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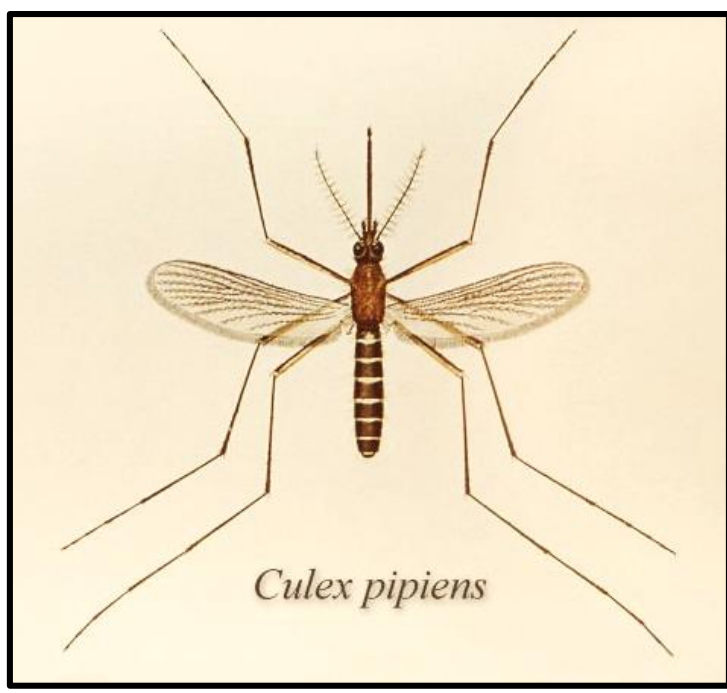
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A Surveillance Study on *Culex* sp. in Southeast Kansas and Prevalence of West Nile Virus in the Mosquito Population

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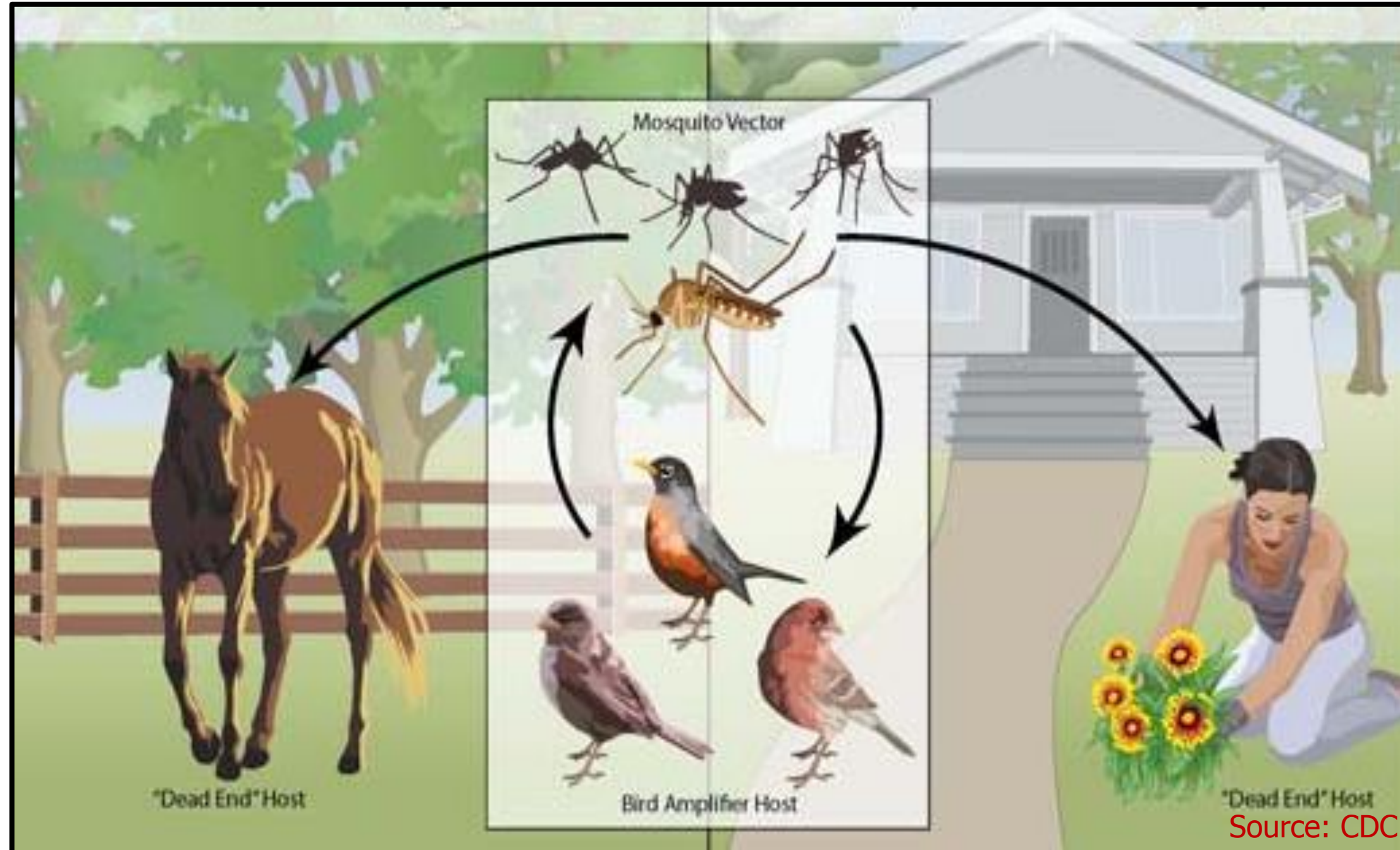


