West Nile Story

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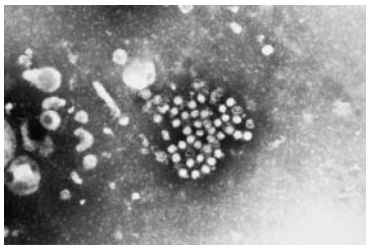
West Nile Virus

West Nile Province of Uganda

- First identified in 1937 in the West Nile Province of Uganda in the blood of a febrile woman
- Known at first as a cause of a febrile fever with spontaneous recovery
- Later caused neurologic disease with a spread to the Middle East and Southern Europe
- First recorded epidemic in Israel in 1950's
- Soon recognized as one of the most widespread Flaviviruses
- Distributed through Africa, West Asia, Europe and the Middle East

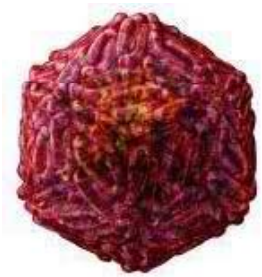


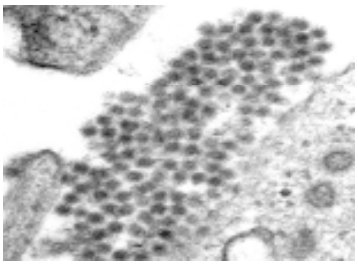
Lanciotti et al. 1999. Origin of the West Nile virus responsible for an outbreak of encephalitis in the northeastern U.S. [Science 286:2333-337.]

Major Outbreaks

- Israel - 1951-1954, 1957(Nursing homes)
- France - 1962
- South Africa - 1974
- Romania – 1996 (393 cases)
- Italy - 1998
- Russia - 1999
- United States - 1999-2002
- Israel - 2000
- France - 2000

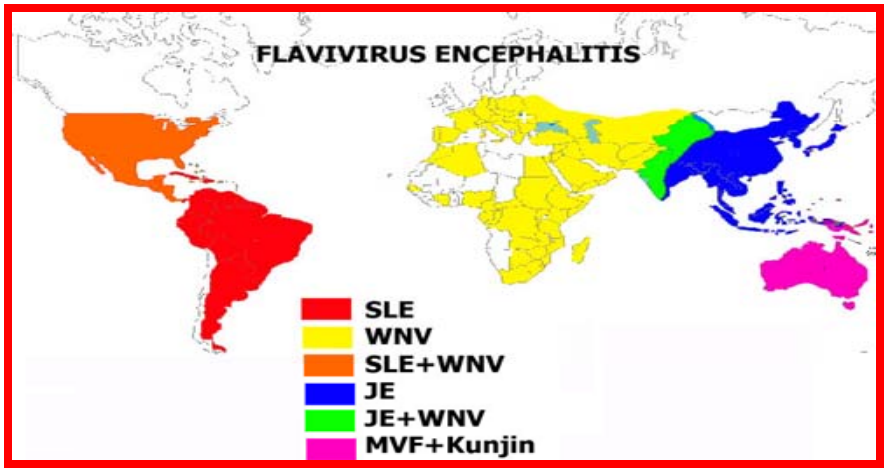
FlaviViruses (Flaviviridae family)





- **Flaviviruses are named from the yellow fever virus, the type virus for the family; *flavus* means yellow in Latin.**
- **Flaviviruses share a common size (40-65 nm), symmetry (enveloped, icosahedral nucleocapsid), nucleic acid (positive-sense, single stranded RNA approximately 10,000–11,000 bases), and appearance in the electron microscope.**
- **These viruses are transmitted by the bite from an infected arthropod (mosquito or tick). Human infections with these viruses are typically incidental, as humans are unable to replicate the virus to high enough titres to reinfect arthropods and thus continue the virus life cycle.**
- **In Flavivirus group:**
- **Other viruses in the group: Alfuy, Japanese encephalitis, Kokobera, Koutango, Kunjin, Murray Valley, St. Louis, Stratford, Usutu**
- **All transmissible by mosquitoes, reservoir in birds**
- **Most cause aymptomatic infections, benign fever or neuro-invasive disease**
- **The genome encodes 3 structural proteins (Capsid, prM, and Envelope) and 8 non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, 2K, NS4B and NS5).**

- **2 clades: vector borne viruses and no known vector**
- **Vector clade: mosquito borne and tick borne**
- **Mosquito group : 2 branches:**
  - **Neurotropic viruses: encephalitic disease in humans or livestock. Spread by Culex species. Bird reservoirs.**
  - **Hemorrhagic disease viruses: Aedes species as vectors and primate hosts.**
- **Tick-borne viruses:**
  - **Associated with seabirds**
  - **Tick-borne encephalitis associated primarily with rodents.**
- **No known vector:**
  - **Bat group I**
  - **Bat group II**
  - **Rodent group**



Dengue virus group
-Dengue virus (DENV)
-Kedougou virus (KEDV)
-Japanese encephalitis virus group
-Japanese encephalitis virus (JEV)
-Murray Valley encephalitis virus (MVEV)
-Rocio virus (ROCV)

St. Louis encephalitis virus (SLEV)
-Usutu virus (USUV)
-West Nile virus (WNV)
-Yaounde virus (YAOV)

Yellow fever virus group
-Sepik virus (SEPV)
-Uganda S virus (UGSV)
-Wesselsbron virus (WESSV)
-Yellow fever virus (YFV)

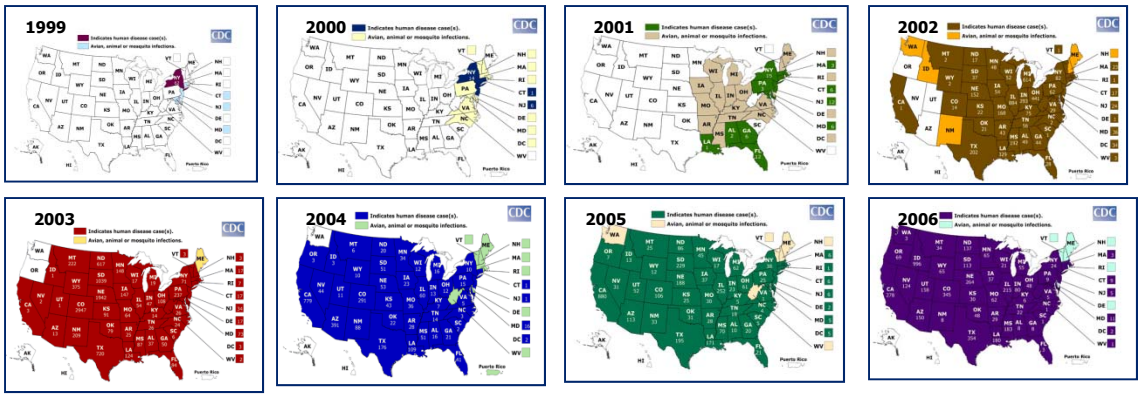
WNV in the U.S.

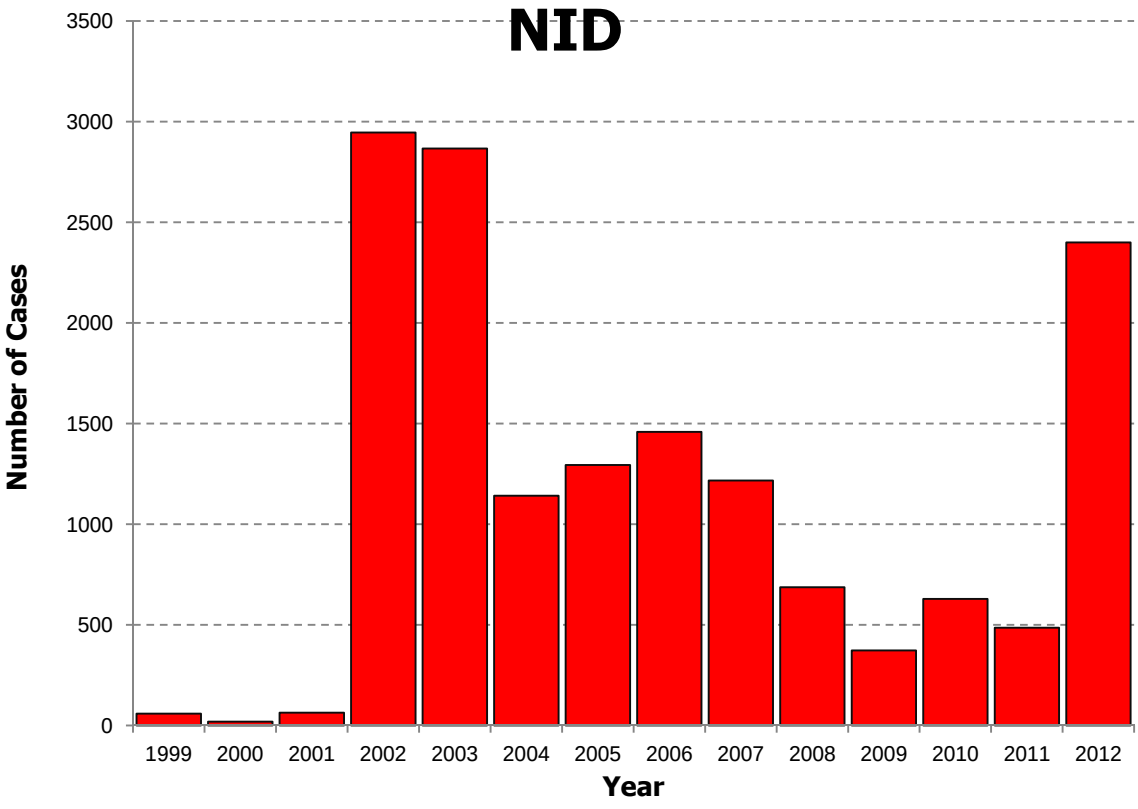
WNV TRANSPORTED IN THE USA 1999:

HOW ? We do not know

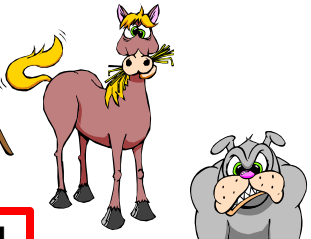
- **Infected human host**
- **Human-transported vertebrate host**
  - **Legal**
  - **Illegal**
- **Human-transported vector(s)**
- **Storm-transported vertebrate host (bird)**
- **Intentional introduction (terrorist event)**

