Acta Tropica 137 (2014) 123–129

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

# Risk assessment of flavivirus transmission in Namibia

Bruce H. Noden[a,*], Milka Musuuo[b], Larai Aku-Akai[b], Berta van der Colf[a], Israel Chipare[c], Rob Wilkinson[c]

[a] Department of Biomedical Science, School of Health and Applied Science, Polytechnic of Namibia, Windhoek, Namibia
[b] Department of Environmental Health, School of Health and Applied Sciences, Polytechnic of Namibia, Windhoek, Namibia
[c] Blood Transfusion Service of Namibia, Windhoek, Namibia

ARTICLE INFO

*Article history:*
Received 23 January 2014
Received in revised form 12 May 2014
Accepted 15 May 2014
Available online 25 May 2014

*Keywords:*
Dengue virus
Flavivirus
Seroprevalence
*Aedes*
*Culex*
Namibia

ABSTRACT

The role of arboviruses causing acute febrile illness in sub-Saharan Africa is receiving more attention. Reports of dengue in tourists were published nearly 10 years ago in Namibia, but the current epidemiology of arboviruses is unknown and surveys of mosquito vectors have not been carried out since the 1950s. To begin addressing this knowledge gap, a prospective cross-sectional study was conducted using samples from volunteer blood donors linked to questionnaire. Serum samples were tested using a Dengue IgG Indirect ELISA which measured exposure to dengue virus/flaviviruses. Entomological samples were collected from tires during the rainy season (February–March 2012) in six locations across Namibia's capital city, Windhoek. Among 312 blood donors tested, 25 (8.0%) were positive for dengue virus/flavivirus exposure. The only significant risk factor was age group with high exposure rates among those older than 50 (29%) compared with those below 40 years old (between 2.9% and 8.3%) ($P < 0.002$). Larvae and pupae of *Aedes aegypti* and *Culex pipiens* complex accounted for 100% of the 2751 samples collected, of which only 12.2% ($n = 336$) were *Ae. aegypti*. Each site demonstrated high variability of species composition between sampling times. While the significant dengue virus/flavivirus exposure rate among those above 50 years old is likely indicative of the West Nile epidemic in the 70s and 80s, the low exposure among those under 50 suggests that flaviviruses are still circulating in Namibia. While *Ae. aegypti* and *C. pipiens* sp. may play a role in future epidemics, the significance of presence may be reduced due to short rain periods, dry, arid, cold winters and policies and social understandings that limit non-structured storage and use of tires in low income areas. Future studies should further characterize the circulating arboviruses and investigate mosquito ecology nationally to map areas at higher risk for future arbovirus outbreaks.



## 1. Introduction

The role of arboviruses in acute febrile illness in sub-Saharan Africa is receiving more attention, especially as countries make measurable strides toward malaria elimination (Hertz et al., 2012; Maina et al., 2012; Sokhna et al., 2013; Vairo et al., 2012). This is especially critical in Southern Africa where four countries (Namibia, Botswana, South Africa, and Swaziland) are actively working to eliminate malaria (Cotter et al., 2013). Information on arbovirus epidemiology is needed to develop local diagnostic and treatment algorithms, especially in regions with large numbers of immune compromised individuals, such as persons living with HIV (Hertz et al., 2012; Kasper et al., 2012).

The epidemiology of arboviruses in Namibia has not been evaluated since the country gained independence in 1990. In the late 1950s (Kokernot et al., 1965) and mid-1980s (reviewed by Noden and van der Colf, 2013), arbovirus exposure to Chikungunya, Germiston, Rift Valley Fever, Crimean-Congo Hemorrhagic Fever, and West Nile viruses was reported among people living in northern Namibia. Since the 1980s, outbreaks of Rift Valley Fever virus and Crimean-Congo Hemorrhagic Fever virus have occurred but no published follow-up has characterized the epidemiology or ecology of these pathogens with a goal to prevent future epizootics (Noden and van der Colf, 2013).

In 2011, a review of dengue virus epidemiology in Africa indicated that *Aedes aegypti* mosquitoes were found in Namibia and dengue cases had been diagnosed in traveling tourists from the early to mid-2000s (Amarasinghe et al., 2011; Were, 2012). The

* Corresponding author. Current address: Department of Entomology and Plant Pathology, Oklahoma State University, Stillwater, OK, United States.
*E-mail addresses:* bruce.noden@okstate.edu, noden4@gmail.com (B.H. Noden), milkamusuuo@gmail.com (M. Musuuo), lakuakai@polytechnic.edu.na (L. Aku-Akai), bvandercolf@polytechnic.edu.na (B. van der Colf), Israel@bts.com.na (I. Chipare), robwilkinson795@gmail.com (R. Wilkinson).

http://dx.doi.org/10.1016/j.actatropica.2014.05.010
0001-706X/

dengue data was reported by Wichmann et al. (2003), who summarized confirmed and probable (IgM positive serology) dengue infections among German travelers reported by the European Network on Imported Infectious Disease Surveillance (TropNetEurop) and the German Surveillance Network on Imported Infectious Diseases (SIMPID). While *Ae. aegypti* was reported in Namibia in the 1940s (Noden and van der Colf, 2013), the Namibian Ministry of Health and Social Services has not been actively tracking dengue infections. With the recent outbreak of dengue in Angola (Schwartz et al., 2013), there is a critical lack of any baseline data in Namibia on which to base risk assessment.

