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Changing epidemiology of dengue in Southern Sri Lanka

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Introduction

Dengue is a major public health hazard in Sri Lanka. Transmitted by the Aedes mosquitoes, Dengue virus (DENV) is a flavivirus, which has four distinct serotypes (DENV 1-4) which can cause disease in humans. Dengue may present as a spectrum of disease ranging from undifferentiated fever, dengue fever, to severe dengue. Sequential infection from a different serotype, known as secondary dengue, is more likely to progress to severe disease, with a greater potential to develop plasma leakage, bleeding or organ impairment than primary dengue.

The first serological confirmation of dengue in Sri Lanka was in 1962 and the first outbreak was recorded in 1965[1]. Although all four serotypes were known to be co-circulating in the country causing smaller outbreaks, dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) were rare in Sri Lanka before 1989[2]. From 1989 through 2008, periodic epidemics with increasing magnitude and severity occurred every few years, all associated with a new clade of DENV-3. However, since 2009, there has been a dramatic increase in dengue transmission, with 35000-45000 cases reported per year. The predominant virus associated with these recent outbreaks has been a newly introduced genotype of DENV-1[3]. Epidemic Dengue is reported mostly from the heavily urbanized Western Province of Sri Lanka even though the disease has been spreading throughout the country for decades. Southern Province, which is 117 km away from the capital city, is regularly plagued by dengue and has significantly contributed to the nationwide burden of the disease.

This oration is based on two large research studies carried out in the Southern region of Sri Lanka during two epidemiologically distinct periods, and is focused to describe the changing epidemiology and virology of dengue. The results of our studies also possibly represent the behavior of dengue within the entire island.

Methodology

We conducted two large, prospective cohort studies on dengue in Southern Sri Lanka in two distinct periods corresponding to periods before (2007) and after (2012) the beginning of nationwide large annual epidemics.

Setting and subjects in the two studies: 2007 and 2012

Both studies were cross sectional surveillance studies carried out at Teaching Hospital Karapitiya (THK), Galle, the only tertiary referral center for the entire Southern Province which provides care for one million people in the Galle district.

Ethical clearance was obtained from University of Ruhuna, Duke University Medical Center and Johns Hopkins University USA.

Adults and children with documented fever (temperature ≥ 38°C tympanic or axillary, or ≥ 38.5 °C oral) at presentation or within 48 hours of hospital admission were enrolled after obtaining informed consent or assent. The clinical and epidemiological data were recorded and blood samples were collected, the first on enrollment and the second 2-4 weeks later at the time of convalescent visit. Patients who were hospitalized within previous two weeks, who have had surgery in the previous 7 days, with focal bacterial infections and unwilling or unable to give consent were excluded from the study.

MBBS-qualified physicians recorded epidemiologic and clinical data and trained phlebotomists collected the blood samples. The laboratory testing was supported by University of Ruhuna, Duke University and Johns Hopkins University USA.

Statistical analyses were performed using STATA, version 11 (STATACorp, College Station, Texas USA). Categorical variables were compared using the Chi square test or Fisher exact test and continuous variables were compared using the t-test or Kruskall-Wallis test.

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2007 study[4]

A total of 1079 consecutive febrile patients were enrolled from March to October 2007. Paired sera were available for 859 (79.6%) patients.

Paired serum samples were tested for DENV IgG ELISA and IgM ELISA. Acute sera were tested for PCR and virus isolation. We defined acute dengue as IgG seroconversion or as a four-fold increase in antibody titre. Primary and acute secondary dengue was distinguished by the absence or presence of IgG in acute-phase serum samples, respectively. The presence of IgG in acute-phase serum samples without a substantial increase in titre in the convalescent sample defined past dengue infection. Seroprevalence was defined as the presence of IgG in acute-phase serum samples. Dengue virus was isolated in C6/36 cells and serotyped with reverse transcription PCR. The seropositives were confirmed by neutralization assay.

Results: 2007 study

Among 859 with paired sera 61.2% were male, and the median age was 30.7 years. The median duration of fever before seeking medical care was 3 days. Majority (90.2%) reported rural residence. Acute dengue was confirmed in 54 (6.3%) of 859 patients[4].