FROM WHERE: ISRAEL / PALESTINE





Cycle: Mosquitoes & Birds



Incidental Hosts

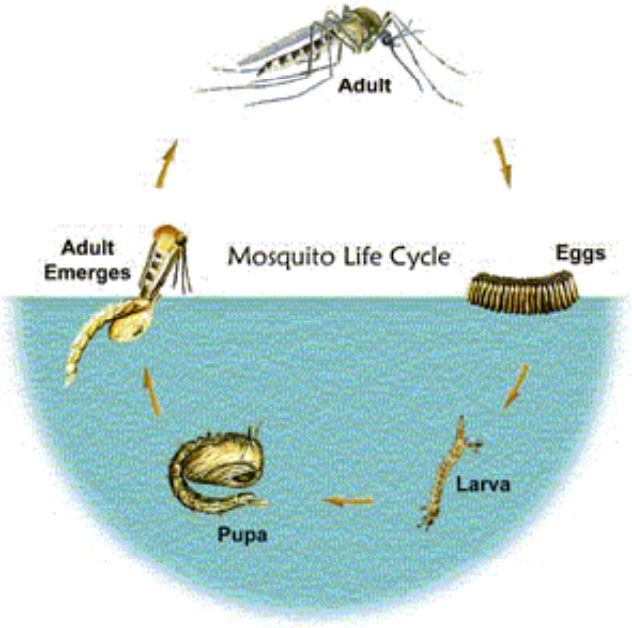
CYCLE

- **WNV in the blood of the bird (Viremic phase), depending on the species**
- **Female mosquito feeds on bird blood to mature her eggs**
- **At the same time, she ingests WNV**
- **WNV multiplies in the stomach cells; X by 100,000 times**
- **WNV migrates to the mouth parts of the mosquito; This intrinsic period lasts from 7 to 14 days depending on outside temperature;**
- **When ready, WNV injected into another bird or incidental hosts**

MAMMALS AS DEAD END HOSTS

- **Infected with WN virus**
  - **Mortality (big brown bat, little brown bat, rabbit, chipmunk, gray squirrel, horse, cat)**
  - **Seropositive dog**
- **Valuable as surveillance indicators**
  - **Identification of species for use as sentinels?**
- **Role of vertebrates not known**

Mosquitoes



Female mosquitoes need to feed on blood every few days as they need blood to mature their eggs

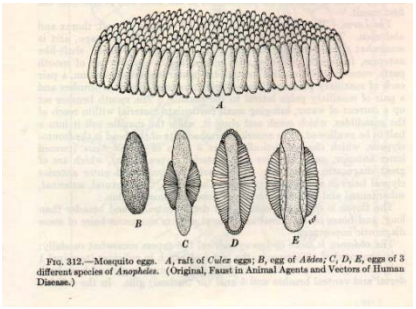
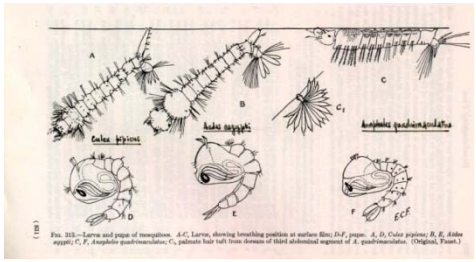


Fig. 312.—Mosquito eggs. *A*, raft of *Culex* eggs; *B*, egg of *Aëdes*; *C*, *D*, *E*, eggs of 3 different species of *Anopheles*. (Original, Faust in Animal Agents and Vectors of Human Disease.)



When a mosquito bites - saliva, containing anticoagulants and enzymes that produce hematomas, is injected

If undisturbed, the female will feed up to 4x her weight. If disturbed, she will seek out another host until blood volume is sufficient for egg development

- **Isolated from over 40 mosquito species, 29 in the USA**
- **Only few play a major role in transmission**
- **Mostly *Culex* species:**
  - ***Cx. univittatus, Cx. perixiguus, Cx. pipiens, Cx. modestus, Cx. quinquefasciatus, Cx. tritaeniorhynchus, and Cx. vishnui***
- **Other mosquito species in a variety of genera:**
  - ***Aedes, Aedeomyia, Anopheles, Coquillettidia, Mansonia, Mimomyia***
  - **Other species as accessory vectors?**
- **Isolated from ticks:**
  - **Soft tick genera: *Argas, Ornithodoros***
  - **Hard tick genera: *Amblyomma, Dermacentor, Hyalomma***

Mosquitoes:
Culex quinquefasciatus

Ubiquitous species and abundant in tropical and subtropical countries

Egg Laying: Females lay a single raft of 140-340 eggs on heavily polluted small water collection after each blood meal. Eggs hatch in 1-2 days

Egg to Adult: 8-12 days after laying

Breeding place: all types of large man-made containers and collections of ground water, storm sewer catch basin, ground pools, ditches, run-off from sewage plants, small artificial containers, cesspits, drains, septic tanks, unused wells, storm water canals.

Flying range: up to 3,600 feet /night

Life span: 2 weeks

Biting activity: usually attack humans towards the middle of the night indoors and outdoors; indoor biting

Preferred food: more attracted to birds > mammals and humans; 40% mammalian DNA in stomach
Six avian species most frequently fed on by Q more frequently than expected based on their abundance:
- **Northern cardinal**
- **Northern mockingbird**
- **Common grackle**
- **Brown thrasher**
- **House sparrows less frequently.**

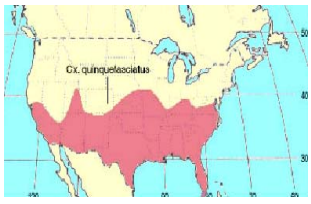
These data support the conclusions of previous studies that *Cx. quinquefasciatus* is the most important vector for both the enzootic amplification and transmission of West Nile virus to humans in southern Louisiana.

Vector:
Avian malaria (*Plasmodium relictum*)
Dog heartworm (*Dirofilaria immitis*)

***Wuchereria bancrofti* in Oriental & Afrotropical**

Japanese encephalitis virus in Vietnam

AKA Quink or Southern House Mosquito



QUINKS ARE HARD TO CONTROL
1-Asynchronous life cycle
2- Cryptic habitats, resting & breeding
3-Active ALL night long
4.-Ability to become tolerant to insecticides

Mosquitoes:
Culex quinquefasciatus

Breeding Sites & Hiding Places

Storm Sewer

Modular Waste Water Treatment Plant

Drainage Ditch

Storm Sewer

Mosquitoes:
Aedes albopictus

Oriental mosquito: recently invaded US (Houston 1985)
Morphology: white bands on legs; medium narrow band on thorax

Egg Laying: Eggs of *Aedes albopictus* are laid along the side of artificial or man-made containers and will hatch when water levels rise above the location of the egg, submerging it. The eggs will hatch in water with low turbidity and a pH ranging from 5.2 to 7.6 (optimal range from 6.8 to 7.6). The ideal pool of water has a high organic nitrogen content for feeding upon.

Egg to Adult: 10-14 days

Breeding place: all types of small peridomestic sites; man-made containers, tin cans, jars, bottles, dishes, wading pools, flower vases, clogged roof gutters, tires+++

Flying range: ~500 yards

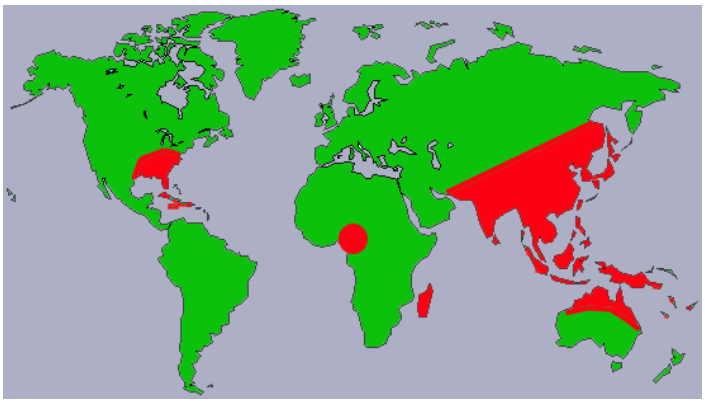
Life span: 2 weeks

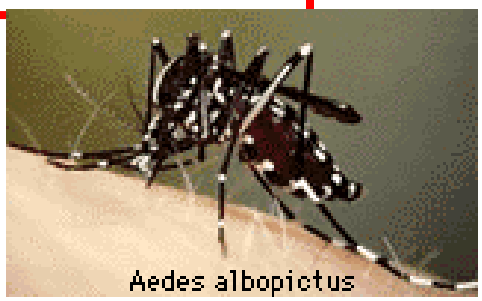
Biting activity: aggressive, lands & bites immediately, day time

Preferred food: humans

Vector: urban yellow fever, dengue, dog heartworm

AKA Asian Tiger Mosquito





Mosquitoes: *Culex salinarius*

AKA Salt Water Marsh Mosquito

Mostly Louisiana coastal areas

Morphology: Brown species, proboscis and tarsi dark, irregular narrow bands on abdomen

Breeding place: fresh and brackish water in marshes with emergent or decaying vegetation; ponds, pools, ditches, barrels, bilge water from boats, artificial containers around homes

Flying range: 1 mile

Biting activity: mostly outdoors, occasionally indoors; dusk, first hours of darkness

Season: Spring and Fall (contrary to other Cx continue during fall); colder months; predominant Cx in winter

Preferred food: 83% mammalian DNA in their stomach, not very discriminate

Vector: secondary vector of SLE, EEE

Overwintering in parous state, may harbor virus during winter

Mosquitoes: *Culex erraticus*

Morphology: Tiny dark mosquito

Breeding place: grassy shallow margins of ponds, lakes, marshes, and streams.

Flying range: 1 mile

Biting activity: mostly outdoors, occasionally indoors; dusk, first hours of darkness

Season: July to Sept.