To address this gap between tourist-based studies, possible cross-border infection from the Angolan epidemic and the lack of any information on the local epidemiology of arboviruses and vector mosquitoes in Namibia, this study was initiated to assess the risk of possible dengue virus transmission. This was done by: (1) evaluating the exposure of a healthy population of Namibians to dengue virus/flavivirus using an indirect enzyme immunoassay (EIA); and (2) surveying discarded auto tires for vector mosquito species around the capital city of Windhoek. The focus was to determine whether dengue virus or other flaviviruses were present in a population of permanent Namibian residents, and to evaluate whether an outbreak could occur in Windhoek during the rainy season through mosquitoes breeding in tires.

## 2. Methods and materials

### 2.1. Ethical considerations

The study was approved by the Permanent Secretary and the Research Committee of the Ministry of Health and Social Services (MoHSS) of Namibia, and by the Blood Transfusion Service of Namibia (NAMBTS). All participants provided written informed consent and the parent/guardian of any participant under 18 provided written informed consent on their behalf. Blood donors' identifying information was held by NAMBTS. The research team only received the completed surveys and two vials of donor serum, both of which were linked by a unique donor ID number.

### 2.2. Study population and study design

A prospective cross-sectional sample of volunteer blood donors was collected between September 2011 and February 2012. All samples were collected by The Blood Transfusion Service of Namibia (NAMBTS) at one of 20 fixed or mobile donation clinics throughout Namibia. The samples collected were part of a broad sero-survey for exposure to viral, bacterial and protozoan zoonotic pathogens among Namibian volunteer blood donors. Due to a focus on exposure to various zoonotic pathogens, we attempted to oversample for volunteer blood donors with rural or farm experience. However, most Namibian blood donors live in urban centers and frequently visit farms and rural areas. As such, it was appropriate to test the donor samples for exposure to what is normally considered to be an 'urban' associated virus because the majority of donors live in urban areas most of the time. The study sample size ($n = 319$) was established using EpiInfo 6.0 (CDC, Atlanta, GA, USA), using an estimated prevalence of 30% (based on flavivirus exposure in Kavango region, Namibia (Joubert et al., 1985), an absolute error of 0.5 and a 95% confidence interval (95% CI). While exposure can vary by region and could, thus, have affected sample size, the prevalence used is considered one of the highest in the region and therefore, the sample size is sufficient for such a study. A questionnaire was used to capture information about blood donors' risk factors for exposure to dengue virus or other flaviviruses. Only those samples that included an accompanying questionnaire were tested for exposure to pathogens.

### 2.3. Sample selection

Inclusion criteria included healthy individuals (first time or repeat donors) who passed the NAMBTS selection criteria (Vardas et al., 1999). Volunteer blood donors are commonly used to evaluate exposure to bacterial pathogens (Dupont et al., 1995; Hogema et al., 2012; Kelly et al., 1991; Kilic et al., 2008; Niang et al., 1998; Sun et al., 2010), however, prevalence estimates from these studies are considered conservative since blood donors are normally younger, healthier and screened for other significant pathogens (Letaief et al., 1995; Mansueto et al., 2012; Negri et al., 2013).

A study consent form in English or Afrikaans was provided to each volunteer before donating blood. After reading and discussing the form with NAMBTS staff, donors were asked if they wanted to participate. If they agreed, a short questionnaire (English or Afrikaans) was completed which included questions on demographics such as gender, age, region, and the general area where they lived (city, peri-urban or rural) and an additional 4 ml of blood was drawn during their donation session.

### 2.4. Serum samples

After donation, blood samples were transported to the NAMBTS headquarters in Windhoek where they were processed and serum components were divided into two 2 ml vials, each labeled with a unique patient identification number, and stored at −20 °C until picked up for testing at the Polytechnic of Namibia.

### 2.5. Serological testing

Serological testing on the serum samples was performed using a Dengue IgG Indirect ELISA kit (PanBio, Inverness Medical, Queensland, Australia) for the qualitative detection of IgG antibodies to dengue antigen serotypes 1–4 in clinical samples as well as for past exposure. Protocols followed manufacturer's instructions, including the cutoff calibrator instructions. Following instructions, all samples were screened at 1:100 dilution. All positives and equivocals were confirmed with a second round of ELISA testing. While recognizing that RNA amplification by PCR is normally used to prove dengue infection (Hertz et al., 2012), as per the manufacturer's instructions ("in areas where multiple flaviviruses co-circulate, the presence of crossreactive flavivirus antibodies should be considered"), we recognize that a positive sero-sample is only indicative of prior flavivirus exposure and not necessarily dengue. Confirmatory tests (PCR and/or more specific antibody tests) on the samples and tests for other possible flaviviruses such as Usutu or West Nile viruses were not run.

### 2.6. Study area and collection sites

Entomological surveys were conducted between February and March during the peak of the rainy season in 2012 in Central Namibia (Namibia Weather, 2014) in six urban and peri-urban locations in Windhoek. Sites were chosen based on the presence of unused tires in the vicinity, the willingness of the property owners to allow sampling to take place, as well as their location and environment (proximity to riverbeds and human dwellings) within the city boundaries. While other containers types were considered, other studies have demonstrated that tires provide a good indicator of major mosquito species in the sites with minimal engagement of sampling communities (Yee, 2008; Yee et al., 2010). All sites were open to direct sunlight. Three peri-urban sites were identified in Katutura (Havana settlement, Singles

**Table 1**
Characteristics of the Namibian study population and bivariate logistic regression analysis of risk factors associated with exposure to dengue/flaviviruses.

