

Dengue virus detection in Lao PDR and Colombia: Comparative evaluation of PCR tests

Simone Kann¹ | Joerg Blessmann² | Yvonne Winkelmann³ | Jessica Hansen³ |
Leonardo J. Maya Amaya⁴ | Gadith E. Rivera Salcedo⁴ | Hussein El Halas⁵ |
Jonas Schmidt-Chanasit⁶ | Latdamone Keoviengkhone⁷ | Vatsana Sopraseuth⁷ |
Christina Deschermeier³ | Angela Mika³

¹Medical Mission Institute, Würzburg, Germany

²Department for Implementation Research, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany

³Diagnostics Development Laboratory, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany

⁴Hospital Eduardo Arredondo Daza, Valledupar, Colombia

⁵Altona Diagnostics GmbH, Hamburg, Germany

⁶Department of Arbovirology, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany

⁷Savannakhet Provincial Hospital, Savannakhet, Lao PDR

Correspondence

Angela Mika, Diagnostics Development Laboratory, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany. Email: mika@bnitm.de

Abstract

Objectives: Dengue virus (DENV) detection by polymerase chain reaction (PCR) facilitates diagnosis of dengue fever, which is the most frequent arboviral disease globally. Two studies were performed in countries with high dengue incidence, to assess the diagnostic performance of different PCR techniques.

Methods/results: Two hundred and seventy-nine acute phase blood samples from febrile patients were analyzed for DENV by the RealStar Dengue RT-PCR kit (Altona Diagnostics) as gold standard in comparison with the Tropical Fever Core multiplex PCR (Fast Track Diagnostics). In total, 102 samples collected in Savannakhet Province (Lao PDR, Southeast Asia) in 2013 and 35 samples from Valledupar (Colombia, South America) tested positive for DENV by RealStar RT-PCR. In comparison, the Tropical Fever Core multiplex PCR detected 65.0% (65/102) and 68.6% (24/35) of these samples as positive for DENV in Savannakhet and Valledupar, respectively. Diagnostic sensitivity of the multiplex PCR strongly correlated with viral load. A subset of DENV PCR-confirmed samples was additionally tested by BNITM in house Dengue Type RT-PCR in comparison with two commercial test kits (RealStar Dengue Type RT-PCR [Altona Diagnostics], Dengue differentiation PCR [Fast Track Diagnostics]). The leading dengue serotype in Savannakhet was DENV-3 (58% [29/50]), while DENV-1 (53.8% [14/26]) was the predominant serotype found in samples collected in Valledupar by BNITM-type PCR. However, three DENV serotypes were circulating in Valledupar and in Savannakhet. In 2015, additional studies found predominantly DENV-4 (71% [12/17]) in Savannakhet.

Conclusions: Both studies emphasized that routine diagnostics in both regions will benefit from an expanded use of highly sensitive pan-dengue PCRs.

KEYWORDS

Colombia, dengue infections, dengue serotypes, Lao PDR, PCR diagnostics

Sustainable Development Goal: Good health and wellbeing

This is an open access article under the terms of the Creative Commons Attribution NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2021 The Authors *Tropical Medicine & International Health* Published by John Wiley & Sons Ltd

INTRODUCTION

Dengue fever (DF) is one of the most important infectious diseases in tropical and subtropical regions and the most common arbovirus infection globally [1]. Numbers of infections increase more and more, due to population growth, progression of the urbanization, globalisation and climate change (e.g. heavy rains, increasing temperatures) [2]. Poor infrastructure (e.g. water supplies, sewage systems) and inadequacies of current methods to reduce transmission [3] further contribute to DF infections. Today about half of the world's population is at risk, an estimated 390 million infections occur per year, with 96 million having clinical signs and symptoms [1,4]. DF is present in about 129 countries, whereby the actual burden seems to be the highest in Asia (70% of the infections) [5].

DF is an arthropod-borne viral disease caused by four serologically related, but antigenically distinct dengue virus serotypes (DENV-1–4). Dengue viruses are single-stranded RNA viruses from the family *Flaviviridae*. Primary vectors are *Aedes* spp. mosquitos [5]. Dengue infections can be asymptomatic or symptomatic. The most common symptoms are fever, severe headache, retro-orbital pain, myalgia, arthralgia and rash [6]. Without further complications, it is categorized as (classic) DF. Severe cases are defined as dengue haemorrhagic fever (DHF) or dengue shock syndrome (DSS), both life-threatening complications of DF. According to WHO [7] the revised case definitions of DF are categorized in dengue, dengue with warning signs and severe dengue. As there is no specific treatment, early detection of the disease and access to proper medical care are essential. Only by these means can fatality rates be reduced and countermeasures against the expansion of the infection be successful [6].

