Chikungunya (*Togaviridae*) and dengue 2 (*Flaviviridae*) viruses detected from *Aedes aegypti* mosquitoes in Burkina Faso by qRT-PCR technique: preliminary results and perspective for molecular characterisation of arboviruses circulation in vector populations

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Dengue 2 and Chikungunya viruses were detected within natural populations of *Aedes aegypti* in Burkina Faso

Abstract

In 2016, an entomological study was carried out in a railway transect between Banfora and Ouagadougou, Burkina Faso. The objective was to assess the risk factors of arbovirus outbreaks, including vector-borne infection status within representative regions of the country. *Aedes aegypti* mosquitoes were collected at larval stage from their natural rearing habitats in four study sites when estimating the main larval index, then reared until adult stage and kept in RNAlater for detection of arbovirus RNA. In the lab, mosquito samples were tested for dengue virus (DENV) and Chikungunya virus (CHIKV) using a real-time qRT-PCR stage. A DENV-2 positive pool was detected in Ouagadougou with a minimum infection rate (MIR) of 16.67 and other 6 CHIKV positive pools with a MIR of 66.67 in Ouagadougou, Banfora and Boromo. This qRT-PCR approach, if validated with various samples also comprising wild blood-fed adults, is a useful tool for arbovirus circulation and disease monitoring in Burkina Faso.

Keywords: arboviruses; Dengue; Chikungunya; molecular biology; qRT-PCR; *Aedes aegypti*; Burkina Faso

1 Introduction

Arboviruses are a major public health concern worldwide and have been a source of many outbreaks and epidemics. Over the past few years, some arboviruses like Yellow Fever Virus (YFV), Dengue Virus (DENV), Chikungunya Virus (CHIKV) and Zika Virus (ZIKV) have rapidly expanded globally [1,2]. CHIKV is now disseminated all over the world due to the prevalence of vectors and their effectiveness in the transmission of the virus. Another potential reason for the spread of CHIKV is displacement. Travel habits have increased the importation of the virus to new geographic areas by individuals with viraemia [3]. The epidemiological pattern of CHIKV includes sporadic and epidemic cases in West Africa, from Cameroon to Senegal, as well as in several other African countries (Central African Republic, South Africa, Angola, Nigeria, Democratic Republic of the Congo, South Africa, Malawi, Guinea, and Uganda) [3]. Dengue fever is endemic in over 128 countries according to the World Health Organization [4]. In sub-Saharan Africa, the situation of the arbovirus is mainly characterized by dengue fever epidemics located in certain West African countries [4]. The number of dengue cases reported to WHO increased over 8-fold over the last two decades, from 505,430 cases in 2000, to over 2.4 million in 2010, and 5.2 million in 2019 [4]. CHIKV has spread to almost 40 countries worldwide.

From 1983 to 1986, a study conducted in the western region of Burkina Faso reported a number of arboviruses, primarily flaviviruses, associated with sylvatic *Aedes* species including *Aedes furcifer*, *Aedes luteocephalus*, *Aedes cumminsi*, *Aedes opok*, and *Aedes aegypti* [11]. Since then, in 2016, an outbreak of dengue virus was reported in the human population within Ouagadougou without any clearly incriminated vector [12]. Arboviral infection can be effectively controlled using safe and effective vaccines. Unfortunately, except for the yellow fever vaccine and Rift Valley fever vaccine (available for livestock) no effective vaccine is available for the other forms of arboviruses such as dengue [13]. Preventing or minimizing vector–human contact and/or early detection of virus in the vectors or humans (for *Ae. aegypti*–borne arboviruses) can be key parameters to preventing or minimizing outbreaks. Detecting the presence of arboviruses in natural vector populations has been attempted several times with inconclusive results in some cases suggesting that this approach to arboviruses detection in natural mosquito populations can be challenging [14]. However, to predict