| | *N* (%) | Exposure (1:100 dilution) | OR (95% CI) | *P*^ |
|---|---|---|---|---|
| Gender | | | | |
| Males | 196 (62.8%) | 20/196 (10.2%) | 2.52 (0.92–6.92) | 0.072 |
| Females | 116 (37.2%) | 5/116 (4.3%) | 1 | |
| Age groups | | | | |
| Under 20 | 39 (12.5%) | 2/39 (5.1%) | 0.14 (0.02–0.75) | 0.022 |
| 20–29 | 103 (33.0%) | 3/103 (2.9%) | 0.08 (0.02–0.33) | 0.001 |
| 30–39 | 96 (30.8%) | 8/96 (8.3%) | 0.23 (0.07–0.75) | 0.015 |
| 40–49 | 53 (17.0%) | 6/53 (11.3%) | 0.32 (0.09–1.14) | 0.078 |
| 50 and above | 21 (6.7%) | 6/21 (28.6%) | 1 | |
| Areas[a] | | | | |
| North | 23 (7.4%) | 2/23 (8.7%) | 1.32 (0.17–10.43) | 0.796 |
| Central | 273 (87.5%) | 22 (8.1%) | 1.43 (0.12–17.23) | 0.779 |
| South | 16 (5.1%) | 1/16 (6.3%) | 1 | |
| Residence | | | | |
| Rural | 54 (17.6%) | 6/54 (11.1%) | 1.48 (0.56–3.91) | 0.424 |
| Urban | 252 (82.4%) | 19/252 (7.5%) | 1 | |
| Interacted with animals on a regular basis? | | | | |
| Yes | 301 (96.5%) | 25/301 (8.3%) | 0.00 | 1.00 |
| No | 11 (3.5%) | 0/11 (0%) | 1 | |
| Worked with animals on farm/hunting? | | | | |
| Yes | 114 (36.5%) | 10/114 (8.8%) | 1.16 (0.5–2.7) | 0.829 |
| No | 198 (63.5%) | 15/198 (7.6%) | 1 | |

[a] North (Caprivi, Ohangwena, Oshikoto, Oshana, Omusati, Kunene); central (Otjozondjupa, Erongo, Khomas, Omaheke); south (Hardap).
^ *P* values less than 0.05 were considered statistically significant.

Quarters, and Malaka Dry), and three urban sites were identified within the Windhoek city limits (Khomasdal, Ausspannplatz, and Eros).

The Katutura sites were chosen for their location and proximity to human dwellings. The Havana site was in a small shrub-filled valley next to a primary school on the edge of the settlement area. The tires at this site were part of an erosion control plan for the primary school property, so the tire numbers changed as they were used. The Singles Quarters site was inside the central commercial area of Katatura with piles of tires arranged in the front fenced-in area of a homestead. The Malaka Dry site was also centrally located in Katatura with randomly arranged tires in a fenced-in property with small houses located around a main house.

The Khomasdal site was chosen for its higher degree of urbanization and location near a riverbed. The Ausspannplatz site was in downtown Windhoek in a busy business district. The tire pile sampled at a used car dealership was located in an alley behind an office building. The Eros site was residential property next to a wilderness area on the edge of a middle-income area.

### 2.7. Mosquito sampling

Samples were taken only from discarded tires filled with water that could serve as mosquito breeding sites. The tires sites were within 10 m of the houses, schools or businesses connected to the properties. To maximize the chances of finding larvae and pupae, collections were made during the rainy season. An additional visit to each site was made after the winter months at the beginning of the dry season to confirm the absence of mosquitoes after the cold, dry winter. During each visit, 10 tires were randomly selected for sampling using a hand pump. Tires were agitated before sampling to maximize collection of bottom dwelling larvae. Containers containing water from each site were labeled. At the laboratory, larvae and pupae from each site were placed into plastic containers and labeled according to the sampling location. Water used for rearing came from the tires from each site and flakes of commercial fish food were sprinkled every two days for a food source. Larvae and pupae were grown to adults before identification. Emerging adults were identified using Walter Reed Biosystematics Unit online keys (WRBU, 2011).

### 2.8. Statistical analysis

All data were entered into Microsoft Excel spreadsheets and analyzed using SPSS (version 21, IBM, Armonk, NY, USA). For categorical data, we used Pearson's $\chi^2$ tests or Fisher's exact tests (when values were less than five). Bivariate logistic regression analyses were used to assess associations between population characteristics (from the survey) and seropositivity results. Odds ratios (OR) and 95% confidence intervals (CI) were calculated. *P* values less than 0.05 were considered statistically significant.

## 3. Results

### 3.1. Study population

The majority of people tested were male and lived in the Central regions of Namibia (Table 1). Most lived in urban areas and were between the ages of 20–29 and 30–39.

### 3.2. Prior flavivirus exposure

Anti-DENV IgG serological testing was performed for 312 participants, of which 25 (8.0%) were positive (Table 1). The only significant risk factor was age group. Blood donors 50 years old and older had significantly higher exposure than those below 50 years old ($P < 0.002$). The mean age of positive samples was $40.2 \pm 12.4$, which differed significantly from those not exposed ($31.4 \pm 10.8$) ($P < 0.001$). Gender, region, or residence were not significant.

