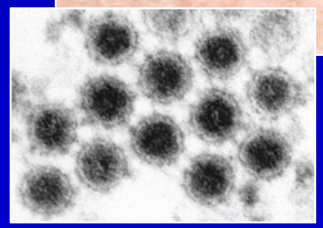
Research and Surveillance Activities on Mosquitoes and Mosquito-Borne Disease

Theodore G. Andreadis

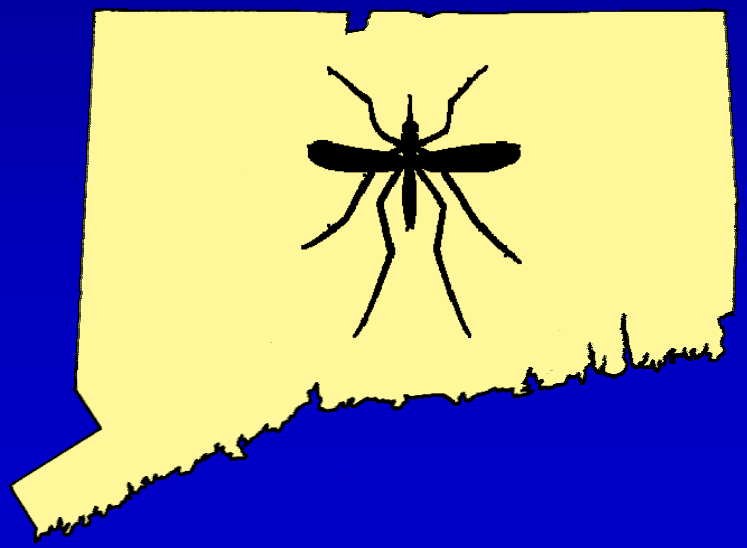
The Connecticut Agricultural Experiment Station
New Haven, CT



Mosquito Arbovirus Surveillance Program

- Established in 1997 to monitor Eastern Equine Encephalitis (36 sites)
- Expanded in 2000 with the introduction of West Nile virus (91 sites)
- Primary Objectives
  - Provide an early warning system to detect EEE and WNV
  - Assess human risk
  - Guide control measures
- Goal

  Prevent a sustained local outbreak



Mosquito Trapping Stations

NORTH CANAAN
SALISBURY
CANAAN
NORFOLK
COLEBROOK
HARTLAND
BARKHAMSTED
GRANBY
SUFFIELD
ENFIELD
SOMERS
STAFFORD
UNION
WOODSTOCK
THOMPSON
EAST GRANBY
WINDSOR LOCKS
EAST WINDSOR
ELLINGTON
TOLLAND
WILLING-TON
ASHFORD
EASTFORD
PUTNAM
POMFRET
KILLINGLY
WINCHESTER
SHARON
CORNWALL
GOSHEN
TORRINGTON
NEW HARTFORD
CANTON
SIMSBURY
WINDSOR
BLOOMFIELD
SOUTH WINDSOR
VERNON
CHAPLIN
MANSFIELD
COVENTRY
HAMPTON
BROOKLYN
AVON
WEST HARTFORD
HART-FORD
EAST HARTFORD
MANCHESTER
BOLTON
KENT
WARREN
LITCHFIELD
HARWINTON
BURLINGTON
FARMINGTON
ANDOVER
COLUMBIA
WINDHAM
SCOT-LAND
PLAINFIELD
STERLING
CANTERBURY
MORRIS
WETHERS-FIELD
GLASTONBURY
HEBRON
WASHINGTON
THOMAS-TON
PLYMOUTH
BRISTOL
PLAIN-VILLE
NEW BRITAIN
NEWINGTON
ROCKY HILL
MARL-BOROUGH
LEBANON
SPRAGUE
FRANKLIN
LISBON
GRISWOLD
VOLUN-TOWN
BETHLEHEM
NEW MILFORD
WATERTOWN
WOLCOTT
SOUTHINGTON
BERLIN
CROMWELL
PORTLAND
EAST HAMPTON
COLCHESTER
BOZRAH
NORWICH
PRESTON
WOODBURY
ROXBURY
SHERMAN
BRIDGE-WATER
MIDDLE-BURY
WATERBURY
MERIDEN
MIDDLE-FIELD
MIDDLETOWN
NEW FAIRFIELD
BROOK-FIELD
SOUTHBURY
NAUGATUCK
PROSPECT
CHESHIRE
WALLINGFORD
DURHAM
HADDAM
EAST HADDAM
SALEM
MONTVILLE
LEDYARD
NORTH STONINGTON
DANBURY
BETHEL
NEWTOWN
OXFORD
BEACON FALLS
BETHANY
HAMDEN
NORTH HAVEN
CHESTER
DEEP RIVER
ESSEX
LYME
EAST LYME
WATERFORD
NEW LONDON
GROTON
STONINGTON
SEYMOUR
WOOD-BRIDGE
ANSONIA
DERBY
NORTH BRAN-FORD
KILLING-WORTH
MONROE
REDDING
RIDGEFIELD
SHELTON
NEW HAVEN
EAST HAVEN
BRANFORD
GUILFORD
MADISON
CLINTON
WEST-BROOK
OLD SAYBROOK
OLD LYME
EASTON
TRUMBULL
ORANGE
WEST HAVEN
MILFORD
WILTON
WESTON
STRATFORD
BRIDGE-PORT
FAIRFIELD
NEW CANAAN
WESTPORT
NORWALK
STAMFORD
DARIEN
GREENWICH

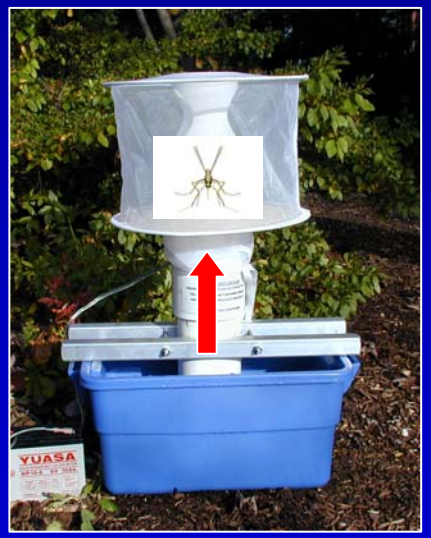
Mosquito Collection Sites

- Urban / Suburban Sites
  - Neighborhood parks and schools
  - Along waterways and streams
  - Sewage treatment plants
  - Horse stables
  - Tire dumps

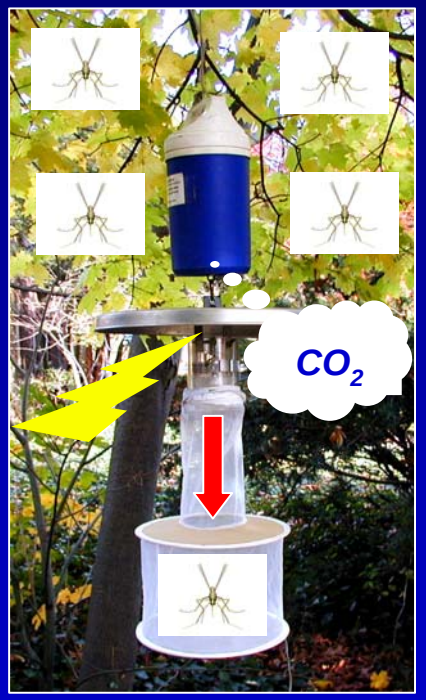
- Rural Areas
  - Permanent swamps and bogs
  - Marshes (fresh and salt)

Mosquito Surveillance

- Mosquito trapping from June - October
- 91 permanent trapping stations (trap weekly)
- 2 Mosquito trap types:
  - CO_2-baited CDC light trap
    All species
  - Gravid mosquito trap
    (Hay infusion)
    Culex mosquitoes



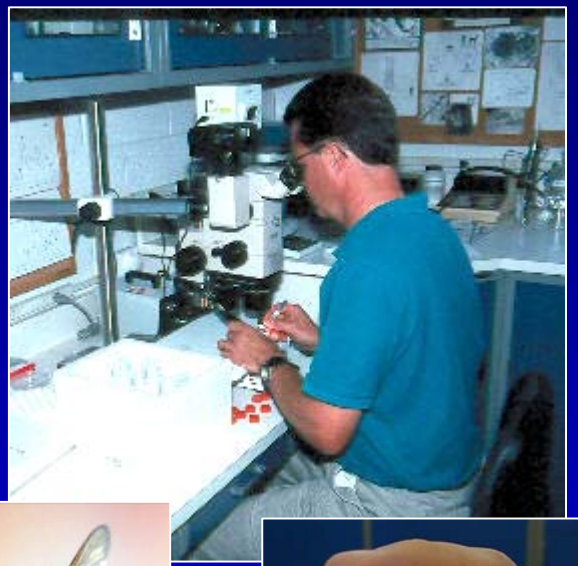
Gravid trap



CDC light trap

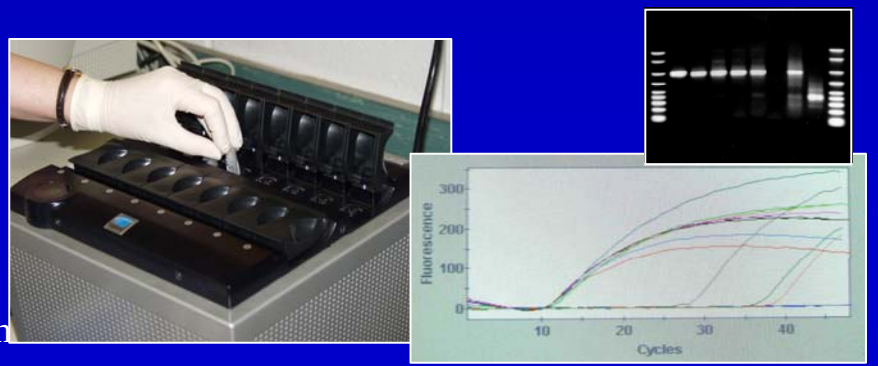
Mosquito Identification

- Mosquito identification to species
  - 50 species in CT
  - Routinely collect 35 species
- Completed on day of collection
- Pooled by species and site
  - Maximum of 50 / pool
- All species tested