On analysis of symptoms in those with dengue, headache was the most frequent (75.9%) followed by lethargy and muscle and joint pain (>50%); however, these symptoms were just as frequent in patients without dengue. Patients with dengue were less likely to report cough and sore throat and to have lymphadenopathy. Although gastrointestinal symptoms and signs were uncommon, overall diarrhea, jaundice, hepatomegaly, and abdominal tenderness occurred more frequently in dengue. Patients with dengue had significantly lower leucocyte and platelet counts than those without dengue ($p<0.001$). Clinical illness was similar in patients with primary and secondary dengue, but diarrhoea, jaundice and hepatomegaly were more common in secondary dengue ($P<0.05$). Out of all confirmed dengue cases, only 14% were clinically diagnosed by the care providers.

PCR Testing confirmed DENV-2 serotype (2 primary cases), DENV-3 (8 primary, 7 secondary), and DENV-4 (1 primary, 1 secondary). No cases of DENV-1 serotype were identified.

The overall seroprevalence of the cohort was 50.9%. The proportion of patients who were seropositive at enrollment increased from 9% in those <5 years old to 72% in those 40-44 years old. Overall, seropositivity was more likely in male than in female patients (55.9% vs. 42.9%; $p<0.0001$), and in urban than in rural dwellers (75.3% vs. 52.0%; $p<0.0001$).

Conclusions: 2007 study

The prevalence of dengue fever in this undifferentiated febrile cohort was lower than the other infections such as leptospirosis (17.7%) and rickettsial fevers (13.5%)[5,6]. In 2007 there were 7331 cases of dengue fever reported from the country, with 100 cases from the Galle district. The low prevalence of dengue fever during the study period is in line with the occurrence of smaller outbreaks in Sri Lanka before the year 2009[7].

The seroprevalence of dengue in the cohort increased with age; however, half of those aged 20-25 years were seronegative. In contrast, 50% of children in Colombo city were positive for dengue IgG antibody by 5 years of age, and >70% have been found to be seropositive by 12 years of age[8]. The high proportion of susceptible adults in Galle suggests the recent emergence of DENV in this area. Serotypes DENV-2-4 caused illness, with DENV-3 being the most frequent. According to the study only a very low percentage (14%) of patients with confirmed dengue were clinically diagnosed. This finding emphasizes the difficulties with clinical diagnosis, particularly in unselected patients with recent onset of fever (median 3 days) in the absence of a recognized epidemic.

2012 study[9,10]

Total of 976 consecutive febrile adults and children admitted to THK from June 2012 - May 2013 were enrolled, paired sera were available for 877 (89.6%) patients[9,10].

Acute dengue was confirmed by IgG paired ELISA, quantitative RT-PCR and Dengue virus isolation in acute-phase serum on C6/36 *Aedes albopictus* cells. Indirect immunofluorescence assay was performed to serotype isolated dengue virus. Full genome sequencing of dengue virus isolates was done to identify the genotype.

Acute dengue was confirmed as follows: IgG seroconversion, PCR and/or virus isolation positive with a positive convalescent IgG, virus isolation and dengue-specific PCR positive, and/or PCR positive with 2 targets [dengue-specific and pan-flavivirus]. Among those with acute dengue, the presence of IgG in the acute sample was considered as evidence of secondary infection and its absence as evidence of primary infection.

Results and conclusions: 2012 study

Acute dengue was confirmed in 388 (39.8%) of the 976 hospitalizations for fever during the study period. Out of the total 976 study cohort, 39 patients had inconclusive results for dengue and therefore were excluded from the analysis. From the 388 confirmed dengue, 100 were confirmed by IgG seroconversion. Most were virologically confirmed by multiple methods. Adults were more likely to have acute dengue than children, 47.3% versus 28.4%, p <0.001). Median age in patients with dengue was 28.3years versus 26.1 years in patients without dengue (p<0.001). Dengue accounted for over half (52.7%) of fevers in adolescents and young adults who were 15 to 39 years of age (Figure1). Among 120 children below 5years, 11% had acute dengue, equally distributed in males and females.

Among 351 patients with acute dengue who were dengue PCR or virus isolation-positive, 320 (91.2%) were DENV-1, 25 (7.1%) were DENV-4, and 6 (1.7%) were DENV-2. The phylogeographic analysis of the major serotype DENV-1 confirmed that the DENV-1 genotype in Southern Sri Lanka is similar to what is circulating in the Western Province, China and Thailand[11].