### 3.3. Mosquito survey

Larvae and pupae of *Ae. aegypti* and *Culex pipiens* complex accounted for 100% of the 2751 individual larvae or pupae collected, of which only 12.2% ($n = 336$) were *Ae. aegypti*. Each site demonstrated high variability of numbers of mosquitoes and proportions

**Table 2**
Comparison of *Aedes* and *Culex* mosquito densities from six sites[a] during February–March 2012.

| Area of Windhoek | Neighborhood | Month | *Aedes aegypti* N (% total) | *Culex pipiens* N (% total) |
|---|---|---|---|---|
| Katatura | Havana | February 2012 | 5 | 692 |
| | | March 2012 | 0 | 314 |
| | | Total | 5 (0.5%) | 1006 (99.5%) |
| | Singles Quarters | February 2012 | 21 | 109 |
| | | March 2012 | 64 | 34 |
| | | Total | 85 (37.3) | 143 (62.7%) |
| | Malaka Dry | February 2012 | 51 | 0 |
| | | March 2012 | 36 | 41 |
| | | Total | 87 (68.0%) | 41 (32.0%) |
| Khomasdal | Khomasdal | February 2012 | 11 | 0 |
| | | March 2012 | 102 | 0 |
| | | Total | 113 (100%) | 0 |
| Windhoek central | Ausspannplatz | February 2012 | 2 | 1 |
| | | March 2012 | 1 | 42 |
| | | Total | 3 (6.7%) | 43 (93.3%) |
| Eros | Eros | February 2012 | 35 | 0 |
| | | March 2012 | 10 | 1183 |
| | | Total | 45 (3.7%) | 1183 (96.3%) |

[a] Sampling from 10 tires in each site.

of species collected between sampling times (Table 2). It was common to find different larval stages of both *Ae. aegypti* and *Culex* in the same tires at many of the sites. The Havana and Eros sites contained large populations of *C. pipiens*, while Ausspannplatz totals were considerably lower for both species. Differences between populations at each site demonstrated possible competitive relationships (Fig. 1; Table 2). The Havana, Eros, and Ausspannplatz sites had limited numbers *Ae. aegypti* in February but none or smaller numbers in March. No Culex sp. were identified in the Khomasdal site during any sampling period. The Malaka Dry site yielded no *Culex* in February but, by March, *Culex* accounted for almost half of the larvae and pupae collected at the same location.

## 4. Discussion

This is the first study to evaluate exposure of a healthy population to flavivirus in Namibia since the country gained independence in 1990. It is also the first since the 1950s to focus on mosquitoes breeding in Windhoek (De Meillon and Hardy, 1953; Muspratt, 1956). Prompted by a recent cross-over border case of dengue

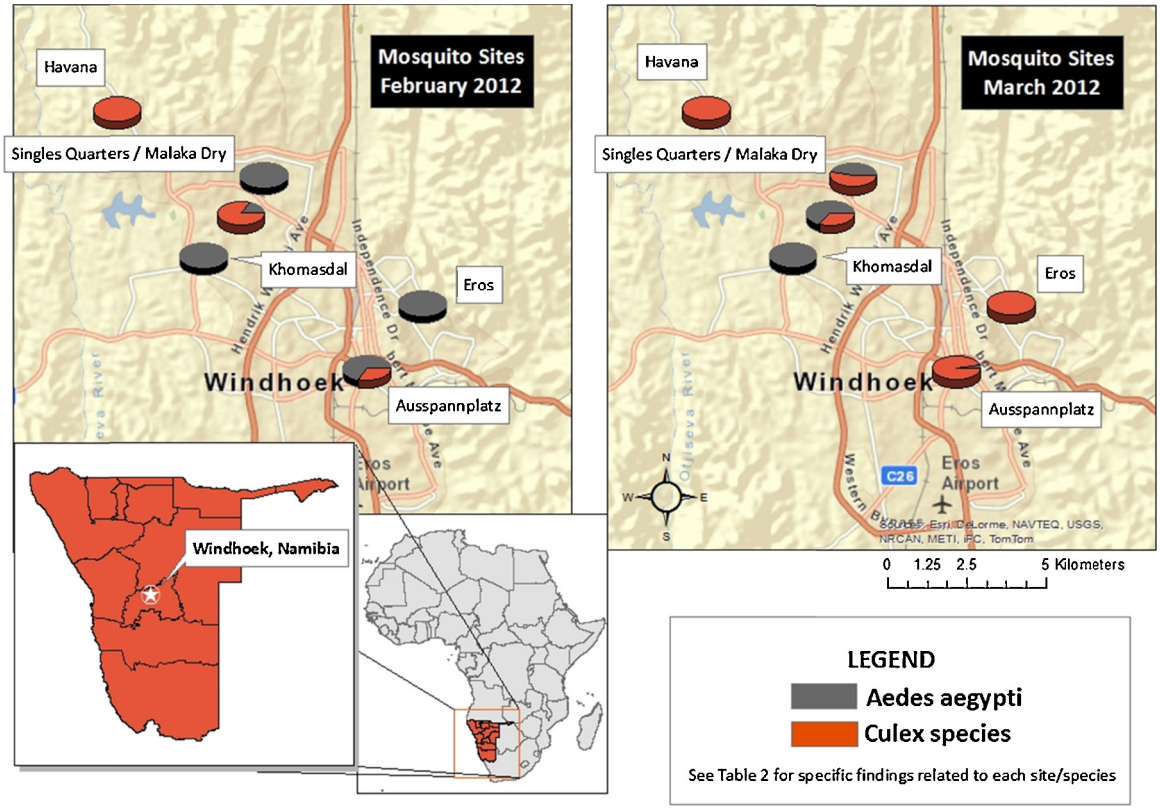


**Fig. 1.** Distribution of *Aedes aegypti* and *Culex* sp. larvae collected from six surveillance sites in Windhoek, Namibia (February–March 2012).

infection in a Namibian infected in a recent dengue outbreak in Angola (Kapitako, 2013) and recent publications of 'tourist' dengue from Namibia (Amarasinghe et al., 2011; Were, 2012), the discovery of 25 dengue virus/flavivirus-positive specimens from healthy permanent residents of Namibia was surprising. The use of an ELISA with lower specificity than a combined IgG/IgM assay meant that the positive samples could be actual dengue virus or, as the manufacturer's instructions described, could be cross-reacting antibodies with local flaviviruses. Therefore, the results are presented as dengue virus/flavivirus until future studies confirm which viruses are being recognized.