the emergence of arboviral epidemics, the vector monitoring and control programmes should be established in order to monitor and assess the prevalence of virus infection in natural and wild local vector populations. During the dengue outbreak of 2016, the monitoring and control strategy in Burkina Faso had consisted of passive detection of symptomatic human cases and spraying insecticides targeting adult *Ae. aegypti*, the presumed vector for DENV in this outbreak. Targeting the aquatic stages of *Ae. aegypti* was included in the national dengue vector control strategy and this consisted primarily of the systematic removal of artificial breeding sites. As a result, that ultimately leads to the reduction of the adult vectors' population and the possibility of vertical transmission, as revealed by several studies of *Ae. aegypti* populations [15–18]. Indeed, adult stages resulting from the development of aquatic stages being infected by their mothers through transovarian transmission (TOT) are potential reservoirs for arboviruses and may initiate transmission and outbreaks. The aquatic stages that are not reached by spray campaigns can continue residual and autochthonous arbovirus transmission especially around the end of the rainy season, when the adult vector abundance is low [19]. Therefore, the need to incriminate the vector species responsible for the transmission of a particular arbovirus, and to enhance disease monitoring in the vector population, are both important to develop successful outbreak responses.

Within the genus *Aedes*, *Ae. aegypti* is probably the most important vector, it has been incriminated in a large number of arboviral outbreaks [1]. Recent studies conducted in Burkina Faso indicated that of one in four cities along a railway transect, *Ae. aegypti* was the most common vector species collected in the container in domestic and peri domestic areas [20,21]. The abundance of *Ae. aegypti* is associated with a high risk of transmission of DENV and other arboviruses in Ouagadougou, Bobo-Dioulasso, Boromo, and Banfora cities. All stegomyian indices such as the house index (HI), the container index (CI) and the Breteau index (BI) exceeded the risk level associated with arboviral outbreak [20]. However, the mosquitoes have not been processed in the presence of arboviruses.

Real time quantitative polymerase chain reaction (qRT-PCR) in a molecular approach has been used in recent years to detect the presence of arboviruses in Aedes mosquitoes and has proved to be a sensitive tool [22, 23]; we have used this technique to screen the mosquito samples collected during previous entomological monitoring [20] for their infection profile. The ultimate objective is first to validate this approach with our previous samples from 2016 collections and then to extend this approach with wild adults including blood fed females. For entomological monitoring, determining the infection rate in the wild population is crucial and the outbreak risk within the study area could be better understood.

2 Materials and Methods

2.1 Study sites and samples preparation

Samples of *Ae. aegypti* collected in August 2016 during dengue outbreaks in Ouagadougou (12°21′56″N, 1°32′01″W), Bobo-Dioulasso (11° 10' 41.16"N, 4° 17' 30.38"W), Boromo (11° 45' 0.00"N, 2° 55' 60.00"W) and Banfora (10° 37' 60.00"N, 4° 45' 0.00"W), as previously reported in [22] and [23], were analysed (Figure 1). Ouagadougou and Bobo-Dioulasso are urban whereas Banfora and Boromo are peri-urban. In short, at all four study sites, mosquito larvae were sampled from June to November 2016 to determine the stegomyian indices [23]. In each study area and in each house sampled, all containers found inside and outside the houses were inspected for the presence of water, larvae and mosquitoes. Each container was recorded based on type, presence of water, and presence or absence of larvae. From positive containers, the mosquito larvae were pipetted and placed into plastic cups containing water and bearing the house identification number. The larvae

have been enumerated and categorized as *Culicinae* genus. The mosquito reproductive habitats have been grouped into five types: plastic containers, metal containers, terra cotta pots, discarded tyres, and other containers [23]. Subsequently, the extirpated adults were identified at the level of the species genus under binocular magnification (10X). Despite the fact that other species of Aedes have been identified, such as *Ae. vittatus* (found in very small number), only *Ae. aegypti* adults has been analysed for this study. Hence, the females *Ae. aegypti* mosquitoes were segregated into pools depending on the site and placed in a cryo-tube containing RNA later (Qiagen RNAlater RNA Stabilization Reagent, 250 mL, lot No 151026237, Qiagen Straße 1, 40724 Hilden Germany) and stored at -20°C for further laboratory analysis. A total of 580 females grouped in 33 pools of 10 and 20 mosquitoes were screened for the detection of dengue viruses 2 and chikungunya virus in this preliminary data analysis. The analysis focused on DENV2 detection due to the reported results of serological surveys from positive cases detected during the 2016 outbreak in Burkina Faso [23]. The other arboviruses (WNV, DENV1, etc.) will be subjected to subsequent analyses. Samples were prepared for shipment to the Insect Pest Control Laboratory (IPCL), Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, Seibersdorf, Austria for the RNA extraction and qRT-PCR analysis.

