



ABSTRACT

Dengue fever caused by the dengue viruses (DENVs), the most common arboviral infection transmitted by mosquitoes of the genus *Aedes* globally. One novel approach to control DENVs is to use the endosymbiotic bacterium, *Wolbachia* to limit DENV replication inside the primary mosquito vector, *Aedes aegypti*. Various *Wolbachia* strains transfected into *Ae. aegypti* block the transmission of Dengue viruses from *Ae. aegypti* to humans forms the basis of *Wolbachia* based biocontrol strategy. The strength of blocking appears to correlate with *Wolbachia* density where higher density associated with greater viral inhibition. The main objective of the current study was to determine the relationship of wAlbB *Wolbachia* density in transfected *Ae. aegypti* and DENV-2 viral inhibition in whole body and various somatic tissues such as salivary gland, midgut and head. Furthermore, it is important to access the effect of variability of tissue density on pathogen blocking in insect host. The current study explore the role of tissue specific *Wolbachia* density in DENV-2 interference in major vector *Ae. aegypti* of dengue from Lahore, Pakistan. For this purpose three mosquito groups wCL (wAlbB *Wolbachia* induced), wCLT (*Wolbachia* removed with tetracycline) and cL (control without *Wolbachia*) were fed on human blood with initial DENV-2 viral titer 2×10^7 PFU/mL. The *Wolbachia* density and viral titer in the whole body and above mentioned tissues of mosquitoes at various days post infection (0, 4, 8 and 12) of viremic blood was measured by q-PCR and qRT-PCR respectively. *Wolbachia* density was evaluated with the ratio of WSP/RPS6 (*Wolbachia* surface protein genomic copies normalized with mosquito housekeeping gene) while, viral titer with the ratio of NS1/RPS6 (viral non-structural protein genomic copies with mosquito housekeeping gene). The results indicated that *Wolbachia* wAlbB strain induced a strong inhibition of DENV-2 replication in wCL mosquitoes associated with *Wolbachia* density in whole body and various somatic tissues at 12DPI of viremic blood. DENV-2 genome copy number in *Wolbachia* infected group (wCL) was significantly reduced ($p < 0.001$) in salivary gland, midgut, head and whole body compared with wild control (cL) respectively. There was 1.38×10^5 (99.99%), 5.07×10^4 (98.57%), 1.8×10^4 (98.32%) and 1.2×10^4 (97.99%) times inhibition of virus replication in salivary gland, midgut, head and whole body respectively in *Wolbachia* infected (wCL) group as compared to control (cL) group at



12DPI. Maximum inhibition DEN-2 was observed in Salivary gland (1.38×10^5) that was compared with other tissues midgut (5.07×10^4), head (1.8×10^4) and whole body (1.2×10^4). In addition significant increase ($p < 0.001$) in *Wolbachia* density was observed from 0-12DPI in salivary gland (1.8×10^1 - 4.7×10^1), midgut (0.31×10^1 - 0.92×10^1) that was , head (0.6×10^1 - 2.7×10^1) and whole body (1.12×10^2 - 2.11×10^2). It has been found that there was strongly inverse relationship between *Wolbachia* density and viral titer from 0-12DPI, in salivary gland ($r = -0.998$; $p = 0.041$), midgut ($r = -0.998$; $p = 0.041$), Head ($r = -0.998$; $p = 0.043$) and whole body ($r = -1.000$; $p = 0.016$) with the increase in time post infection. In conclusion the potential usefulness of *Wolbachia*-based control strategies for pathogen blocking and onward transmission to human are important implications for the future vector-borne disease control.