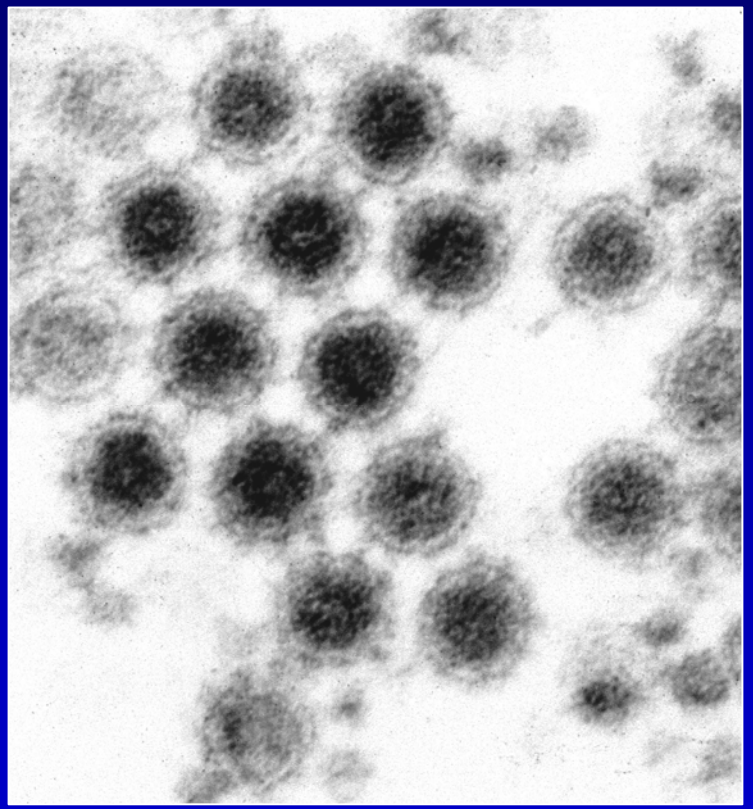
Virus Isolation & Identification

- Biosafety Level 3 Laboratory
- Virus isolation in Vero cell cultures (African Green Monkey)
  - Incubate for 7 days at 37 ^{o}C in 5% CO_2
  - Examine daily for virus growth
- Virus identification by
  Real time PCR, RT-PCR,
  or PRNT



Mosquito-Borne Viruses in Connecticut

- *West Nile Virus*
- *Eastern Equine Encephalitis*
- *Jamestown Canyon*
- *Cache Valley*
- *Trivittatus*
- *La Crosse*
- *Highlands J*
- *Potosi*
- *Flanders*



Cause Human Disease

Human Disease Causing Mosquito-Borne Viruses in CT

Virus	*Reservoir*	*Age Group*	*Human disease*
West Nile	Bird	Elderly	Moderate to severe, fever, encephalitis
Eastern Equine Encephalitis	Bird	Children	Severe, encephalitis
La Crosse	Squirrel, chipmunk	Children	Severe, encephalitis
Jamestown Canyon	White-tailed deer	Young adults	Mild, flu-like, respiratory involvement
Cache Valley	Deer, horse, sheep	All ages	Febrile illness, fever
Trivittatus	Rabbit, squirrel, raccoon, opossum	All ages	Febrile illness, fever

EEE and WNV - Human Clinical Features

	EEE	*WNV*
Risk group	Children < 15 yrs Adults > 50 yrs	Adults > 50 yrs
Incubation period	3 – 10 days	3 – 14 days
Symptoms	Mild flu-like, fever, headache, body aches, nausea, neck stiffness, disorientation, inflammation of brain, convulsions, coma, death	
Treatment	Supportive care – intravenous fluids, breathing assistance	
Vaccine	None	
Mortality rate	30%	3 - 4% (Adults > 75yr)
Long-term effects	Mild to severe brain damage (30% of patients)	Fatigue, weakness, memory impairment up to 15 months

Mosquitoes Trapped and Tested for Arboviruses in CT 1997 - 2007

1.5 million trapped and tested

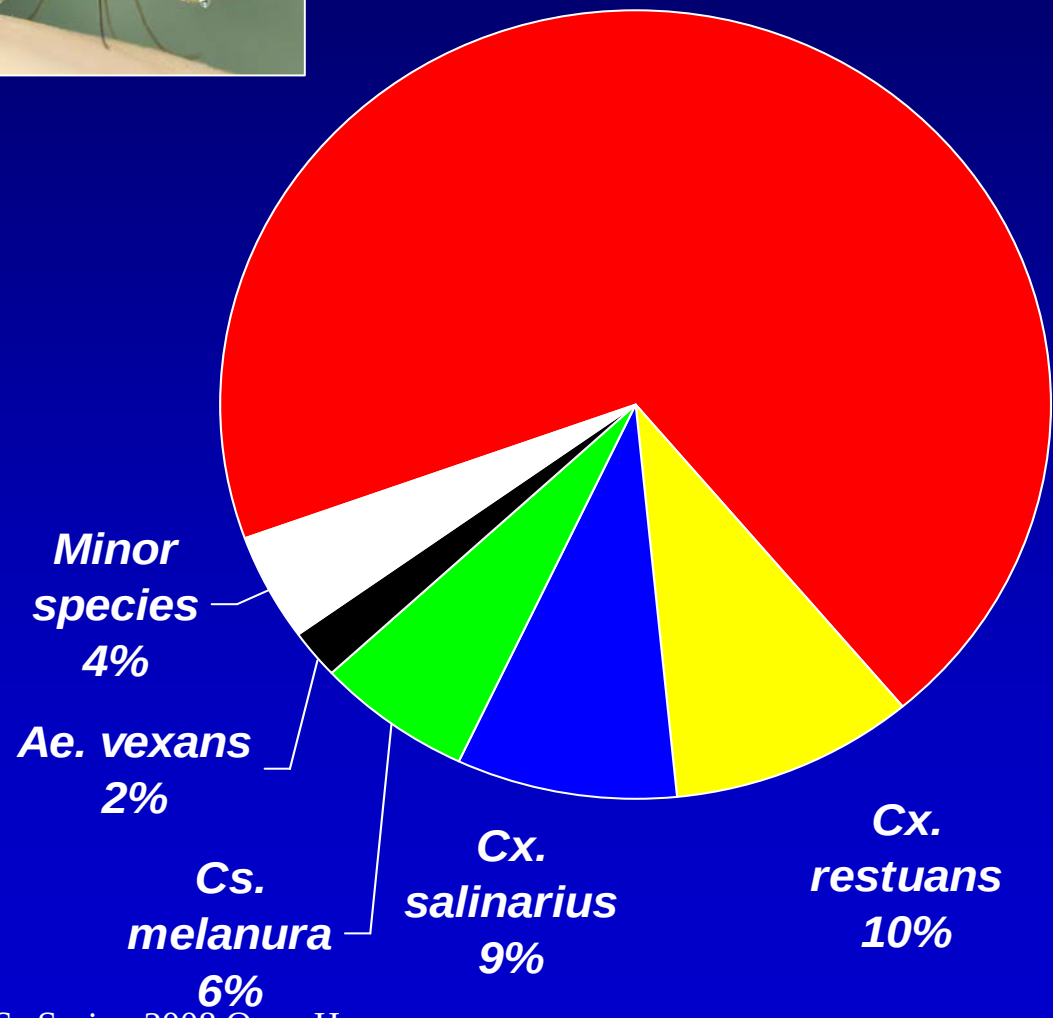
West Nile Virus

300,000
250,000
200,000
150,000
100,000
50,000
0

1997 *1998* *1999* *2000* *2001* *2002* *2003* *2004* *2005* *2006* *2007*

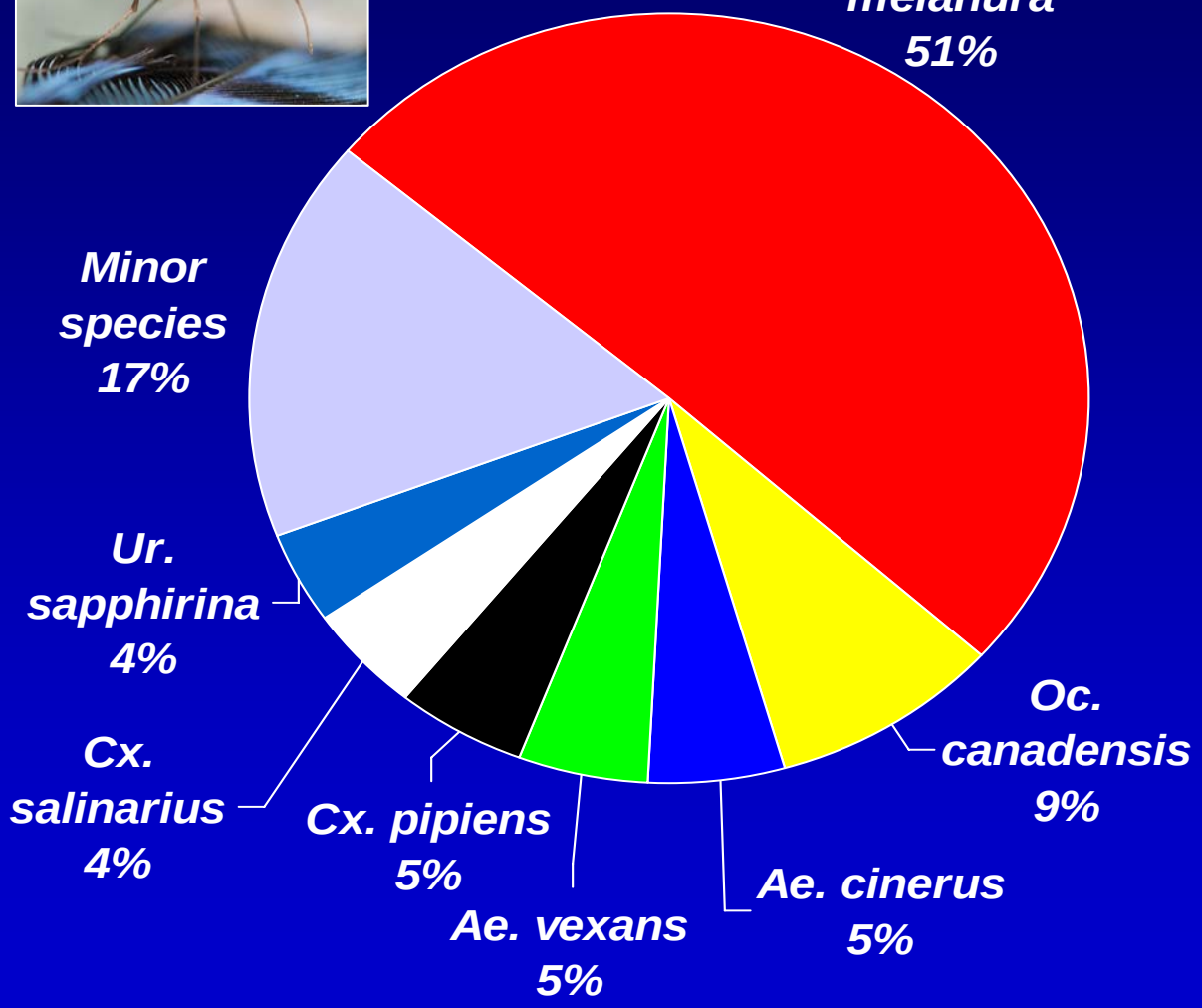
WNV Positive Mosquitoes in Connecticut 1999 – 2007