West Nile Virus and Public Health

West Nile virus (WNV) is the most common mosquito-borne disease in Kansas and the United States. Several species of mosquitoes can carry WNV but *Culex* is the most common in the U.S. According to the U.S. Centers for Disease Control and Prevention (CDC), 80% of people infected with WNV show no symptoms; but for the remaining 20%, symptoms can include headaches, body aches, joint pains, vomiting, diarrhea, or rash. Even less commonly, about one in every 150 people develop serious symptoms affecting the central nervous system, including brain & spinal inflammation, resulting in death in ~10% of those cases. There is no vaccine or cure for WNV currently, so avoiding being bitten is the best course of action.

The Kansas Department of Health and Environment (KDHE) announced that Kansas and neighboring states are becoming more and more hospitable to the *Culex* mosquitoes as average temperatures rise. KDHE reported 600 cases of the **worst** form of the disease in KS, including 30 deaths (1999-2017). The KDHE announced recently that based on historical human cases of the disease, half of the state is at a high risk for WNV infection, while the rest of the state is at a moderate risk (Fig. 1). Our study area will be landscaped vegetation at residential & commercial properties, parks, cemeteries, and wetland habitats in urban and suburban Crawford County and adjacent area in southeast Kansas.

West Nile Virus Transmission Cycle



WNV is considered **neuroinvasive** when a more severe onset occurs. Symptoms include high fever, confusion, coma, tremors, stiffness, convulsions, and muscle weakness. West Nile Neuroinvasive Disease (**WNND**) is very serious and should be treated right away.

History of WNV Strains in the U.S.

