

# Species-specific salivary antigen ELISAs as biomarkers of exposure to La Crosse virus vectors in North Carolina

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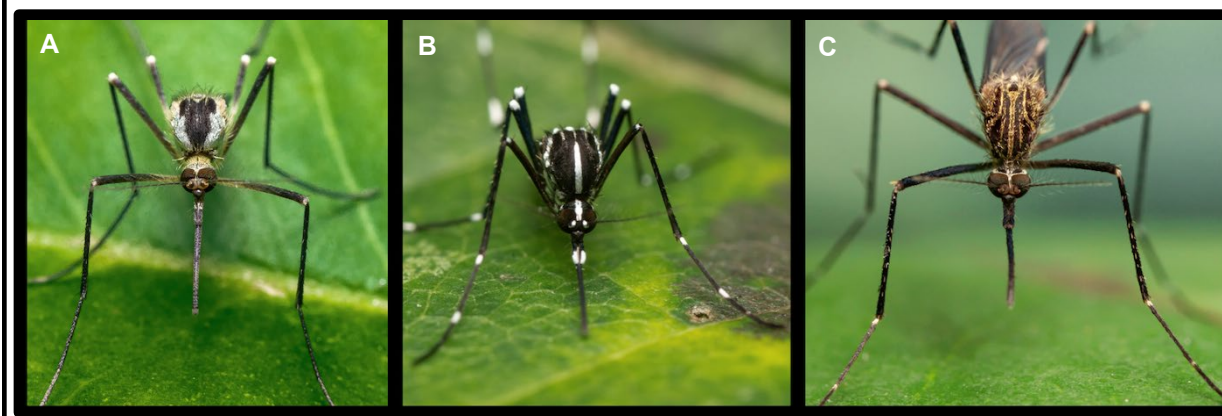


Figure 1. A) *Aedes triseriatus*, B) *Aedes albopictus*, C) *Aedes japonicus*

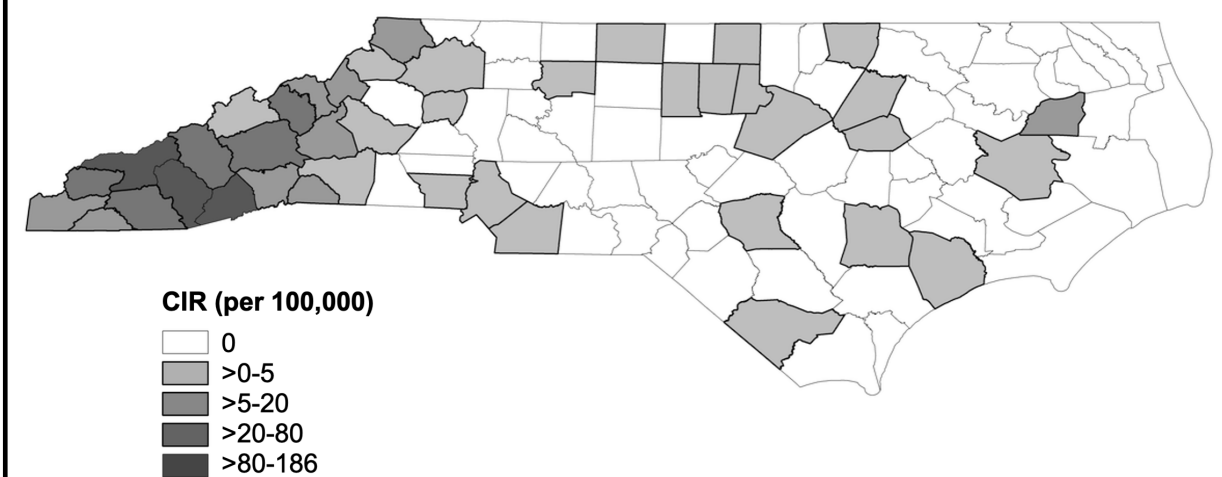


Figure 2. Probable and confirmed neuroinvasive La Crosse disease in North Carolina 2000-2020 (Davis et al. 2024). Western NC has a higher cumulative incidence rate than the other two regions (Piedmont and Coastal) of the state, suggesting geographic persistence.