Figure 1. Study sites located on railway transect in Burkina Faso (Banfora, Bobo-Dioulasso, Boromo, Ouagadougou).

2.2 Total RNA virus isolation

Total RNA was extracted from mosquitoes using the TRIzol ™ Reagent (Invitrogen, Waltham, MA, USA, Catalog Numbers 15596026 and 15596018) as directed by the supplier. Depending on the size of the pool, adult mosquitoes were homogenized into 1 ml TRIzol using a sterile pestle. All pools were individually homogenized by vertexing for 15 s and incubated for 5 min at ambient temperature to allow complete dissociation of the nucleoprotein complex. Chloroform (200 µl per 1 ml of TRIzol™ Reagent) was added to the mixture, and homogenization was performed by shaking the tubes vigorously for 15 s by hand. The mixture was then incubated at ambient temperature for 2–3 min. The samples were centrifuged at 12.000 g for 15 min at 4 °C. The mixture splits into a weaker red phenol-chloroform, and interphase, and a colourless superior aqueous phase. The aqueous phase of each sample was removed and transferred to a new tube containing 500 µl of 100% isopropanol by angling the tube at 45° and pipetting the solution out. This mixture was incubated at ambient temperature for 10 min and then centrifuged at 12.000 g for 10 min at 4 °C. Total RNA precipitate formed a white gel-like pellet at the bottom of the tube. The supernatant was removed, and the RNA pellet was washed with 1 ml of 75 % ethanol mixing occasionally by gentle inversion. The samples were homogenized briefly and then centrifuged at 7,500 g for 5 min at 4 °C. The supernatant was discarded, and the RNA was then air-dried for 15 min. The RNA pellet was resuspended in 50 µl of RNase-free water by pipetting up and down; then incubated in a water bath or heat block set at 55 °C for 10-15 minutes. The quantity and quality of RNA samples were determined using a Synergy™ H1 microplate reader (BioTek, Winooski, Vermont, USA). The RNA samples were serially diluted in ten-fold steps from 10 to 0.0001 ng/µl for a concentration that would consistently give the same amount per well in the qRT-PCR and were stored at − 80 °C.

2.3 Generation of RNA standard for the qRT-PCR assays

The standard curve for DENV-2 virus was conducted according the method described by Tang et al. [24]. In brief, the RNA sequences containing and flanking the sequence regions of the virus specific primers and probes of CHIKV and DENV2 were amplified using the primers listed in Table 1 and

cloned into pGEM-T vector (Promega, Madison, Wisconsin, USA). The primers in Table 1 have been selected using the primer3 software with the default setting (http://bioinfo.ut.ee/primer3-0.4.0/) and the sequence of CHIKV (GenBank: NC004162), and DENV2 (GenBank: MN294937). These plasmids were linearized through digestion with EcoRI and the target sequences were amplified using the in vitro RNA transcription kit (Roche Diagnostics, IN, USA). The plasmids containing the cDNA of two arboviruses collected from blood samples from positive patients were treated with the Fast-Media® LB Amp. They were extracted and purified using the QIAprep® Miniprep purification kit (Qiagen). The SuperScript® III First-Strand Synthesis System for RT-qPCR (Invitrogen) was used to synthesize first-strand cDNA from purified poly(A)+ or total RNA. The targeted sequences were amplified by Taq PCR Master Mix (Qiagen) with the following PCR conditions: 5 min at 94 °C; 35 cycles of 30 s at 94 °C, 30 s at 58 °C and 1 min at 72 °C; and 10 min at 72 °C. The PCR product was purified using the High Pure PCR Clean-up Micro Kit (Roche, Basel, Switzerland). The cDNA was quantified using a Synergy™ H1 microplate reader (BioTek, Winooski, Vermont, USA). The copy number of the cDNA was calculated based on the concentration and its molecular weight using NEBioCalculator (https://nebiocalculator.neb.com/#!/dsdnaamt) then 7 concentrations with known DNA copy numbers/µl were prepared by serial dilutions and used as standard in all qRT-PCRs. A series of dilutions of seven known concentrations were used as a positive control for each qRT-PCR reaction. Sterile, nuclease-free water was used as a no template control (NTC).

