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

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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

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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