The proportion of patients who were seropositive at enrollment increased with age from 30.7% in those <5 years old to 85.4% in those 45-49 years old. Presence of IgG at enrollment was similar in male and female patients (56.9% versus 54.1%, respectively) and in rural and urban dwellers (54.9% versus 56.4%, respectively).

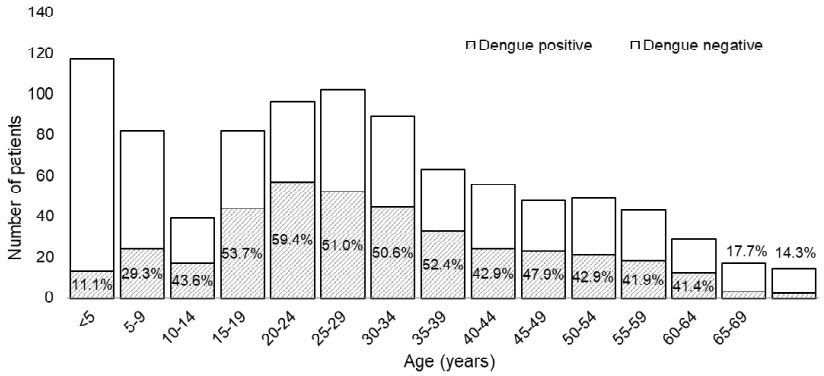

Figure 1. Number and percentage of patients admitted with acute dengue as cause of acute fever by age group at a tertiary care hospital in Southern Sri Lanka, June 2012 - May 2013.

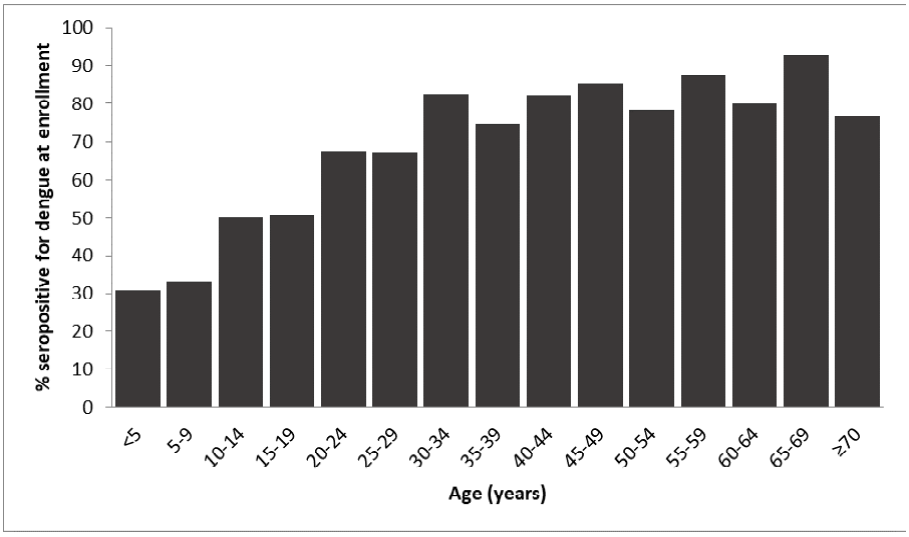

Figure 2. Proportion seropositive for dengue by age group, Southern Sri Lanka, July 2012 - May 2013.

During the study period, dengue accounted for at least 10% of acute fevers during each month, with a peak of 82% in October 2012 and a nadir of 10% in March 2013. Notably, October 2012 was the month with the greatest rainfall during our study period (519.5mm), whereas March 2013 had a relatively lower rainfall (114.1mm)[13].

A history of travel within the country in the previous 30 days was shown to be significantly associated with acute dengue than no dengue (27.9% versus 17.2%) (P<0.001). Residence in a rural area (38.7% versus 24.4%), and traveling a longer distance to the hospital (median 25km versus 18km) were more common in those with dengue than in those without dengue (P<0.001).