2.4 ARBOV RNA detection by qRT-PCR assays

ARBOV detection was performed by quantitative RT-PCR using a C1000 Touch^{R} Thermal Cycler machine (CFX96R Real-Time System from BIO-RAD). One step real-time qRT-PCR was conducted using ARBO virus-specific primers and TaqMan probes previously reported to detect each specific virus were synthesized by the Eurofins Genomics with 5-FAM, HEX as the reporter dye for the probe. The details of the primers and probes sequences and their characteristics are summarized in Table 2 [25,26]. Using Quantitect Probe qRT-PCR kit (Qiagen), qRT-PCR reactions were performed in a 20 µl volume mixture containing 2 µl of RNA template, 10 µl of 2× QuantiTect Probe qRT-PCR Master Mix, 8 µM of forward primer, 8 µM of reverse primer, 2.5 µM of probe, 0.25 µl of QuantiTect RT Mix and 4.95 µl RNase-free water. The primers and probes have been double tested. qRT-PCR cycling included a single cycle of reverse transcription of 30 min at 50 °C, followed by 15 min at 95 °C for reverse transcriptase inactivation and DNA polymerase activation, and then 45 cycles of 15 s at 94 °C and 60 s at 60 °C (annealing-extension step). The quantity of viral RNA in each sample was estimated by comparing the threshold values for cycle quantification (Cq) to the standard curve for each qRT-PCR test. The number of viral copies in each positive sample was estimated by qRT-PCR using absolute quantification by the standard curve method and reported as viral RNA copies/µL (of the eluted RNA). For each qPCR test, both negative (RNA-free water) and positive sample (RNA sample tested positive for each virus) were added to ensure that no contamination occurred.

2.5 Data analysis

Real-time data was analysed using the CFX managed software version 3.1 supplied by Bio-Rad. Negative and positive controls were included in all PCR reactions conducted. A sample was determined empirically to be positive if the cycle quantification (Cq) value was lower than 36, based on background cross-reactivity of the primers and probes in non-template control reactions. Positive results were determined according to the amplification cycle at which the relative fluorescence unit (RFU) was detected below the cycle quantification.

The Excel add-in PooledInfRate v.4 statistical software package was used to calculate the minimum infection rate (MIR) of arboviruses in mosquitoes with a 95% confidence interval [27]. Only the positive pools of female *Aedes* mosquitoes have been calculated for their MIR. The MIR was estimated based on the number of female mosquitoes in a positive pool divided by the number of female mosquitoes tested multiplied by 1000. The MIR uses the assumption that a positive pool contains only one infected mosquito. The statistical analysis was carried out by the means of the exact Fischer test. Data was compared by sites and also following two types of area such as urban representing the two big cities (Ouagadougou and Bobo-Dioulasso) and peri-urban, which are also cities but considerably smaller than the first ones mentioned above (Banfora and Boromo).

3 Results

3.1 Quantification of RNA isolated and ARBOV detection from fields mosquitoes

The RNA concentrations were 11.53 ng/µl and 490.98 ng/µl in pool of 10 and 20 mosquitoes respectively. The ratio of A260:A280 was approximately 2 values with RNA purity extracted from the samples.