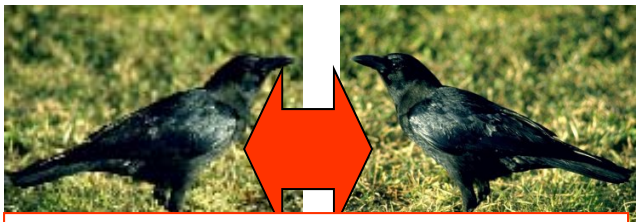
Preferred food: Birds (20% DNA in stomach), reptiles, mammals

Vector: EEE

Overwintering in parous state, may harbor virus during winter

Birds

- **Isolated from numerous wild birds**
- **Wetland and terrestrial species**
- **Birds are primary amplifier hosts**
- **Migratory bird role in distribution**
- **Viremia lasts several days**
- **Migratory birds travel far in a few days**
- **Strain highly infectious for North American birds**
- **Causing mortality and high viremia**



Direct transmission from crow to crow in cages: Virus in urine and feces, pecking

In the early years of the U.S. epidemic, WN was highly infectious and lethal for many American bird species

Dead birds were an early indicator of the presence of WN in an area

- **The virus is found in oropharyngeal and cloacal swabs of birds.**

- **The prototypical amplifying host for most bird-maintained arboviruses, is a species**
  - **that is locally abundant,**
  - **readily accessible to arthropod vectors,**
  - **develops a high level of viremia for an extended duration,**
  - **does not develop clinical disease.**

- **Species that had viremia levels of $<10^5$ PFU/mL were considered to be noninfectious for two enzootic vectors, *Cx.pipiens and Cx. quinquefasciatus,* and hence deemed incompetent hosts.**

- **The seasonal discrepancy in seroprevalence between late winter/spring collections (37.4%) versus summer collections (15.6%) may be partially ascribed to the influx of naïve juveniles into the population during months of quiescent, or reduced virus activity before the onset of peak transmission in late summer.**

Blue jays & crows were the main birds affected by WN

DEAD BIRDS WERE AN EASY WAY TO COLLECT AN INDICATOR OF WN PRESENCE
Direct bird surveillance became an important tool. Dead birds were reported to health departments, maps were made.

Birds

The order Passeriformes is by far the most successful group of birds on earth. They account for about 5,100 species, compared with 3,500 species in all the other orders combined.

Passerines, or perching birds, have three toes facing forward and one facing backward, allowing the bird to grasp branches for perching. Includes: sparrows, robins, finches, ravens or crows, yellow headed blackbirds, chickadees, red winged blackbirds, cowbirds, wrens, swallows, goldfinches, thrushes, jays, blue jays
Charadriiformes are gulls and shore birds, including: oystercatchers, stilts, avocets, plovers, sandpipers, jaegers, gulls , terns, skimmers , auks
Anseriformes are waterfowl, including ducks, geese, swan
Columbiformes are doves and pigeons, including rock doves
Galliformes are domestic fowl and gamebirds, including grouse, quail, pheasants, turkeys, chickens
Gruiformes are cranes, gallinules, and hemipodes, including whooping cranes, sandhill cranes, limkens, rails , coots
Piciformes are woodpeckers and toucans and their relatives.
Psittaciformes are parrots, including budgerigars or parakeets, cockatoos, cockatiels, macaws, Amazon parrots, keas

Passerine birds (blue jays, crows, sparrows, finches), Charadriiform birds (shorebirds such as gulls), and at least two species of raptors (specifically the kestrel and the great horned owl) are the most competent species to be considered candidates as reservoirs for WNV in the environment.
Most reported WNV in the wild has occurred in crows, but 150 species of birds have been found positive for WNV
•Birds can experimentally contract the disease orally in water, orally from ingesting infected mosquitoes, from other sick birds that are ingested, from small infected rodents (if ingested, this may explain large number of crow deaths, often carrion feeders), from a cage-mate by unknown means. Viremias are similar to those acquired by mosquito bite in all modes of transmission.
•Passerines and charadriiforms exhibit the highest viremias; Psitticine (parrots) and gallinaceous birds (chickens, turkeys, quail, pheasant) exhibited the lowest viremias
•Corvids (blue jays and crows) and ring billed gulls were the most likely species to die from the virus (among the species tested)
•Surviving birds produced neutralizing antibodies for 4 - 9 weeks post inoculation
•Birds shed the disease in the feces or from the cloaca (highest levels in crows and blue jays), and also in the oral cavity (highest levels in great horned owl, crows, and kestrel). Psittaciformes (parrots) and Piciformes (woodpeckers and toucans) did not shed the disease orally
•Viral load in organs of dead birds was highest in crows, blue jays, fish crows, and black billed magpies; almost all organs were infected, highest titers in kidney, brain and skin, also relatively high in eye, heart, spleen, lung, intestines, gonads, esophagus, lowest in liver.
•Ranking orders on competence for transmission of the virus to mosquitoes:
- **•1st passerines (the best)**
- **•2nd anseriformes (ducks, geese, and swan) and rock doves, weakly competent**
- **•incompetent to transfer to mosquitoes: galliformes and most columbiformes.**

•presence of high skin viremia may explain how virus can be transmitted to ticks

Baton Rouge Wild Birds	% Inf
Cardinal	67%
House Sparrows	21%
Blue Jay, Mocking Bird, Brn Trasher, Cmn Trasher, Morning Dove	22%

Phases of WN Transmission

Jan-Mar	**Maintenance Phase**
Apr-Jun	**Amplification Phase**
Jul-Aug	**Early Transmission**
Oct-Dec	**Late Transmission**

http://fmel.ifas.ufl.edu/buzz/jdavian.shtml

Culex **mosquitoes spend the winter hibernating in protected structures such as root cellars, bank barns, caves, abandoned tunnels and other subterranean locations. Overwintering adult mosquitoes do test positive for WNV**

Maintenance Phase (January-March) is when arboviruses survive the winter. It is difficult to document virus transmission or isolate viruses during the Maintenance Phase.

Amplification Phase (April-June) corresponds with a major portion of the avian nesting season. During this period, mosquitoes and non-immune nestling birds come into contact and initiate the first rounds of avian and mosquito infections. This is called "arboviral amplification."

The Early Epidemic Phase (July-September), is the hot, wet, humid period that includes the worst of the Atlantic hurricane season. During this phase arboviral transmission increases dramatically, human cases appear, and arboviral transmission to humans rapidly increases with epidemics usually peaking in late September.

Finally, during the Late Epidemic Phase (October-December) the number of new human cases gradually declines as epidemics burn themselves out.

Of the four phases, the Amplification Phase is the most important in determining the relative risk of human infection and the intensity of an arboviral epidemic later in the year.

Full-blown arboviral epidemics require large numbers of infected mosquitoes. WN outbreaks have typically reported mosquito infection rates ranging from 1:1,000 to 1:200. To realize mosquito infection rates of this magnitude, extremely efficient viral amplification must occur between local avian populations and vector mosquitoes. The Amplification Phase in mosquitoes corresponds with the avian nesting season. This is particularly important because nestling birds provide a relatively easy source of blood for infected mosquitoes and also provide a large population of non-immune birds that can efficiently amplify virus.

Epidemic arboviral amplification requires more than just an unusually productive avian nesting season. Large numbers of adult birds also need to be non-immune. Once a bird is infected with an arbovirus and survives, that bird is immune to re-infection for life. If, for example, 75% of the birds in a population have been infected with an arbovirus and are immune, then 3 out of 4 mosquito blood meals are wasted relative to arboviral amplification.

Infective mosquitoes feeding on immune birds do not re-infect that bird. Likewise, uninfected mosquitoes feeding on previously infected immune birds do not become infected. In order to have efficient amplification, a large population of non-immune birds must encounter infective vectors. When this happens in the presence of increasing vector populations, explosive amplification will occur.

Amplification Phase

The most important factors contributing to explosive arboviral amplification are:
1) **an extensive source of non-immune wild birds and**
2) **a steady supply of infective vector mosquitoes.**

There are 3 major sources of birds:
1-Exotic birds that are imported into the state and include pets, zoo animals, breeding animals in colonies, and birds used as agricultural commodities
2-Wild migrants that pass through, or take up temporary residence during autumn and spring migrations to and from wintering grounds in the Caribbean and Central and South America
3-Resident birds that remain in the state throughout the year

Exotic birds probably play only a minor role in arboviral amplification, mainly because they are so focal (flamingos and parrots in zoos, emus and ostriches in farms), and patchy in their distribution.

Huge numbers of migrant birds pass through, or overwinter. Obligate insectivores, such as swifts and swallows, pass through during southward migrations starting in August. Other migrant species follow, with the peak autumn migration occurring between September and October. This places large numbers of migrant birds during the Early and Late Transmission phases of the arboviral transmission cycle. The nonimmune portions of these migrant populations thus have the potential of adding to epidemic amplification. During spring migration, birds move through Louisiana heading north between February and May with peak activity in March and April. This places spring migrants in the state when mosquitoes are relatively rare. It is unlikely that the spring migrants contribute significantly to arboviral amplification that will result in epidemic transmission later in July-September.

Resident avian populations are therefore the most likely source for the arboviral amplification. Resident populations can be divided into those that are rare, focal, or widespread.

Rare birds may become infected and, in turn, infect vector mosquitoes. However, because these birds are rare and very patchy in their distribution, it is unlikely that they are responsible for infecting large numbers of vector mosquitoes.

Focal bird population: some avian populations are extremely focal in their distribution. For example, common grackles are focally distributed geographically and temporally. Common grackle populations are also very cyclic. During some years these birds are extremely abundant and produce huge numbers of offspring. The populations usually build slowly, peak over a 2-3 year period and then crash. During peak population years common grackles can be found nearly everywhere, but they are especially abundant around dumpsters associated with fast-food restaurants and grocery stores.

Finally, some avian populations are widely distributed both geographically and temporally. Species like mourning doves, blue jays, northern mockingbirds, and northern cardinals are found in large numbers in virtually every habitat, especially suburban residential neighborhoods. Because these 4 avian species are so widely distributed, they can efficiently amplify arboviruses over a large geographic area.

