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Detection of circulating mosquito-borne arboviruses in Brazil using travellers as sentinels

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ABSTRACT

Travellers visiting countries endemic for arboviral diseases may introduce arboviruses to new areas where competent vectors are already present, potentially leading to disease outbreaks in populations that have not previously been exposed. To assess exposure risks for travellers and identify which arboviruses they might import, we explored the use of international travellers to collect mosquito saliva samples at their travel destinations to map locally circulating arboviruses. We recruited 73 travellers from Switzerland visiting Brazil between August 2018 and June 2019 and provided each of them with five kits to collect viral RNA on Flinders Technology Association cards. We have chosen Brazil as a study area for a proof of concept for reasons of convenience, as this is a popular travel destination. We asked the travellers to place the kits at their destinations and use a smartphone app to track their locations. We received 155 kits back and screened the cards for arboviral RNA using reverse transcriptase PCR with consecutive sequencing of amplicons from positive samples. Due to RNA degradation during an extended storage period, 129 cards produced no positive signal. In contrast, among 26 cards that could be processed shortly after receipt, seven were positive for dengue virus serotype 4 and three for West Nile virus lineage 2 (WNV-2). To our knowledge, this is the first time WNV-2 has been reported from Brazil. Our 'citizen science' approach has the potential to contribute to arbovirus surveillance, extending the geographic range of sampling – particularly in regions lacking an arbovirus surveillance programme.

1. Introduction

Arthropod-borne viruses (arboviruses), especially those transmitted by mosquitoes such as dengue, chikungunya and Zika, cause significant morbidity in endemic countries [1]. Similarly, travellers visiting countries endemic for arboviral diseases are also at risk [2]. Moreover, being viraemic upon return, they may introduce arboviruses to new areas where competent vectors are already present, potentially leading to disease outbreaks in populations that have not previously been exposed. The increasing number of autochthonous transmission events following the introduction of dengue and chikungunya to Europe in recent years [3], in connection with the spread of the competent mosquito vector *Aedes albopictus* [4,5], illustrates this aspect.

Traditionally, arbovirus surveillance is carried out through case detection, sentinel animals, mosquito monitoring or a combination of these approaches. However, each approach has its limitations [6] and overall, surveillance of locally circulating arboviruses in endemic countries is resource intense, costly, and thus often neither available nor implementable for many tropical and subtropical countries.

An innovative way to assess the presence of mosquito-borne viruses is based on the use of Flinders Technology Association (FTA) cards (Whatman International Ltd, Maidstone, UK) [7]. These cards are

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specialised filter papers designed for the long-term storage of nucleic acids from biological samples. When biological material contacts the FTA cards, cells are lysed and proteins are degraded, effectively inactivating bacteria and viruses, while stabilising DNA and RNA for future detection [8]. When feeding, mosquitoes expectorate saliva that may contain viruses, a behaviour exploited in arbovirus surveillance [9]. Honey-baited FTA cards have been integrated into mosquito traps to detect viruses from mosquito saliva [10,11]. Once a positive FTA card is identified, further analysis can be performed on the mosquitoes caught in the same trap. This method has proven effective even in areas with low arbovirus circulation when used alongside box gravid traps [12]. Additionally, FTA cards have been employed for detecting malaria sporozoites, expanding their utility in vector-borne disease surveillance [13].

Since ill-returning travellers can serve as sentinels to detect the circulation of arboviruses or even ongoing outbreaks in regions where their presence previously went unrecognised [14–16], we considered it worth exploring how international travellers could further contribute to global arbovirus surveillance. In this study, we explored the feasibility of engaging travellers equipped with custom-made mosquito saliva sampling kits, based on FTA card technology, to map circulating arboviruses – the local 'arbovirome' – in mosquito populations at their destinations in Brazil.

2. Materials and methods

2.1. Study design

The study was conducted as an international observational study. The primary objective of this study was to assess the feasibility of engaging international travellers in establishing a surveillance system using fructose-baited, FTA card–based kits for the collection of mosquito saliva in Brazil, with the aim of characterising the circulating arboviruses in local mosquito populations at their destinations.