The significant variation in prevalence rates observed between age groups (Table 1) is striking on two accounts. First, the 28% seroprevalence among volunteer blood donors over 50 where all other age groups were below 10% is most likely indicative of exposure to West Nile virus during a documented epidemic 30 years ago. The only known flavivirus epidemic in the region in the past 30 years occurred with West Nile virus in the Karoo area of northwestern South Africa (just south of Namibia) in 1974, in which 18,000 persons were recorded with acute febrile illness (Venter and Swanepoel, 2010). Ten years later in northern Namibia in the mid-1980s, Joubert et al. (1985, 1991) reported flavivirus rates of 30.2% in Kavango region (Joubert et al., 1985) and 28.9% in Katima Mulilo (Caprivi region) (Joubert et al., 1991). They also discussed the dramatic increase of 16% in West Nile prevalence in the Caprivi region in one year between 1983 and 1984 (Joubert et al., 1991). The timing of these studies also correlates to the isolation of West Nile virus from a febrile patient in northern Namibia (Burt et al., 2002). Based on the timing of the South African outbreak and testing results in northern Namibia, it appears that the West Nile epidemic moved south to north in Namibia during that 10 year period (Jupp, 2001; Weaver and Reisen, 2010). That 10 year period is the same period that this group of Namibian volunteer blood donors over 50 years old would have been in their 20s and 30s, a prime age for exposure to a moving vector-borne arbovirus epidemic due to working outside and exposure to mosquitoes.

The second interesting part of the age distribution occurred among those under 50 years old (but older than 16, the first year of eligibility for blood donation in Namibia) with exposure ranges between 2.5% and 10%. This suggests that flaviviruses are still circulating and potentially contributing to non-malaria febrile illnesses in Namibia – but are not captured by national public health surveillance systems. Among Namibia's neighbors, dengue has only been reported in Angola (Filipe et al., 1975). Other flaviviruses, however, such as West Nile (Filipe et al., 1975; Kokernot et al., 1965; Jupp, 2001; Sharp et al., 1987; Venter and Swanepoel, 2010; Zaayman and Venter, 2012), Wesselsbron virus (Kokernot et al., 1965, Sharp et al., 1987; Weyer et al., 2013) and Usutu virus (Nikolay et al., 2011) are widespread in Southern Africa. While the dengue-positive blood donors may have also been exposed to other areas of the southern African sub-region, we believe the ecological and geographical proximity of central Namibia to these areas of reported endemnicity is suggestive that these limited findings represent a conservative estimate of what is actually occurring in the central regions of Namibia. Future studies should focus on active surveillance and the classification of specific viruses. This information would be important for the safety of the national blood supply (Petersen and Busch, 2010; Pierro et al., 2011), and to help revise diagnostic and treatment algorithms for acute febrile illnesses (Hertz et al., 2012; Punjabi et al., 2012).

The survey of tire sites in Windhoek revealed the presence of two principal mosquito vectors known to transmit flaviviruses – *Ae. aegypti* and *C. pipiens* sp. Surveys from the 1940s and 1950s, reported *Ae. aegypti* throughout Namibia while *C. pipiens* complex was reported mainly in Central Namibia (reviewed by Noden and van der Colf, 2013). *Ae. aegypti* are known vectors for dengue virus and Wesselsbron virus, in addition to a local species of dog heartworm, *Dirofilaria repens* (Noden et al., 2011; Schwan, 2009). *C. pipiens* sp. are a known vectors of West Nile (Hayes et al., 2005; Kramer et al., 2008). The presence of both species in large numbers throughout Windhoek (at times found within the same tire) has significant epidemiological ramifications. While Windhoek's climate (mostly dry and arid) and elevation (>1000 m above sea level) have likely prevented or limited vector-borne outbreaks in the past, the presence of known vectors during the rainy season could drive an arbovirus outbreak in the future, particularly that of *Ae. aegypti* which are more anthropophilic than *C. pipiens*. The absence of *Culex* mosquitoes in the Khomasdal site, a more middle-class neighborhood, is a possible indicator that socio-economics may play a role in mosquito breeding sites throughout the city. This aspect requires further attention in future surveys.

There are several aspects that may limit the possibility of a future arbovirus outbreak in Windhoek. Unlike other African cities, piles of unused tires are rare, even in the low income areas of Windhoek. This is due to the provision of a service and a managed site for tire disposal in the City to reduce mosquito breeding (personal communication, Milka Musuoo). Also, conversations with local residents indicated that there is a strong social pressure in the low-income communities to not have piles of under-utilized tires. If present, tires are almost always used for erosion control or boundary demarcation. As proof, no tires remained at the Malaka Dry site (the largest tire site) one year after the survey. While mosquitoes continue to breed in other containers or in seeping rivers, an aggressive municipal sanitation strategy, coupled by a strong cultural bias against tire dumping may naturally limit the risk for an arbovirus outbreak in the city.

There was considerable variation among mosquito populations in the tires between the two sampling times. Some sites contained almost exclusively one species while proportions of the two species changed in other sites during the month between sampling times. Several aspects may play a role in the ecology of these two vector species. First, tire-breeding mosquitoes are known to be heavily influenced by abiotic and biotic factors (Kling et al., 2007; Yee, 2008; Yee et al., 2010). The detritus found in the tires, as well as other environmental factors and competitive relationships between species, could have affected population densities during the study (Kling et al., 2007; Yee et al., 2010). Additionally, the ecology of vector species colonization in certain areas of Windhoek is completely unknown. Where and how the vectors survive the region's cold winters (below 0 °C) and 4–5 months of arid, dry conditions between rainy seasons also needs further study.