Lao People's Democratic Republic (Lao PDR) and Colombia are hyperendemic countries for DF. Lao PDR has an estimated population of seven million people, who live mostly in rural areas with all 18 provinces being at risk by dengue infections [8,9]. Savannakhet in the south is the largest province with approximately one million inhabitants. Shepard et al [9] estimated an annual average of 2.9 million dengue infections and 5,906 deaths annually for a region of 12 Southeast Asian countries (including Lao PDR), resulting in annual economic costs of approx. US\$ 950 million [9,10]. In Asia, outbreaks occur on a regular basis, approximately every 3–5 years during the rainy season [8,9].

In Colombia, dengue has shown re-emergence and intense transmission with increasing tendency in frequency and severe outbreaks over the years. Recently, the frequency of epidemic cycles increased from every 3–5 years to every 2–3 years [11,12]. Colombia has about 46 million inhabitants, and dengue is endemic in most parts of the country [3]. The economical loss related to DF was estimated to be about US\$130 million in 2012 [3,13]. Valledupar, department César, belongs to one of the three regions in Colombia with the highest incidence for DF according to health officials [3].

Although DF has been a large public health issue for the last decades, the precise magnitude of infections and the

epidemiological direction and parameters driving epidemics have only been partially understood; extensive serotype surveillance data to predict epidemics are scarce [14]. The gold standard for dengue diagnosis is confirmation by PCR [7]. Unfortunately, in many countries only very few reference laboratories are able to routinely perform PCRs, mostly due to lack of tools and/or specialized staff. Hence if any tests are on offer at all, they are mainly point-of-care tests or ELISA. Insufficient access to tests and a reporting system based on clinical interpretation of unspecific dengue symptoms leave many cases undetected [3]. Reliable PCR-based diagnostics, with high sensitivity and specificity, can contribute to a correct diagnosis, differentiation from other diseases, immediate intervention to prevent fatalities, surveillance and preventive measures.

In this study, we compared and analysed the performance of two pan-dengue PCR techniques and three serotype-specific dengue PCRs in two highly endemic countries of Asia and South America for the detection of DF and identification of circulating dengue serotypes.

METHODS

ETHICS STATEMENT

This study complies with the Declaration of Helsinki. Ethical approval for the study in Lao PDR was granted by the Lao National Ethics Committee for Health Research (No. 030/NECHR). Ethical approval for the study in Colombia was given by the Ethics Committee of Valledupar, Hospital Rosario Pumarejo de López (César, Colombia, Acta No. 0022013). Both approvals included the permission to export selected anonymized samples for further analysis at the Bernhard-Nocht Institute for Tropical Medicine (BNITM, Hamburg, Germany). Written informed consent was obtained from each participant or in case of children by his/her parent or legal guardian before enrolment.

Study design, sample collection and processing

In this study, we investigated patients with fever of unknown origin admitted to Savannakhet Provincial Hospital in Savannakhet Province (Lao PDR, Southeast Asia) and to Hospital Rosario Pumarejo de López, as well as Hospital Laura Daniela and the Hospitals Eduardo Arredondo Daza in Valledupar (Department César, Colombia, South America). A diagnostic laboratory was set up in Valledupar and Savannakhet, and local staff was trained in the new diagnostics, especially in the performance of real-time PCRs.

Acute-phase samples from 172 febrile patients were collected in the Savannakhet Provincial Hospital in 2013, and acute samples from 107 febrile patients were collected in the hospitals in Valledupar during from May to August 2014. Samples were taken on the day of admission. A nucleic acid extraction was made from serum or EDTA blood (200 µl)

and finally eluted in 60 μ l elution buffer with the RTP Pathogen Kit (Stratec Molecular). Extracts were tested with the RealStar Dengue RT-PCR Kit 1.0 (Altona Diagnostics [Altona], Hamburg, Germany; [15]) and the Tropical Fever Core multiplex Real-time PCR (Fast Track Diagnostics [FTD], Luxembourg). All procedures were performed according to the manufacturers protocols.