Other Modes of Transmission

- **Blood from infected donors**
  - **PCR on blood donors → few positives**
  - **Surveillance of Presumptive Viremic Donors (PVD):**
    - **84% develop no disease**
    - **15% WN Fever**
    - **1% NeuroInvasive disease**

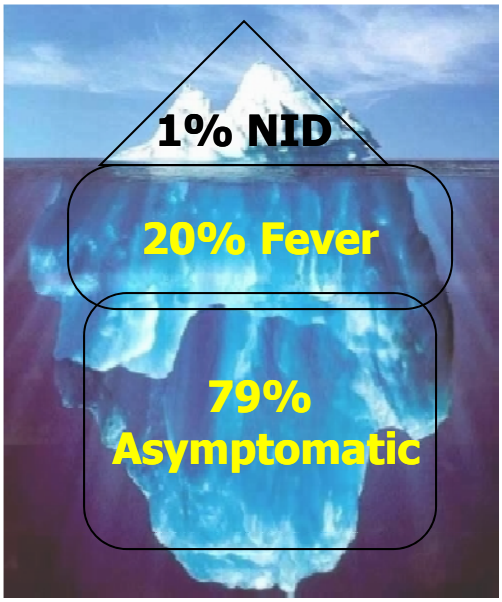
- **Transmitted in RBCs, plasma & platelets**
- **Transplant Associated:**
  - **Donor was negative on PCR and IgM**
  - **Became positive after 36 hrs**
  - **Infected 4 recipients: 1 Kidney →NID & death, 1 Kidney → NID**
    1 Heart → NID, 1 Liver →WNF

- **Transplacental**
  - **Late 2nd trimester infection, prolonged clinical illness in mother**
  - **Child with chorio-retinitis & bilateral white matter loss in temporal /occipital lobe, Temporal lobe cyst**
  - **Cord and heel: WNV IgM Positive**
  - **CSF: WNV IgM Positive**
  - **TORCH, CMV, LCMV Neg**
  - **HSV, WNV PCR negative**

- **Milk**
  - **In 2002: 6 infants < 1 year old**
  - **1 asymptomatic (breast feeding associated)**
  - **5 WNV NID: 1 breastfed BUT mother not infected, 4 not breast fed in month before illness**

WNV Human Infection

- **Incubation Period**: 1-2 weeks
- **Asymptomatic** : No symptoms
- **WNV-Fever (mild illness)**
  - Fever
  - Headache, body and joint aches
  - Fatigue, tiredness
  - Mild rash
  - Adenopathy: few, slightly enlarged
- **WNV-NID (neuroinvasive disease)**
  - Meningitis, neck stiffness
  - Encephalitis,
  - Flaccid paralysis, severe muscle weakness, Disorientation
  - Tremors
  - Altered mental status, coma
  - Similar to Guillain-Barre Syndrome
- **Case fatality rate:**
  - Romania/1996: 4%
  - Avg. U.S. Case Fatality Rate 11%
  - Higher in age > 70 years old and hospitalized (up to 25%)



<65 yr old NID= 1/300

65+ yr old NID= 1/50

Long term sequelae very common (>1 year after illness); Alan Ou, Louisiana

Pains	Chronic Headache	31.4%
	Joint Pain	46.6%
	Muscle Ache	49.2%
Sens-Motor	Paresthesia	43.2%
	Tremor	35.6%
	Weakness	50.0%
Mental State	Altered Mental Status	38.1%
	Confusion	36.4%
	Seizures	6.8%
	Coma	0.0%
Other CNS	Slurred Speech	17.8%
	Visual Impairment	43.2%
	Auditory Impairment	26.3%
	Balance Problems	41.5%
Mood	Depression	40.7%
	Anxiety	37.3%
	Hyperactivity	16.9%
	Emotional Lability	39.8%
	Aggressivity	33.9%
	Agitation	45.8%
	Panic Attack	20.3%
Chronic Fatigue		54.2%
Urinary Incontinence		18.6%

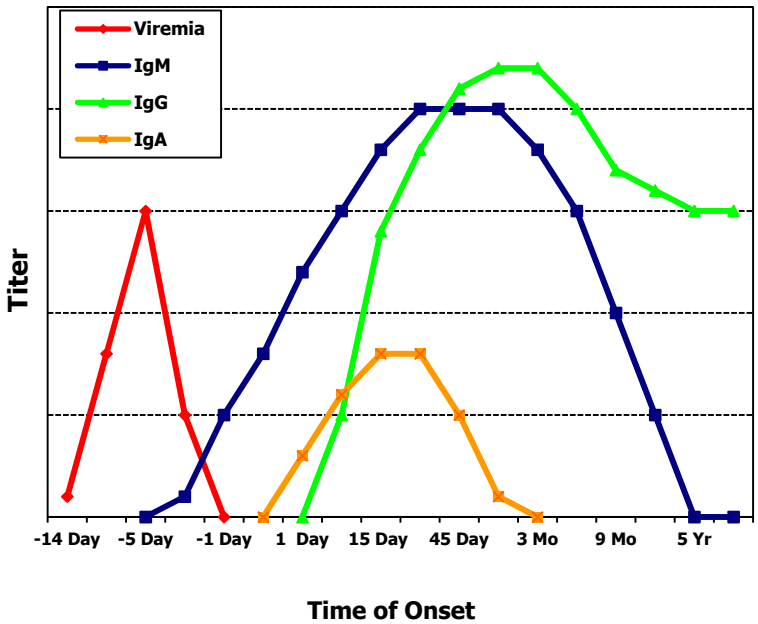
Viremia & Antibodies

COLLECT CSF IS NID SUSPECTED
- Lymphocytic pleocytosis <1,000 cells/mm^3
- Neutrophils may predominate in early infections
- Protein – moderately increased
- Glucose – normal

SEROLOGIC ASSAYS in serum and CSF
- IgM ELISA (MAC-ELISA)
- IgG ELISA
- PRNT
- Complement Fixation
- Hemagglutination Inhib
- Imuno Fluorescence

The IgM antibody capture enzyme-linked immunosorbent assay (MAC–ELISA) is the most conclusive laboratory method for diagnosis of WNV infection of the CNS. Most large private labs offer:
- -IgM MAC–ELISA
- -IgG ELISA
- -Real-time polymerase chain reaction (PCR)
- -Nucleic acid amplification test i(NAT) for donor testing.

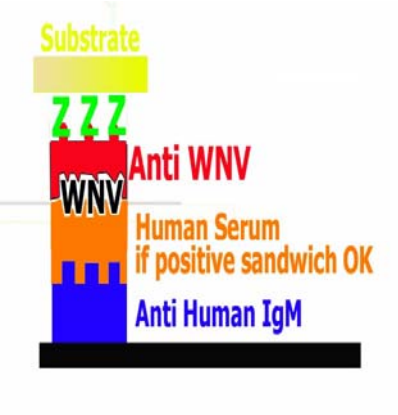
The methods can be performed using CSF or blood (ie, serum/plasma) samples.



TIMING
PCR: 1-14 days before onset, less sensitive
IgM: Does not cross BBB, pos at NID onset, last for months /years: 40% positive at 1 year
IgG: Start 7-21 days, last life
Cross Reaction: SLE, Dengue, JE

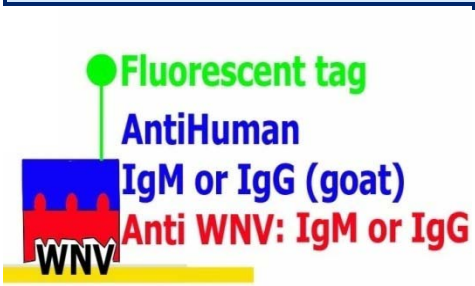
Diagnostic Tests

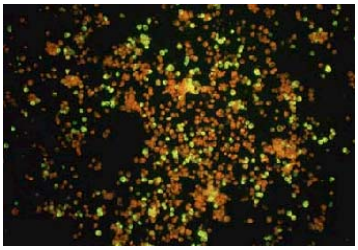
EIA: IgM Antibody Capture Enzyme Linked Immuno Absorbent Assay

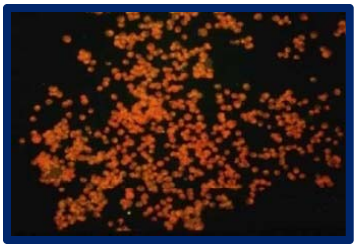


- The bottom of the tube is coated with an Anti-Human IgM. The serum of the patient is then added, then the antigen (extract from WNV), then an anti-WNV antibody tagged with an enzyme, then a substrate that will change color in the presence of the enzyme.
- If the serum contains anti-WNV antibodies, the sandwich is complete and the substrate will change color: this is a <u>positive</u> reaction.
- If the serum does NOT contains anti-WNV antibodies, one of the layers of the sandwich is missing and the upper layers of the sandwich do not stick. When the substrate is added, there is no change in color. This is a <u>negative</u> reaction.
- The optical density of the reaction measures the intensity of the reaction

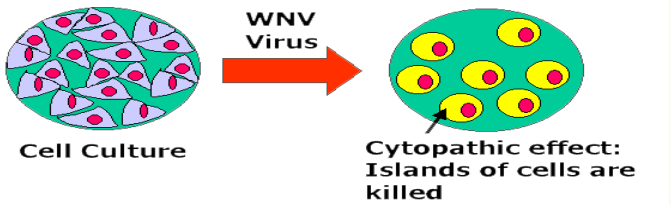
IFA: Immuno Fluorescence Test

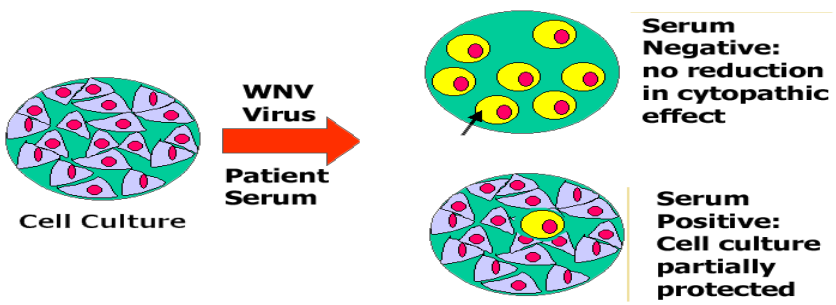




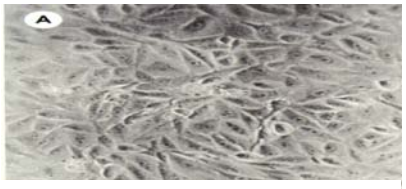


Plaque Reduction Neutralization Tests





- Tested serum added to live virus and cell culture
- If there are antibodies against the virus in the test serum
- reduction in virus damage
- compared to control with no antibodies
- Best test for differentiating WNV from SLE, dengue or Yellow Fever
- BUT
- It requires handling cell cultures and live virus
- It takes several days to evaluate plaque reduction



Vero cell culture

WN in Horses

- Weakness (94%)
- Ataxia (72%)
- Abnormal Mentation (67%)
- Nonresponsiveness
- Somnolence
- Low numbers of seizures
- Persistent or intermittent
- Increased Body Temperature (65%)
- Fasciculation (61%)
- Anorexia (57%)
- Cranial Nerve Deficits (44%)
- Teeth Grinding (20%)
- Case Fatality Rate 30%

Ataxia

Flaccid Paralysis

VACCINE

- Killed virus product given conditional license by APHIS / VS / CVB on 1 August 2001, renewed 2002
- Full license February 2003
- Over 6 million doses distributed
- Administration: 2 doses IM 3-6 weeks apart; one annual booster.