18 SPECIES	TOTAL
Culex pipiens	385
Culex restuans	53
Culex salinarius	48
Culiseta melanura	34
Aedes vexans	11
Aedes cinereus	4
Ochlerotatus canadensis	3
Uranotaenia sapphirina	2
Ochlerotatus sticticus	2
Ochlerotatus taeniorhynchus	2
Ochlerotatus triseriatus	2
Ochlerotatus trivittatus	2
Psorophora ferox	2
Other species (5)	5

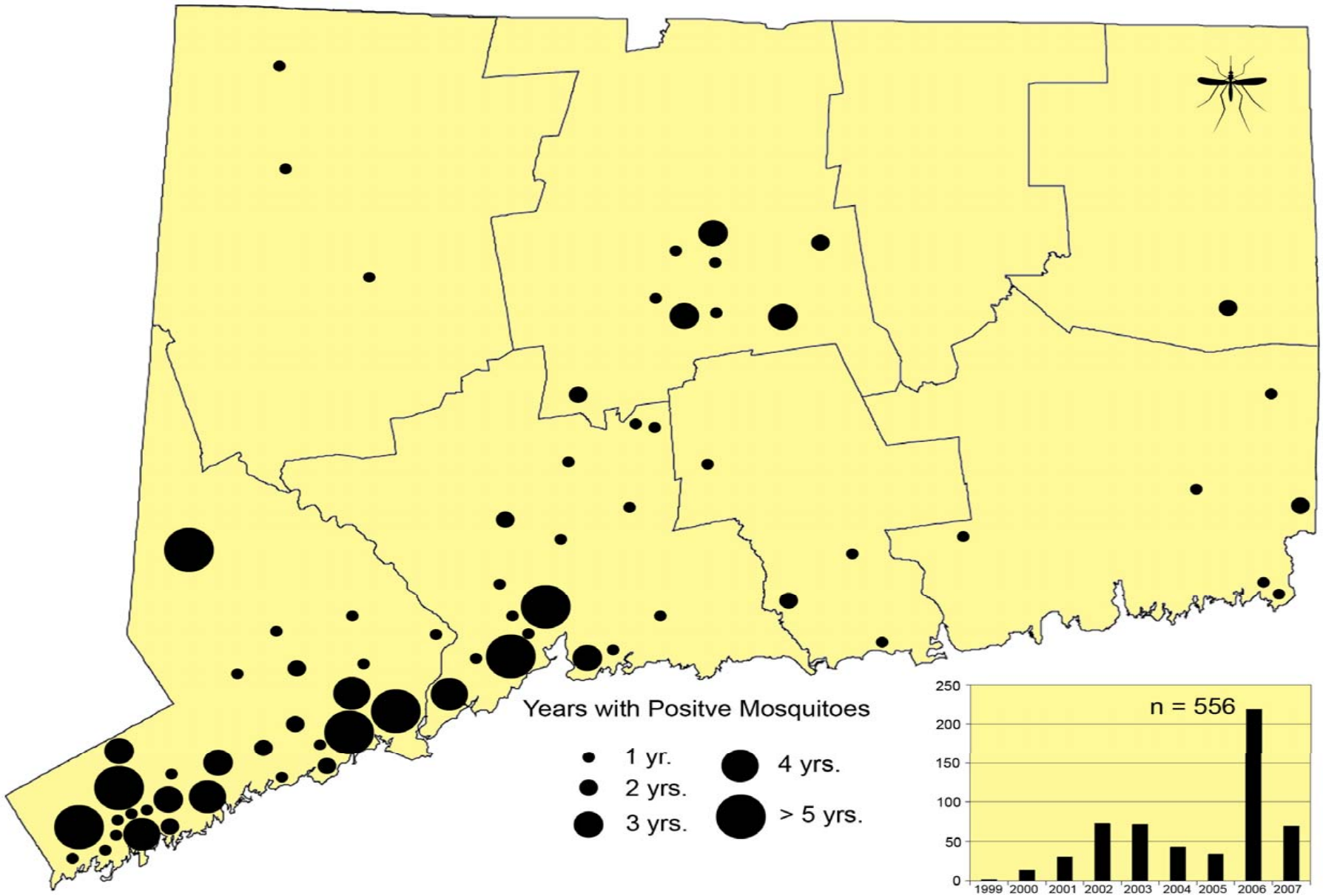


EEE Positive Mosquitoes in Connecticut 1996 – 2007

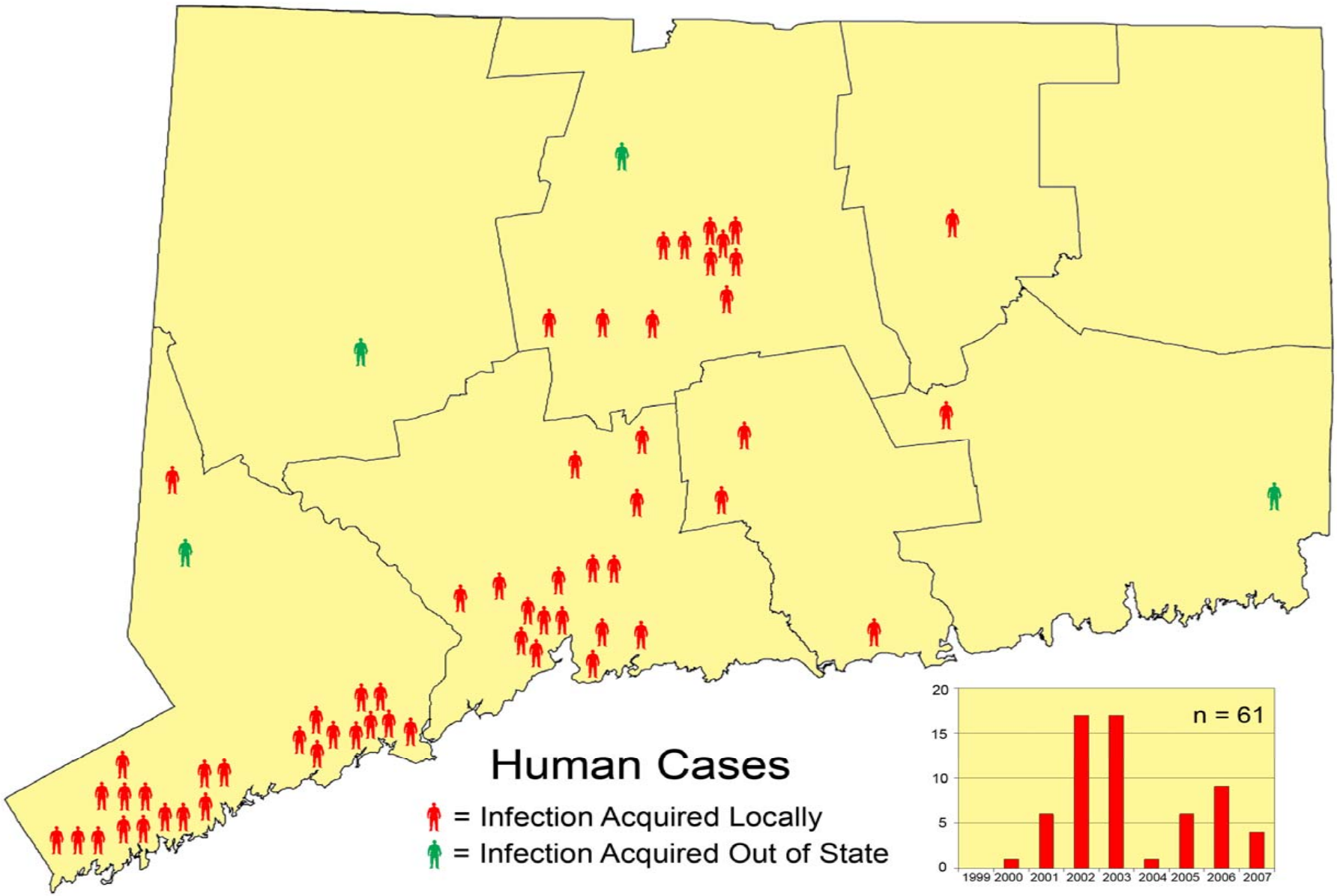
18 SPECIES	TOTAL
Culiseta melanura	102
Ochlerotatus canadensis	18
Aedes cinereus	11
Aedes vexans	10
Culex pipiens	10
Culex salinarius	9
Uranotaenia sapphirina	8
Ochlerotatus trivittatus	6
Culiseta morsitians	5
Anopheles punctipennis	4
Coquillettidia perturbans	4
Ochlerotatus cantator	4
Ochlerotatus sollicitans	3
Other species (5)	8