NY99

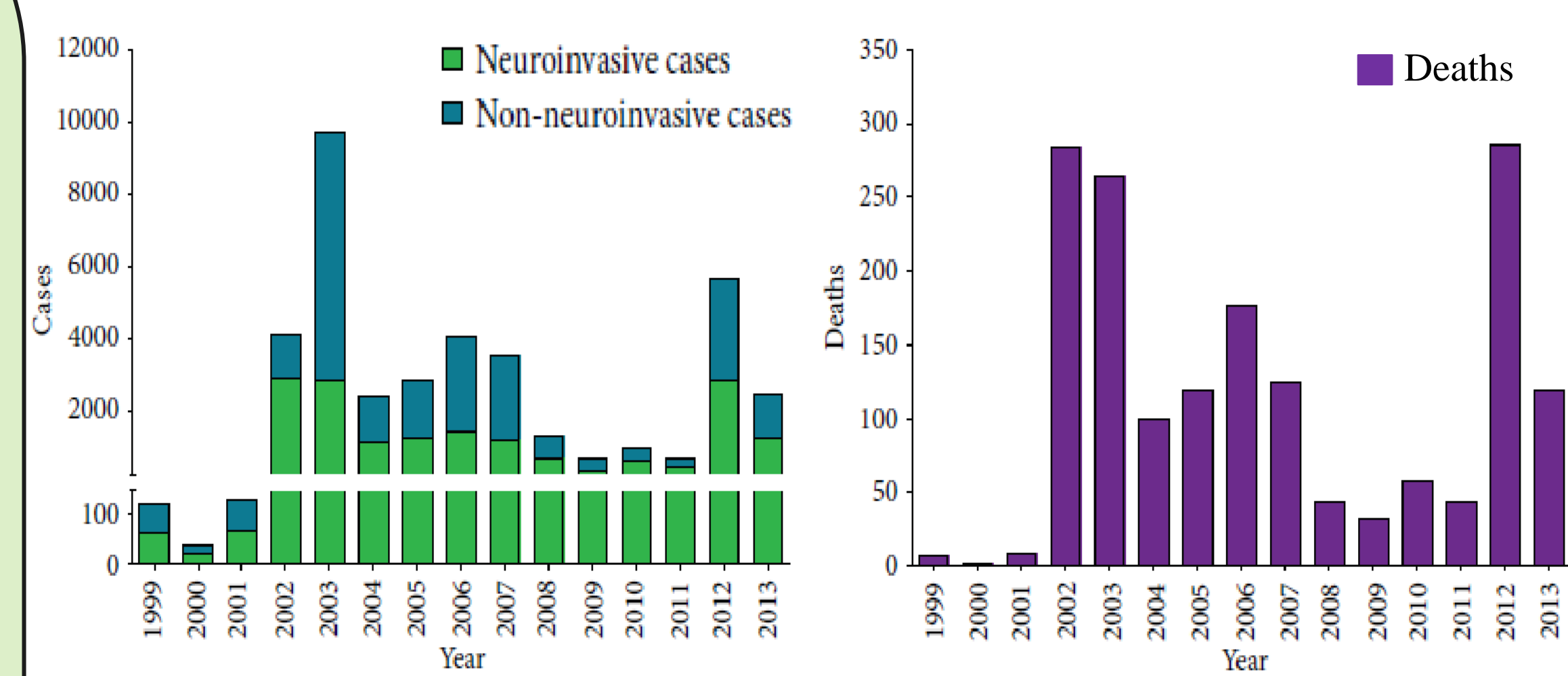
U.S. 1999: The strain involved in an outbreak in New York was named NY99. This was first major occurrence of the virus in the U.S. 59 out of 62 cases being WNND, leading to 7 deaths.