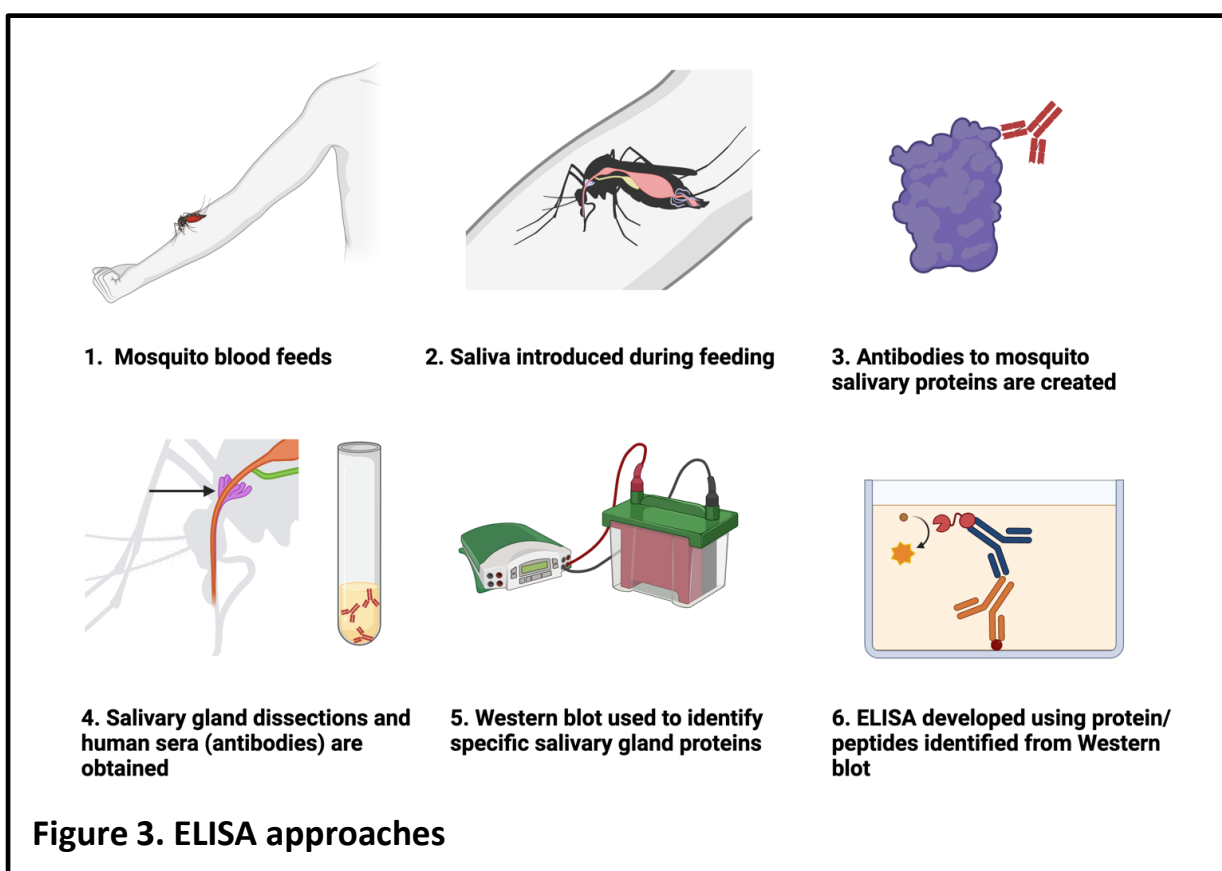


Figure 3. ELISA approaches

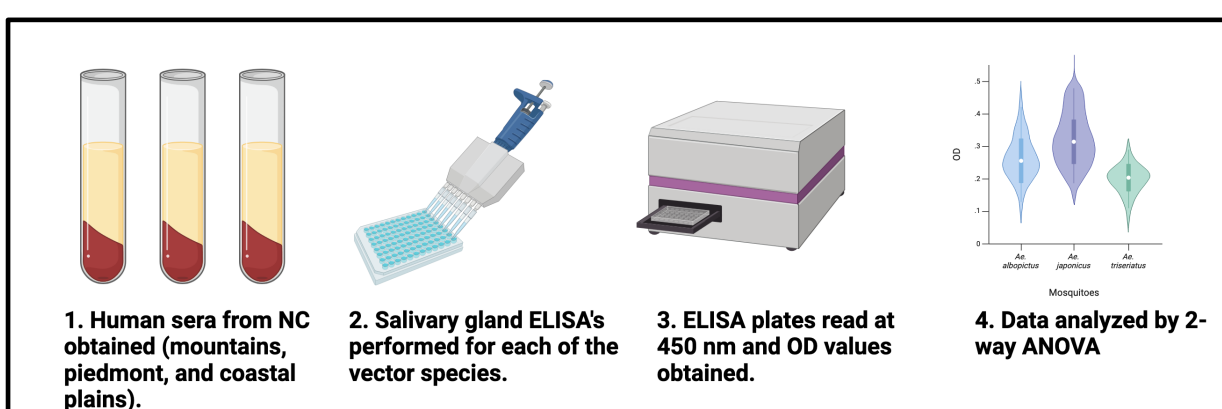


Figure 4. Experimental design: analysis of human sera for anti-LACV vector IgG from three regions in NC (Mountains, Piedmont, and Coastal Plains).

**BACKGROUND/PROBLEM STATEMENT:** La Crosse virus (LACV) is a leading cause of pediatric arboviral neuroinvasive disease in the United States. Three mosquito species are likely responsible for the majority of LACV transmission – *Aedes triseriatus*, the primary, endemic vector and two invasive, secondary vector species: *Ae. albopictus* and *Ae. japonicus* (Figure 1). The risk of LACV disease is geographically persistent in North Carolina (Figure 2); however, current estimates of disease risk do not accurately reflect exposure risk or the genuine burden of disease. Furthermore, low incidence and poor detection rates grossly limit the evaluation of potential public health interventions.

**APPROACH:** We seek to use IgG antibodies against mosquito salivary glands detected by ELISAs to measure the level of exposure to LACV vectors as a proxy for mosquito bite intensity/disease risk. Here we share the development of salivary gland (crude extract) IgG ELISAs for *Ae. triseriatus*, *Ae. albopictus* and *Ae. japonicus* (Figure 3).

**RESULTS:** Using convenience human sera (n=132 individuals) from North Carolina, we compared optical density (OD) values for all three species (Figure 4). Pairwise comparisons (ANOVA, Tukey's HSD comparisons) detected higher mean OD values for *Ae. albopictus* as compared to *Ae. triseriatus* and *Ae. japonicus* ( $P < 0.0001$ ) (Figure 5). This is consistent with prior knowledge that *Ae. albopictus* is the more common peridomestic container *Aedes* in the areas where the human samples were obtained (Figure 6). This general trend was conserved upon analysis of OD values for each species grouped by region where the human sample was collected (Figure 7). Two-way ANOVA showed a significant effect from both species and region on OD value (Species:  $F = 58.86$ ,  $df = 2$ ,  $P < 0.001$ ; Region:  $F = 6.48$ ,  $df = 2$ ,  $P < 0.01$ ).

**FUTURE WORK:** Similar approaches will be used with field collected human sera from ongoing epidemiological studies of LACV disease in western NC; improvements to the assays using antigenic peptides are underway.