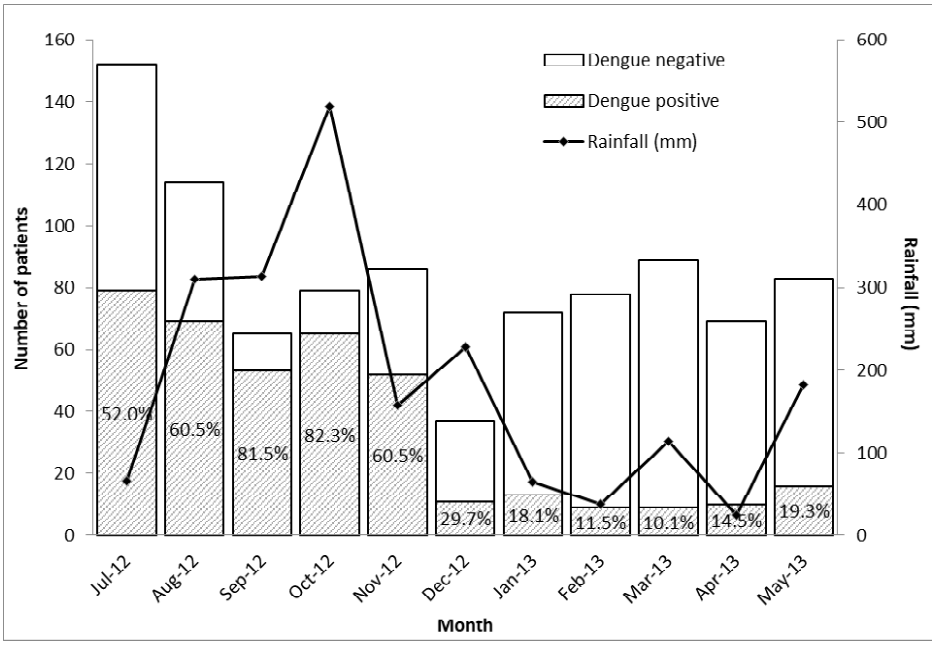

Figure 3. Proportion with acute dengue among patients hospitalized with acute febrile illness at a tertiary care hospital in Southern Sri Lanka, June 2012 - May 2013. Monthly rainfall for the Galle district is depicted by the line graph.

Table 1. Demographic characteristics of febrile patients with acute, past, and no evidence of dengue, Southern Sri Lanka, 2012-13 [* >18 years; ^Proportions (%) except median (IQR) age and distance. IQR, interquartile range.]

*Demographic Characteristics^*	*Acute dengue^ (n=388)*	*Past dengue^ (n=255)*	*No dengue (n=294)*	*P-Value*
Age, years	28.3 (19.5-41.5)	36.6 (23.4-51.5)	13.6 (4.4-29.5)	<0.001
Male	250 (64.4)	173 (67.8)	183 (62.)	0.39
Rural residence	150 (38.7)	55 (21.6)	79 (26.9)	**<0.001**
Travel, past 30d	107 (27.9)	54 (21.3)	40 (13.7)	**<0.001**
Distance km to hospital	25 (10- 50)	20 (6- 30)	18 (8- 30)	**<0.001**
Education ≥12th grade*	111 (36.6)	66 (31.3)	41 (32.5)	0.42
Occupation*				
Unemployed/ retired	69 (23.9)	64 (32.2)	30 (24.4)	0.10
Laborer	99 (34.3)	72 (36.2)	54 (43.9)	0.17
Merchant/office	52 (18.0)	33 (16.6)	12 (9.8)	0.11
Other	69 (23.9)	30 (15.1)	27 (22.0)	0.06

We analyzed the clinical features and laboratory criteria, which recognize dengue in adults and children (Table 2).

Table 2.