WN02

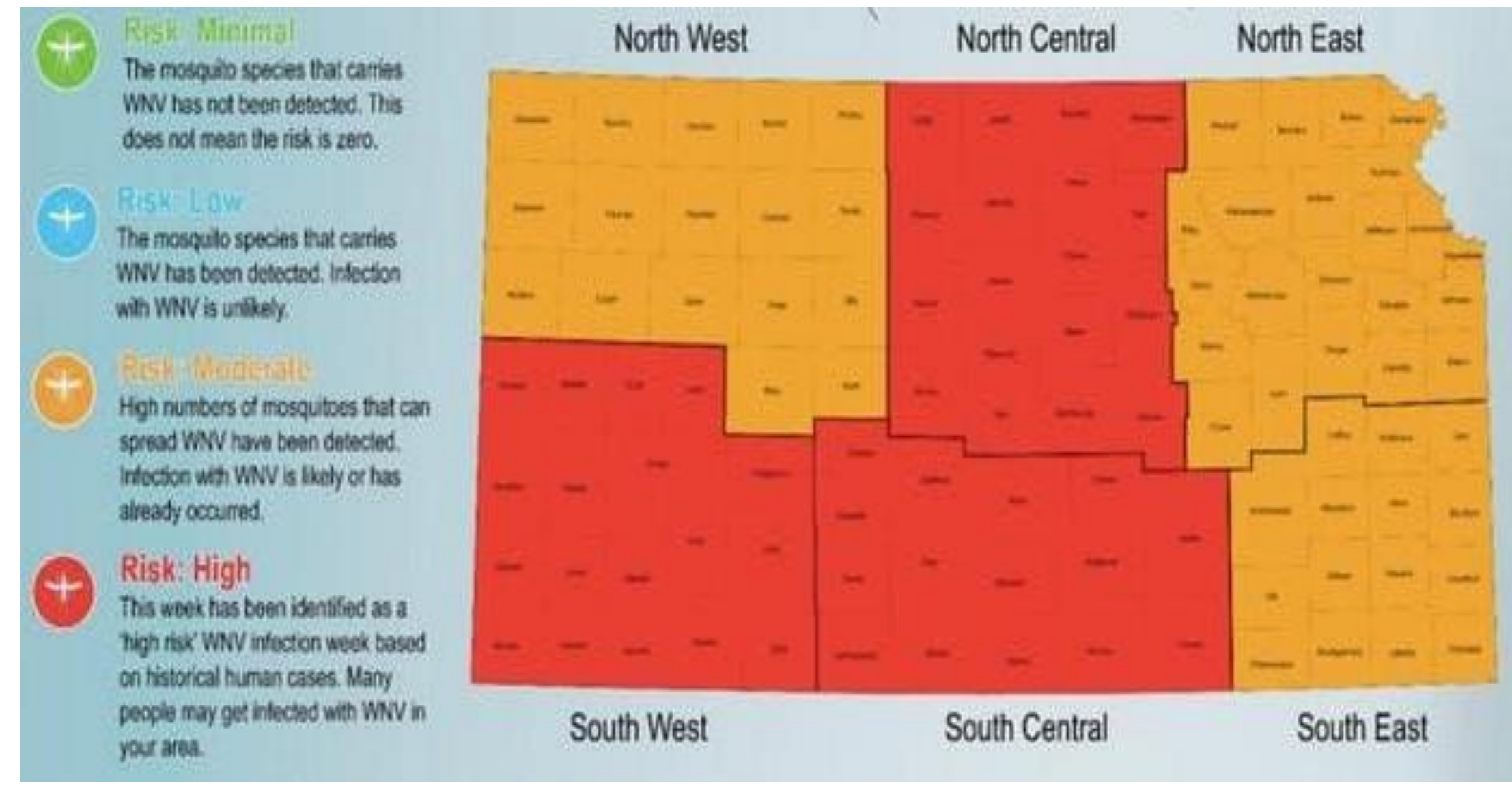
U.S. 2002-2003: During this outbreak a new viral genotype WN02 replaced the original genotype NY99; WN02 was observed to disseminate more efficiently in North American *Culex pipiens* and *Culex tarsalis* mosquitoes than the NY99 genotype.

U.S. 2012: A large outbreak in the U.S., causing 5,764 cases; of those, 2,873 were WNND and 286 deaths occurred. This was the most deaths ever reported in a single WNV season.

WNV Cases & Deaths from WNV Infections in the U.S. (CDC, 1999-2013)



Kansas WNV Risk Level & Surveillance Results (as of July 2018, KDHE)



Human WNV Outbreaks in Kansas (data provided by KDHE)

Year	Total cases	WNND cases	Non-WNND Cases
2015	34	12	22
2016	37	17	20
2017	27	11	16

