with a ZIKV neutralizing titer ≥8 fold of DENVs neutralizing titer. While anti-IgG ZIKV antibody tests may have lower specificity than other laboratory assays, the test is useful for identifying probable ZIKV cases during an outbreak.[23,32] Considering the ZIKV PRNT as a gold standard, the sensitivity is 60% and the specificity is 93.3% for the ZIKV ELISA kit. While further studies using a larger number of patients are needed, the findings of this study suggest that these assays were useful in seroprevalence studies of the current DENV situation and a combination of tests that determine ZIKV neutralizing antibodies and ZIKV-specific IgG antibodies would be useful for long-term seroprevalence studies in Sri Lanka. The fact that 4/32 ZIKV PRNT positives were missed by the ZIKV IgG kit suggests that the antibody positivity rate may even be as high as 19/149 (13%), if we had tested all the samples, but this cannot be confirmed without comprehensive testing. Although the incidence of dengue increased 20-fold from the year 2000 to 2012 and a further 3-fold from 2012 to 2019, this increase is not reflected in a similar increase in the age-stratified seropositivity rates for dengue, with the annual seroconversion rates being 0.76% in 2013 and 0.91% in 2017.[7] In addition, although a 13-fold increase in dengue was seen in those who were <19 years of age, a 52.4-fold increase was seen in the 40- to 59-year age group.[7] According to our study, 94.6% were positive for anti-DENV antibodies which comprised an adult population (18–77 years). This shows that our results are similar to previous findings with an increase during this time. As dengue is endemic in Sri Lanka, there is a potential for it to reach a 100% seroprevalence rate, as every individual may get dengue at least once during their lifetime. Therefore, based on these data, although the incidence of dengue infections

Table 2: Comparison of plaque-reduction neutralization test 50 and immunoglobulin G enzyme-linked immunosorbent assay OD values

Original sample ID	IgG OD values	$PRNT_{50}$
4	0.263	1280
5	0.324	320
19	0.261	320
73	0.233	320
145	0.303	320
106	0.329	160
32	0.270	40
139	0.231	<20
89	0.179	320
138	0.029	320
13	0.140	160
144	0.142	160
6	0.175	80
142	0.115	80
147	0.051	80
40	0.000	80
28	0.148	40
62	0.141	40
39	0.081	40
149	0.167	<20
128	0.126	<20
91	0.020	<20
116	0.018	<20
127	0.007	<20
112	0.003	<20
125	0.001	<20
8	−0.038	40
47	−0.033	40
18	−0.058	<20
3	−0.051	<20
78	−0.041	<20
2	−0.034	<20
34	−0.033	<20
15	−0.033	<20
7	−0.026	<20
11	−0.022	<20
1	−0.021	<20
37	−0.015	<20
98	−0.013	<20
35	−0.003	<20

***Original sample numbering system is given in the first column and only 40 out of 149 were selected due to budget constraints. $PRNT_{50}$: Plaque-reduction neutralization test 50: cutoff for positive was determined as ≥160, IgG: Immunoglobulin G, O.D.: Optical Density: cut-off for positive (>0.200)

Table 3: Sensitivity, specificity, positive and negative predictive values for Zika IgG ELISA were obtained using formulas and calculations compared to Zika PRNT Gold standard as described in Supplementary material

	Zika PRNT (Gold standard)	
Zika Ig G ELISA	True positive (a=06)	False positive (b=02)
	False negative (c=04)	True negative (d=28)

ELISA: Enzyme-linked immunosorbent assay, PRNT: Plaque-reduction neutralization test, IgG: Immunoglobulin G

has increased over the years in all age groups, the steepest rise has been in the older age groups. According to our data, age groups 18–27 (84%), 28–37 (91%), 38–47 (100%), 48–57 (96%), 58–67 (93%), and 68–77 (100%) were positive for anti-DENV antibodies. Although the reasons for the rise in dengue cases in the adult population are not clear, it could be due to multiple factors such as the differences between infection with different DENV serotypes, change in host factors (increase in comorbid illnesses), and the intensity of transmission of the DENV.

