West Nile Virus (WNV) is an RNA arbovirus in the family *Flaviviridae*. While birds are the primary reservoir for the virus, humans can be a dead end host. In Wyoming, the *Culex tarsalis* mosquito acts as the primary vector. Most human infections are asymptomatic. In less than 1% of infections, however, severe symptoms including paralysis, meningitis, encephalitis, and death may occur. Fremont County is a hot spot of WNV with 64% of all cases in Wyoming reported in 2007. Our study proposes the testing of a percentage of the human population of Fremont County to determine the number of people exposed to WNV. Sera will be tested using commercially available IgG and IgM antibody kits.

**Materials and Methods**

Serum samples are derived from whole blood samples taken via venipuncture. Whole blood is spun for 15 minutes in a centrifuge and the serum is separated and frozen for future study. The sera are then put through an enzyme-linked immunosorbent assay (ELISA). IgG and IgM antibody surveys are then completed on dilute samples of the collected serum. A microplate reader is used to assess the presence of either IgG or IgM antibodies in the serum, determining if the individual providing the sample was or was not previously infected with WNV. WNV antibodies will adhere to either the WNV IgG antigen (direct) or to the WNV IgM anti-antibody (indirect) adhered to the wells of the ELISA test plate. The microplate reader generates an OD value for each serum sample. This value is then divided by the OD value of the calibrator to derive an Index Value. Index Values that are greater than 1.5 indicate a positive presence of WNV antibodies in the serum sample. Index Values lower than 0.8 indicate a negative presence of WNV antibodies in the serum sample.

**Results**

To date, this serosurvey has conducted two different ELISA studies. The second ELISA included four serum samples from 3 individuals with unknown history of WNV infection and one individual with diagnosed WNV infection. The results in the above table are consistent with the expected outcomes: the sample from the previously diagnosed individual (subject 4) was positive for the presence of WNV antibodies.

**Future Direction**

At this point in the serosurvey, the methods and materials have been proven to work successfully. Additionally, the protocols for conducting the ELISA have been refined and reproduced. The direction of this study will now turn to the broader population of Fremont County. Blood samples from the population will be solicited this summer (2010) for serum separation, storage, and study. We estimate that a minimum of 200 unique samples are needed to adequately study the Fremont County population (approximately 35,000). The study will perform ELISA studies on all samples. Individuals volunteering samples of their blood will answer a questionnaire that will determine if the individual has been positively diagnosed for WNV, vaccinated against other flaviviruses, and if the individual has traveled in regions where other flaviviruses are endemic. Results of this study will help inform public policy decisions about continued mosquito eradication efforts and provide data to help Wyoming Department of Health better predict the future of WNV human cases.

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Resources:

- http://www.badskeeter.org/