2.2. Study participants and study procedures

Between August 2018 and June 2019, we recruited study participants among Swiss residents travelling to Brazil that were seeking health advice at the Centre for Tropical and Travel Medicine at the Swiss Tropical and Public Health Institute in Basel, Switzerland. The inclusion criteria were: (i) being Swiss resident, (ii) being at least 18 years old, (iii) consenting to study enrolment, (iv) travelling to Brazil, and (v) travelling with a smartphone. For convenience in the proof of concept, we selected Brazil because it has a high prevalence and co-circulation of various arboviruses [17,18], and because it is one of the five most popular holiday destinations among Swiss travellers [19].

2.3. FTA card kit

Upon enrolment, we provided the study participants with the study material, consisting of an information leaflet, five FTA card kits, a prepaid mobile phone SIM card for Brazil, packaging material for returning the kits and access to a smartphone app (Supplementary File S1). We instructed each study participant on how to place the kits at the place where they would stay for at least 24 h and how to return them at the end of their stay. We also provided the study participants with packaging material and postage stamps so that they could return the kits to a laboratory at Fiocruz in Recife, Brazil. The team at Fiocruz collected the incoming kits and forwarded them in bulk to the Spiez Laboratory, where we processed and analysed the FTA cards from the kits. The Spiez Laboratory is a Swiss governmental institution providing services related to arms control, protection measures, health and incident management for international organisations, authorities and the general population.

The custom-made FTA card kits consisted of five elements: a plastic case, an FTA card, a sleeve for the FTA card, a sponge and a unique bar code (Supplementary File S1). The plastic case (Sunshing Plastic and Packaging Co., Ltd, Guangdong, China) measured 10 mm × 65 mm x 95 mm. Inside the case, a plastic coin sleeve (Leuchtturm Gruppe GmbH & Co., Geesthacht, Germany) contained a 30 mm × 30 mm piece of FTA card and was attached to the back of the case with double-sided adhesive tape. The plastic sleeve had a circular opening, providing mosquitoes access to the FTA card baited with a fructose solution. To keep the card moist, we put a 30 mm × 30 mm piece of moist sponge behind it that was soaked in an aqueous solution containing 50 % fructose (m/v). In previous studies, the FTA cards were baited with honey to exploit its antibacterial properties [20]. Due to import restrictions on honey in Brazil, we replaced the honey with fructose. In a preliminary laboratory experiment comparing honey- and fructose-baited FTA cards, feeding rates were comparable.

Before handing out the FTA card kits to the travellers, we kept them at 4 °C. Each study participant received a zip lock bag containing five kits. We asked the study participants to activate the kits upon arriving at their destinations by opening the case and placing them outside their residence (e.g. window frame) with one kit per location.

2.4. Mobile phone app

To track the locations of the kits, we gave the study participants access to an advanced version of the mobile phone app 'TOURIST' that was initially developed in the frame of another study involving travellers [21]. Using the app on their smartphone, the study participants scanned a unique QR code on the back of each kit. Using the phone's global positioning system (GPS) as well as a time stamp, the app tracked where, when and for how long a kit was placed.

2.5. Sample processing and molecular analysis

We screened the FTA cards from the returned kits for arboviral RNA using reverse transcriptase PCR with consecutive sequencing of amplicons from positive samples.

We extracted RNA from the returned FTA cards from Brazil at Spiez Laboratory, Switzerland, by following the manufacturers' protocols for the FTA elution buffer (GE Healthcare Life Sciences, Freiburg, Germany) and the RNeasy Plus Universal Mini Kit (Qiagen, Hilden, Germany). Briefly, we eluted RNA from the FTA card by adding 1 ml of Whatman™ FTA elution buffer and shaking it on ice for 20 min. After elution, we extracted the RNA using the RNeasy Plus Universal Mini Kit.