	Adults			***Children***		
Characteristic	*Primary dengue n=69*	*Secondary dengue n=203*	*P value*	*Primary dengue n=34*	*Secondary dengue n=42*	*P value*
Age	30.3 (24.2-43.8)	34.1 (26.5-46.1)	0.11	10.2 (5.0-15.9)	13.3 (8.2-16.1)	0.19g
Male	45 (65.2%)	135 (66.5%)	0.85	24 (70.6%)	22 (52.4%)	0.11
Symptoms						
Days of fever	4 (3- 5)	5 (3-6)	**0.01**	4 (2-5)	5 (5-6)	**<.001**
Rhinitis/Congestion	10 (14.5%)	15 (7.4%)	0.08	12 (35.3%)	6 (14.3%)	**0.03**
Sore throat	11 (15.9%)	33 (16.3%)	0.95	11 (33.3%)	8 (19.1%)	0.16
Cough	33 (47.8%)	65 (32.5%)	**0.02**	14 (41.2%)	15 (35.7%)	0.63
Joint pain	41 (59.4%)	163 (81.1%)	**<0.001**	13 (40.6%)	26 (61.9%)	0.07
Muscle pain	40 (58.5%)	157 (77.3%)	**0.003**	14 (43.8%)	28 (66.7%)	**0.049**
Anorexia	64 (92.8%)	186 (92.1%)	0.86	29 (85.3%)	38 (90.5%)	0.49
Abdominal pain	20 (29.0%)	46 (22.8%)	0.30	14 (41.2%)	18 (43.9%)	0.81
Vomiting	29 (42.7%)	110 (56.7%)	**0.046**	18 (52.9%)	28 (70.0%)	0.13
Diarrhea	7 (10.1%)	29 (14.4%)	0.37	3 (8.8%)	0 (0%)	0.05
Dysuria	5 (7.3%)	25 (12.3%)	0.25	5 (14.7%)	4 (9.8%)	0.51
Oliguria	10 (14.7%)	25 (12.3%)	0.61	2 (5.9%)	9 (22.0%)	0.05
Headache	60 (87.0%)	166 (82.2%)	0.36	21 (65.6%)	32 (78.1%)	0.24
Fatigue	56 (82.4%)	158 (78.6%)	0.51	26 (78.8%)	40 (97.6%)	**0.01**
Signs						
Temperature	100.6 (99.2-101.0)	99.8 (98.8-100.8)	**0.04**	99.5 (98.4-00.8)	100.3 (99.0-100.8)	0.27
Heart rate/min	80 (76-88)	80 (72-92)	0.76	98 (88-110)	96 (82-100)	0.26
Systolic BP	110 (110-120)	110 (100-120)	0.15	100 (90-110)	110 (100-110)	0.04
Diastolic BP	70 (70-80)	70 (70-80)	0.44	60 (60-70)	70 (60-70)	0.24
Conjunctiva injection	16 (23.2%)	30 (14.8%)	0.11	6 (17.7%)	0(0%)	0.005
Pharyngeal/erythema/ exudate	4 (11.8%)	1 (2.4%)	0.10	7(10.1%)	17(8.4%)	0.65
Lymphadenopathy	9 (13.4%)	18 (9.2%)	0.32	9 (26.5%)	7(16.7%)	0.30

(Continued)

(Table 2 Continued)

	Adults			***Children***		
Characteristic	*Primary dengue n=69*	*Secondary dengue n=203*	*P value*	*Primary dengue n=34*	*Secondary dengue n=42*	*P value*
Jaundice	0 (0%)	2 (1.0%)	0.41	0	0	-
Lung crackles	6 (8.7%)	4 (2.0%)	0.01	2 (5.9%)	1 (2.4%)	0.44
Right upper abdominal tenderness	8 (11.8%)	32 (15.9%)	0.41	8 (24.2%)	6 (14.6)	0.29
Hepatomegaly	6 (8.8%)	15 (7.5%)	0.73	3 (8.8%)	1 (2.4%)	0.22
Rash	6 (8.7%)	41 (20.2%)	0.03	6 (17.7%)	12 (28.6%)	0.27
Flushing	3 (4.4%)	28 (13.8%)	0.03	4 (11.8%)	10 (23.8%)	0.18
Laboratory parameter	**Median IQR or Mean +/-SD**					
WBC per µL	3.5 (2.5-6.7)	3.4 (2.4-5.1)	0.29	7.3 (4.0-10.8)	2.5 (2.2-3.8%)	**<.001**
Leukopenia*	39 (56.5%)	127 (62.9%)	0.35	7 (23.3%)	32 (78.1%)	**<.001**
ANC per µL	2.5 (1.5-4.7)	2.1 (1.3-3.7)	0.09	4.8 (1.7-6.2)	1.3(0.9-2.0)	**<.001**
ALC per µL	0.7 (0.5-1.1)	0.7 (0.5-0.9)	0.67	1.2 (0.7-2.8)	0.8 (0.6-1.3)	**0.009**
Hemoglobin(g/dL)	13.7 (12.5-14.7)	13.6 (12.4-14.7)	0.63	12.9 (11.8-13.9)	12.9 (12.2-13.5)	0.91
Hematocrit	41.0 (37.0-44.1)	40.3 (37.7-43.7)	0.65	38.8 (35.5-40.6)	38.6 (37.2-41.0)	0.71
Platelets (×1000/ µL)*	130 (88-175)	103 (60-150)	**0.003**	204 (139-261)	84 (59-124)	<.001
Thrombocytopenia*	21 (30.4%)	99 (49.0%)	**0.007**	6 (20.0%)	23 (56.1%)	0.002
Elevated transaminases*	2 (2.9%)	17 (8.4%)	0.12	1 (2.9%)	3 (7.1%)	0.42
Antibiotics at enrollment	34 (49.3%)	79 (38.9%)	0.13	11 (32.4%)	15 (35.7%)	0.76