Above the cycle threshold (Cq) of 36 of the amplified RNA samples showing curves were considered to be very low concentrations and, as result, the samples were not considered to be infected by the targeted virus. Following the protocol described by Tang et al. [24], the purified plasmid was used for each virus and a DNA concentration with a known copy number of 4.7 × 109 was prepared. Subsequently, 10-fold serial dilutions in water were used to prepare seven DNA concentrations with copy numbers ranging from 4.7 × 108 to 4.7 × 102 per ml, which were used to prepare the calibration curves for primer and probe for DENV2. Viral DNA detection has been a success for both viruses and standard curves have shown a linearity of more than 7 orders of magnitude (Figure 2). The correlation coefficient (R2 value) was 0.998, for DENV2. The calibration curves for CHIKV were previously described per Tang et al. [24], where the correlation coefficient (R2 value) was 1.000. The regression coefficients (R2) of 0.998 and 1.000 for DENV2 and CHIKV, respectively, indicated the high reproducibility of the test.

In addition, the quantification cycle (Cq) values for the standard DENV2 concentration of seven series dilutions ranged from 11±0.02 to 33.5±0.065 Cq (Figure 2). Samples taken in Ouagadougou for DENV-2 showed a Cq amplifying curve of 32 indicating that the samples were infected with DENV2 (Figure 3). Otherwise, the quantitative cycle values of the standard CHIKV concentration of seven dilutions ranged from 10±0.05 to 30±0.21 Cq as previously described by Tang et al. [24]. The amplification curves of RNA samples tested for detecting CHIKV started at 28 Cq and reached 35 Cq (Figure 4) indicating the presence of RNA virus at different densities varied based on samples tested. All samples analysed for CHIKV detection were less than 36 Cq (Figure 4), therefore were all considered positive.

Figure 2. Amplification and standard curves of serial dilution of plasmid containing the sequence targeted by the primers and probes for the qRT-PCR for DENV2 detection: A) the correlation between the relative florescent unit (RFU) and the quantification cycle (Cq) and B) the virus log 10 copy number and the Cq. The regression equations and correlation coefficients (R) are given for DENV2 plot.

Figure 3. Positive RNA samples from Ouagadougou for detection of dengue virus 2 (DENV2) RNA by one step real-time qRT-PCR assay.

Figure 4. Positive RNA samples from Ouagadougou, Boromo and Banfora for detection of chikungunya virus (CHIKV) by real-time qRT-PCR assay.

3.2 Spatial distribution and infection rates of arbovirus

A total of 27 pools of *Aede*s mosquitoes were tested for arboviruses. The mosquitoes were collected in August 2016, corresponding to the time of a dengue outbreak in Burkina Faso. Overall, 480 *Ae. aegypti* were tested (**Table 3**). The qRT-PCR analysis showed the presence of arboviruses with one pool positive for DENV-2 (3.7%) and six pools positive for CHIKV (22.22%); but density varied depending on locality (**Table 3**). Indeed, the minimum infection rates (MIR) of CHIKV in *Aedes* mosquitoes were estimated at 66.67 (95%CI:41.2—72.6) (4 infected pools/60 samples tested); 0 (0 infected pool/140 samples tested); 7.14 (95%CI :4.3—10.2) (1 infected pool/140 samples tested) and 7.14 (95%CI:4.3—10.2) (1 infected pool/140 samples tested) in Ouagadougou, Bobo-Dioulasso, Banfora and Boromo, respectively (**Table 4**). The MIR of CHIKV was significantly higher in Ouagadougou compared to the other locations (P = 0.0219). The MIR of DENV-2 was estimated at 16.67 (95%CI: 14.7—19.4) (1 infected pools/60samples tested) in Ouagadougou while no detection has been recorded in the other sites (**Table 3 and 4**). When the study sites were categorised based on their predominant land use type (urban or peri-urban), MIR differed. In urban areas, the MIRs of DENV-2 was 5 (95%CI :3.7—6.9) and for CHIKV was 20 (95%CI :17.5—26.1), both higher than in peri-urban sites (P<0.01) (**Table 4**). The data also showed a co-infection of arboviruses (DENV/CHIKV) in one pool from Ouagadougou (**Table 3 and 4**).