We performed family-specific PCRs to screen for alpha- and flaviviruses and sequenced any resulting amplicons. As an internal control to ensure effective extraction, we added Mengovirus to each FTA card before extraction and ran a Mengovirus-specific reverse transcription quantitative PCR (RT-qPCR) on a Light Cycler 96 System (Roche Diagnostics, Rotkreuz, Switzerland) as described in Wipf et al. [12].

To identify alphaviruses, we ran a reverse transcriptase PCR (RT-PCR) in duplex on a Light Cycler 96 System using the master mix from the OneStep RT-PCR Kit together with primers from the VIR966 chikungunya (CHIKV) reference genome (NCBI Reference Sequence: NC_004162.2) [22]. These primers provide broad coverage for distinguishing alphaviruses [23]. In addition to a negative control ('RNA' extracted from an unused FTA card), we used a CHIKV S27 Petersfield strain, cultured at Spiez Laboratory, as a positive control. We ran the RT-PCR with the following cycling conditions: 30 min at 50 °C, 15 min at 95 °C, followed by 45 cycles of 20 s at 94 °C, 20 s at 55 °C and 20 s at 72 °C. Using the Qiagen QIAxcel DNA Screening Kit, we selected 98 bp amplicons on the nsP1 gene when clear and distinct bands were visible. Prior to the sequencing reaction, we purified the amplicons with the Qiagen QIAquick PCR Purification Kit.

For flavivirus detection, we ran a hemi-nested PCR to increase sensitivity [24]. In addition to a negative control, we included a yellow fever virus 17D strain, cultured at the Spiez Laboratory, as a positive

control. In the first round, we used the Pan-Flavi primers MAMD and cFD2 with the master mix from the Qiagen OneStep RT-PCR Kit. MAMD and cFD2 primers amplify a 263 bp sequence in the NS5 gene. We performed the PCR on a Light Cycler 96 System with the following conditions: 30 min at 50 °C, 15 min at 95 °C, followed by 45 cycles of 1 min each at 94 °C, 50 °C and 72 °C, and finally 7 min at 72 °C. Following the first round of PCR, we diluted the products 1:1000 with nuclease-free water. We then used this dilution as the template for the heminested PCR, performed with the Qiagen Fast Cycling Kit using the external primer cFD2 and the internal primer FS778, which yielded a 215 bp product under the following conditions: 5 min at 96 °C, followed by 45 cycles of 5 s at 96 °C, 5 s at 50 °C and 30 s at 68 °C, and 1 min at 72 °C. Using the Qiagen QIAxcel DNA Screening Kit, we selected 215 bp amplicons on the NS5 gene when clear and distinct bands were visible. Prior to the sequencing reaction, we purified the identified amplicons with the QIAquick PCR Purification Kit.

For alphaviruses, we sequenced PCR fragments using the forward and reverse VR966 primers, whereas for flaviviruses we employed the forward primer FS778 and reverse primer cFD2. We performed sequencing with the BigDye™ Terminator v3.1 Cycle Sequencing Kit (ThermoFisher Scientific, Massachusetts, USA) on a LightCycler 96 System with the following conditions: 1 min at 95 °C, followed by 25 cycles of 10 s at 95 °C, 5 s at 50 °C and 2 min at 60 °C. Subsequently, we purified the amplicons with the Qiagen DyeEx 2.0 Spin Kit and sequenced them on a 3130 Genetic Analyzer (Applied Biosystems, Muttenz, Switzerland).

2.6. Data analysis

To create summary tables with arboviruses identified from the FTA cards and their geolocations, we transferred the data from the TOURIST app server to R 4.1.0 [25]. We produced the map showing the geolocations of the placed traps with ArcGIS Desktop 10.6.1 (Environmental Systems Research Institute, Inc., California, USA). To analyse the virus sequences from the positive samples, we used the blasting software CodonCode Aligner version 9 (CodonCode Coorporation, Centerville, Massachusetts, USA).