In Sri Lanka, the outbreak of CHIK fever started in October 2006 in parallel with Maldives and Andaman and Nicobar Islands. More than 100,000 chikungunya cases were diagnosed in Sri Lanka in 2006 and 2007.[31,33,34] The previous outbreak reported in Sri Lanka was in 1969 and the next reported outbreak was in 2007. It is unknown whether there were any other minor outbreaks or clusters in Sri Lanka during this time. There have been very few cases of CHIKF reported in recent times in some regions and the question remains as to why there are very few cases of CHIKF while dengue is prevalent at high levels. In our study, we conducted IgG ELISA to detect past infections to chikungunya and a 37.5% seropositivity rate was found in the selected population. There are no past surveillance studies done in Sri Lanka, but there were acute infection studies done during the 2006–2007 outbreak. A study done in Brazil has reported 18.3% IgG positives for CHIKV among a selected rural community in 2015.[35] According to Teixeira *et al.,*[36] the global seroprevalence was 22.1%, ranging from 2.0% to 70.5%. According to the studies done in India,[37] their prevalence of IgG antibodies against CHIKV in the study population was 18.1% which is relatively low compared to our data. Chikungunya cases were not reported in Sri Lanka in recent times, and therefore, these antibodies detected against chikungunya may be due to the outbreak in 2006 and 2007.[31] Further, the seroprevalence in the age group of 18–45 years of the study done by Kumar *et al*., 2021, in India[37] was 21.6% and our result was 32.2% which is relatively high compared to the region. Our findings will aid understanding of population susceptibility for CHIKV, help in the design of surveillance strategies, predict future outbreaks, and plan control measures. Estimating the CHIKV seroprevalence will be helpful for vaccine developers in understanding the population-level immunity and deciding appropriate target age groups and sites for future CHIKV vaccine trials in Sri Lanka.

Even though dengue and chikungunya have been widely reported, ZIKV has not been reported in Sri Lanka. Therefore, we conducted our seroprevalence study to determine past infections of ZIKV in our cohort in Sri Lanka. We assumed that since DENV, ZIKV, and CHIKV share the same mosquito vector, ZIKV should also be present in Sri Lanka. Our results revealed that 6.7% (10/149) of the study population

were positive for ZIKV-specific antibodies and hence have encountered the virus. Although it is a low percentage, it confirmed that ZIKV has been or is present in Sri Lanka. Since all the ZIKV-positive cases were also positive for dengue, we had to confirm that this is not due to the cross-reactivity of dengue antibodies.

We could not confirm the presence of ZIKV infections in Sri Lanka by using an IgG ELISA kit alone and the results were confirmed by PRNT assays which are considered the gold standard for identifying the presence of neutralizing antibodies.[23] Therefore, we carried out PRNT assays to confirm the ZIKV presence in Sri Lanka. When analyzing PRNT results, in some samples, we noticed a high level of plaque even with a high level of dilution (>640) which strongly suggests that these people had specific antibodies against ZIKV and had been previously infected. There were some mismatching data between IgG ELISA and PRNT assays. Most were compatible while two of them were ELISA+ and PRNT− and four were ELISA− and PRNT+. But clearly, enough evidence is present to suggest the presence of ZIKV in Sri Lanka for the very first time. According to the data, it was suggested that ZIKV may have been present in Sri Lanka before the 1980s, since all the positive cases reported were >38 years in age [Supplementary Material Table 1]. Due to unknown reasons, ZIKV has not been reported in Sri Lanka. It may be due to the lack of diagnostic tools to identify ZIKV, and especially in the 1980s, there were very few tools available, as our data suggest that it was probably present a long time ago. DENV has been predominant in the country and most/all of the common screening has been to detect the presence of DENV, unless there is a clear epidemic as was the case for chikungunya in 2006.[31] There is abundant evidence now to show that responses elicited by DENV infection can cross-react with other members of the genus *Flavivirus*, particularly ZIKV.[38] Cohort studies have shown that prior DENV immunity is associated with protection against Zika.[38] The neighboring country India also reported 16.8%.[14] The studies done in Vietnam by Nguyen *et al*., 2020,[23] showed seropositive rates of 1.1% for ZIKV. In Sri Lanka, according to our results, there is low ZIKV seroprevalence (6.7%), and no reported cases up to date. This protection may be a reason for not having any ZIKV epidemics in Sri Lanka.