Workflow of the Proposed Study

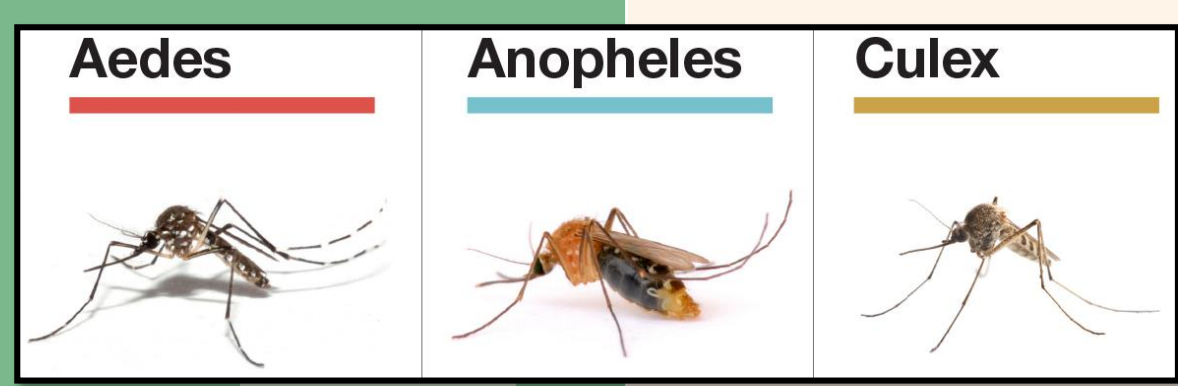
Collection

- Dry ice baits and gravid traps will be placed weekly at collection sites during peak season for a two year span
- Crawford county and surrounding areas will be studied
- Collected mosquitos will be transferred to laboratory live and will be knocked-down with dry ice or trimethylamine



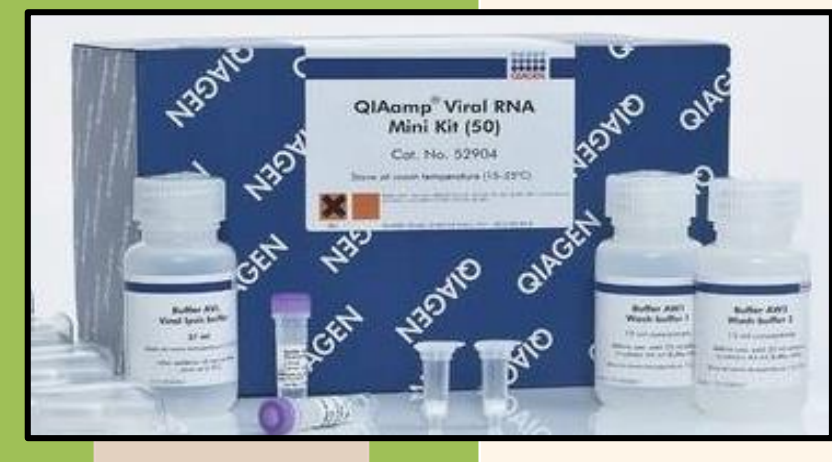
Identification

- Taxonomic keys will be used to identify species
- Males and females will be separated
- Females will be grouped into blood-fed and non blood-fed categories



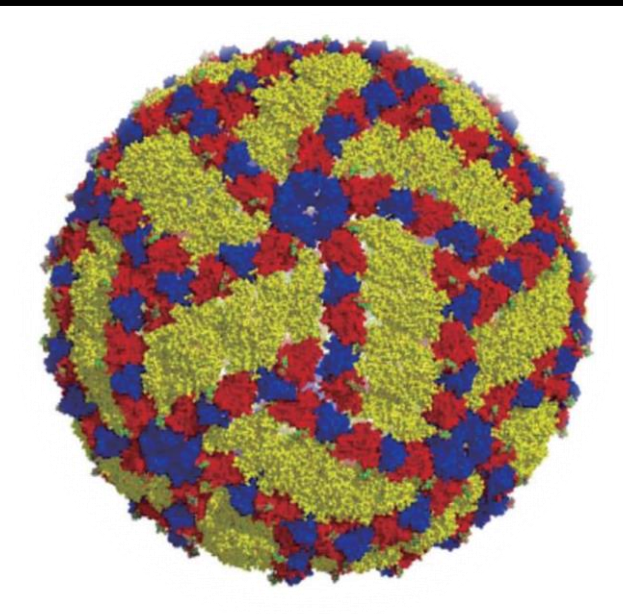
Laboratory

- Blood-fed females will be tested for WNV
- Head and thorax tissue will be inoculated and RNA will be extracted using a viral RNA kit
- The NS5, envelope and NS5 partial 3'UTR regions will be amplified using flavivirus consensus or West Nile specific primers.