3. Results

We enrolled 73 study participants (53 % female) with a median age of 36 years (range: 21–77 years) who spent a median of 21 days (range: 2–100 days) in Brazil. Of the 365 FTA kits handed out, 155 found their way back to us and were available for molecular analysis. We had geographic location data from 121 of the returned kits. For the remaining 34 kits, either the study participants did not scan the QR code, or there were issues with the scanning and data transfer process. The 121 returned FTA card kits with known geographic locations had been placed in 80 different locations across Brazil (Fig. 1). The median placement time of an FTA card kit was 29 h (range: 4–156 h). Due to COVID-19 pandemic-related restrictions, we had to postpone the screening of 129 FTA card kits by over two years. All FTA cards from this second screening block were negative for arboviruses. Before the COVID-19 pandemic, the RNA of all FTA cards was eluted, and in a first screening block, we selected 26 FTA cards from kits that had been in the field the longest and for which we had GPS data. Seven of these FTA cards were positive for dengue virus serotype 4 (DENV-4) and three for West Nile virus lineage 2 (WNV-2) (Table 1 and Supplementary File S2). The DENV-4-positive FTA cards were set in the states of Ceará (twice in Jijoca de Jericoacoara), Bahia (Lençóis), Mato Grosso (Várzea Grande), Amazonas (Careiro) and Paranà (Foz do Iguaçu) in November 2018 and in Bahia (Lauro de Freitas) in January 2019. The WNV-2-positive cards were set in the states of Mato Grosso do Sul (Corumbá) and Amazonas (Amatari) in November 2018 and in São Paulo (São Paulo) in April 2019 (Fig. 1 and Table 1).

4. Discussion

Using FTA card technology for sampling and detecting arboviruses in mosquito populations has methodologically enriched routine mosquito surveillance [26,27]. We took a step further by using the technology in combination with a customised kit and a mobile phone app, enabling travellers to detect circulating arboviruses at their destinations. Among 26 kits received before the COVID-19 pandemic, seven were positive for DENV-4 and three for WNV-2.