Pan-dengue PCR-positive samples were further evaluated for serotyping with the BNITM in house Dengue Type PCR for dengue serotype detection (version 2013, [16]), the Dengue Differentiation PCR (FTD) and the RealStar Dengue Type RT-PCR 1.0 RUO (Altona). With RealStar Dengue Type RT-PCR, serotypes were distinguished if (i) only one serotype channel was positive or, in case (ii) several channels showed positive PCR curves, the dominant CT of one serotype was lower by at least six CT values (cycles) than signals in other detection channels. All procedures were performed according to the manufacturers protocols.

Diagnosis

A dengue diagnosis was confirmed by PCR-positive result using the RealStar Dengue RT-PCR Kit 1.0 (Altona) as described above. Patients were treated by hospital personnel according to WHO guidelines [7]. While all patients in Valledupar were residents, three dengue patients in Savannakhet were travellers, one each from Germany, Japan and the USA.

Data analysis

Significance calculations were performed using unpaired t-test or Fisher's exact test by Prism software (GraphPad, San Diego, California, USA). Confidence intervals and kappa agreements were calculated using QuickCalcs (GraphPad).

RESULTS

Strongly different diagnostic sensitivity of pan-dengue PCRs

We analyzed 279 acute-phase blood samples from febrile patients collected in two highly endemic regions for DENV by RealStar Dengue RT-PCR kit (Altona) as gold standard in comparison with the Tropical Fever Core multiplex PCR (FTD).

In Savannakhet (Lao PDR), 102 PCR-positive dengue cases from 172 (59.3%) febrile patients were found in 2013 by RealStar Dengue RT-PCR (Altona), while 65.0% (65/102) of the patients found by RealStar PCR were also detected as DENV-positive by the multiplex PCR (FTD; Figure 1a). Samples were taken between day 1 and 12 after onset of symptoms (median day 4).

In Valledupar (Colombia), 35 of 107 febrile patients (32.7%) whose samples were collected in 2014 were diagnosed with dengue using the RealStar PCR (Figure 1b). Samples were taken between day 1 and 8 after onset of symptoms (median day 4). Multiplex PCR (FTD) detected 68.6% (24/35; Figure 1b) of these, thereby revealing a similar ratio as in Lao PDR. Thus, concordance (Cohen's kappa 0.639; 95% confidence interval 0.554–0.725) between RealStar and multiplex PCR was substantial but not high (Figure 1c).

A significant difference was visible in both study groups: the higher the CT value found in the RealStar Pan-dengue PCR, the lower the likelihood for dengue detection by the FTD multiplex PCR (Figure 2). Thus, the diagnostic sensitivity of the FTD test strongly correlated with viral load. Notably 42.9% (15/35) of the PCR-positive cases in Valledupar were below the age of 18, in contrast to 12.7% (13/102 cases) in Savannakhet in 2013.

Dengue serotypes

A subset of 127 pan-dengue PCR-positive samples (found by RealStar Dengue RT-PCR) was additionally tested for dengue serotypes by BNITM's in-house Dengue Type RT-PCR as gold standard. In Savannakhet in 2013, 58% (29/50) of the cases detected positive by BNITM Type PCR were DENV-3, 40.0% (20/50) DENV-2 and 2% (1/50) DENV-1, while no DENV-4 patients were detected (Table 1). In Valledupar, 53.8% (9/26) were DENV-1, 34.6% (9/26) DENV-2 and 11.5% (3/26) DENV-4 (Table 1).

Dengue serotype results found by BNITM Dengue Type PCR used as gold standard for samples from Lao PDR and Colombia were compared with test results of two commercial test kits RealStar Dengue Type RT-PCR (Altona Diagnostics) and Dengue Differentiation PCR (Fast Track Diagnostics). In total, the RealStar Dengue Type RT-PCR (Altona) showed a high sensitivity for DENV-3 and DENV-4 (100%), while sensitivity was lower for DENV-1 and DENV-2 (86.2–86.7%; Table 2). Specificity was high for all serotypes (97.9%–100%). Thus concordance was high between the RealStar Type RT-PCR and the gold standard (Cohen's kappa 0.885, 95% confidence interval 0.799–0.971, Table 2).

The Dengue Differentiation kit (FTD) also revealed a high specificity (100%) for the four dengue serotypes and was tested with a smaller subset of samples in Lao PDR only. However, sensitivity for DENV-2 was zero (0% [0/16]), whereas it was 100% for the other three serotypes (Table 2). Thus, concordance between the Dengue Differentiation kit (FTD) and the gold standard was lower (Cohen's kappa 0.372, 95% confidence interval 0.271–0.473, Table 2).

