

indicated that in-country variations existed with higher WNV seroprevalence rates in the Western and central areas (19.7% and 13.8% respectively) and lower rates of 4.2% in the Eastern parts¹⁷.

WNV human infections had been reported in many African countries. Studies conducted in Egypt in the 1950s and 1970s showed WNV antibodies in serum samples of more than 60% of the study population and 74% in Upper parts of Egypt and 28% around Cairo¹⁸. In Tunisia, in the 1960s and some areas in North Africa showed a seroprevalence of 5%, while Senegal reported higher rates of up to 98%². Prevalence rates of 3-65% were reported in Kenya between 1966 and 1968² and 9.2% in 2011¹⁹. Studies in South Africa reported seroprevalence of 13-24% in 1961-1965 PubMed and up to 45% in Madagascar².

Several risk factors for WNV infection emerged from the study in America such as climate and transmission even after adjusting for other environmental factors²⁰ and the results were comparable to results obtained in other studies which confirmed an association between urban/sub-urban versus a more rural area^{21,22}. However other studies reported significant association when there was less population density and rural. Temperature changes determining human activity and mosquito replication was also significantly associated with risk of infection. It was also concluded that these factors could differ yearly due to changes in bird populations with high bird population as being protective²⁰. It is possible that when there are more birds the virus could be concentrated in bird mosquito bird cycle whereas if there are fewer birds the mosquito could resort to human meals. In Arizona staying at home and not attending school was found to be a risk factor to WNV infection, although it was argued that the possibility that those who stayed at home were the elderly who were already known to be at high risk of infection with WNV¹¹. Seroprevalence studies conducted in Cameroon had indicated that iron roofed houses were protective for the infection compared to grass thatched houses²³.

Studies had also confirmed that age had a bearing on the severity of the infection with the older age group, 50 years and above, being most at risk²⁰. However other studies reported that WNV affects all age groups; In Israel and other parts of Africa infection was reported

in children and healthy adults^{2,24}. In Kenya the seroprevalence tended to increase with age¹⁹.

Although WNV infection has been reported worldwide, there has been no information on the extent of the problem in Zambia. Therefore, this study was conducted to determine the seroprevalence of WNV IgG and IgM antibodies and its correlates in Zambia during a Yellow fever risk assessment survey.

Methods

Study site, design and population

The study was conducted in North-Western and Western provinces of Zambia. The selection of the two provinces was based on the classification as low potential risk areas for yellow fever transmission by World Health Organization (WHO) in 2010²⁵. North-Western province which borders with Angola on the western side and Democratic Republic of Congo (DRC) on the northern side has a population of 706,462²⁶. It is the most sparsely populated province in the country and the provincial capital is Solwezi. The province comprised of 8 districts which were divided into 1178 Standard Enumeration Areas (SEAs) and the main economic activities were mining in one district and general pineapple growing. Western province with a population of 881,524, borders with Angola and has seven districts (Census 2010) divided into 1902 SEAs. The main economic activities were crop farming, livestock production and fishing. A cross sectional study was carried out among individuals aged nine months or older.

Sample size, inclusion/exclusion criteria and sampling

The sample size calculation was based on the assumption that the seroprevalence was 7% based on the study conducted by Robinson²⁷. In estimating the sample size for persons aged 5 years or older, the following parameters were considered: a prevalence of 7%, desired precision or confidence interval (d) of +3%, and a design effect (DE) of 2 and an 80% response rate. Considering sex, we aimed to recruit 700 male and 700 female participants in each province. Assuming an average of 4 persons aged 5 years or older in each household, a total of 12 households in each of the 30 cluster was to be recruited in the survey. The total number of persons that would be recruited from each province was 1806, totaling 3612 from both provinces.

The seroprevalence of children was about half that for older children, and in estimating the sample size for persons aged below 5 years, the following parameters were considered: a prevalence of 3.5%, desired precision or confidence interval (d) of +3.4%, and a design effect (DE) of 2 and an 80% response rate. The computed sample size was 282. Therefore, in each province 406 children would be recruited for the survey.

Included in the study were individuals aged 9 months or older who were found in a sampled household at the time of the study and were resident in the study site for at least seven days. Individuals who had received YF vaccination in the last ten years to the survey were also included in the survey. Excluded from the study were persons who were aged less than 9 months, or individuals regardless of age who resided in the study site for less than 7 days prior to the survey. Children under the age of 9 months were excluded based on the fact that at this age children could still carry maternal antibodies from exposed mothers which could pose the risk of false positive results.

The sample was drawn using a two-stage cluster sampling technique using probability proportional to size. A list of the standard enumeration areas (SEAs) in each province constituted the sampling frame. The line lists of the SEAs were provided by the Government's Central Statistics Office (CSO). The sampling was designed to achieve fairly good estimates at the provincial level of analysis, and not representing the sub divisions of the province.

Study Variables

Dependent variable: A case with evidence of WNV exposure was defined as any individual aged 9 months or older whose blood sample was confirmed to have WNV exposure. WNV exposure status was positive if IgG or IgM antibodies were determined in the serum sample by laboratory testing and negative if the IgG or IgM antibodies were absent by laboratory testing.

Independent variables: The independent variables included demographic data: age, sex, occupation, education, roof type and use of mosquito preventive measures such as insecticide treated nets (ITNs) and indoor residual spray (IRS).

Laboratory procedures

Three to 5 millilitres of blood was collected by ve-

nepuncture into an EDTA vacutainer tube and transported on cold chain to the local laboratories for serum separation and storage.