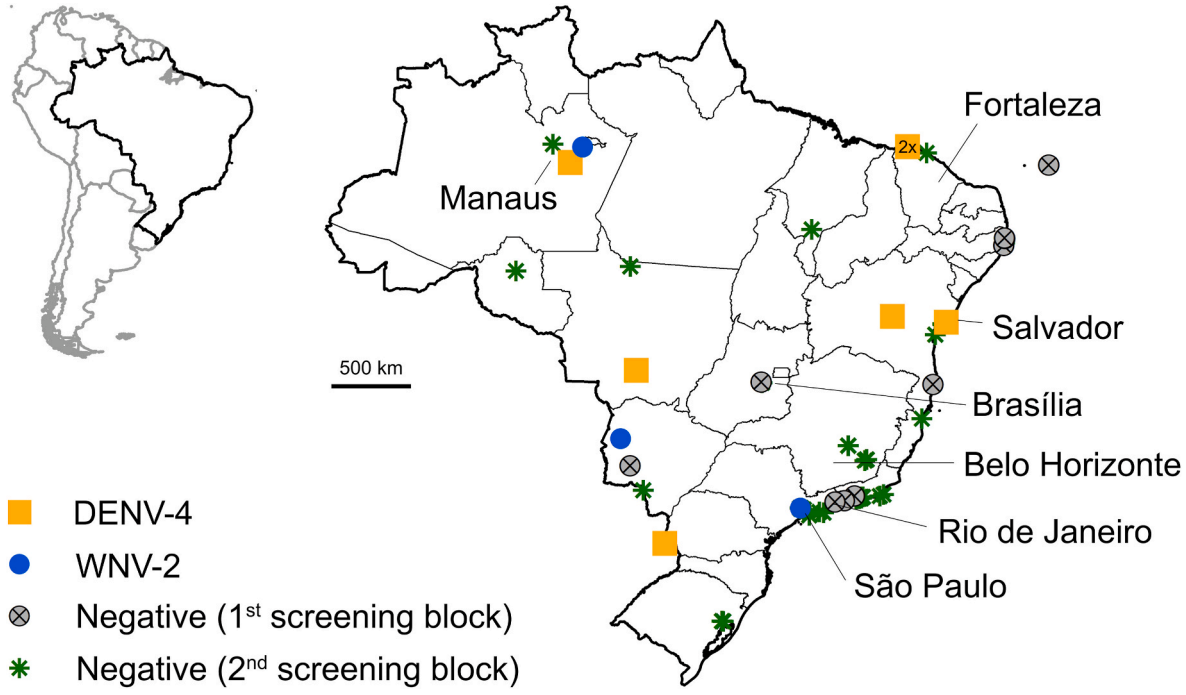

Fig. 1. Travel destinations of the study participants and results from molecular diagnostics of arboviruses on FTA cards. Two of the WNV-2-positive cards originated from the same location, as indicated by '2x' on the map.

Table 1
BLAST sequencing alignment results of FTA cards positive for DENV and WNV.

Identified virus	Location[a]	Sample[b]	Sampling date	Query coverage (%)	E-value	Identity (%)	Accession no.
DENV-4	Amazonas (Careiro)	AKA2	15 November 2018	91	$1e^{-74}$	96.1	MK506266.1
	Bahia (Lauro de Freitas)	ASA1	4 January 2019	92	$1e^{-73}$	95.0	MK506266.1
	Bahia (Lençóis)	AAT3	26 November 2018	77	$3e^{-86}$	97.9	MK506266.1
	Ceará (Jijoca de Jericoacoara)	AKA4	14 November 2018	85	$2e^{-86}$	98.4	MK506266.1
		AKA3	22 November 2018	86	$7e^{-87}$	98.4	MK506266.1
	Mato Grosso (Várzea Grande)	AAT4	20 November 2018	98	$6e^{-67}$	90.0	MK506266.1
	Paraná (Foz do Iguaçu)	AAT1	17 November 2018	54	$8e^{-89}$	96.1	MK506266.1
WNV-ln2	Amazonas (Amatari)	AAS2	16 November 2018	94	$3e^{-70}$	92.9	ON813218.1
	Mato Grosso do Sul (Corumbá)	AAS4	10 November 2018	94	$8e^{-81}$	97.3	ON813218.1
	São Paulo (São Paulo)	PAA1	26 April 2019	31	$6e^{-72}$	95.5	EF429197.1

[a] Federal state and place in brackets.
[b] The sample code corresponds to the sequences in Supplementary File S2.

All four DENV serotypes circulate in Brazil, with DENV-4 re-emerging in 2010 [28] after 30 years of absence [29] and becoming the predominant serotype in the 2014–2015 outbreaks [30]. DENV-4 was also found in subsequent years across the country [31,32]. In 2018 and 2019, DENV-4, along DENV-1 and DENV-2, was also reported to the Brazil's Notifiable Disease System (SINAN) from the states of Paraná, Mato Grosso and Bahia [33]. Although not detected in the present study, DENV-1 was intriguingly the most frequently reported serotype in the SINAN database. However, given the low number of positive FTA cards and as for 99.5 % of dengue cases reported to SINAN the serotype is not available, meaningful comparison is not feasible. Therefore, the detection of DENV-4 in all DENV-positive tested FTA cards is not unexpected and indicates that this serotype continues to circulate widely in Brazil.

WNV was first detected in North America in the late 1990s [34] and subsequently spread to Central and South America [35]. Recent studies from Brazil have reported serological evidence of WNV circulation among various hosts and regions [36–38], including the first documented case of human WNV encephalitis in 2014 [39]. The most prevalent strain in North and South America, WNV-1a, was also recently isolated in Brazil for the first time [40]. WNV-2, primarily present in sub-Saharan Africa, has also recently emerged in Eastern and Western Europe [41]. However, to our knowledge, this is the first detection of WNV-2 circulating in Brazil.

A recent review estimated a monthly incidence of 0.5–0.8 % for dengue, the arbovirus most frequently infecting travellers, among non-immune travellers to endemic areas [42]. In the present study, most of the virus-positive FTA cards were also dengue-positive, consistent with our hypothesis that the positivity rate of FTA cards from a given region correlates with local arbovirus transmission. However, confirming such a correlation would require a substantially larger dataset than is available in the present study [43].