Follow-up study

In an additional study in Savannakhet in 2014–2015, we screened 144 febrile patients for DENV. No dengue infections were found in 2014, whereas 18 PCR-confirmed

(a) Lao PDR

PCR positive (Altona, FTD)	RealStar Dengue RT-PCR (Altona)	Tropical Fever Core (FTD)	CT Pan-DENV Altona	Age	Gender	Days after onset
Status	Cases (%)	Cases (%)	Median (Range)	Median (Range)	m/f (%)	Median (Range)
+ -	37 (36.3)	0	34.9 (19.7-40.8)	23 (15-46)	21/16 (57/43)	5 (2-12)
+ +	65 (63.7)	65 (100)	28.1 (19.3-36.7)	22 (15-57)	32/33 (49/51)	4 (1-7)
Total	102 (100)	65 (65.0)	31.7 (19.3-40.8)	22 (15-57)	53/49 (52/48)	4 (1-12)

(b) Colombia

PCR positive (Altona, FTD)	RealStar Dengue RT-PCR (Altona)	Tropical Fever Core (FTD)	CT Pan-DENV ADT	Age	Gender	Days after onset
Status	Cases (%)	Cases (%)	Median (Range)	Median (Range)	m/f (%)	Median (Range)
+ -	11 (31.4)	0	32.9 (30.1-41.0)	20 (14-61)	5/6 (45/55)	5 (3-8)
+ +	24 (68.6)	24 (100)	30.2 (22.9-41.8)	18 (12-46)	14/10 (58/42)	4 (2-8)
Total	35 (100)	24 (68.6)	30.7 (22.9-41.8)	18 (12/61)	19/16 (54/46)	4 (2-8)

(c) Total

RealStar Dengue RT-PCR (Altona)	n	Tropical Fever Core multiplex PCR (FTD, result for dengue)	
		% (n)	[95% CI]
Positive	137	65.0 (89/137)	[0.567-0.725]
Negative	142	98.6 (140/142)	[0.947-0.999]
% agreement		82.1	
Cohen's kappa		0.639	[0.554-0.725]

FIGURE 1 Detection of DENV by RealStar Dengue RT-PCR (Altona) in comparison with the Tropical Fever Core Kit multiplex PCR (FTD). (a) Dengue cases found in Savannakhet (Lao PDR) in 2013 and (b) Valledupar (Colombia) in 2014, with demographic parameters. (c) Comparison of the test performance of the RealStar Dengue RT-PCR as gold standard with the multiplex PCR (FTD) using the febrile samples collected in both regions ($n = 279$). CT, threshold cycle; DENV, dengue virus; pos, dengue-positive (by RealStar RT-PCR); neg, dengue-negative