-Given a reported vaccine efficacy of 94% in properly vaccinated horses:
- On average, ~100,000 horses in a State

-If 20 percent are vaccinated (20,000) and if clinical attack rate is about 4 per 1000, then:
-80 vaccinated horses should have become ill, but 94% (75) would be protected;
-thus, 5 vaccinated horses would still become clinically ill (vaccine "failures") in an "average" State in 2002.
-The more vaccinated horses, or the higher the attack rate, the more potential vaccine failures.

WN in Other Mammals

- Infected with WN virus
  - Mortality (big brown bat, little brown bat, rabbit, chipmunk, gray squirrel, horse, cat)
  - Seropositive dog
- Valuable as surveillance indicators
  - Identification of species for use as sentinels?
- Role vertebrates not known

Other Species

- Epizootic characterized by neurologic disease which occurred at a 9,000-head alligator farm in Florida.
- Approximately 300 alligators (*Alligator mississippiensis*) died during this outbreak
- *Information provided by University of Florida, preliminary data

- High hatchling mortality at 4 LA farms (700)
- Isolated to 1 building
- Alligators bitten around eyes & mouth
  - Most likely cause of initial infection
  - Then spread by feces
- Large refuse ponds, mosquito breeding sites
- NO threat to public
  - Heating kills virus in meat
- Threat to workers?
  - Turkey farmers
  - Skinning process

<u>Clinical signs</u> associated with WNV in alligators -

• Primarily neurological in origin: Swimming in circles, muscle tremors, lack of coordination, head tilts

<u>Transmission</u> of WNV to alligators

• Certain species of mosquitoes feed on alligators and reptiles from the areas of soft skin such as around the eyes and inside the mouth.

• Alligators get high levels of the virus in the blood and consequently shed the virus in the feces.

• Possibility for other alligators within the pen or building to become infected by contact with alligator feces

<u>Potential risks to humans</u> in contact with alligators -

No studies on direct risks of contact with infected alligators, their organs, blood, or meat. Because it appears that alligators can have high levels of WNV in their blood and shed it in the feces, exposure to these could be a way of possible human infection.

Human Surveillance (IDEpi)

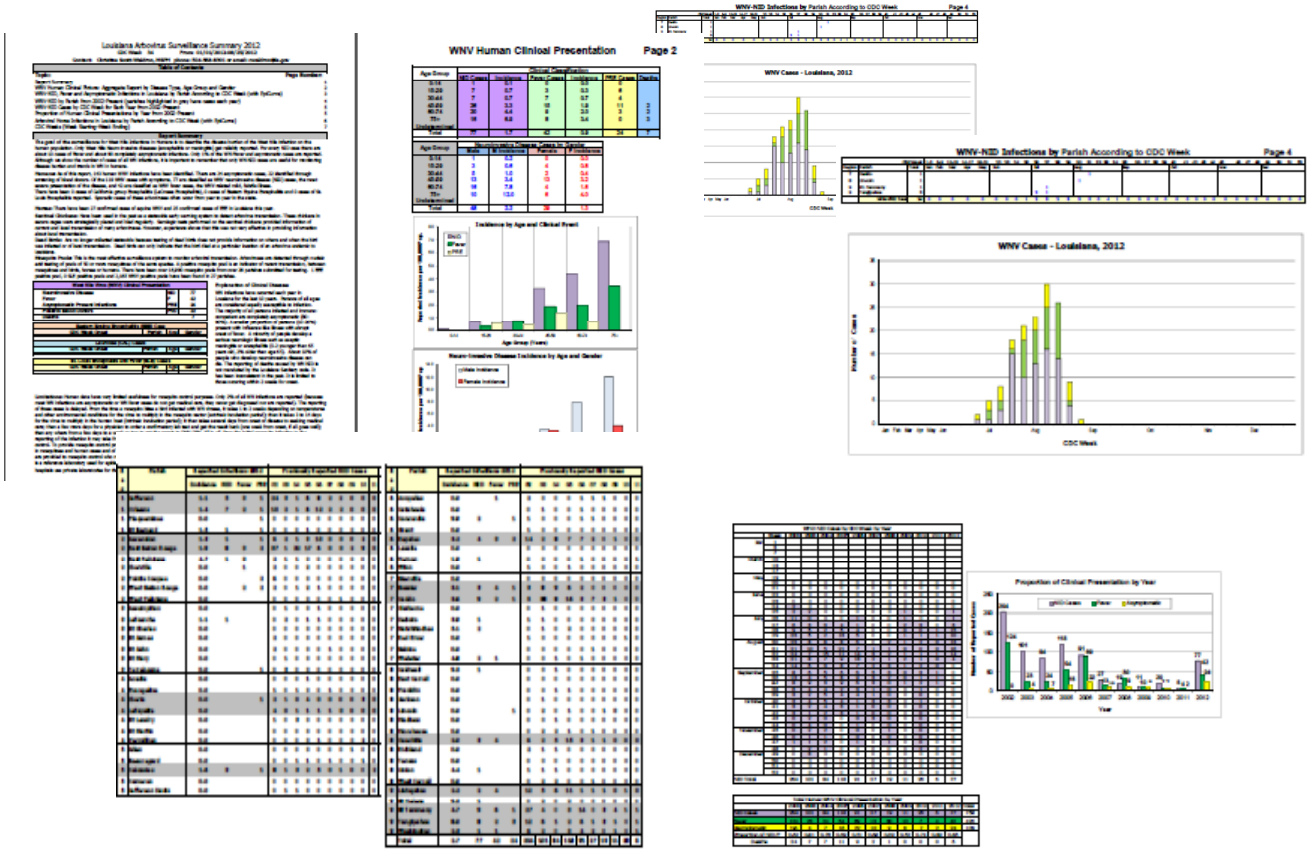
HUMAN SURVEILLANCE

Reporting through:

•Web-based Infectious Disease Reporting Information System (IDRIS),
•Electronic Laboratory Reporting (ELR),
•Phone, fax, hearsay, media and public.

Collection of basic demographics, onset date, clinical status for classification, lab tests for verification of case definition.

•Analyzes data, studies trends
•Cases attributed to week of onset and parish of residence
•Weekly summary sheet posted on web, e-mailed to ID Physicians, Infection Preventionists, Public Health Regions
•Reported to Mosquito Abatement Districts once weekly: Only address at 100th block level
•Reported weekly to CDC ArboNet



ROLE OF THE SECTION OF INFECTIOUS DISEASE EPIDEMIOLOGY

- **Provides consultation to physicians regarding diagnosis of arboviral encephalitis**
- **Provides medical and epidemiologic expertise during outbreaks**
- **Develops guidelines for surveillance**
- **Evaluates the encephalitis situation to make recommendations for the development of a comprehensive encephalitis control program for the whole state**
- **Communicates with the Department of Agriculture, the LSU Veterinary laboratory and the veterinarian community**

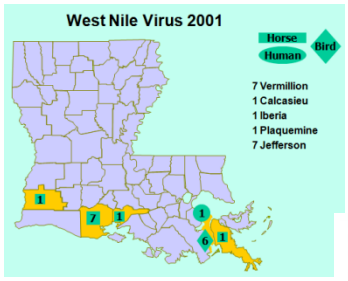
Reg	Parish	Reported Infections 2012				Previously Reported NID Cases									
		Incidence	NID	Fever	PRE	02	03	04	05	06	07	08	09	10	11
1	Jefferson	1.1	5	3	1	24	3	1	6	8	2	2	0	0	0
1	Orleans	1.4	7	2	1	10	2	1	6	12	2	2	0	0	0
1	Plaquemines	0.0			1	0	0	0	0	0	0	0	0	0	0
1	St Bernard	1.5	1		1	0	0	0	1	0	0	0	0	0	0
2	Ascension	1.3	1		1	6	2	1	3	10	0	0	0	2	0
2	East Baton Rouge	1.9	8	3	2	37	1	22	17	6	0	0	2	9	0
2	East Feliciana	4.7	1	3		2	1	1	0	0	0	0	0	0	0
2	Iberville	0.0		1		2	0	0	2	0	0	0	0	0	0
2	Pointe Coupee	0.0			2	6	0	0	0	0	0	0	0	0	0
2	West Baton Rouge	0.0		2	2	2	0	1	2	1	0	0	0	0	0
2	West Feliciana	0.0				0	0	0	0	0	0	1	0	0	0
3	Assumption	0.0				0	1	0	0	1	0	0	0	0	0
3	Lafourche	1.1	1			0	2	0	1	1	0	0	0	0	0
3	St Charles	0.0				0	0	0	0	0	0	0	0	0	0
3	St James	0.0				2	0	0	0	0	0	0	0	0	0
3	St John	0.0				2	0	0	0	0	1	0	0	0	0
3	St Mary	0.0				0	1	0	0	0	0	0	0	0	0
3	Terrebonne	0.0			1	0	3	0	0	0	0	0	0	0	0
4	Acadia	0.0				0	0	0	1	0	0	0	0	0	0
4	Evangeline	0.0				1	0	1	0	0	1	0	0	0	0
4	Iberia	0.0			1	2	1	0	4	0	0	0	0	3	0
4	Lafayette	0.0				4	0	1	1	1	1	0	0	0	0
4	St Landry	0.0				1	0	3	0	0	0	0	0	0	0
4	St Martin	0.0				0	0	0	0	0	0	0	0	0	0
4	Vermillion	0.0				0	0	0	0	1	0	0	0	2	0
5	Allen	0.0				0	0	0	0	0	0	0	1	0	0
5	Beauregard	0.0				0	0	1	1	0	1	0	0	1	0
5	Calcasieu	1.6	3		1	8	1	3	2	5	0	1	0	0	2
5	Cameron	0.0				0	0	0	0	0	0	0	0	0	0
5	Jefferson Davis	0.0				0	1	1	0	0	0	0	0	0	0
6	Avoyelles	0.0		1		2	0	0	0	1	1	1	0	0	0
6	Catahoula	0.0				0	1	0	0	1	0	0	0	0	0
6	Concordia	9.9	2		1	1	0	0	0	1	1	0	0	0	0
6	Grant	0.0				1	0	0	0	0	0	0	0	0	0
6	Rapides	3.2	4	3	2	14	2	8	7	7	2	0	1	0	0
6	Lasalle	0.0				0	0	0	0	0	0	0	0	0	0
6	Vernon	1.9	1			0	0	0	0	1	0	0	0	0	1
6	Winn	0.0				1	0	0	1	0	0	0	0	0	0
7	Bienville	0.0				0	0	0	0	0	0	0	0	0	0
7	Bossier	3.1	3	4	1	3	8	9	6	2	0	0	0	0	0
7	Caddo	3.6	9	2	1	5	38	8	16	3	7	3	1	0	0
7	Claiborne	0.0				0	1	0	0	0	0	0	0	0	0
7	DeSoto	3.9	1			1	1	0	0	0	0	0	0	0	0
7	Natchitoches	5.1	2			0	1	0	2	0	0	0	0	0	0
7	Red River	0.0				1	0	0	0	0	0	0	0	1	0
7	Sabine	0.0				0	0	0	0	0	1	0	0	0	0
7	Webster	4.8	2	1		0	0	1	0	1	0	0	0	0	0
8	Caldwell	9.5	1			0	0	1	0	0	0	0	0	0	0
8	East Carroll	0.0				0	0	0	0	0	0	0	0	0	0
8	Franklin	0.0				0	0	1	1	0	0	0	0	0	0
8	Jackson	0.0				0	1	0	0	0	0	0	0	0	0
8	Lincoln	0.0			1	0	2	0	1	0	0	1	0	0	0
8	Madison	0.0				0	0	1	0	0	0	0	0	0	0
8	Morehouse	0.0				0	2	2	1	0	1	0	0	0	0
8	Ouachita	2.0	3	4		6	2	5	15	3	1	1	0	0	0
8	Richland	0.0				2	1	1	0	0	0	0	0	0	0
8	Tensas	0.0				0	0	0	0	0	0	0	0	0	0
8	Union	4.4	1			1	1	1	0	0	0	0	0	0	0
8	West Carroll	0.0				0	2	2	0	0	1	0	0	0	0
9	Livingston	2.2	2	4		12	5	6	11	1	1	1	0	1	0
9	St Helena	9.5	1			0	2	0	2	0	0	0	0	0	0
9	St Tammany	4.7	9	6	1	27	4	0	3	14	0	3	4	1	1
9	Tangipahoa	8.0	8	2	3	12	6	1	2	6	1	3	1	0	1
9	Washington	2.3	1	1		6	2	0	3	4	2	0	1	0	1
	Total	2.7	77	42	24	204	101	84	118	91	27	19	11	20	6