Subsequently serum samples were transported on cold chain and thereafter subjected to primary testing (YFV specific IgG and IgM) at the University of Teaching Hospital in Zambia virology unit and Institute Pasteur, Dakar. All presumptive YFV-specific IgG and IgM samples were subjected to IgG and IgM antibodies testing against other flaviviruses known to cause haemorrhagic fever-like disease including WNV. The testing was carried out using IgG capture enzyme-linked immunosorbent assay (ELISA).

Data management and analysis

Data collected from the field was entered in an Epi-Info data entry screen that had consistency and range checks embedded in it. Further editing was conducted by running frequencies during the analysis stage. Epi data files were exported to SPSS for data analysis. The data was summarized to describe the occurrence of WNV exposed individuals in absolute numbers and percentage by place (residence, travel or work) and person (age, sex, occupation, education). Further analysis was conducted to determine independent factors associated with WNV sero-positivity. Odds ratios were used to estimate the magnitude of associations.

Ethical considerations

Ethical clearance was sought from the Tropical Diseases Research Centre Research Ethics Committee, and ethical standards were adhered to throughout this study. Informed consent was sought from study participants. Guardians provided assent for the participation of the persons under the consenting age. They were asked to read or have read to them, understand and sign/thumbprint an informed consent form. Responsible adults in the household were identified to give proxy consent on behalf of minors.

Results

Table 1 provides a description of the respondents in the two provinces. A total of 3,625 persons participated in the survey. About 1 in 5 of the participants was in the age groups 5-14 years (23.9%) and 15-24 years (20.6%). The sample comprised more female participants (53.3%). Male participants tended to be more educated than females ($p < 0.001$) with 35.9% of male and 26.6% of female participants having attained secondary level of education or higher.

Participants who had attained primary level of education were 32% (AOR=1.32; 95% CI [1.01, 1.72]) more likely to have infection compared to participants who had attained higher level of education. Farmers were 20% (AOR=0.80; 95% CI [0.64, 0.99]) less likely to have infection compared to students. Meanwhile, participants who lived in grass roofed houses were 2.97 (AOR=2.97; 95% CI [1.81, 4.88]) times more likely to be infected than those who lived in asbestos roofed houses. Compared with participants who did not have their houses sprayed those who had IRS in their houses were 19% (AOR=0.81; 95% CI [0.69, 0.94]) less likely to have infection. Participants who had travelled to DRC were 72% (AOR=0.28; 95% CI [0.10, 0.74]) less likely to have infection compared with those who did not travel there. Travelling to Angola was associated with the infection [AOR=1.40; 95% CI [1.09, 1.81]].

Discussion

This study presents the first documentation of the seroprevalence of WNV infection and its correlates in Zambia. The results of this survey revealed a seroprevalence of 10.2%. The following factors were statistically significantly associated with the infection: age, education, occupation, roof type, living in a house with IRS, sleeping under a mosquito net and visiting Congo DR and Angola.

The seroprevalence results obtained in this study were consistent with results from surveys conducted in other parts of the world including Africa although in some areas very high seroprevalence rates of WNV infection were obtained. The seroprevalence in USA varied across the country with rates ranging from 4.2%-19.7%¹⁹. In Africa higher rates of (60%-74%) in Egypt, (3%-65%) in Kenya and (13%-24%) in South Africa, were reported as way back as the 1950s to 1970s^{2,17}. It is also important to note that most of these studies were conducted on blood donors following an outbreak, which could explain the marked difference in the rates obtained in this study.

Disease prevalence is dependent upon a wide range of risk factors, climate and the environment affecting the activities of the virus and the humans^{20-22,28}. This study has shown that being younger than 5 years old was protective to the WNV infection while literature indicates that all age groups are at risk for the disease²⁰. This

study however indicates that those aged between 35-44 years of age were more likely to acquire the infection compared to those who were 45 years older. Contrary to other literature which suggests that those above 50 years of age were more at risk for the disease compared to those below the age of 50 years²⁰.

Regarding vector management and control the study has shown that participants, who had a house with IRS, were less likely to be infected with the virus compared to those who did not have their houses sprayed. Therefore the national malaria control program should sustain efforts to attain higher IRS coverage as this provides protection against arthropod-borne viruses, including the WNV, in addition to malaria, to the beneficiaries. CDC guidelines on WNV surveillance control and prevention call for active surveillance of the disease in humans and integrated vector management. The guidelines emphasize that since WNV outbreaks are non-predictable there is a need for active surveillance to monitor and detect increases in the disease rates and to have control measures readily available for outbreak response.

There is evidence of arboviral outbreaks in Africa with recent reports of dengue outbreaks in Angola, DRC and Tanzania, which are neighbors with Zambia. Travel between Zambia and these countries is inevitable, increasing the risk of viral importation. This study provides evidence that participants who had a history of travelling to Angola were at an increased risk of being infected with WNV. Human movement has been implicated in the spread and transmission of vector borne diseases including WNV²⁹. It should therefore be emphasized that travelers take personal protective measures against mosquito bites as a preventive measure.

Concerning education level of the participants, this study demonstrates that compared to participants who had attained tertiary level of education, those who had attained primary school level of education were more likely to be infected with WNV. This finding contradicts studies in Arizona which indicated that those who did not attend school were more at risk of the disease than those who attended school¹¹. This is not surprising as literacy is generally a symbol of improved socioeconomic status leading to increased chances of access to medical care resulting in reduced chances of exposure to the disease²⁹.

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