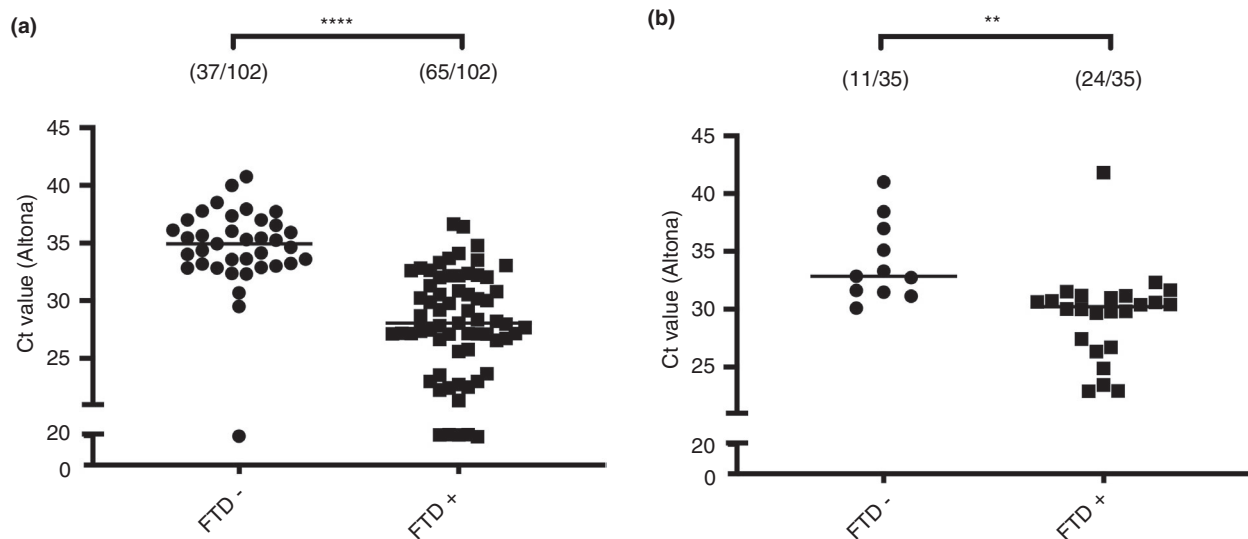


FIGURE 2 CT-dependent comparison of pan-dengue PCR techniques. Correlation of CT values of dengue patients found by RealStar Dengue RT-PCR (Altona) with dengue-positive or -negative result by Tropical Fever Core multiplex PCR (FTD) in Lao PDR (a) and Colombia (b). **, $\rho < 0.01$; ****, $\rho < 0.0001$; straight line, median

TABLE 1 DENV serotype results

		DENV-1	DENV-2	DENV-3	DENV-4	Total
Lao PDR	Positives (%)	1 (2.0)	20 (40.0)	29 (58.0)	n.d.	50 (100)
Colombia	Positives (%)	14 (53.8)	9 (34.6)	n.d.	3 (11.5)	26 (100)

Note: A subset of pan-dengue PCR-positive samples found by Altona RealStar Dengue RT-PCR in Lao PDR ($n = 92$) and Colombia ($n = 35$) was analysed by BNITM Dengue Type PCR for serotype differentiation.

TABLE 2 Test performance of dengue serotype PCRs

		RealStar Dengue Type RT-PCR (Altona)				Dengue differentiation (FTD)		
		Total n	Lao PDR % (n)	Colombia % (n)	Total % (n)	Total [95% CI]	Lao PDR % (n)	Lao PDR [95% CI]
BNITM Dengue Type PCR								
DENV-1	Sensitivity	15	100 (1/1)	85.7 (12/14)	86.7 (13/15)	[0.609–0.975]	100 (1/1)	[0.168–1.000]
	Specificity (D2,D3,D4)	61	100 (49/49)	91.7 (11/12)	98.4 (60/61)	[0.905–0.999]	100 (40/40)	[0.896–1.000]
DENV-2	Sensitivity	29	95.0 (19/20)	66.7 (6/9)	86.2 (25/29)	[0.688–0.951]	0 (0/16)	[0–0.227]
	Specificity (D1,D3,D4)	47	100 (30/30)	100 (17/17)	100 (47/47)	[0.910–1.000]	100 (25/25)	[0.842–1.000]
DENV-3	Sensitivity	29	100 (29/29)	n.d.	100 (29/29)	[0.861–1.000]	100 (24/24)	[0.837–1.000]
	Specificity (D1,D2,D4)	47	95.2 (20/21)	100 (26/26)	97.9 (46/47)	[0.879–0.999]	100 (17/17)	[0.784–1.000]
DENV-4	Sensitivity	3	n.d.	100 (3/3)	100 (3/3)	[0.383–1.000]	n.d.	
	Specificity (D1,D2,D3)	73	100 (50/50)	100 (23/23)	100 (73/73)	[0.940–1.000]	100 (41/41)	[0.898–1.000]
% Agreement					92.1		52.9	
Cohen's kappa					0.885	[0.799–0.971]	0.372	[0.271–0.473]

Note: Dengue serotype results found by BNITM Dengue Type PCR as gold standard for samples from Lao PDR and Colombia were compared with test results of the RealStar Dengue Type PCR (Altona) and the Dengue Differentiation PCR (FTD). $N = 73$ samples were tested by RealStar Dengue Type PCR. The FTD test was analysed with a smaller subset of samples and in Lao PDR only ($n = 41$). D1, D2, D3, D4, DENV-1,2,3,4 samples; CI, confidence interval.

dengue cases were detected in 2015 by RealStar Dengue RT-PCR (Altona, Supporting Information S1). In 2015, dengue serotype distribution was significantly different ($p < 0.0001$) from 2013. The predominant serotype was DENV-4 (71% [12/17]; Supporting Information S1).