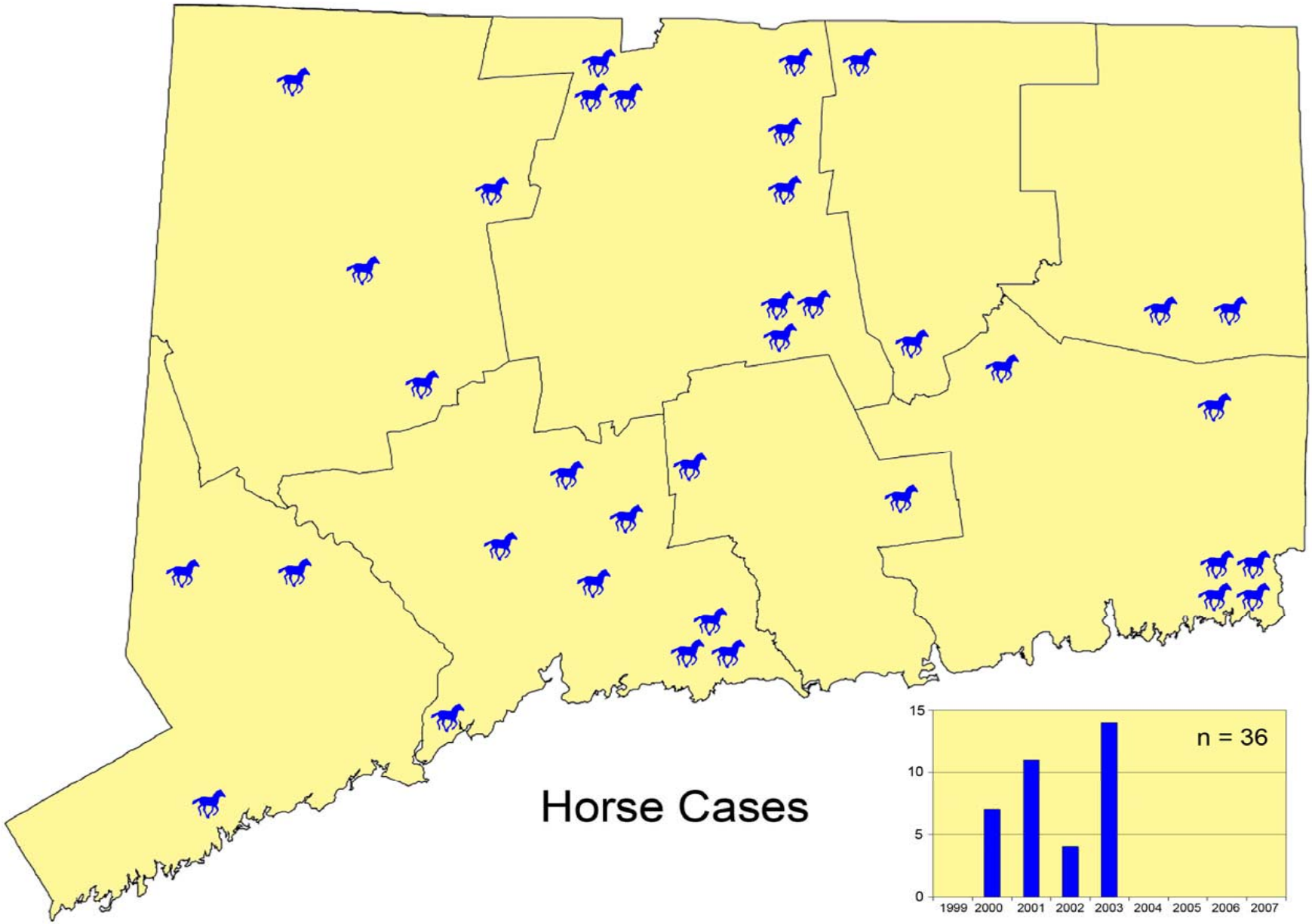
West Nile Virus in Connecticut 1999 – 2007: Mosquitoes