The scope and specific objectives of the study may have produced some limitations which need to be noted. The use of samples from a wider study design focused on exposure to zoonotic pathogens normally encountered in a rural or farming environment could have introduced a possible bias into our data. However, the high proportion of urban dwellers (82%) increases the likelihood of encountering mosquito-borne flaviviruses normally associated with urban areas such as dengue. The focused sampling approach using only tire communities, due to difficulties in establishing sampling sites within several communities, may have limited the diversity of mosquito species recovered. Future studies need to consider all possible mosquito breeding sites and include a more comprehensive approach to sampling throughout the city. Finally, the purpose of this paper was to begin the process of evaluating the whether Namibians are at risk for flavivirus outbreaks. It is envisioned that this information will be used together with environmental, climatic and ecological variables in future to develop spatial risk maps which will help to inform policy and sustainable prevention and control efforts.

## 5. Conclusion

While the findings of this study provide serological evidence of past epidemics, suggest current flavivirus exposure in a healthy cohort of Namibians, and identified mosquito vectors breeding in Windhoek, future studies are needed to classify and quantify specific circulating arboviruses in Namibia. With Rift Valley Fever virus and Crimean-Congo Hemorrhagic Fever virus potentially in the epidemiological mix in Namibia (Noden and van der Colf, 2013), it is imperative to know which arboviruses are present and how (and where) they interact with human and animal populations. In addition to public health surveillance for arbovirus illnesses, an updated national mosquito survey is needed to anticipate where future outbreaks could occur. The last mosquito surveys were carried out in the 1950s, but research elsewhere has shown that mosquito populations can shift dramatically, especially in areas where malaria elimination has been a focus (Noor et al., 2013a,b). By linking arbovirus surveillance with mosquito surveys, it would be possible to easily identify potential 'hot spots' of transmission and effectively update national diagnostic and treatment algorithms for fevers of unknown etiology.

## Acknowledgements

The research team would especially like to thank the blood donation team at The Blood Transfusion Service of Namibia for their hard work in recruiting volunteers for this study as well as processing and storing the samples. Special thanks to Dr. Antony Marfin who provided assistance in interpreting the results, John Pitman for the maps and editorial assistance. We would also like to thank those who allowed us to sample mosquitoes in their household areas and to Mr. Ethan Noden for assistance in collecting and counting mosquitoes at the Eros site. Funding for this project came from a grant awarded by the Institutional Research and Publications Committee at the Polytechnic of Namibia with equipment purchased through a CDC Cooperative Agreement (5U2GPS002902). The article was approved for publication by the Director of Oklahoma Agricultural Experiment Station and BHN was partially supported by the Oklahoma Agricultural Experiment Station (OKL-02909). Contents of this publication are solely the responsibility of the authors and do not necessarily represent the official views of the Centers for Disease Control and Prevention.

## References

Amarasinghe, A., Kuritsk, J.N., Letson, G.W., Margolis, H.S., 2011. Dengue virus infection in Africa. Emerg. Infect. Dis. 17 (8), 1349–1354.

Burt, F.J., Grobbelaar, A.A., Leman, P.A., Anthony, F.S., Gibson, G.V., Swanepoel, R., 2002. Phylogenetic relationships of southern African West Nile virus isolates. Emerg. Infect. Dis. 8 (8), 820–826.

Cotter, C., Sturrock, H.J., Hsiang, M.S., Liu, J., Phillips, A.A., Hwang, J., Gueye, C.S., Fullman, N., Gosling, R.D., Feachem, R.G., 2013. The changing epidemiology of malaria elimination: new strategies for new challenges. Lancet 382 (9895), 900–991.

De Meillon, B., Hardy, F., 1953. New records and species of biting insects from the Ethiopian region: IV. J. Entomol. Soc. S. Afr. 16 (1), 17–35.

Dupont, H.T., Brouqui, P., Faugere, B., Raoult, D., 1995. Prevalence of antibodies to *Coxiella burnetti*, *Rickettsia conorii*, and *Rickettsia typhi* in seven African countries. Clin. Infect. Dis. 21 (5), 1126–1133.

Filipe, A.R., De Carvalho, R.G., Relvas, A., Casaca, V., 1975. Arbovirus studies in Angola: serological survey for antibodies to arboviruses. Am. J. Trop. Med. Hyg. 24 (3), 516–520.

Hayes, E.B., Komar, N., Nasci, R.S., Montgomery, S.P., O'Leary, D.R., Campbell, G.L., 2005. Epidemiology and transmission dynamics of West Nile virus disease. Emerg. Infect. Dis. 11 (8), 1167–1173.

Hertz, J.T., Munishi, O.M., Ooi, E.E., Howe, S., Lim, W.Y., Chow, A., Morrissey, A.B., Bartlett, J.A., Onyango, J.J., Maro, V.P., Kinabo, G.D., Saganda, W., Gubler, D.J., Crump, J.A., 2012. Chikungunya and dengue fever among hospitalized febrile patients in northern Tanzania. Am. J. Trop. Med. Hyg. 86 (1), 171–177.

Hogema, B.M., Slot, E., Molier, M., Schneeberger, P.M., Hermans, M.H., van Hannen, E.J., van der Hoek, W., Cuijpers, H.T., Zaaijer, H.L., 2012. *Coxiella burnetii* infection among blood donors during the 2009. Q-fever outbreak in The Netherlands. Transfusion (Paris) 52 (1), 144–150.

