West Virginia Mosquito-Borne Disease Surveillance Report, 2010

Introduction

Mosquito-borne diseases are transmitted to humans from the bite of an infected mosquito. Most mosquito-borne diseases are viruses and are known as arboviruses, but parasites, such as malaria, can also be transmitted by mosquitoes. The major arboviruses in the United States include West Nile Virus (WNV), La Crosse Encephalitis virus (LAC), St. Louis encephalitis virus (SLE), Eastern Equine encephalitis virus (EEE), and Western Equine encephalitis virus (WEE). In West Virginia, LAC has historically been the major arbovirus to cause illness in humans. However, human cases of WNV have also been identified. Human cases of SLE have previously been identified in WV, mostly during the 1970's, and EEE-infected birds were identified in the state during 2002.

Surveillance for arboviruses and other mosquito-borne diseases is important in understanding the public health impact of these diseases and monitoring for changes in disease activity, particularly because arboviral outbreaks are difficult to predict. Surveillance among sentinel species, such as birds and horses, and mosquito vectors can help assess disease activity in an area. In West Virginia, surveillance efforts focus mainly on arboviruses and involve four components: mosquitoes, dead birds, horses (equines), and humans. Systematic mosquito surveillance began in West Virginia during 2007 with trapping sites in several counties. Since 2007, the methods for mosquito surveillance have remained similar but the number and location of trapping sites have varied each year. Prior to 2007, mosquito surveillance was conducted based on location of human cases and mosquito complaints. Dead bird and equine arbovirus surveillance are passive, relying on submissions from local health departments and veterinarians. Surveillance for human cases of arbovirus is based on enhanced passive surveillance methods, while surveillance for other mosquito-borne diseases is passive. This report summarizes mosquito-borne disease surveillance efforts for 2010, with primary focus on arboviral surveillance, and the number of cases identified among mosquitoes, birds, equines, and humans.

Methods

Mosquito surveillance

Trapping for mosquitoes began on May 7, 2010 and ended on August 11, 2010. Four summer interns and the public health entomologist conducted mosquito trapping at 7 sites — 5 in Jackson county and 2 in Kanawha county. All sites were previously used during the 2009 trapping season. One local health department, Ohio county, also conducted mosquito trapping and sent specimens to the Office of Laboratory Services (OLS) for testing.

CDC gravid traps were used to collect mosquitoes. The traps were set on Monday and mosquitoes were collected daily Tuesday–Friday. The traps were disassembled on Friday for cleaning. CDC gravid traps use a net to collect mosquitoes and the daily mosquito collections were returned to OLS in these nets. The nets were placed in a minus 80 degree Celsius freezer to kill the mosquitoes. After freezing, all mosquitoes from a single trap were placed in a large six inch Petri dish. Mosquitoes were sorted into two groups: *Culex* and non-*Culex* species. All *Culex* mosquitoes (regardless of species) were pooled into groups with a maximum of 150 mosquitoes per pool. The pooled groups of mosquitoes were then placed in two milliliter Sarstedt micro tubes with two copper or glass beads. A buffer solution was

added to the micro tubes and the tubes were placed on a mixer mill for four minutes. The buffer solution together with the action of the beads, ground the mosquitoes into slurry, which was centrifuged and extracted. Using reverse transcriptase-polymerase chain reaction (RT-PCR), the samples were analyzed for WNV and SLE. Pools containing non-*Culex* species were tested for WNV, SLE, LAC, and EEE using the same procedures.

All collected pools were recorded in a Microsoft Excel spreadsheet that included date of collection, site, species, pool size, and arbovirus testing result. A report was submitted to CDC through ArboNet for all mosquito pools that tested positive for an arbovirus.

As a supplement to the mosquito arbovirus testing results, pooled infection rates were calculated in aggregate for all species and sites by week of collection using the CDC-developed Microsoft Excel add-in "Pooled Infection Rate." The pooled infection rate provides an estimate of the infection prevalence among mosquitoes tested and is expressed as the number of infected mosquitoes per 1,000 mosquitoes tested. In addition to the estimated infection rate point estimates, 95% confidence intervals were also calculated. For more information on the methodology used to calculate pooled infection rates, see http://www.cdc.gov/ncidod/dvbid/westnile/software.htm

Avian surveillance

Local health department personnel submitted oral swabs from dead birds to OLS for testing of WNV and EEE by RT-PCR. Reports from citizens were often important in locating dead birds for surveillance purposes. A report was submitted to CDC through ArboNet if any avian specimen tested positive for an arbovirus.