West Nile Virus in Connecticut 1999 – 2007: Human Cases

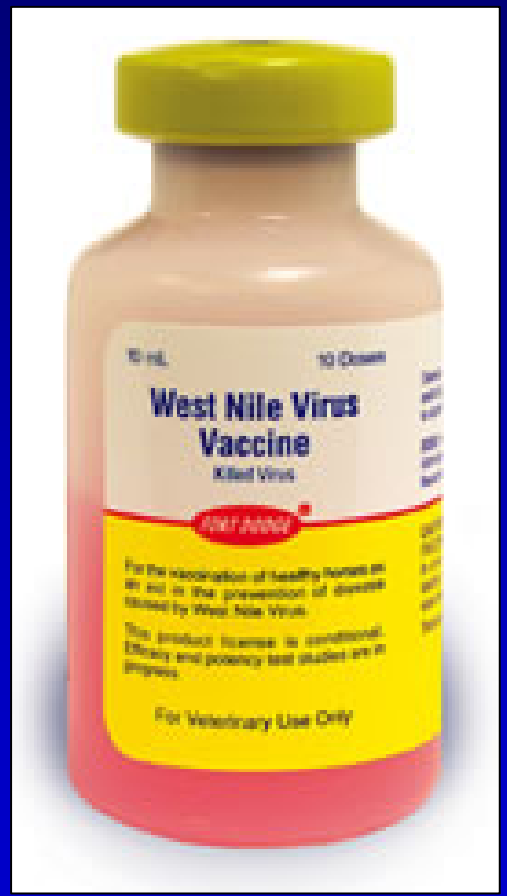


West Nile Virus in Connecticut 1999 – 2007: Horse Cases

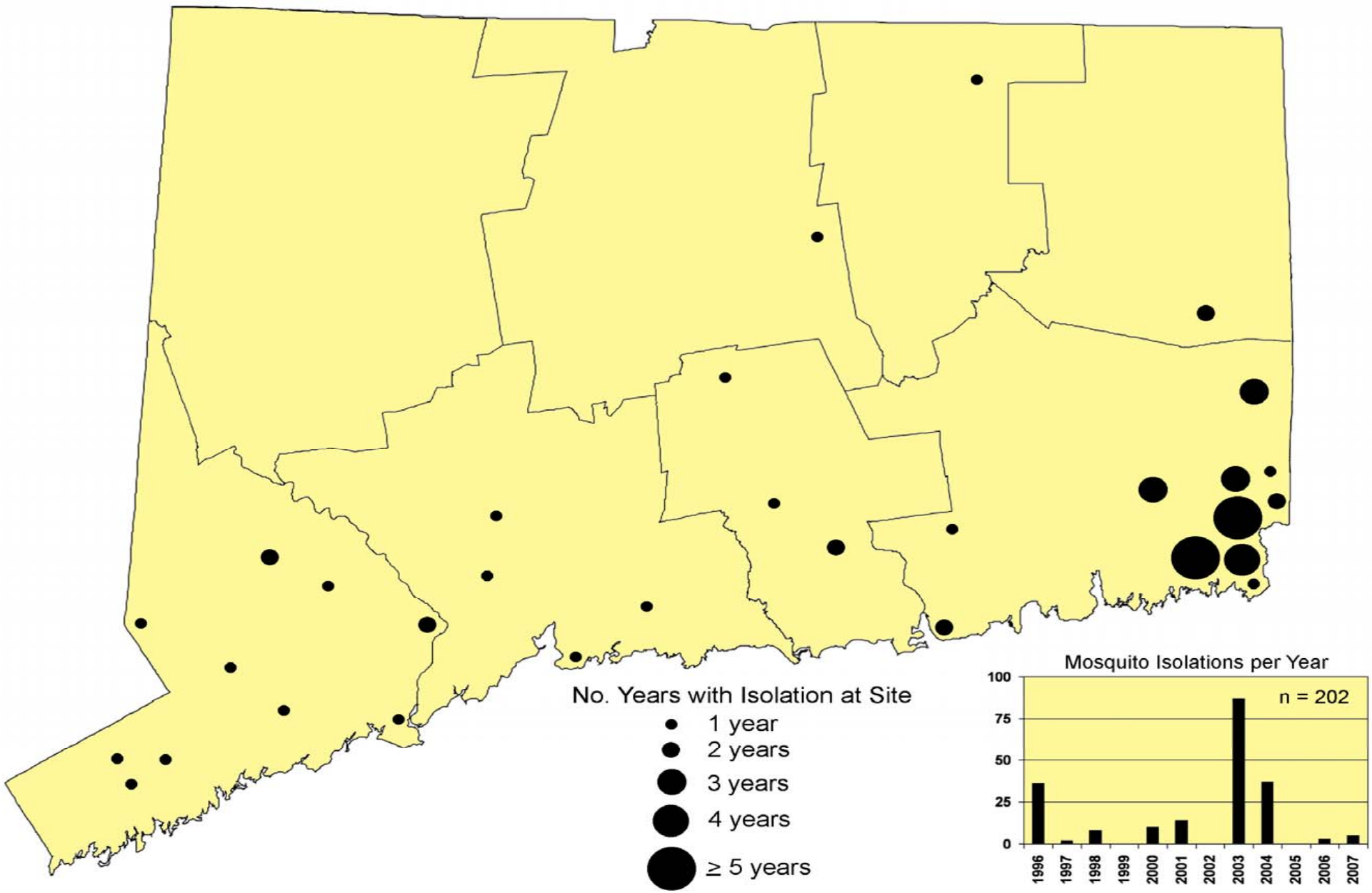


West Nile Virus Horse Vaccine

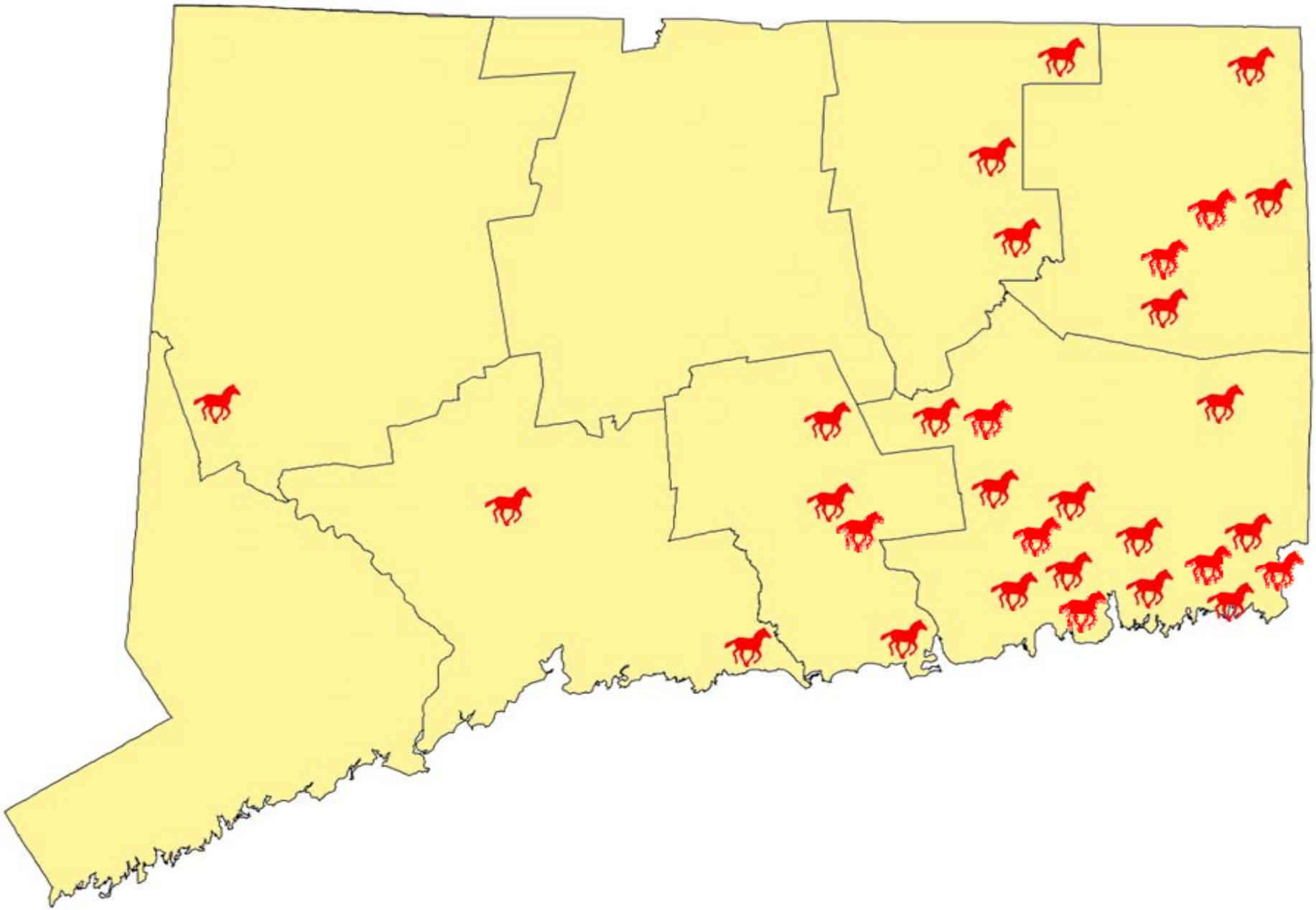
- Killed virus product given full license by USDA (APHIS) February, 2003
- Available from veterinarians only
- Administration: 2 doses 3 to 6 wks. apart in spring
- Annual booster recommended
- 94% of vaccinated horses protected with 2 doses



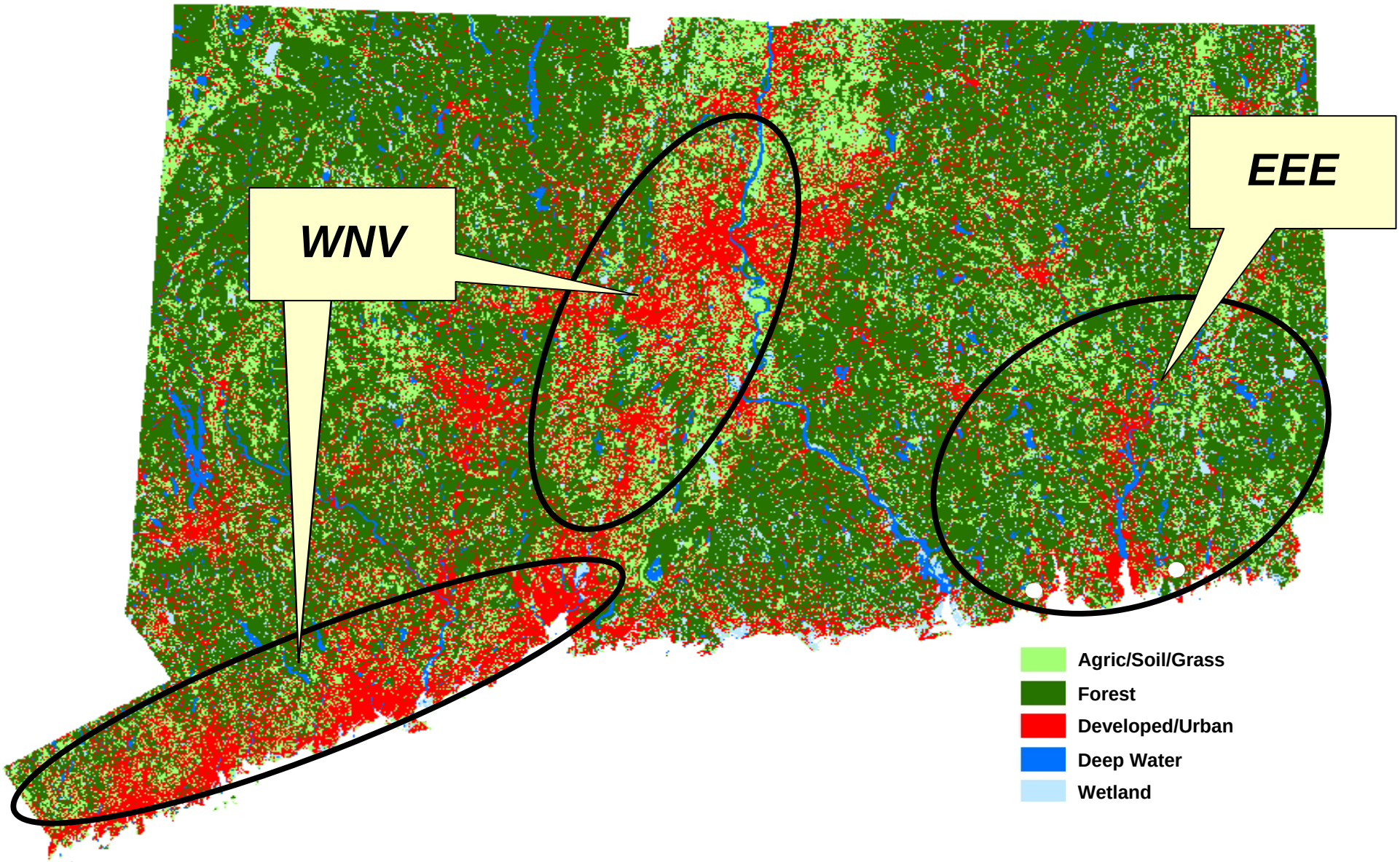
EEE Virus in Connecticut 1999 – 2007: Mosquitoes