Kapitako, A., 2013. First suspected 'dengue fever' case. NewEra newspaper, Available: http://allafrica.com/stories/201306261105.html (accessed 18.12.13).

Kasper, M.R., Blair, P.J., Touch, S., Sokhal, B., Yasuda, C.Y., Williams, M., Richards, A.L., Burgess, T.H., Wierzba, T.F., Putnam, S.D., 2012. Infectious etiologies of acute febrile illness among patients seeking health care in south-central Cambodia. Am. J. Trop. Med. Hyg. 86 (2), 246–253.

Kelly, P.J., Mason, P.R., Matthewman, L.A., Raoult, D., 1991. Seroepidemiology of spotted fever group rickettsial infections in humans in Zimbabwe. J. Trop. Med. Hyg. 94 (5), 304–309.

Kilic, S., Yilmaz, G.R., Komiya, T., Kurtoglu, Y., Karakoc, E.A., 2008. Prevalence of *Coxiella burnetii* antibodies in blood donors in Ankara, Central Anatolia, Turkey. New Microbiol. 31 (4), 527–534.

Kling, L.J., Juliano, S.A., Yee, D.A., 2007. Larval mosquito communities in discarded vehicle tires in a forested and unforested site: detritus type, amount, and water nutrient differences. J. Vector Ecol. 32 (2), 207–217.

Kokernot, R.H., Casaca, V.M., Weinbren, M.P., McIntosh, B.M., 1965. Survey for antibodies against arthropod-borne viruses in the sera of indigenous residents of Angola. Trans. R. Soc. Trop. Med. Hyg. 59 (5), 563–570.

Kramer, L.D., Styer, L.M., Ebel, G.D., 2008. A global perspective on the epidemiology of West Nile virus. Annu. Rev. Entomol. 53, 61–81.

Joubert, J.J., Prozesky, O.W., Lourens, J.G., van Straten, A.M., Theron, J.W., Swanevelder, C., Meenehan, G.M., van der Merwe, C.A., 1985. Prevalence of hepatitis virus and some arbovirus infections in Kavango, northern SWA/Namibia. S. Afr. Med. J. 67 (13), 500–502.

Joubert, J.J., van der Merwe, C.A., Lourens, J.H., Lecatsas, G., Siegrühn, C., 1991. Serological markers of hepatitis B virus and certain other viruses in the population of eastern Caprivi, Namibia. Trans. R. Soc. Trop. Med. Hyg. 85 (1), 101–103.

Jupp, P.G., 2001. The ecology of West Nile virus in South Africa and the occurrence of outbreaks in humans. Ann. N.Y. Acad. Sci. 951, 143–152.

Letaief, A.O., Yacoub, S., Dupont, H.T., Le Cam, C., Ghachem, L., Jemni, L., Raoult, D., 1995. Seroepidemiological survey of rickettsial infections among blood donors in central Tunisia. Trans. R. Soc. Trop. Med. Hyg. 89 (3), 266–268.

Maina, A.N., Knobel, D.L., Jiang, J., Halliday, J., Feikin, D.R., Cleaveland, S., Ng'ang'a, Z., Junghae, M., Breiman, R.F., Richards, A.L., Njenga, M.K., 2012. *Rickettsia felis* infection in febrile patients, western Kenya, 2007–2010. Emerg. Infect. Dis. 18 (2), 328–331.

Mansueto, P., Pepe, I., Cillari, E., Arcoleo, F., Micalizzi, A., Bonura, F., Seidita, A., Palillo, L., Di Gregorio, M.F., Affronti, M., Di Rosa, S., Rini, G., Vitale, G., 2012. Prevalence of antibodies anti-*Bartonella henselae* in western Sicily: children, blood donors, and cats. J. Immunoassay Immunochem. 33 (1), 18–25.

Muspratt, J., 1956. The Stegomyia mosquitoes of South Africa and some neighboring territories: including chapters on the mosquito-borne virus diseases of the Ethiopian zoo-geographical region of Africa. Memoirs Entomol. Soc. S. Afr. 4, 1–138.

Namibia Weather, 2014. Windhoek Yearly Rainfall Summary, Available: http://weather.namsearch.com/wdhrainsummary.php (accessed 2.04.14).

Negri, E.C., Santarém, V.A., Rubinsky-Elefant, G., Giuffrida, R., 2013. Anti-*Toxocara* spp. antibodies in an adult healthy population: serosurvey and risk factors in Southeast Brazil. Asian Pac. J. Trop. Biomed. 3 (3), 211–216.

Niang, M., Parola, P., Tissot-Dupont, H., Baidi, L., Brouqui, P., Raoult, D., 1998. Prevalence of antibodies to *Rickettsia conorii*, *Ricketsia africae*, *Rickettsia typhi* and *Coxiella burnetii* in Mauritania. Eur. J. Epidemiol. 14 (8), 817–818.

Nikolay, B., Diallo, M., Boye, C.S., Sall, A.A., 2011. Usutu virus in Africa. Vector Borne Zoonotic Dis. 11 (11), 1417–1423.

Noden, B.H., Morkel, C., Manyarara, R., Tubbesing, U., Soni, M., 2011. Emerging zoonotic diseases of companion animals in Namibia [abstract 98]. ASTMH 31. http://www.astmh.org/AM/Template.cfm?Section=Abstracts_and_Education1&Template=/CM/ContentDisplay.cfm&ContentID=3715 (accessed 18.12.13).

Noden, B.H., van der Colf, B.E., 2013. Neglected tropical diseases of Namibia: unsolved mysteries. Acta Trop. 125 (1), 1–17.