On bivariable analysis, anorexia, arthralgia, and myalgia and absence of cough were common in children and adults with dengue than in those without dengue (p<0.001). Leucopenia, thrombocytopenia, and transaminitis within 48 hours of admission were more common in both children and adults with dengue, than in those without dengue (p<0.001). Overall disease severity was low, with two dengue-positive and four dengue-negative patients being transferred to an intensive care unit. Patients with secondary dengue were more likely to be adults and to be older than those with primary dengue (median age 31.3 years versus 24.2 years; p <0.001), and the duration of fever prior to enrollment was longer in secondary dengue. Patients with secondary dengue were also more likely to have arthralgia (77.8% versus 53.5%, p<.001), myalgia (75.5% versus 54.0%, p<.001) leucopenia (65.4% versus 46.7%, p<.001) and thrombocytopenia (50.2% versus 27.3; p<0.001) but less likely to have rhinitis/ congestion and cough than primary dengue.

The overall sensitivity and specificity of clinical diagnosis of dengue at discharge were 57.7% (95% CI 52.6-62.7) and 92.7% (95% CI 90.2-94.7), respectively. The positive predictive value (PPV) and negative predictive value (NPV) were 84.9% (95%

CI 79.9-89.0) and 75.6% (95% CI 72.2-78.8), respectively. Sensitivity of clinical diagnosis of dengue was higher in secondary dengue than in primary dengue (66.9%, 95% CI 60.7- 72.8 versus 37.9%, 95% CI 28.5- 48, p<.001).

In a subset of 409 patients, who had cost analysis performed, patients with dengue spent more on travel (Rs. 607 versus Rs. 329, P=0.02), and to stay ≥4 days in-hospital (p<0.001) than patients without dengue. The median duration of hospitalization was greater in dengue-positive patients than in dengue-negative patients (5 versus 4 days; p<0.001). Median cost (expenses, work lost) to an employed adult with dengue was 3800 Sri Lankan rupees.

In a subset of 409 patients analyzed from the study population 262 (64%) received antibiotics: 108 (26.4%) before admission, 173 (42.3%) after admission to hospital and 47 (11.5 %) after discharge. Out of the 173 patients who received antibiotics in hospital, 37 (21.3%) received intravenous antibiotics. Fifty-four patients (40%) who were clinically suspected as dengue and 68 (36.1%) who were laboratory confirmed received antibiotics. Commonest in-hospital antibiotics included penicillin (72, 41%) and 3rd generation cephalosporin (32, 18%).

Discussion

Our studies confirm a remarkable increase in dengue in 2012 contributing to 40% of hospitalized acute febrile illnesses, in contrast to 6.3% in 2007. We detected a new epidemic of DENV-1 in 2012 in a susceptible population who had no prior DENV-1 infection identified according to the findings from the 2007 study. The virologic confirmation (dengue virus isolated or PCR positive or both) provided conclusive evidence for DENV-1 as the cause of the dengue epidemic. In 2012, there were 44,461 reported cases of dengue Island wide and 1513 cases were from Galle district[7].

Though traditionally, dengue has been considered an urban disease, in both studies, rural residence was surprisingly more common in those with acute dengue. There have been increasing reports of dengue from rural areas worldwide, but this pattern of transmission is not yet fully understood. Travel to urban centers that have a heavy burden of disease, secondary vectors such as *Aedes albopictus*, and stored water containers have all been implicated as reasons for the increasing prevalence of dengue in rural areas.