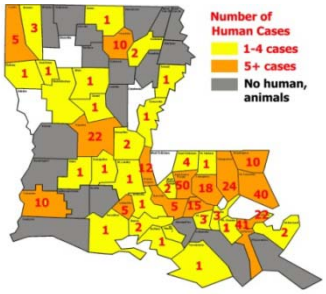
Human Surveillance 2002-2012

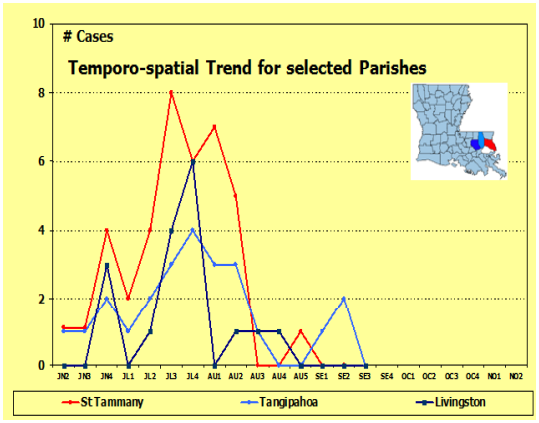
WNV-NID Cases by CDC Week by Year												
	Week	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
Jan	1											
	3											
	7											
March	10											
	13											
	17											
May	19											
	20	0	0	0	0	0	0	0	0	0	0	0
	21	0	0	0	0	0	0	0	0	0	0	0
June	22	0	0	0	0	0	0	0	0	0	0	0
	23	0	0	0	0	0	0	0	0	0	0	0
	24	2	0	0	0	0	0	0	0	0	0	0
	25	2	2	0	0	0	0	0	1	0	0	1
July	26	11	0	0	0	1	0	0	1	0	0	1
	27	6	3	3	4	1	0	0	2	3	0	3
	28	9	5	2	5	4	0	0	0	0	1	15
	29	23	5	2	13	5	0	0	1	1	1	10
August	30	23	8	8	8	6	0	2	1	2	0	13
	31	21	10	5	21	7	1	1	0	0	0	16
	32	24	7	15	11	14	3	2	1	1	1	14
	33	21	8	7	9	13	2	1	2	1	0	4
	34	14	6	3	8	7	2	3	1	2	0	0
September	35	8	6	5	6	6	5	3	0	3	1	0
	36	13	4	5	8	9	3	2	0	1	1	0
	37	8	9	3	9	6	3	0	1	2	1	0
	38	6	4	4	2	3	1	0	0	1	0	0
	39	3	2	5	4	4	1	0	0	0	0	0
October	40	3	4	5	4	1	3	3	0	1	0	0
	41	3	2	4	3	1	0	0	0	0	0	0
	42	3	1	2	3	1	0	0	0	0	0	0
	43	0	2	0	0	0	3	0	0	0	0	0
	44	0	4	0	0	1	0	0	0	0	0	0
November	45	0	2	2	0	0	0	1	0	0	0	0
	46	0	1	1	0	0	0	0	0	0	0	0
	47	1	1	2	0	1	0	1	0	0	0	0
	48	0	2	1	0	0	0	0	0	2	0	0
December	49	0	3	0	0	0	0	0	0	0	0	0
	50	0	0	0	0	0	0	0	0	0	0	0
	51	0	0	0	0	0	0	0	0	0	0	0
	52	0	0	0	0	0	0	0	0	0	0	0
NID Total		204	101	84	118	91	27	19	11	20	6	77

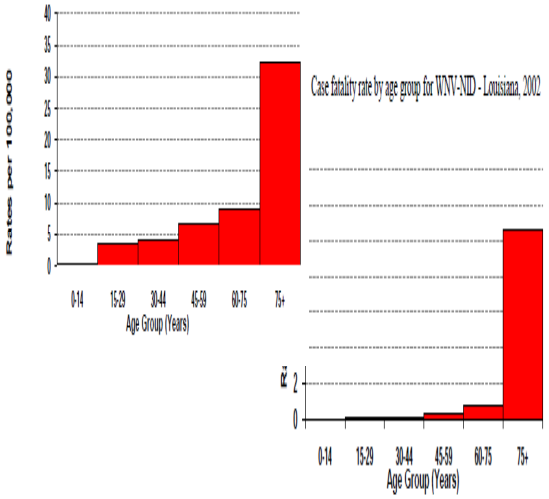
Total Human WNV Clinical Presentation by Year												
	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	Total
NID Cases	204	101	84	118	91	27	19	11	20	6	77	758
Fever	124	23	24	54	89	13	30	10	7	4	42	420
Asymptomatic	NA	4	7	16	22	10	9	8	7	2	24	109
Deaths	24	7	7	11	9	2	1	0	0	0	6	
Total Disease	328	128	115	188	202	50	58	29	34	12	143	

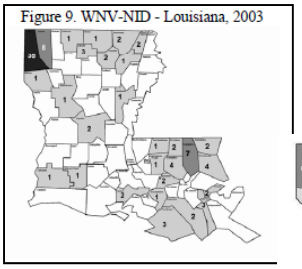
Human Surveillance-Louisiana

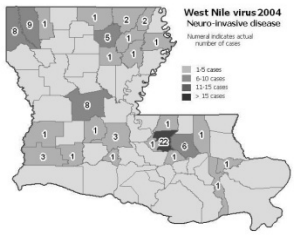








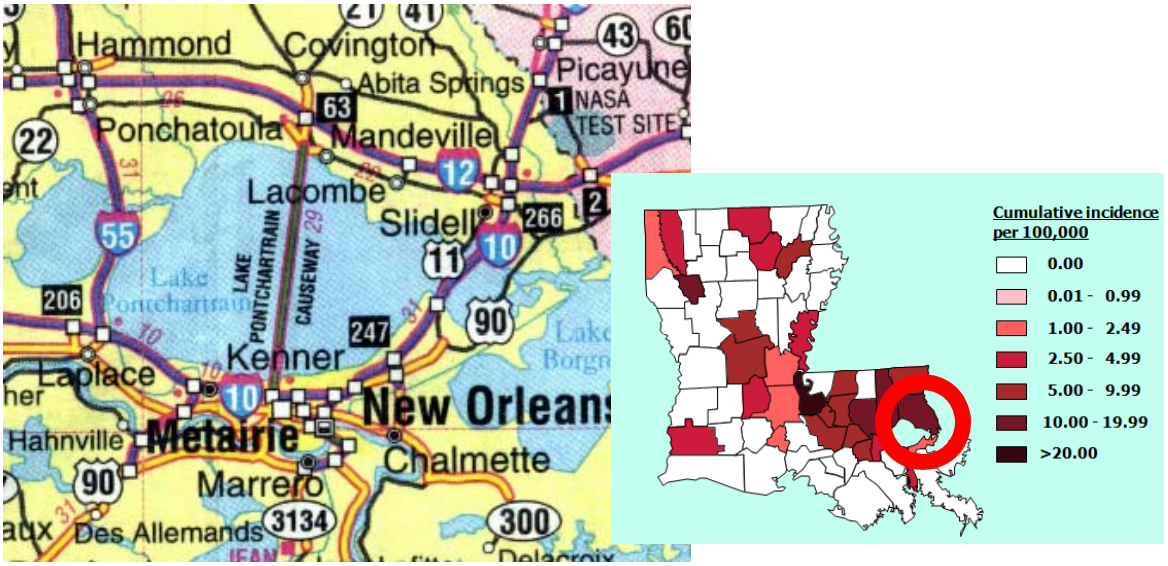




STOP & THINK

Limitations: Human data have very limited usefulness for mosquito control purposes. Only 2% of all WN infections are reported (because most WN infections are asymptomatic or WN fever cases do not get medical care, they never get diagnosed nor are reported). The reporting of those cases is delayed. From the time a mosquito bites a bird infected with WN viruses, it takes 1 to 2 weeks depending on temperatures and other environmental conditions, for the virus to multiply in the mosquito vector (extrinsic incubation period); then it takes 3 to 14 days for the virus to multiply in the human host (intrinsic incubation period); it then takes several days from onset of disease to seeking medical care; then a few more days for a physician to order a confirmatory lab test and get the result back (one week from onset, if all goes well); then any where from a few days to a week or two to get the report to DHH OPH. All in all, from the initial mosquito infection to the reporting of the infection it may take from 3 to 6 weeks. In summary, human data are too little too late to be of major use for mosquito control. To provide a mosquito control program with data on location of human cases that may be of limited use for correlating infection rates in mosquitoes and human cases and of use to address public and media concern, general geographical location of cases and weeks of onset are provided to mosquito control who request the information. This information must remain strictly confidential. The DHH OPH Laboratory is a reference laboratory used for epidemiologic purposes. Its role in diagnosis of cases is limited since the great majority of physicians and hospitals use private laboratories for their diagnosis.