4 Discussion

This study is the result of entomological investigations conducted in 2016 in urban and peri-urban areas along a rail transect from Banfora to Ouagadougou during a dengue epidemic in Burkina Faso. In the first published article on this study, we found that all the larval indices that have been examined had largely exceeded the critical level for outbreaks in the four cities where the study has been conducted. This implies an increased transmission risk for at least DENV including the peri-urban areas such as Boromo and Banfora [20]. Current evidence builds on the previous findings by identifying and quantifying the presence of arbovirus in wild *Ae. aegypti* populations. In fact, DENV-2, and CHIKV have been detected in certain pools with pronounced rates of infection recorded especially in Ouagadougou for DENV-2. Although CHIKV infection was also detected in Boromo and Banfora, its prevalence was higher in Ouagadougou indicating probably that these viruses were more concomitantly circulating in the capital city than in the other study areas.

Globally the infection features described in *Ae. aegypti* populations confirmed the autochthonous urban transmission of dengue in urban cities including Ouagadougou. They were more or less consistent with human infection patterns where serotypes DENV-2 and DENV-3 were most detected in humans [28–32]. However, in exceptional cases, DENV-4 was reported by Ridde et al. [23] at the

same time as DENV-2 and DENV-3. The presence of DENV-2 was also found in the blood of febrile travellers returning from Burkina Faso to France during this period [28].

The findings also showed the occurrence of CHIKV in the vector populations from three of the four sites sampled. This was more prevalent in Ouagadougou than the other areas. No data has previously confirmed their presence in human patients, probably due to the lack of specific serological tests targeting these viruses during the diagnosis process.

This is the first study confirming the circulation of arboviruses in vector populations both from urban and peri-urban areas in Burkina Faso. In 1993, the sylvatic circulation of arbovirus was confirmed by the isolation of both DENV-2 and CHIKV in the surrounding forest gallery of the Bobo-Dioulasso region from wild *Aedes* mosquitoes such as *Ae. furcifer*, *Ae. luteocephalus*, *Ae. cumminsi*, *Ae. opok*, and in Bobo-Dioulasso city for DENV-2 from *Ae. aegypti* [13]. The epidemiology of arboviruses in Africa in the last five years has shown an expansion of certain arboviruses such as West Nile, Chikungunya, Zika in West Africa [29–34].

Our findings confirm a vertical transmission of arboviruses [16-17] as well as DENV-2 and CHIKV in *Ae. aegypti* populations of urban and peri-urban settlements which has been reported from earlier studies [18-19]. Vertical transmission (infected female mosquito-infected offspring) is assumed to be a mechanism ensuring the maintenance of the virus during conditions that would be adverse for horizontal transmission (harsh winters, inter-epidemic stages) [35] and can also potentially accelerate the epidemiology of arboviruses if the monitoring system is not sufficiently reliable. Although it is mainly the horizontal transmission (human-mosquito-human) of arboviruses that determines the epidemiology of the disease, health authorities should therefore be vigilant for future outbreaks. Furthermore, a co-infection (DENV / CHIKV) was detected in a pool of *Aedes* from Ouagadougou highlighting the co-circulation of both arboviruses. Even if, our control system focused on the larval stages providing vertical transmission, it can support autochthonous transmission if the larval indices and the domestic index reach the threshold. In addition, it should be noted that while using field-collected larvae and keeping them in the lab until the adult emergence for the detection of arboviruses was successful, it may not be the best approach for disease surveillance because it may lose infection in dead larvae or pupae before adult emergence. The greatest limitation of this study is that no sample of mosquitoes directly collected in the field at adult stage that can include blood fed females was considered in the analysed samples due to the collection method. As such, the data presented here are more observational and preliminary and the conclusion needs to be observational and not yet predictive until we expand the testing for adult field mosquitoes. Furthermore, the annual recurrence of dengue outbreaks in Burkina Faso over the last five years increases the interest in developing such a molecular diagnostic approach for vector surveillance. Indeed, the growth of international travel to and from Burkina Faso, especially from passengers arriving from other countries where arboviruses are endemic, should be seen as a potential reservoir of virus. These conditions supports the circulation of arbovirus in Burkina Faso. During the rainy season, this vertical transmission could be the source of a resurgence of dengue.