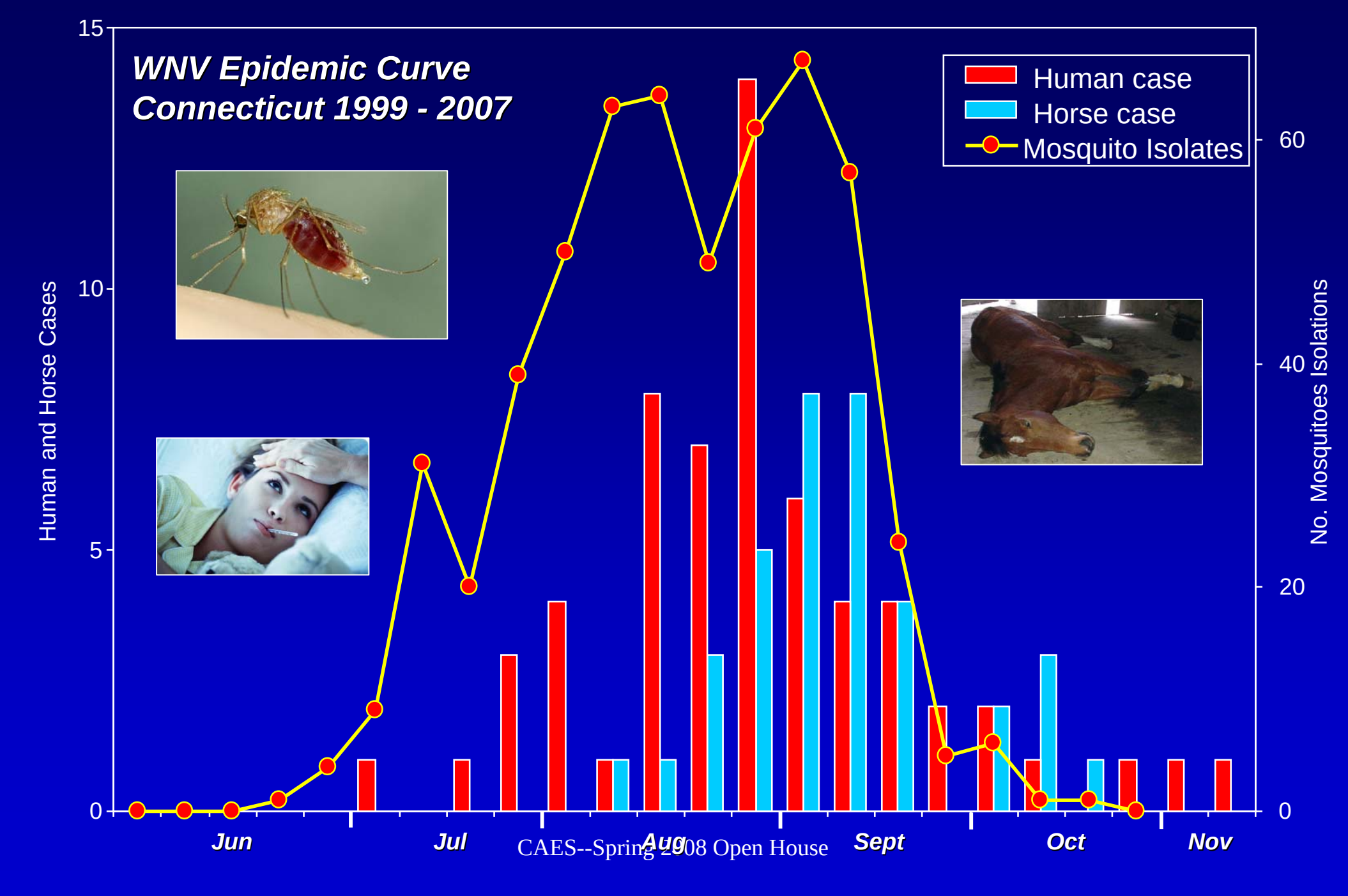


EEE Virus in Connecticut : Equine Outbreaks 1938 - 2007



Connecticut Land Use Map and High Risk Areas




WNV Epidemic Curve
Connecticut 1999 - 2007
Human case
Horse case
Mosquito Isolates
Human and Horse Cases
No. Mosquitoes Isolations
15
10
5
0
60
40
20
0
Jun
Jul
Aug
Sept
Oct
Nov
CAES--Spring 2008 Open House

EEE Epidemic Curve Connecticut 1996 – 2007

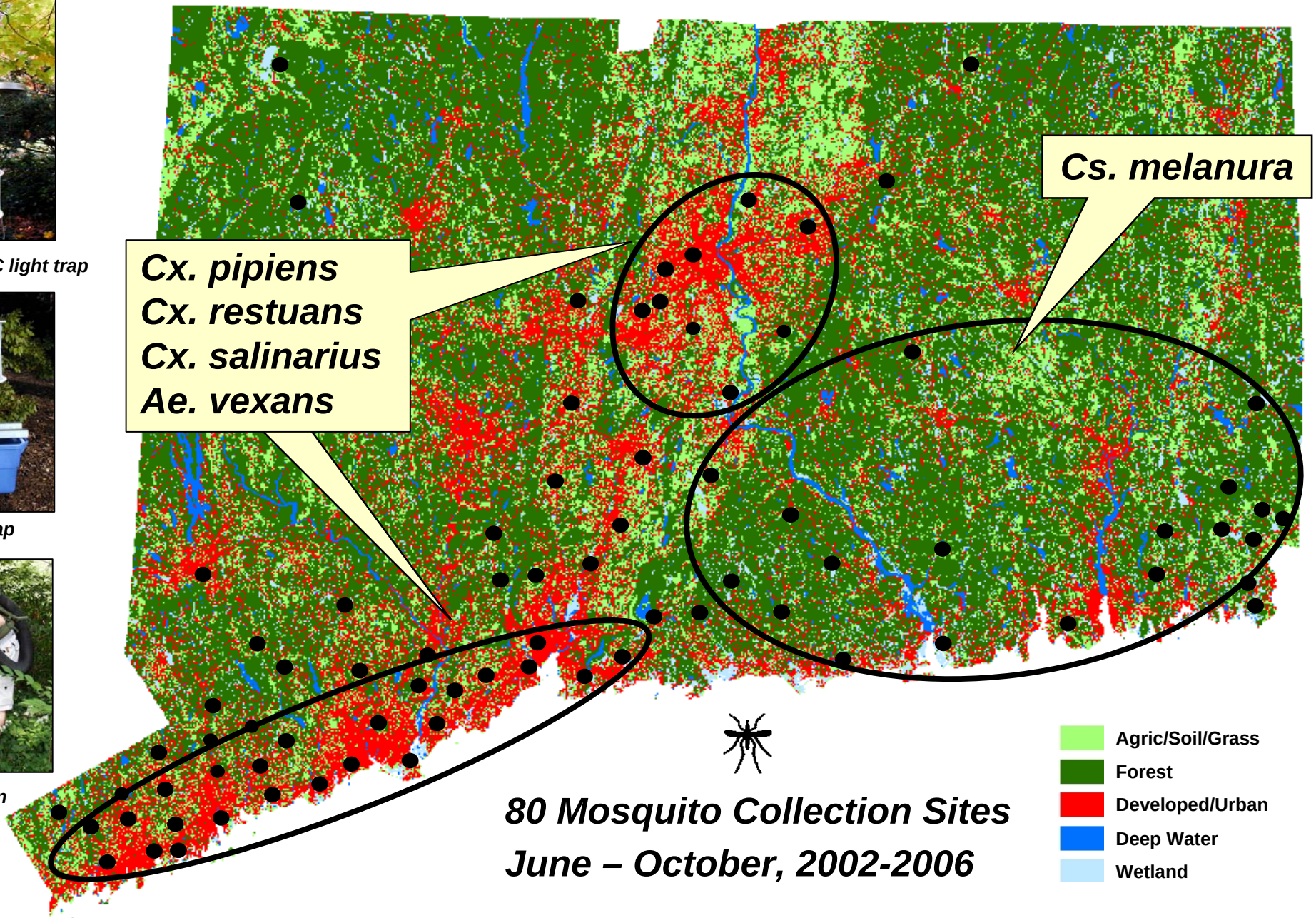
Mosquito Blood Feeding Studies - Objectives

- To characterize the host-feeding patterns of the principal mosquito vectors of WNV in Connecticut
- Evaluate their contribution to enzootic amplification of these viruses in wild bird populations and epidemic transmission to mammalian hosts including humans
- Identify specific avian and mammalian hosts
- Clarify the role of these hosts in the ecology and epizootiology of WNV in the region