This study confirms ZIKV infection in the Ratmalana area of Sri Lanka and suggests that ZIKV has been present in Sri Lanka. The prevalence of ZIKV-specific antibodies was 6.7% in this study, suggesting that Zika could be present in other parts of the country, and due to unknown circumstances, it has not created an outbreak within the country. While DENV seroprevalence remains high in the region, the overall low ZIKV seroprevalence indicates limited Zika spread within the population. There is no recorded history of the presence of ZIKV in Sri Lanka, and to the best of our knowledge, this is the first report. According to the neutralization assays, it confirms the presence of ZIKV, strongly suggesting that ZIKV was present in the past in Sri Lanka. Further studies of seroprevalence in the general population and continuous surveillance are needed to better understand the extent of the outbreak in the general population and to define the potential risk of ZIKV transmission in the region.

Research quality and ethics statement

This study was approved by the Ethical Review Committee of the Faculty of Medicine, General Sir John Kotelawala Defence University (RP/2017/11).

The authors followed applicable EQUATOR Network (http://www. equator-network.org/) guidelines during the conduct of this research project.

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Conflicts of interest

There are no conflicts of interest.

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Supplementary Material Table

Supplementary Material Table 1: Immunoglobulin G positives of DENV, CHIKV, and ZIKV against selected socioeconomic demographic data

Variable	Number of participants	Number of positives		
		DENV Panbio	CHIKV Novatec	ZIKV R and D
Age (years)				
18–27	26	22	7	0
28–37	35	32	10	0
38–47	32	32	13	2
48–57	33	32	17	2
58–67	15	14	5	2
68–77	0	9	4	2
Total	149	141	56	8
Gender				
Male	64	62	25	2
Female	85	79	31	6
Education				
No education	9	7	2	1
Primary	40	37	19	2
Secondary	70	69	28	3
Higher secondary	21	19	5	0
Undergraduate	9	8	1	0
Graduate	0	0	0	0
Occupation				
Service/professional	5	5	2	0
Business	6	6	3	0
Labor/menial workers	40	40	16	2
Student	9	8	1	0
Homemaker	59	56	20	2
Others	8	6	3	1
Nonresponse	22	19	9	1
Observation				
Uncovered water tank outdoor	13	13	7	2
General cleanliness	89	83	32	3
Open drains	44	41	17	2
Having indoor potted plants	19	18	10	0
Types of mosquito control measure				
Bed net	108	101	39	6
Mosquito coil	43	40	15	2
Spray and others	13	11	4	0
Others	12	11	5	0
Attendance in public gatherings in the last 6 months				
No attendance	26	25	5	2
Schools	42	39	18	1
Temple/mosque/church	87	84	40	4
Entertainment places/festivities	45	43	21	0
Whether any family member suffered from febrile illness in the last 6 months				
Yes	24	24	7	2
No	125	116	48	4

Supplementary material formulas and calculations:

Sensitivity = [a/(a + c)] × 100= [06/(06 + 04)] ×100 = 60%

Specificity = [d/(b + d)] × 100= [28/(02 + 28)] ×100 = 93.3%

Positive predictive value = [a/(a + b)] × 100= [06/(06 + 02)] ×100 = 75%

Negative predictive value = [d/(c + d)] × 100= [28/(04 + 28)] × 100 = 87.5%