Conclusion

Our study found that DENV-2 and CHIKV, both in urban and peri-urban areas, were detected from wild *Ae. aegypti* larvae bred to adulthood. This highlighted the occurrence of a vertical transmission and leads us to recommend the urgent need to implement a vector control programme including larval source reduction in Burkina Faso, instead of only fogging adult *Ae. aegypti* populations with insecticides, whose effectiveness in the field has not been assessed after intervention. This molecular approach is the first successful investigation using molecular diagnostic in Burkina Faso to detect and quantify arboviruses within wild mosquito populations during outbreak periods even from adults bred from wild larva collections. This tool is powerful and suitable and should be adopted by the National monitoring programme of arboviruses if extended to adult mosquitoes directly collected in the field including blood fed females, it remains specific and suitable. Nevertheless, the results are preliminary and should be seen as indicative until it becomes validated by wide-scale and large spectrum of samples.

Author Contributions

DKR, VR, EB and AA conceived and designed the experiments. LEPO, IS, SSP, SDD participated in the field collection. AH, AA, CK and AP performed the RT-qPCR to detect arboviruses. AH, CK, LEPO, AA and DKR analysed the data. AH, LEPO, IS and DKR wrote the paper. A. S. H., J.B., F. M. H., A.D., A.A., R. K. D contributed to the paper writing and editing. All authors read and approved the final version of the manuscript.

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Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Table 1. Flanking primers used for viral cloning in pGEM T vector as plasmid.

Oligo name	Sequence (5'-3')	Size (bp)	Genome position
DENV2_1358F	TAACACCTCACTCAGGRGAAGAG	684	1358-1381
DENV2_1667R	TGGGGATTTTTGAAAGTGACCAATG		3939-3959
CHIKV_645F	GTGCCTACCCCTCATACTCG	553	645–664
CHIKV_1198R	CCGTTGCGTTCTGCCGTTA		1180–1198

Table 2. Oligonucleotide primers and fluorogenic probes used in real-time qRT-PCR assay.

Oligo names	Nucleotides sequences	Position in genome	Fluorophore	Reference
DEN-2F (forward)	-CAGGTTATGGCACTGTCACGAT-	1605		(25)
DEN-2C (reverse)	-CCATCTGCAGCAACACCATCTC-	1583		
DEN-2 probe	-CTCTCCGAGAACAGGCCTCGACTTCAA-	1008	HEX/BQ-1	
CHIKV874 (forward)	-AAAGGGCAAACTCAGCTTCAC-	874		(26)
CHIKV961 (reverse)	-GCCTGGGCTCATCGTTATTC-	942		
CHIKV899 probe	-CGCTGTGATACAGTGGTTTCGTGTG-	899	FAM/BHQ-1	

480 **Table 3.** Summary of arboviruses examination of pools (10-20 size) of *Aedes* mosquitoes collected in
481 Burkina Faso.

Localities	Number of pools tested	Total number of mosquitoes	Positive pool after qRT-PCR N (%)	
			DENV2	CHIKV
Banfora	7	140	0	1 (14.28)
Bobo-Dioulasso	7	140	0	0
Boromo	7	140	0	1 (14.28)
Ouagadougou	6	60	1 (16.67)	4 (66.67)
Total number	**27**	**480**	**1 (3.7)**	**6 (22.22)**

482

483 **Table 4.** Minimum infection rates (with 95% Confidence interval) of targeted arboviruses screened
484 in *Ae. aegypti* mosquitoes.

Localities	DENV2 (95%CI)	CHIKV (95%CI)
Banfora	0	7.14 (4.3—10.2)
Bobo-Dioulasso	0	0
Boromo	0	7.14 (4.3—10.2)
Ouagadougou	16.67 (14.7—19.4)	66.67 (41.2—72.6)
Urban	5 (3.7—6.9)	20 (17.5—26.1)
Sub-urban	0	7.14 (5.4—12.8)

485