DISCUSSION

In the present study, we describe the diagnostic performance of the Tropical Fever Core kit (FTD) in comparison with the RealStar Dengue RT-PCR (Altona, [15]) as a gold standard using samples from febrile patients from two highly endemic regions for DF in Lao PDR and Colombia. The gold standard was chosen based on a good test performance evaluated and described elsewhere [15].

Patient sera were collected in 2013 at Savannakhet Provincial Hospital in southern Lao PDR and in 2014 in five hospitals in Valledupar (province César, Colombia). Diagnostic testing was performed on-site for all febrile patients enrolled in this study using the Tropical Fever Core kit multiplex PCR (FTD) simultaneously testing not only for dengue virus, but also chikungunya virus, *Plasmodium* spp., *Rickettsia* spp. and other infections which are described elsewhere [2]. In addition, acute-phase serum samples were

retrospectively tested for Zika virus by PCR (Altona) after the first autochthonous Zika virus infection in Lao PCR had been reported to the WHO in 2016 [17]. No co-infections with chikungunya virus and Zika virus were observed in the DF patients enrolled in our study [2].

Analysis of 279 acute-phase samples (median day 4 post onset of symptoms) from febrile patients by RealStar Dengue RT-PCR (Altona) revealed 102 and 35 dengue patients in Savannakhet and Valledupar, respectively. While the FTD multiplex PCR enables simultaneous screening of 16 infections, the Altona diagnostic was superior in sensitivity for dengue. The multiplex PCR detected less than 70% of the positive samples found by RealStar Dengue RT-PCR in both regions, and concordance between both tests was moderate. One reason for the lower detection rate of the FTD multiplex PCR may be that test sensitivity strongly correlated with viral load. Viral load decreases rapidly over the acute phase of DF infections. In developing countries patients often attend the doctor after having several days of fever, when the viral load may already be declining [2].

In many, especially rural, settings in Lao PDR and Colombia, PCR diagnostics are not available and mostly NS1 antigen rapid tests or ELISA methods are used. NS1 tests, such as the SD Bioline Dengue Duo NS1 rapid test (Alere/

Abott) or the Platelia Dengue NS1 Ag ELISA (Bio-Rad), are known for their good specificity (>98%). However, diagnostic sensitivity was lower than 60% for both NS1 tests in comparison with results by RealStar Dengue RT-PCR using a subset of dengue patient samples from Lao PDR and Colombia [2].

In addition, dengue serotype results measured by two type-specific commercial RT-PCRs were compared with results found by BNITM Dengue Type PCR [16] as gold standard. The specificity of the two commercial serotype PCRs (RealStar Dengue Type and FTD Dengue Differentiation) was high (97.9%–100%). However, sensitivity of the FTD Dengue differentiation PCR was very low for DENV-2 with samples from Lao PDR, although the measurements with test controls for all dengue serotypes were positive. Other studies reported detection of DENV-2 serotype samples after use of the FTD Dengue Differentiation test, e.g. in Singapore and Saudi Arabia [18,19]. With the samples in our study, the RealStar Dengue Type PCR and the BNITM PCR detected DENV-2 serotype cases in samples from both regions. In contrast, the RealStar Dengue Type RT-PCR showed high sensitivity for DENV-3 and DENV-4; however, overall sensitivity for DENV-1 and DENV-2 was less high (86%–87%). Differences in sensitivity may also be caused by the circulation of different genotypes in different regions and viral load. A limitation of the present study regarding the performance of the type-specific PCR tests is the bias to the three DENV serotypes (DENV-1, DENV-2, DENV-3) found, while DENV-4 case numbers were lower.

Our results showed a large dengue outbreak in the Savannakhet region in Lao PDR in 2013, which was part of a large countrywide epidemic, where 48,772 cases and 95 deaths were reported [14], four times more cases than during the same period in the year before. According to Khampapongpane et al [8], this outbreak was the largest registered so far, with transmission patterns possibly connected to increasing urbanization [20]. The latest DF outbreak was in 2010, where 23,000 cases were reported with 46 deaths [8]. From 2007 to 2011 DENV-1 was the predominant serotype detected in Lao PDR, until DENV-3 became the leading serotype in 2012 in 94% of the cases [8]. However, all four serotypes have been circulating in this region for many years. Similarly to 2012, we found DENV-3 and DENV-2 to be the most prominent serotypes in Savannakhet in 2013. However, our latest findings showed a change in 2015, when few dengue cases were observed and newly collected samples suggested a possible switch from DENV-3 to DENV-4.