In our study, a history of travel prior to illness was associated with acute dengue. Galle is located only 116km from the capital Colombo, and frequent travel between the two cities for work and other purposes could have contributed to the epidemic.

The largest number of cases were recorded in October, coinciding with the highest rainfall. The association between dengue and rainfall has been previously described, as rainfall produces conditions that are favorable for reproduction and survival of the vector mosquitoes

A majority of dengue in our 2012 study was caused by DENV-1 serotype, which represents a marked shift from our findings in 2007, when only DENV-2, DENV-3, and DENV-4 were isolated and DENV-3 was the most prevalent. This shift in serotype is similar to what has been observed in the Western Province. Although DENV-1 has been co-circulating in Sri Lanka for decades, large annual epidemics of DENV-1 have only occurred since 2009 with the appearance of a new DENV1 genotype-1 strain that may have been introduced from China or Thailand. The phylogenetic analysis confirmed that the DENV-1 genotype in Southern Sri Lanka is similar to what is circulating in the Western Province, supporting the theory that the travel between the two cities may have contributed to the spread of the disease[12]. Dengue antibodies are generally type-specific. Our 2007 study suggested that the study population lacked DENV-1 antibodies and thus were susceptible to the new DENV-1 strain, In 2007, we found that the dengue seroprevalence reached a plateau of 70% by 40 years of age. In contrast, in 2012 study, nearly 70% were seropositive by age of 20 years and the seroprevalence reached 90% in those aged 60-65 years.

Although both studies included patients with acute undifferentiated fever, the 2012 study was conducted during an epidemic of dengue. Other studies have indicated that clinical suspicion for disease can increase during epidemic times, leading to improved sensitivity of clinical diagnosis.

Patients with dengue had characteristic clinical and laboratory features that distinguished them from patients without dengue, including thrombocytopenia and leucopenia. These laboratory findings were also prominent in dengue-positive patients in our previous study, and have been extensively described by others.

Certain clinical and laboratory features were much more prominent in secondary dengue than in primary dengue, even in the absence of severe disease. Patients with secondary dengue were less likely to have rhinitis/ congestion and cough and were more likely to have arthralgia and myalgia than patients with

primary dengue. In addition, patients with secondary dengue had lower median WBC and platelet counts than patients with primary dengue. To our knowledge, these findings have not been reported previously in the extensive dengue literature. The prominence of these clinical and laboratory findings in this cohort of mainly secondary dengue patients may have helped to improve clinicians' diagnostic impressions. With increasing cases of secondary dengue in countries like Sri Lanka, where dengue is now endemic, it is likely that clinical diagnosis of dengue will improve.

During the epidemic of DENV-1 in Southern Sri Lanka in 2012, the sensitivity of clinical diagnosis of dengue improved greatly (58%) compared to the study at the same hospital during a non-epidemic period in 2007 (14%). The majority of confirmed dengue cases were due to secondary dengue, consistent with the increasing seroprevalence of dengue reported throughout the island. According to the study more prominent symptoms were experienced during secondary dengue and this may have helped to improve clinicians' diagnosis of dengue even in the absence of severe disease which is known to be associated with secondary dengue.

Conclusions

A new epidemic of DENV-1 was confirmed in 2012 in Southern Sri Lanka in a susceptible population who had no prior immunity to DENV-1. Most of the patients were from rural residence indicating the geographical spread of DENV-1 in the Southern province.

DENV-1 in the Southern region was genotypically similar to DENV-1 identified in the Western Province. According to the 2012 study, travel was a newly identified risk factors for dengue, hence further epidemiologic studies should explore the drivers of these trends and control strategies should be adjusted to account for these changes.

Even though the majority of cases recorded were secondary dengue, contrary to expectations, the DENV-1 epidemic in 2012 had a low complication rate. Although improved sensitivity of clinical diagnosis and better management strategies may have contributed, it is also possible that the DENV-1 strain was less virulent.

Rapid increase in the dengue cases in 2012 became a public health concern as majority were adolescents and young. Most of the cases were reported in post-monsoon period indicating a need for acceleration of vector control programmes prior to arrival of monsoon.

Finally, given the magnitude of the disease, there is a growing need to improve laboratory infrastructure, technical expertise and research capacity in Sri Lanka in order to influence dengue surveillance, case management and to develop new approaches in dengue control.

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