Slidell 2002 Serosurvey



- **Serosurvey for West Nile Virus Infection - Slidell, Louisiana, 2002**
- **In 2002, because of high WNV activity, St. Tammany parish was chosen to establish the community-wide prevalence of recently acquired WNV infection. A household-based serosurvey was carried out in and around Slidell, in late October.**
- **Two-stage cluster method to select a representative sample of households in the Slidell area (2000 census population = 45,672), Louisiana. Residents from at least 10 households in each of 70 randomly selected clusters were invited to participate.**
- **Among 1,226 participants, 21 had serological evidence of recent WNV-infection (seroprevalence = 1.7%).**
- **WNV-specific IgM antibodies, positives further tested for WNV-specific neutralizing antibodies.**
- **758 participating households, 1,226 individuals; WNV-specific antibodies detected in 21 participants. Prevalence of WNV infection = 1.7% (95% confidence interval [CI] = 0.8%-2.6%).**
- **Seroprevalence ranged from 3.9% among participants aged 15-24 years to 1.0% among those ≥65 years, but differences were not statistically significant (p = 0.31).**
- **No difference in seroprevalence existed between female and male participants (both 1.7%). The 21 seropositive participants lived in 20 different households; these households were distributed among 17 clusters in no discernable geographical pattern.**
- **Nine (48%, CI = 22%-74%) of 20 seropositive reported a febrile illness between June and October 2002 compared to 212 (18%) of 1,191 seronegative participants.**
- **WNV seropositivity was thus associated with a febrile illness (p = 0.01), and an estimated 30% of the seropositive participants had a febrile illness attributable to a recent WNV infection (95% CI = 7%-53%). In contrast, the percentage of participants reporting symptoms of West Nile fever was similar among seropositive and seronegative participants (4.2% vs. 4.4%, respectively; p = 0.96).**

Vector Surveillance

Virus Detection Assays in Mosquito pools, Tissues

- Virus isolation (cells, mice)
- IFA
- TaqMan RT-PCR**
- Ag-capture ELISA
- RT-PCR / sequencing
- Dipsticks**
- NASBA**

- Implemented by 26 Parishes registered as Mosquito Abatement Districts
- Samples sent to Louisiana Animal Disease Diagnostic Laboratory at LSU
  - Mosquito Pools
  - Sentinel Chickens
  - Wild Birds
  - Dead Birds
  - Varies on Local Parish Mosquito Abatement District or Health Unit
- LA ArboNet and weekly spreadsheets

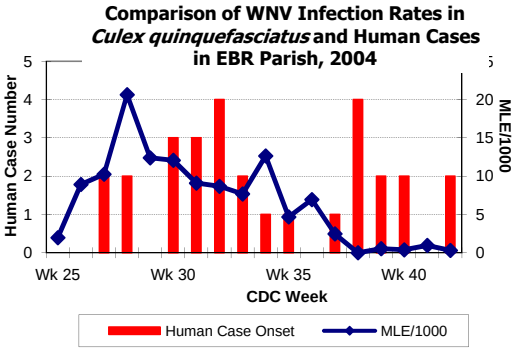
MOSQUITO POOLS
Collect mosquitos (50)
Same species
Test for presence of virus

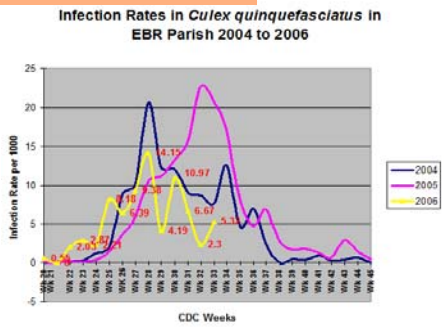
VecTest™ assays for arbovirus antigen

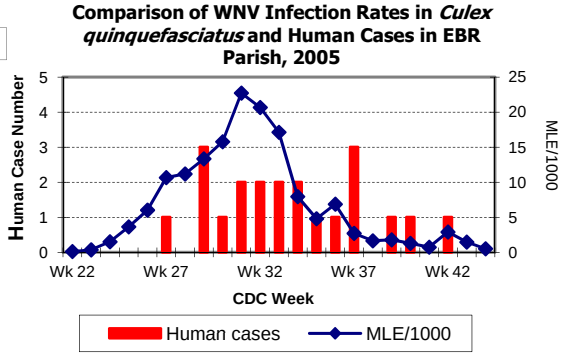
Control Zone: captures unbound Ab-gold complex, confirms migration of sample through test zone.

Test Zone: virus-specific antibodies immobilized on membrane, SLE (top) and WN (bottom) in this assay.

Reagent Zone: virus-specific antibodies conjugated to gold particle.







Chickens are no longer used as sentinels
-Highly susceptible (for development of antibodies), -Resistant to mortality, -Non-infectious to each other, -Non-infectious to handlers, -Non-infectious to mosquitoes
-Chicken flocks placed at known transmission foci
-Birds are bled periodically, serum samples sent for testing
-HI or M-ELISA test used for screen, -N test for confirmation
-Positives replaced for accurate monitoring
BUT EXPERIENCE SHOWED THAT CHICKENs WERE NOT A VERY EFFECTIVE METHOD, MOSQUITO POOLS ARE MUCH BETTER

Environmental Observations

- **Houses with broken windows, broken screens**
- **Houses with porch and outdoor seating**
- **Standing water on property**
- **Abandoned houses or bordered an abandon home, vacant lot, or undeveloped property**
- **Houses within a block of a public drainage canal, with standing water**

Prevention

Main Prevention consists of:

1-An early warning system to detect the presence of virus in an area

VECTOR SURVEILLANCE

2-Reduction of the number of mosquitoes

-by reducing larval site: SOURCE REDUCTION
-by destroying mosquito larvae in standing water: LARVICIDING
-by killing adult mosquitoes before they bite people: ADULTICIDING

3-Prevent mosquitoes from biting people: PERSONAL PROTECTION

4-Diagnose early to minimize the consequences of the disease: particularly Neuro Invasive Disease (NID): EARLY DIAGNOSIS

5-Disseminate information: HEALTH EDUCATION

4-EARLY DIAGNOSIS
Particularly important for Neuro Invasive Disease

1-People need to know the early signs of encephalitis: fever and brain troubles (confused, disoriented, comatosed, difficulty seeing, hearing, speaking - must be checked quickly

2-Suspect WN-NID in case of suspected viral meningitis, Guillain Barré, unexplained paralysis, tremor

5-HEALTH EDUCATION

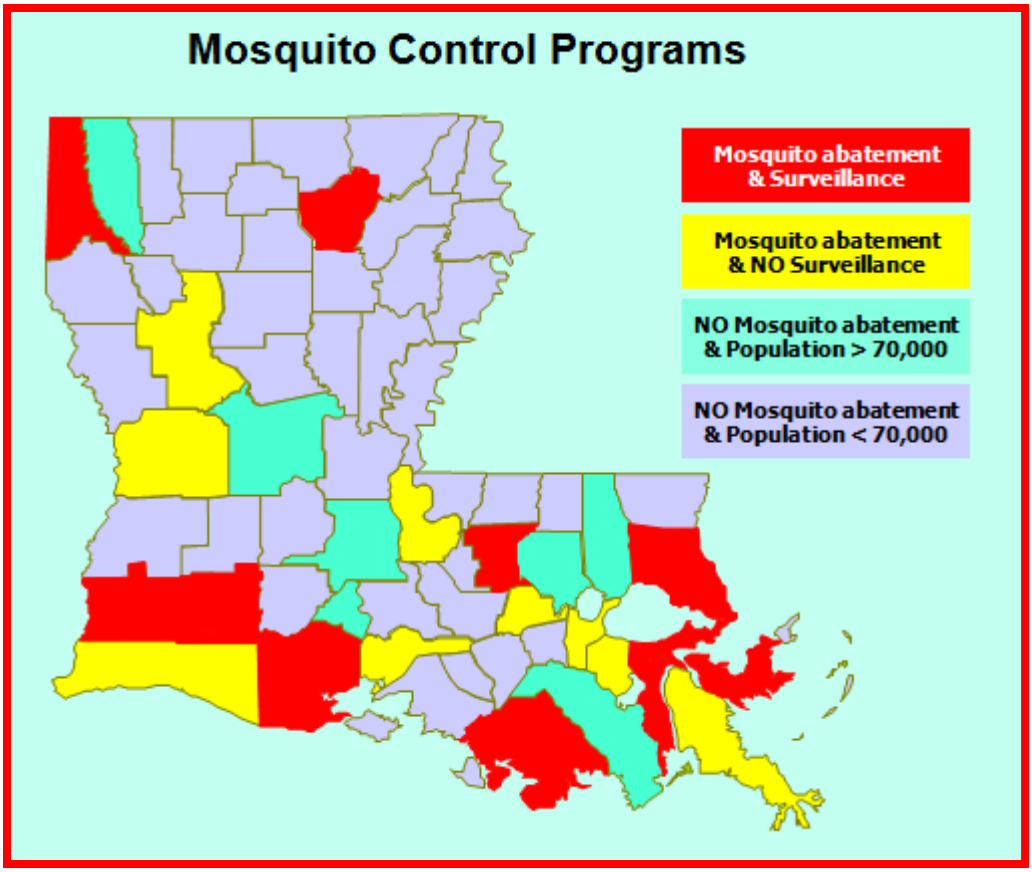
1-Advice on encephalitis prevention communicated through press releases, radio and TV interviews, website and pamphlets

www.oph.dhh.state.la.us

2-Collaboration with local community groups to promote health education and behavior modification

3-Educational programs for medical professionals, conferences, computer facilitated educational programs, lectures