Intriguingly, RNA extraction and detection of arboviruses from the FTA cards were successful even ten months after their deployment in the field and subsequent shipment via regular post, without requiring a continuous cold chain for preservation. However, there seem to be limitations to the long-term storage of RNA extracted from FTA cards even at −80 °C. We attribute the inability to detect arboviral RNA in any of the samples from the post-pandemic round, which were only processed after more than two years, to the degradation of the viral RNA over time. This limitation must be considered if this approach is to be adopted for routine arbovirus surveillance.

A study limitation is that selecting travellers from a single Swiss travel clinic may have introduced bias, as the cohort is unlikely to represent the broader international traveller population. Similarly, the approach should be further validated in contexts other than Brazil to draw more general conclusions. Therefore, additional studies are desirable to validate the approach using the FTA card kit. Future studies should also investigate how results from FTA cards are affected by fluctuations in ambient temperature during shipment and by the duration for which they are stored before and after shipment. It also remains to be explored to what extent the results are influenced by the length of time the kits were deployed in the field, which we were unable to assess owing to the limited sample size. Moreover, we encountered a relatively low return rate of the FTA card kits and an unexpectedly high error rate in accurately scanning and transmitting their barcodes, leading to uncertainty regarding their locations. This technical issue would need to be resolved if this approach is to be employed routinely for arbovirus surveillance, for example by incorporating reminders into the mobile phone app or providing more remote support. Finally, a general limitation of using FTA cards is that they do not enable identification of the mosquito species that fed on an FTA card. However, since both cells and nucleic acids, including mosquito DNA, are collected and stabilised on the FTA cards, there is potential to develop analytical methods to determine the mosquito species that fed on them. Next-generation sequencing technologies could be used to detect both viral and mosquito genomes [44]. However, even with this approach, attributing a specific virus to a particular mosquito species remains challenging if multiple mosquito species feed from the same FTA card.

We conclude that our citizen science approach has the potential to contribute to arbovirus surveillance, extending the geographic range of existing sampling – particularly in regions lacking an arbovirus surveillance programme. Although this project targeted international travellers, a comparable citizen science approach could be applied to other populations to encompass regions beyond tourist destinations.

CRediT authorship contribution statement

Laura Vavassori: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Silvan Hälg:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Nadja Hedrich:** Writing – review & editing, Investigation, Data curation. **Duschinka Guedes:** Investigation. **Marcelo S. Paiva:** Writing – review & editing, Investigation. **Christian Beuret:** Supervision, Resources, Project administration, Methodology. **Ulf Blanke:** Writing – review & editing, Software, Methodology. **Andreas Neumayr:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Pie Müller:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Northwest and Central Switzerland EKNZ (Project ID 2018-00680). All participants provided informed consent, and data protection procedures were implemented in accordance with the approved protocol. As recruitment was conducted exclusively among Swiss residents through the Swiss TPH Centre for Tropical and Travel Medicine, submission to the Brazilian national ethics committee system (CEP/CONEP) was not required.

In line with Brazilian regulations (CNS Resolution 466/2012), ethical approval is mandatory only for studies involving human participants or non-human vertebrate animals. As this study involved only the collection of mosquito saliva from insects naturally present in the hotel environment, it did not fall within the regulatory scope of CEP/CONEP or CEUA. The shipment of FTA cards was registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge in Brazil (SisGen registration no. A62B369).

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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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During the preparation of this work, the authors used ChatGPT (GPT 5) to review grammar and typological errors in parts of the text. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Glossary

CHIKV	Chikungunya virus
DENV	Dengue virus
FTA	Flinders Technology Association
RT-PCR	Reverse transcriptase PCR
RT-qPCR	Reverse transcriptase quantitative PCR
SINAS	Sistema de Informação de Agravos de Notificação (Engl. : Information System for Notifiable Diseases)
WNV	West Nile virus

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tmaid.2025.102936.

Data availability

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

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