Besides Lao PDR, we also analysed samples from Colombia as a country with high incidence for DF. In Colombia, the frequency of epidemics increased from every 3–5 years to 2–3 years recently [12]. A severe outbreak occurred in 2010 with 147,670 DF cases and 9,777 cases of severe dengue disease [3]. As in Lao PDR, dengue case numbers decreased in Colombia in 2011 after the large epidemics in 2010. Our study showed small numbers of DF infections with three dengue serotypes in Valledupar in 2014. Still DF is circulating throughout the Americas and overall numbers steadily increase annually [3]. In Colombia,

DENV-2 and DENV-3 were the most frequently isolated serotypes until 2005, whereas DENV-1 was the predominant serotype from 2006 to 2008 and all serotypes were co-circulating recently [3,21]. We also found DENV-1 to be the most prominent serotype. Both data sets demonstrated clearly the rapid movements and changes of serotypes. Dengue serotype data are often sparse and available for certain years and regions only, and only if specific studies are performed [3].

Comparison of dengue case numbers from different countries and studies is difficult. In many studies in Asian and South American countries, DF infections were confirmed by serology testing and not by PCR techniques [3,8,9,22]. For instance in 2014, more than 105,000 dengue cases in Colombia were diagnosed by syndromic surveillance only. Of these, 46,842 cases were confirmed by laboratory diagnosis [13]. The others were suspected cases although it is known that DF cannot be diagnosed based on DF symptoms as they are unspecific. In contrast, incidence rates in placebo groups of vaccine studies determined by PCR and NS1 antigen diagnostics showed over tenfold under-reporting of dengue infections in Colombia when compared with data from the national epidemiological surveillance system [23].

Overall, the studies conducted in Lao PDR and Colombia demonstrated that without highly sensitive PCR diagnostics many dengue patients in both locations would not have been detected.

CONCLUSIONS

Both studies emphasized that routine diagnostics in both regions will benefit from widespread use of PCR techniques, in particular from sensitive pan-dengue PCR for patients and surveillance measures. Type-specific DENV PCR techniques are valuable tools for serotyping, but their performance may need further improvement for extensive use in highly endemic areas. The shift in serotype distribution and the repetition of epidemics within only a few years emphasize the need for continuous surveillance to counteract outbreaks and to improve DF outcomes. Precise diagnostic case numbers and information on epidemiological data on dengue serotypes can increase awareness and vector control measures. Predictions of regional epidemics after serotype switches may be possible in future after more monitoring.

ACKNOWLEDGEMENTS

The authors thank Alba Luz-Luque Lommel for helpful discussions and her network, Christina Herrde and Claudia Poggensiehl (BNITM) for technical assistance and Karin Rottengatter (Altona Diagnostics) for support of PCR analyses. Particularly, they thank all patients and healthy blood donors who agreed to collaborate in this study.

Funding was provided by the European Regional Development Fund (project no. BWF/H/52228/2012/13.10.10-1/3.4). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of

the manuscript. HEH is an employee of Altona Diagnostics GmbH, which provided support in the form of salary for H.E.H. and research materials but did not have any role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript. Open Access funding enabled and organized by Projekt DEAL.