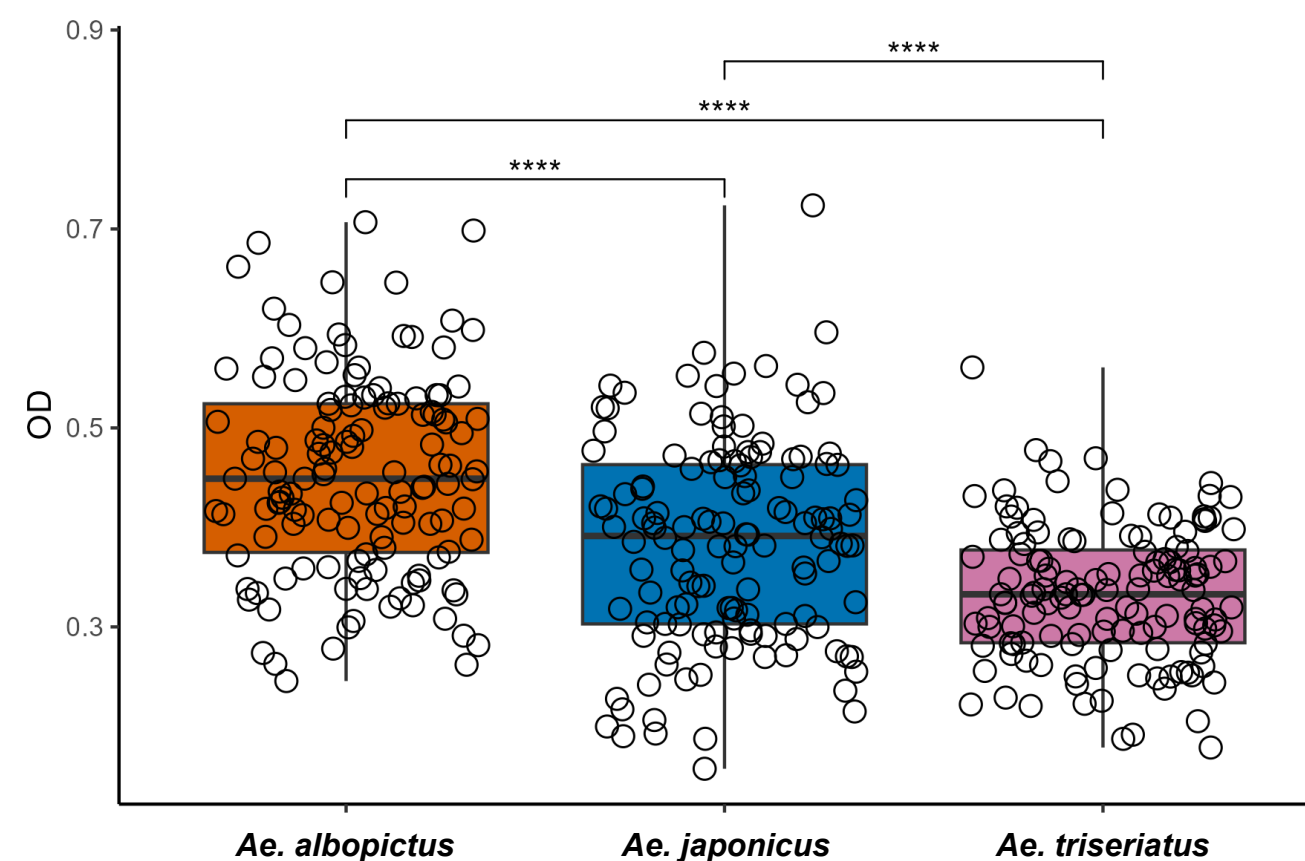


Figure 5. Comparison of OD values for each species using samples from all three NC regions. *Aedes albopictus* has significantly higher OD values than *Ae. japonicus* ( $F = 6.085$ ,  $df = 393$ ,  $P < 0.0001$ ) and *Ae. triseriatus* ( $F = 10.647$ ,  $df = 393$ ,  $P < 0.0001$ ). *Aedes japonicus* has significantly higher OD values than *Ae. triseriatus* ( $F = 4.56$ ,  $df = 393$ ,  $P < 0.0001$ ).

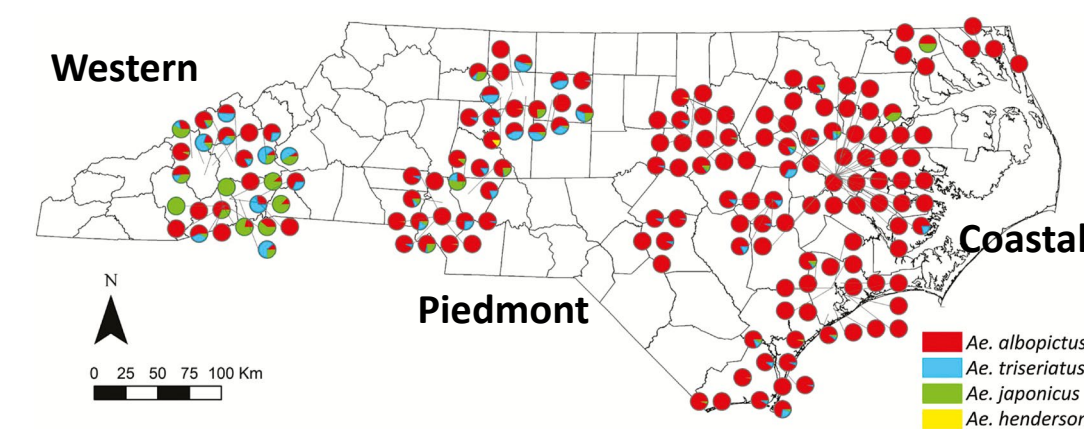


Figure 6. Relative abundance (proportions) for the common peridomestic *Aedes* in NC (Reed et al. 2019).