CO_2-baited CDC light trap

Gravid trap

Vegetation Sweeps

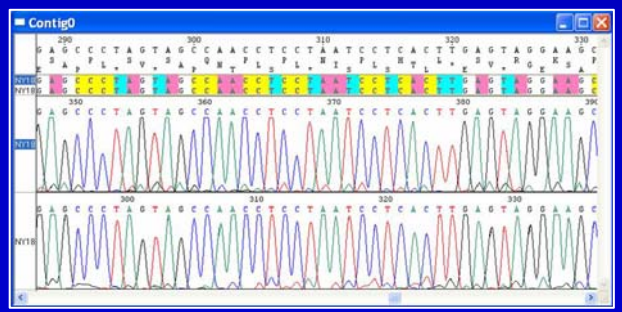


80 Mosquito Collection Sites

June – October, 2002-2006

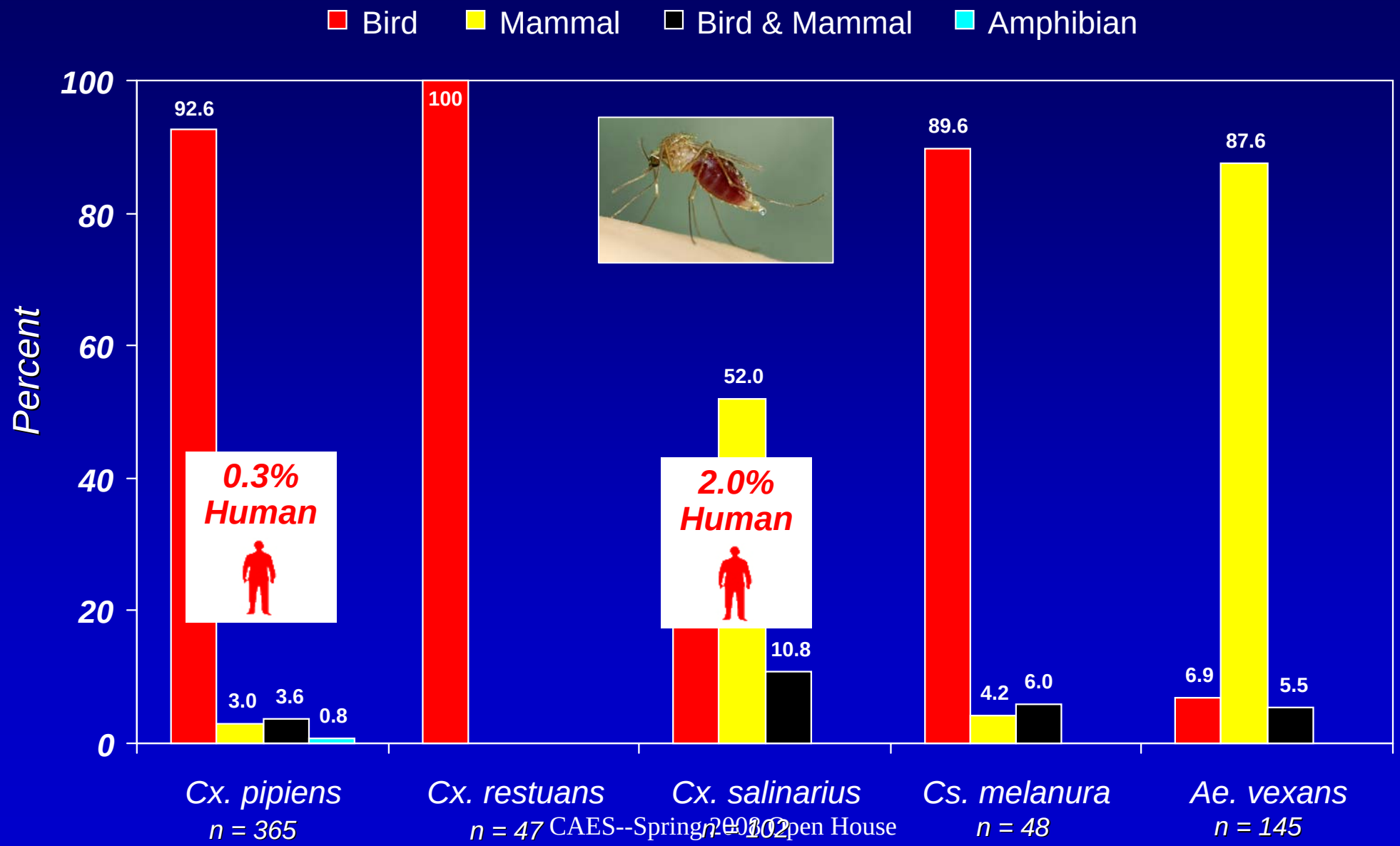
Materials and Methods

- All mosquitoes with fresh or visible blood remnants were individually isolated and stored at -80^{o}C.
- Abdomens were removed under a dissecting microscope and DNA was isolated using DNA-zol®
- The DNA was amplified by PCR using Avian and Mammalian specific primer pairs to the cytochrome b gene
- The PCR amplification products were sequenced and identified to species by comparison to the Genbank® sequence data base



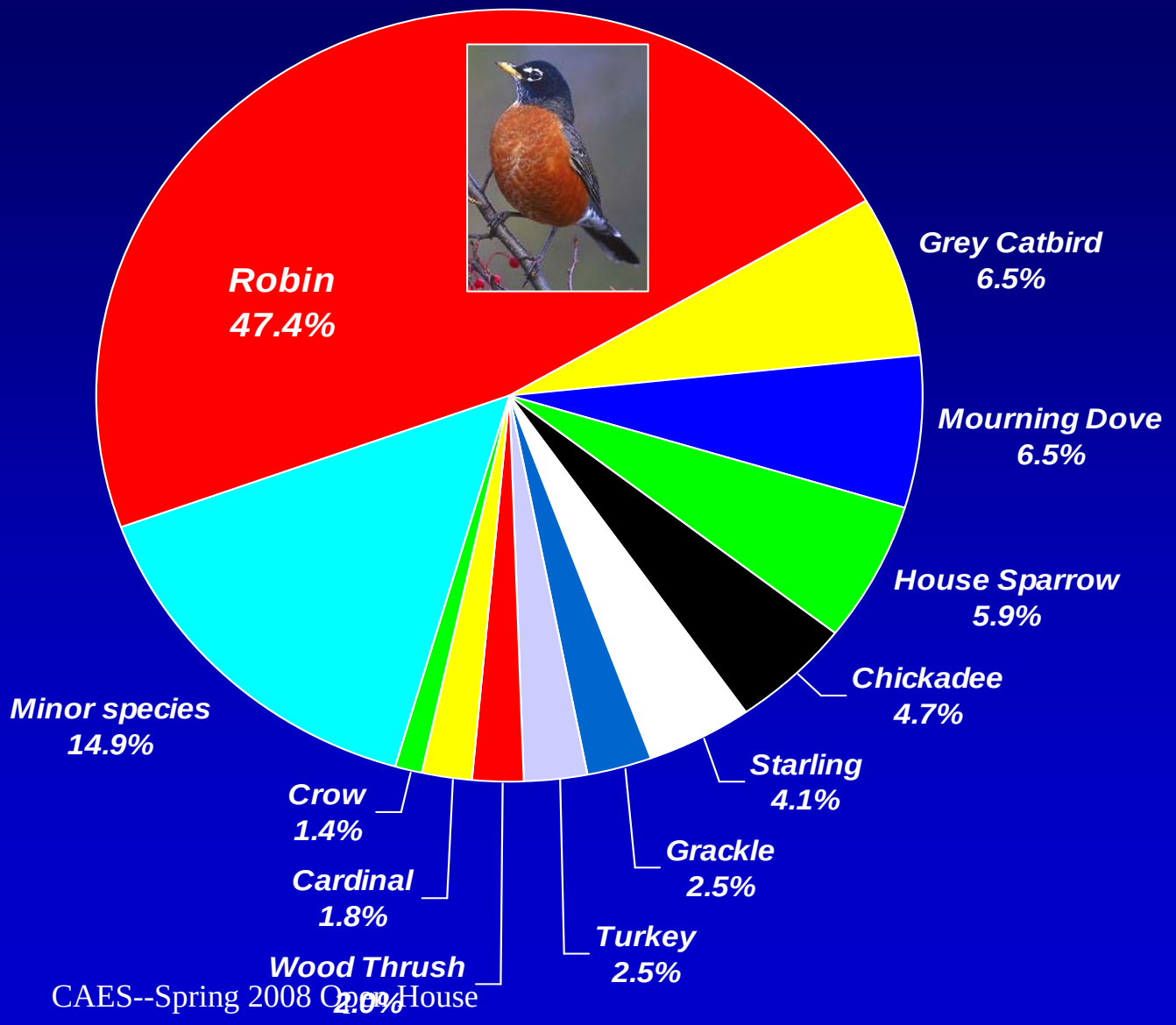
NCBI nucleotide–nucleotide BLAST

Host Feeding Preferences of Mosquito Vectors of WNV in CT



Avian Blood Meal Sources from Mosquitoes in Connecticut

27 BIRD SPECIES	% of TOTAL
American Robin	47.4%
Grey Catbird	6.5%
Mourning Dove	6.5%
House Sparrow	5.9%
Chickadee	4.7%
European Starling	4.1%
Common Grackle	2.5%
Wild Turkey	2.5%
Wood Thrush	2.0%
Northern Cardinal	1.8%
American Crow	1.4%
Minor Species (26)	14.9%



American Robin

- *Short distance migrant*
- *Abundant*
- *2 broods/year*
- *Nest in mid-canopy*

- *Frequently infected*
- *Reservoir competent*
  - *– 4.6 to 8.9 log_{10}PFU/ml*
  - *– Viremia ~ 4.5 days*

Implicate this species as an important reservoir host

Special Issue

Discover

Year in Science

No. 16 Will a New Avian Flu Vaccine Even Work?

No. 2 How Many More Planets Lie Past Pluto?

No. 69 Can Just One Lone Brain Cell Think?

100 Top Science Stories of 2005

No. 1 Does Global Warming Make Hurricanes Worse?

No. 5 Does Too Much Sex Damage Male Genes?

No. 24 Why Do Stupid People Die Young?

No. 7 Dark Galaxies: Could the Cosmos Be Full of Them?

JANUARY 2006

pen House

No part of the continental United States is safe: Robins roam throughout the country.

Mosquito-Borne West Nile Turns Up in an Unsuspected Carrier: the American Robin

EPIDEMIOLOGY—Since West Nile virus arrived in the Western Hemisphere in 1999, people have worried each summer about its spread. Although the virus, carried by mosquitoes, has been detected in more than 200 species of birds, crows have been closely monitored as the primary reservoir. This year medical entomologists at the Connecticut Agricultural Experiment Station learned that we may have been watching the wrong bird and the wrong mosquito.

By extracting blood from the stomach of engorged mosquitoes, Theodore Andreadis and his colleagues found that 40 percent of the infected mosquitoes had feasted on the blood of the American robin, a species that can carry the virus without showing symptoms.

A more important finding questions the strategy of disease control for West Nile, which has focused on eradicating a common, easily controlled, ditch-dwelling mosquito. Andreadis found that these mosquitoes rarely bite mammals, so they are not likely to pass the virus on to people.

Salt marsh mosquitoes, on the other hand, pose a greater risk of disease transmission, says Andreadis, because they feed on birds about a third of the time and on mammals more than half the time. And salt marsh mosquitoes are a challenge to control because they breed in vast stretches of pristine marshland along the coast.

Complicating study of the virus even further, a lab study found that an infected mosquito can pass the virus to nearby mosquitoes while they are feeding on an uninfected animal.