Analysis

- Genotypic comparison:** amplified viral genes will be sequenced and compared with existing NCBI database sequences
- Statistical analysis:** R-statistical software will show presence and absence of WNV among collected mosquitoes



- West Nile virus particle based on cryogenic electron microscopy data
- Genome constituted by ~11,000 bases single-stranded RNA molecule of positive polarity

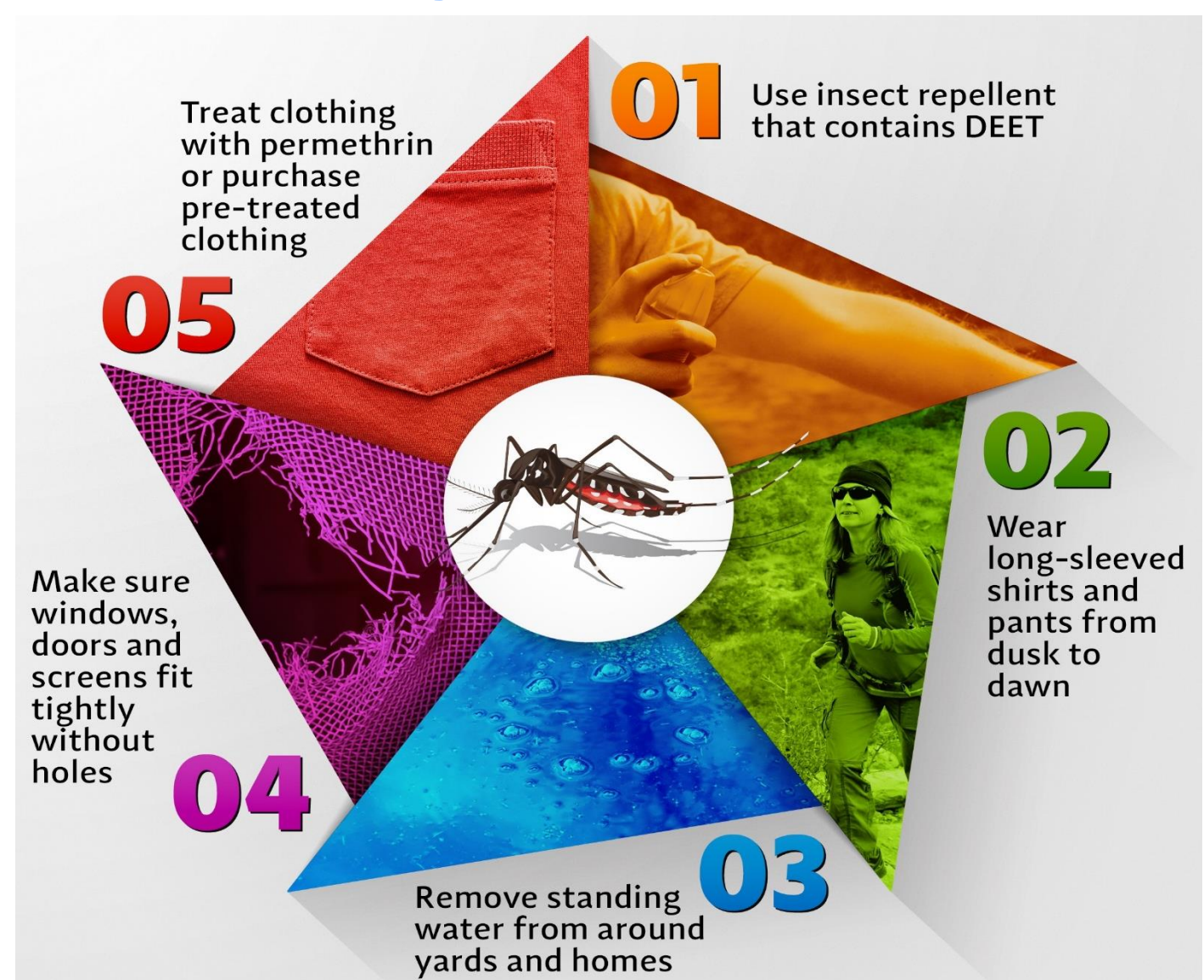
Outcome of the Proposed Study

Based on the recent risk alert on WNV in Kansas with regard to changes in climatic conditions, this proposal aims to establish an efficient and economical surveillance program for *Culex* spp. Although about a hundred neuroinvasive and non-neuroinvasive WNV human cases were reported in Kansas in last three years, there is no data available on the actual prevalence of the virus in mosquitoes. Therefore, this research will also determine the seasonal prevalence of WNV in *Culex* spp. in Crawford County (KS) and adjacent areas.

References

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