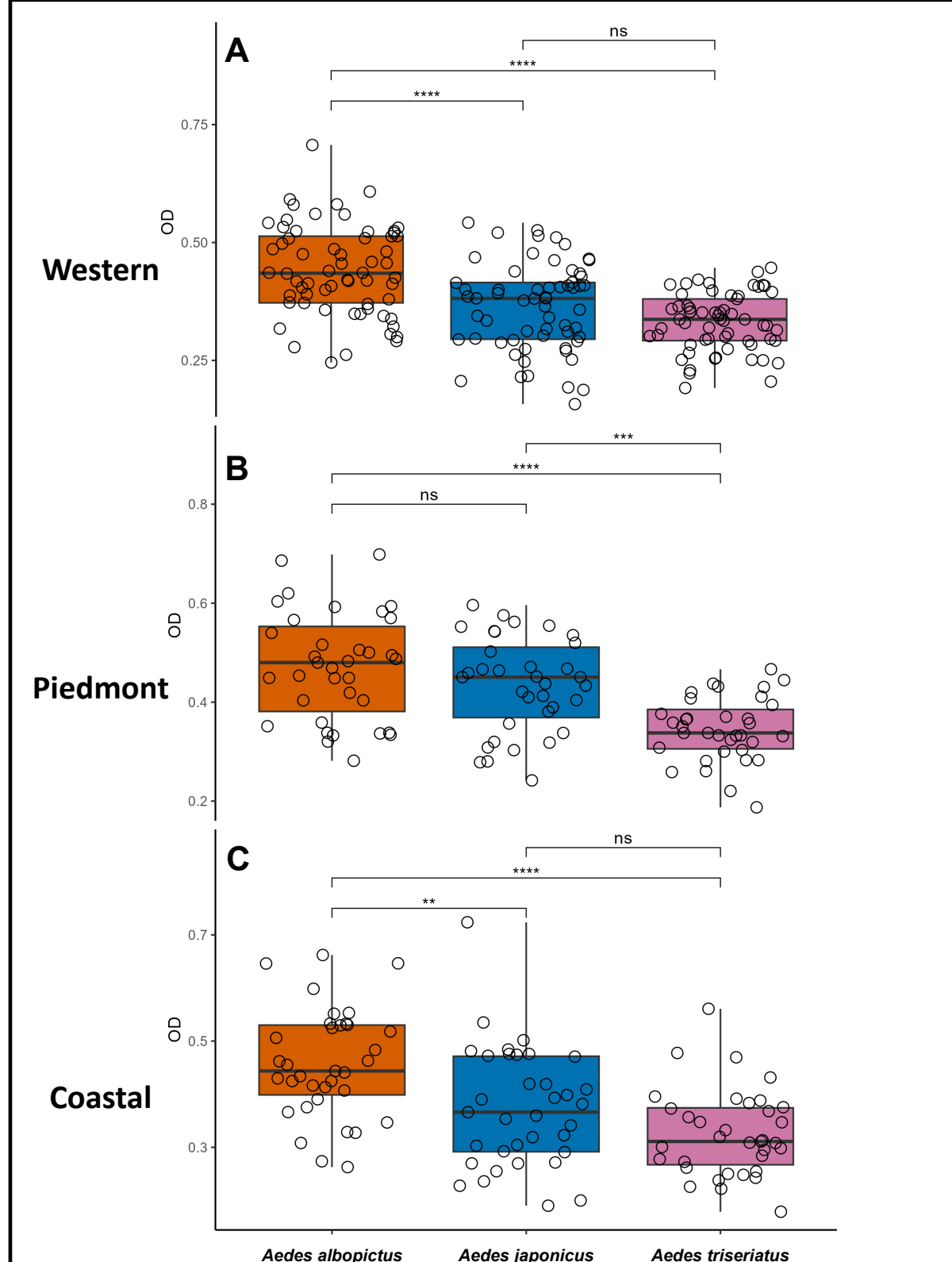


Figure 7. Regional OD values of anti-LACV vectors IgG in human sera (A: Mountain Region, B: Piedmont Region, C: Coastal Plain Region). ns = not significant, \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ , \*\*\*\*  $P < 0.0001$

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