Mosquito Control Programs



Source Reduction

- **Eliminating mosquito breeding areas can be an extremely effective and permanent way to reduce mosquito populations without resorting to insecticides.**
- **HOME OWNERS: Eliminate mosquito breeding grounds by removing unused plastic pools, old tires, or buckets; by clearing clogged gutters and repairing leaks around faucets; by regularly changing water in bird baths; and by filling or draining puddles, swampy areas, and tree stumps.**
- **OPEN WATER MARSH MANAGEMENT (OWMM) involves the use of shallow ditches, about 4 feet wide and 2 feet deep, to create a network of water flow within marshes and to connect the marsh to a pond or canal. The network of ditches drains the mosquito habitat and lets in fish, which will feed on mosquito larvae.**

•"Get rid of standing water around residence"
•"Most of the mosquitoes that bite you are from your neighborhood"

Larviciding

- **Also used as a <u>BIOLOGICAL CONTROL</u> agent is the dead spores of varieties of the natural soil bacterium *Bacillus thuringiensis*, especially *Bt israelensis* (BTI). BTI is used to interfere in the digestion systems of larvae. It can be dispersed by hand or dropped by helicopter in large areas. BTI is no longer effective after the larvae turn into pupae, because they stop eating.**
- **2 species of fungi are currently also being used for killing adult mosquitoes. These are the *Metarhizium anisopliae* fungus, and the *Beauveria bassiana* fungus.**

- **An <u>OIL DRIP</u> can or oil drip barrel was a common and nontoxic antimosquito measure. The thin layer of oil on top of the water prevents mosquito breeding in two ways:**
  - **Mosquito larvae in the water cannot penetrate the oil film with their breathing tube, and so drown and die;**
  - **Adult mosquitoes do not lay eggs on the oiled water.**

- **<u>CONTACT POISONS, GROWTH REGULATORS, SURFACE FILMS, STOMACH POISONS</u> (including bacterial agents), and biological agents such as fungi, nematodes, copepods, and fish. A chemical commonly used in the United States is <u>methoprene</u>, considered slightly toxic to larger animals, which mimics and interferes with natural growth hormones in mosquito larvae, preventing development. Methoprene is frequently distributed in a time-release briquette form in breeding areas.**





Adulticiding

•Adulticides used include malathion, naled, chlorpyrifos, permethrin, resmethrin, sumithrin, and other products.
•The decision about which material to use is based on several factors including the efficacy as determined by scientifically conducted field trials, mosquito species susceptibility, safety, and cost. The insecticide choice is made by each mosquito control agency and varies throughout the state due to differing mosquito species and application requirements.
•Applications are made to coincide with mosquito flight activity so that the insecticide droplets contact the target insects and to avoid the flight activity of non-target insects such as bees and butterflies.

•Local mosquito control kills adult mosquitoes spraying from trucks, plane or even going house to house on special occasions

•Space sprays typically use Ultra Low Volume (ULV) technology, sometimes referred to as cold fogging. Space sprays are applied with specialized spray equipment mounted in aircraft, on the back of trucks, or even carried by hand.

•With space sprays, aerosols are released to drift through a target zone. Chemical concentrates most often are used and, even if diluted, volumes of material used remain low. The aerosol persists in the air column for an appreciable length of time at suitable droplet densities to contact the flying mosquito and is only effective while the droplets remain airborne. Hence, a space spray is short-lived and is not expected to have any residual effect.

•ORGANOPHOSPHATES (OP) generally are acutely toxic and work by inhibiting important enzymes of the nervous system . At the junction between two neurons (a synapse) and at aneuromuscular junction, the neurotransmitter is acetylcholine. Acetylcholine is broken down and inactivated in milliseconds by cholinesterase enzymes. With exposure to OPs, cholinesterase is inhibited, and a build-up of acetylcholine occurs. If acetylcholine is not broken down, the nerve impulse does not stop, ultimately causing paralysis of the insect and eventually death. The organophosphates used in Florida include malathion, naled, and rarely, chlorpyrifos.

•Malathion is used mostly for ground and some for aerial adulticide applications. It is used extensively to control major arthropod disease vectors (Culicidae) in public health programs.

•Naled is the primary chemical used in aerial adulticiding in Louisiana. In Naled breaks down rapidly in the environment. It is highly corrosive and requires special consideration in handling and equipment designs

•Chlorpyrifos is rarely used in mosquito control in Louisiana.

PYRETHROIDS are chemicals whose structures mimic the natural insecticide pyrethrum. Pyrethrins are found in the flower heads of some plants belonging to the family Asteracae (e.g., chrysanthemums). These insecticides have the ability to knockdown insects quickly. Pyrethrums can be degraded very easily by ultraviolet light which oxidizes the compounds. Pyrethroids can pose significant hazards to aquatic organisms. They are highly toxic to insect pests at very low rates (often one order of magnitude less than OPs). Synthetic pyrethroids have been chemically altered to make them more stable and safer to mammals. Pyrethroids are axonic poisons; they poison the nerve fiber by binding to a protein in nerves called the voltage-gated sodium channel.

Permethrin is used for ground adulticiding and is the primary chemical used for adulticiding it is one of the least expensive compounds available for adulticiding. A disadvantage is that it is highly toxic to aquatic organisms.

Resmethrin is used for both ground and aerial adulticiding



Personal Protection

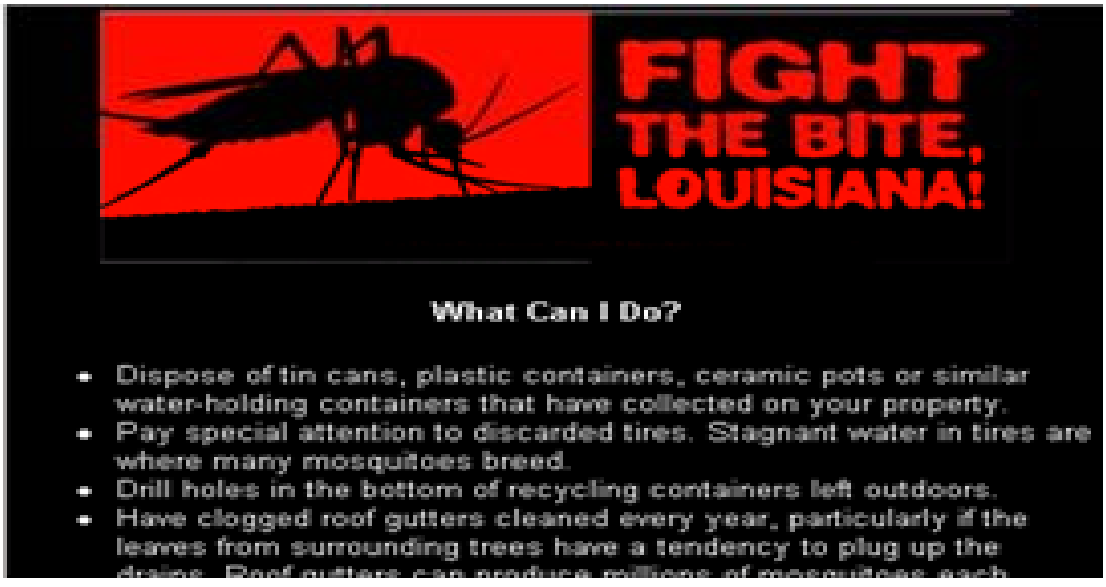
- **PEOPLE CAN PREVENT MOSQUITO BITES**
- **MOSQUITOES ARE NOT WELCOME IN YOUR HOME. The most dangerous mosquitoes bite late at night when you are sleeping. Make sure mosquitoes cannot enter your house. Doors or windows should be closed or have screens in good condition.**
- **DO NOT BREED MOSQUITOES AROUND YOUR HOUSE. Make sure you do not have small containers full of standing water: flower pots, fountains, bird baths, old tires, junk**

EMPTY IT, PITCH IT, PUNCH HOLES AT THE BOTTOM

BUT DON'T LET IT SIT THERE

- **PROTECT YOUR SKIN:**
  - **When you are out - wear long sleeve shirts and long pants and avoid dark colors which attract mosquitoes.**
  - **Do not wear perfume**
  - **Apply insect repellent**

PROTECT YOUR SKIN, PROTECT YOUR LIFE



Repellants

•Types:

• DEET (N,N-diethyl-m-toluamide): Apply according to label instructions may be used along with a separate sunscreen
•Permethrin is different in that it is actually a contact insecticide.
•Icaridin, also known as picaridin, Bayrepel, and KBR 3023
• Essential oil of the lemon eucalyptus (*Corymbia citriodora*) & active compound p-menthane-3,8-diol (PMD)
• Nepetalactone, also known as "catnip oil"
• Citronella oil
• Neem oil
• Bog Myrtle
• Dimethyl carbate
• Tricyclodecenyl allyl ether, a compound often found in synthetic perfumes[6][7].
• IR3535 (3-[N-Butyl-N-acetyl]-aminopropionic acid, ethyl ester)
• Ethylhexanediol or "6-12 repellent," discontinued in the US in 1991 due to evidence of causing developmental defects in animals[8]
•A more recent repellent being currently researched is SS220, which has been shown to provide significantly better protection than DEET.
•Birch tree bark is traditionally made into tar. Combined with another oil (i.e. fish oil) at 1/2 dilution, it is then applied to the skin for repelling mosquitoes

Effectiveness:

•CDC statement in May 2008 recommend equally DEET, picaridin, oil of lemon eucalyptus and IR3535 for skin.
•DEET: H
- **•Heavy application and saturation are unnecessary for effectiveness**
- **•Generally the higher the percentage of DEET, the longer the repellant will last**
- **•Products with 30%-50% DEET do not offer additional protection beyond 3-4 hours**
- **•Use products with low concentration of DEET (10% or less on children 2 - 12 years old)**

•Permethrin is recommended for clothing, gear, or bed nets.
•Oil of lemon eucalyptus is more effective than other plant-based treatments, with a similar effectiveness to low concentrations of DEET. A 2006 published study found in both cage and field studies that a product containing 40% oil of lemon eucalyptus was just as effective as products containing high concentrations of DEET.
•Research has also found that neem oil is a mosquito repellent for up to 12 hours.
•Citronella oil's mosquito repellency has also been verified by research, including effectiveness in repelling *Aedes aegypti*, but requires reapplication after 30 to 60 minutes.

•NEVER use repellents under clothing OR over cuts, wounds or irritated skin
•DO NOT apply directly to face, ONLY sparingly around ears

•Children: Apply to your own hands first and then put it on the child

PROTECT YOUR SKIN,

PROTECT YOUR LIFE