REFERENCES

1. Stanaway JD, Shepard DS, Undurraga EA, Halasa YA, Coffeng LE, Brady OJ, et al. The global burden of dengue: an analysis from the Global Burden of Disease Study 2013. *Lancet Infect Dis.* 2016;16:712–23.
2. Blessmann J, Winkelmann Y, Keoviengkhone L, Sopraseuth V, Kann S, Hansen J, et al. Assessment of diagnostic and analytic performance of the SD Bioline Dengue Duo test for dengue virus (DENV) infections in an endemic area (Savannakhet province, Lao People's Democratic Republic). *PLoS One.* 2020;15:e0230337.
3. Villar LA, Rojas DP, Besada-Lombana S, Sarti E. Epidemiological trends of dengue disease in Colombia (2000–2011): a systematic review. *PLoS Negl Trop Dis.* 2015;9:e0003499.
4. Sarmiento-Senior D, Matiz MI, Jaramillo-Gómez JF, Olano VA, Vargas SL, Alexander N, et al. Knowledge, attitudes, and practices about dengue among pupils from rural schools in an endemic area in Colombia. *Biomédica.* 2019;39:478–90.
5. World Health Organization. Fact sheet: dengue and severe dengue. 2020, 23th June.
6. Hadinegoro SRS. The revised WHO dengue case classification: does the system need to be modified? *Paediatr Int Child Health.* 2012;32:33–8.
7. World Health Organization. Dengue: guidelines for diagnosis, treatment, prevention and control. 2009. p. 1–147.
8. Khamapongpane B, Lewis HC, Ketmayoon P, Phonekeo D, Somoulay V, Khamsing A, et al. National dengue surveillance in the Lao People's Democratic Republic, 2006–2012: epidemiological and laboratory findings. *Western Pac Surveill Response.* 2014;5:7–13.
9. Shepard DS, Undurraga EA, Halasa YA. Economic and disease burden of dengue in Southeast Asia. *PLoS Negl Trop Dis.* 2013;7(2):e2055.
10. Dubot-Pérès A, Vongphrachanh P, Denny J, Phetsouvanh R, Linthavong S, Sengkeopraseuth B, et al. An epidemic of dengue-1 in a remote village in rural Lao. *PLoS Negl Trop Dis.* 2013;7:e2360.
11. Ramos-Castañeda J, Barreto dos Santos F, Martínez-Vega R, Galvão de Araujo J, Joint G, Sarti E. Dengue in Latin America: systematic review of molecular epidemiological trends. *PLoS Negl Trop Dis.* 2017;11(1):e0005224.
12. Rico-Mendoza A, Porras-Ramírez A, Chang A, Encinales L, Lynch R. Co-circulation of dengue, chikungunya, and Zika viruses in Colombia. *Rev Panam Salud Publica.* 2019;43:e49.
13. Martínez-Vega RA, Rodríguez-Morales AJ, Bracho-Churio YT, Castro-Salas ME, Galvis-Ovallos F, Díaz-Quijano RG, et al. A prospective cohort study to assess seroprevalence, incidence, knowledge, attitudes and practices, willingness to pay for vaccine and related risk factors in dengue in a high incidence setting. *BMC Infect Dis.* 2016; 16(1).
14. Lao M, Caro V, Thiberge J-M, Bounmany P, Vongpayloth K, Buchy P, et al. Co-Circulation of dengue virus type 3 genotypes in Vientiane capital. Lao PDR. *PLoS ONE.* 2014;9:e115569.
15. Mat Jusoh TNA, Shueb RH. Performance evaluation of commercial dengue diagnostic tests for early detection of dengue in clinical samples. *J Trop Med.* 2017;2017:4687182.
16. Johnson BW, Russell BJ, Lanciotti RS. Serotype-specific detection of dengue viruses in a fourplex real-time reverse transcriptase PCR assay. *J Clin Microbiol.* 2005;43:4977–83.
17. Lim SK, Lim JK, Yoon IK. An update on Zika virus in Asia. *Infect Chemother.* 2017;49:91–100.
18. Ashshi AM. The prevalence of dengue virus serotypes in asymptomatic blood donors reveals the emergence of serotype 4 in Saudi Arabia. *Virology J.* 2017;14:107.
19. Wee S, Alli-Shaik A, Kek R, Swa HLF, Tien WP, Lim VW, et al. Multiplex targeted mass spectrometry assay for one shot flavivirus diagnosis. *PNAS.* 2019;116:6754–9.
20. Mayxay M, Cui W, Thammavong S, Khensakhou K, Vongxay IL, et al. Dengue in peri-urban Pak-Ngum district, Vientiane capital of Lao: a community survey on knowledge, attitudes and practices. *BMC Public Health.* 2013;13:434.
21. L'Azou M, Moureau A, Sarti E, Nealon J, Zambrano B, Wartel TA, et al. Symptomatic dengue in children in 10 Asian and Latin American countries. *N Engl J Med.* 2016;374:1155–66.
22. Arima Y, Chiew M, Matsui T. Epidemiological update on the dengue situation in the Western Pacific Region, 2012. *WHO/WPSAR.* 2015;6:82–9.
23. Sarti E, L'Azou M, Mercado M, Kuri P, Siqueira J, Solis E, et al. A comparative study on active and passive epidemiological surveillance for dengue in five countries of Latin America. *Int J Infect Dis.* 2016;44:44–9.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Kann S, Blessmann J, Winkelmann Y, Hansen J, Maya Amaya LJ, Rivera Salcedo GE, et al. Dengue virus detection in Lao PDR and Colombia: Comparative evaluation of PCR tests. *Trop Med Int Health.* 2021;26:1296–1302. <https://doi.org/10.1111/tmi.13670>