—Jessa Forte Netting

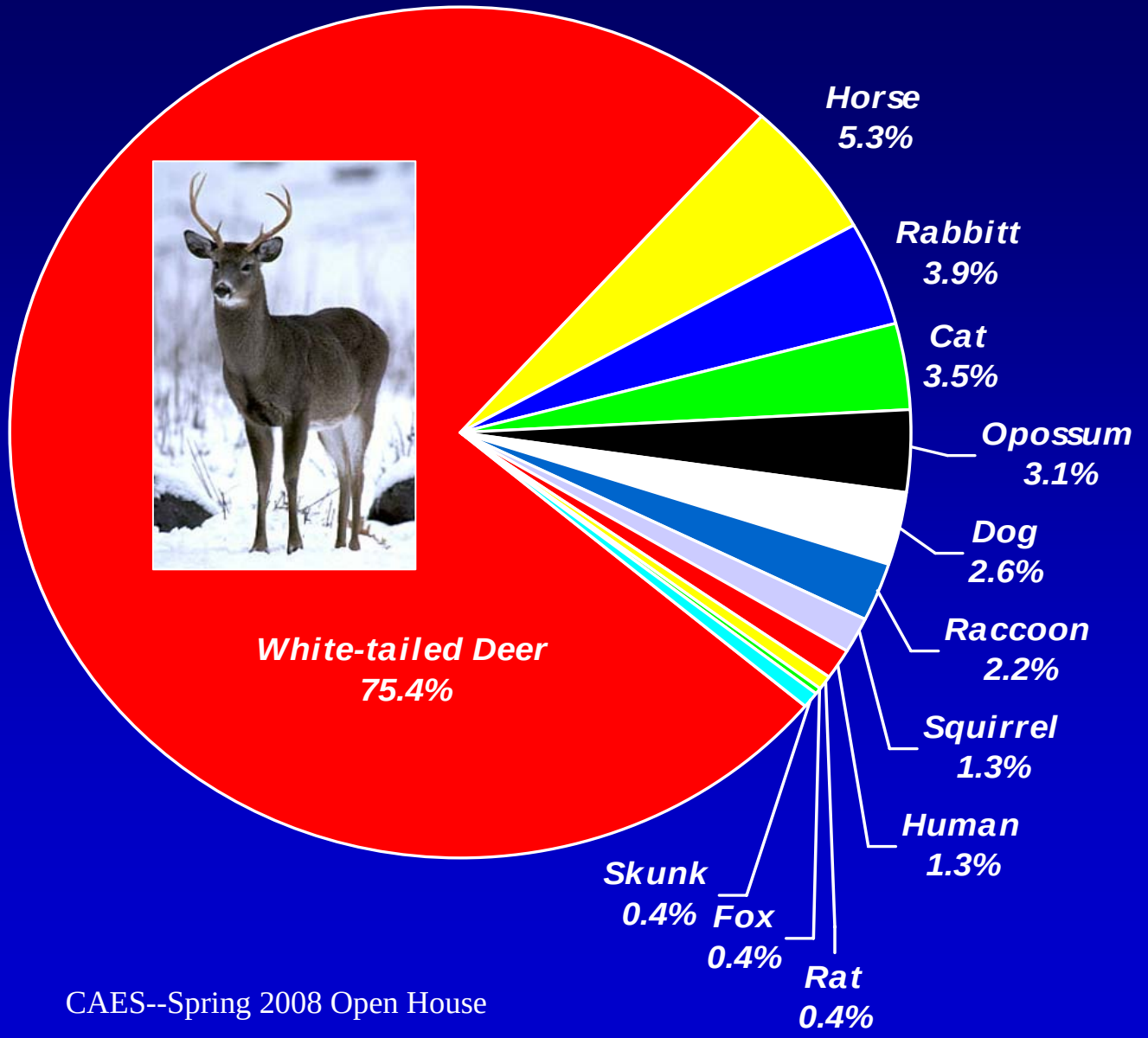
Avian Blood Meal Sources from Mosquitoes in Connecticut

- *Crows likely acquire WNV through means other than mosquito bites*
- *Oral transmission – bird to bird and/or predation*
- *Crows are not the primary amplifying hosts*

Wild Turkey	2.5%
Wood Thrush	2.0%
Northern Cardinal	1.8%
American Crow	1.4%
Minor Species (26)	14.9%

Mammalian Blood Meal Sources from Mosquitoes in Connecticut

12 SPECIES	% of TOTAL
White-tailed Deer	75.4%
Horse	5.3%
Eastern Cottontail	3.9%
Cat	3.5%
Virginia Opossum	3.1%
Dog	2.6%
Northern Raccoon	2.2%
Grey Squirrel	1.3%
Human	1.3%
Brown Rat	0.4%
Red Fox	0.4%
Stripped Skunk	0.4%

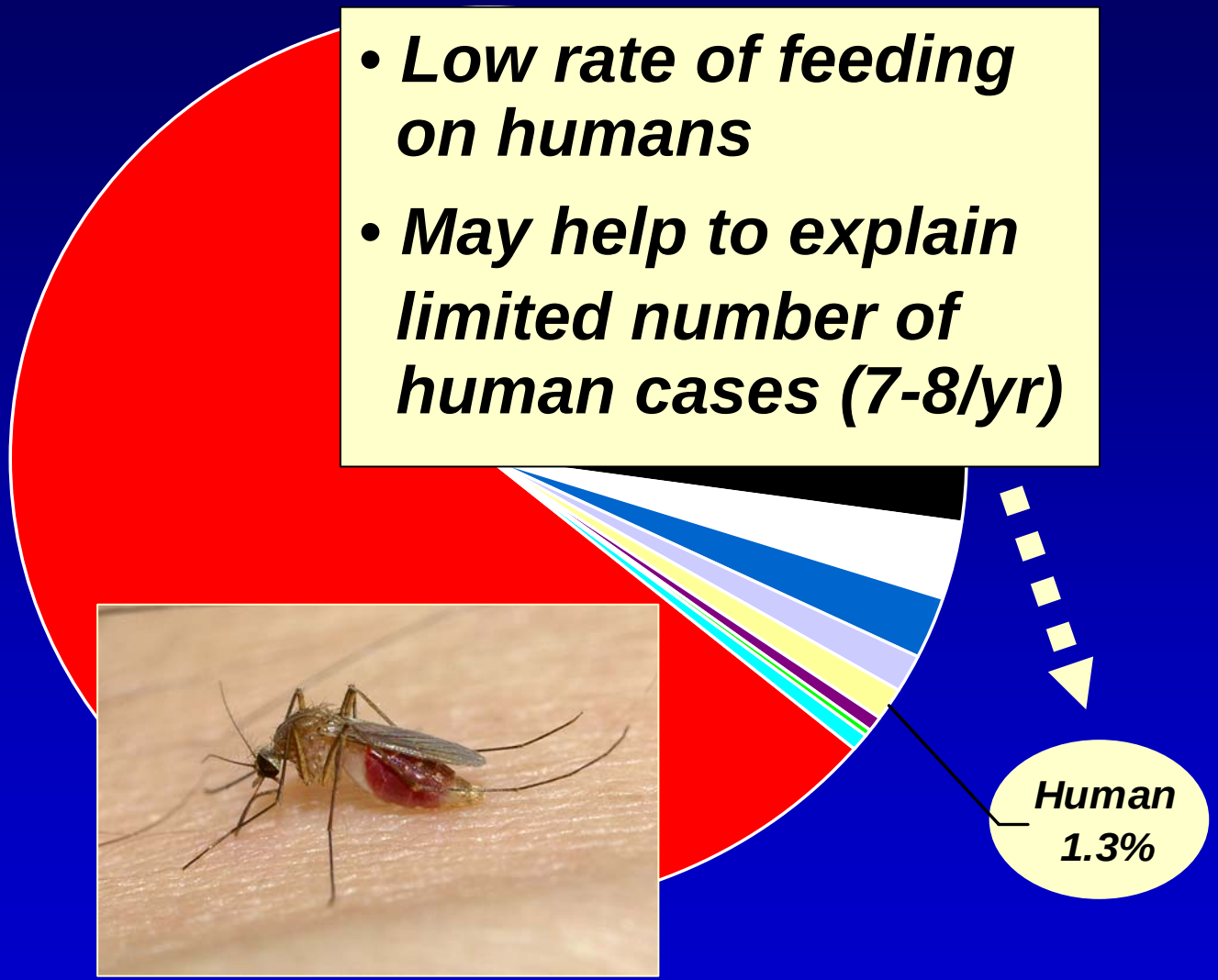


White-tailed Deer

- ***Most abundant large mammal in Connecticut***
- ***Role in transmission of West Nile virus unknown***
- ***May divert feeding from horses and humans***

Mammalian Blood Meal Sources from Mosquitoes in Connecticut

12 SPECIES	% of TOTAL
White-tailed Deer	75.4%
Horse	5.3%
Eastern Cottontail	3.9%
Cat	3.5%
Virginia Opossum	3.1%
Dog	2.6%
Northern Raccoon	2.2%
Grey Squirrel	1.3%
Human	1.3%
Brown Rat	0.4%
Red Fox	0.4%
Stripped Skunk	0.4%



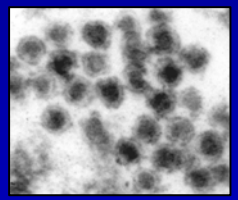
- **Low rate of feeding on humans**
- **May help to explain limited number of human cases (7-8/yr)**

Northeastern US West Nile Virus Transmission Cycle

Overwinter

Culex pipiens
Culex restuans
Culiseta melanura

Enzootic Cycle



Virus

June to October

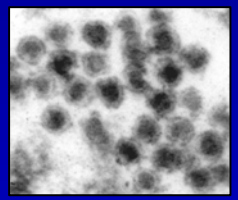
Wild Passerine Bird Reservoir and Amplifying Hosts

Northeastern US West Nile Virus Transmission Cycle

Overwinter

Culex pipiens
Culex restuans
Culiseta melanura

Enzootic Cycle



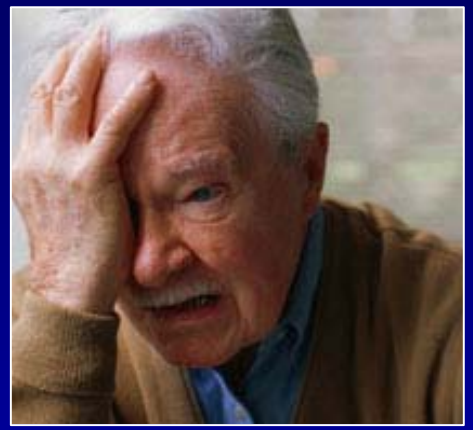
Virus




Wild Passerine Bird Reservoir and Amplifying Hosts

June to October

Epidemic / Epizootic Transmission



Culex salinarius
Culex pipiens
Aedes vexans

Incidental Infections

August to October

Future Expectations

- West Nile virus is now endemic in the US and Canada and is expanding into Central and South America.
- We will continue to see human cases annually with occasional epidemics.
- West Nile virus will continue to be a significant disease factor for the horse industry.
- Bird mortality is likely to moderate as immunity builds up in the population.

CAES Mosquito/Arbovirus Research Group

CAES Mosquito/Arbovirus Research Group