Noor, A.M., Alegana, V.A., Kamwi, R.N., Hansford, C.F., Ntomwa, B., Katokele, S., Snow, R.W., 2013a. Malaria control and the intensity of *Plasmodium falciparum* transmission in Namibia 1969–1992. PLoS One 8 (5), e63350.

Noor, A.M., Uusiku, P., Kamwi, R.N., Katokele, S., Ntomwa, B., Alegana, V.A., Snow, R.W., 2013b. The receptive versus current risks of *Plasmodium falciparum* transmission in Northern Namibia: implications for elimination. BMC Infect. Dis. 13 (1), 184.

Petersen, L.R., Busch, M.P., 2010. Transfusion-transmitted arboviruses. Vox Sang. 98 (4), 495–503.

Pierro, A., Gaibani, P., Manisera, C., Dirani, G., Rossini, G., Cavrini, F., Ghinelli, F., Ghinelli, P., Finarelli, A.C., Mattivi, A., Macini, P.L., Castellani, G., Landini, M.P., Sambri, V., 2011. Seroprevalence of West Nile virus-specific antibodies in a cohort of blood donors in northeastern Italy. Vector Borne Zoonotic Dis. 11 (12), 1605–1607.

Punjabi, N.H., Taylor, W.R., Murphy, G.S., Purwaningsih, S., Picarima, H., Sisson, J., Olson, J.G., Baso, S., Wangsasaputra, F., Lesmana, M., Oyofo, B.A., Simanjuntak, C.H., Subekti, D., Corwin, A.L., Richie, T.L., 2012. Etiology of acute, non-malaria, febrile illnesses in Jayapura, northeastern Papua, Indonesia. Am. J. Trop. Med. Hyg. 86 (1), 46–51.

Schwan, E.V., (PhD Thesis) 2009. Filariosis of domestic carnivores in Gauteng, KwaZulu-Natal and Mpumalanga provinces, South Africa, and Maputo province, Mozambique. University of Pretoria, Available: http://upetd.up.ac.za/thesis/available/etd-12032009-185953/unrestricted/00front.pdf (accessed 18.12.13).

Schwartz, E., Meltzer, E., Mendelson, M., Tooke, A., Steiner, F., Gautret, P., Friedrich-Jaenicke, B., Libman, M., Bin, H., Wilder-Smith, A., Gubler, D.J., Freedman, D.O., Parola, P., 2013. Detection on four continents of dengue fever cases related to an ongoing outbreak in Luanda, Angola, March to May 2013. Eur. Surveill. 18 (21), pii=20488.

Sharp, B.L., Appleton, C.C., Thompson, D.L., Meenehan, G., 1987. Anthropophilic mosquitoes at Richards Bay, Natal, and arbovirus antibodies in human residents. Trans. R. Soc. Trop. Med. Hyg. 81 (2), 197–201.

Sokhna, C., Mediannikov, O., Fenollar, F., Bassene, H., Diatta, G., Tall, A., Trape, J.F., Drancourt, M., Raoult, D., 2013. Point-of-care laboratory of pathogen diagnosis in rural Senegal. PLoS Negl. Trop. Dis. 7 (1), e1999.

Sun, J., Fu, G., Lin, J., Song, X., Lu, L., Liu, Q., 2010. Seroprevalence of *Bartonella* in Eastern China and analysis of risk factors. BMC Infect. Dis. 10, 121.

Vairo, F., Nicastri, E., Meschi, S., Schepisi, M.S., Paglia, M.G., Bevilacqua, N., Mangi, S., Sciarrone, M.R., Chiappini, R., Mohamed, J., Racalbuto, V., Di Caro, A., Capobianchi, M.R., Ippolito, G., 2012. Seroprevalence of dengue infection: a cross-sectional survey in mainland Tanzania and on Pemba Island, Zanzibar. Int. J. Infect. Dis. 16 (1), e44–e46.

Vardas, E., Sitas, F., Seidel, K., Casteling, A., Sim, J., 1999. Prevalence of hepatitis C virus antibodies and genotypes in asymptomatic, first-time blood donors in Namibia. Bull. World Health Organ. 77 (12), 965–972.

Venter, M., Swanepoel, R., 2010. West Nile virus lineage 2 as a cause of zoonotic neurological disease in humans and horses in southern Africa. Vector Borne Zoonotic Dis. 10 (7), 659–664.

Weaver, S.C., Reisen, W.K., 2010. Present and future arboviral threats. Antiviral Res. 85 (2), 328–345.

Were, F., 2012. The dengue situation in Africa. Paediatr. Int. Child. Health 32 (S1), 18–21.

Weyer, J., Thomas, J., Leman, P.A., Grobbelaar, A.A., Kemp, A., Paweska, J.T., 2013. Human cases of wesselsbron disease, South Africa 2010–2011. Vector Borne Zoonotic Dis. 13 (5), 330–336.

Wichmann, O., Mühlberger, N., Jelinek, T., 2003. Dengue – the underestimated risk in travelers. Dengue Bull. 27, 126–137.

WRBU, 2011. Medically Important Mosquitoes: AFRICOM Species, Available: http://wrbu.si.edu/africom MQ.html (accessed 18.12.13).

Yee, D.A., 2008. Tires as habitats for mosquitoes: a review of studies within the eastern United States. J. Med. Entomol. 45 (4), 581–593.

Yee, D.A., Kneitel, J.M., Juliano, S.A., 2010. Environmental correlates of abundances of mosquito species and stages in discarded vehicle tires. J. Med. Entomol. 47 (1), 53–62.

Zaayman, D., Venter, M., 2012. West Nile virus neurologic disease in humans, South Africa, September 2008–May 2009. Emerg. Infect. Dis. 18 (12), 2051–2054.