Equine surveillance

Veterinarians suspecting arboviral infection in an equine patient submitted serum specimens to OLS. These specimens were forwarded by OLS to the National Veterinary Services Laboratory in Ames, IA for testing by IgM capture enzyme-linked immunosorbent assay (ELISA) for WNV and EEE. A report was submitted to CDC through ArboNet if any equine specimen tested positive for an arbovirus.

Human surveillance

A statewide health alert network message (HAN) was disseminated to WV healthcare providers in the spring of 2010. The HAN described the symptoms, diagnosis, and epidemiology of arboviruses. A letter was also sent to hospital laboratory directors describing testing services available at OLS and requesting positive arboviral samples be sent to OLS for further testing. Testing at OLS was performed for LAC, WNV, EEE, and SLE using MAC-ELISA and microsphere-based immunoassay (MIA). A subset of specimens testing positive at OLS were sent to the Arbovirus Disease Branch Laboratory (DVBID/NCID/CDC) in Fort Collins, CO for confirmatory testing by IgM antibody capture ELISA and plaque-reduction neutralization testing (PRNT). Human testing for other mosquito-borne diseases was performed by hospital or reference laboratories.

Patients with a positive test result for a mosquito-borne disease were entered into the West Virginia Electronic Disease Surveillance System for additional follow-up by the local health department. All reported human cases were classified according to the most recent national case definition for each mosquito-borne disease. For arboviruses, the 2004 CDC case definition for neuroinvasive and non-neuroinvasive domestic arboviral diseases was used. Confirmed cases had a clinically compatible illness for arboviral infection and were positive by IgM antibody capture ELISA and PRNT at CDC. Probable cases also had a clinically compatible illness for arboviral infection but only had a single positive LAC

result via IgM antibody capture ELISA. Confirmed and probable cases were reported to CDC through ArboNet. To obtain case counts and basic descriptive epidemiologic characteristics of cases, records were exported from WVEDSS for all mosquito-borne disease cases with a report date of January 1, 2010–December 31, 2010. Data from all surveillance sources (mosquito, avian, equine, and human) were summarized used Microsoft Excel and ArcGIS v.9.3.

Results

Mosquito surveillance

A total of 36,731 mosquitoes from 459 pools were collected and tested for arboviruses. At least 4 unique mosquito species were identified from pooled collections: 444 pools of *Culex* spp. (96.7%), 7 pools of *Ochlerotatus japonicus* (1.5%), 6 pools of *Aedes albopictus* (1.3%), and 2 pools of *Aedes* spp. (0.4%). Of the 459 pools tested, 26 (5.7%) pools were positive for WNV (Table 1). No pools tested positive for SLE, EEE, or LAC. Of the 26 pools testing positive for WNV, 23 (88.5%) were *Culex* spp and 16 (61.5%) were collected in Kanawha County. Positive WNV pools were found in all 3 counties where sampling was performed. In addition to *Culex* spp., the following mosquito species tested positive for WNV: *Ochlerotatus japonicus* and *Aedes albopictus*. The first positive WNV pool was collected on 5/18/2010 and WNV activity, based on pooled infection rates, peaked during the week of 7/11/2010–7/17/2010 (Figure 1). The last WNV positive pool was collected on 8/5/2010, just prior to the end of mosquito trapping in 2010. The number of mosquito pools collected for arboviral testing increased overall from 2004–2008; however, since 2008, the number of collected pools fell by 55% (Figure 2).

Table 1. Mosquito pools testing positive for West Nile virus during the 2010 surveillance season.

County	Collection Date	Species	Pool Size	WNV Result	SLE Result	EEE Result	LAC Result
Kanawha	5/18/2010	*Culex spp.*	23	Positive	Negative	Not Tested	Not Tested
Kanawha	6/2/2010	*Culex spp.*	83	Positive	Negative	Not Tested	Not Tested
Kanawha	6/2/2010	*Culex spp.*	100	Positive	Negative	Not Tested	Not Tested
Kanawha	6/2/2010	*Culex spp.*	150	Positive	Negative	Not Tested	Not Tested
Jackson	6/2/2010	*Culex spp.*	150	Positive	Negative	Not Tested	Not Tested
Kanawha	6/4/2010	*Culex spp.*	5	Positive	Negative	Not Tested	Not Tested
Jackson	6/8/2010	*Culex spp.*	125	Positive	Negative	Not Tested	Not Tested
Ohio	6/14/2010	*Culex spp.*	72	Positive	Negative	Not Tested	Not Tested
Kanawha	7/7/2010	*Culex spp.*	33	Positive	Negative	Not Tested	Not Tested
Jackson	7/7/2010	*Culex spp.*	70	Positive	Negative	Not Tested	Not Tested
Kanawha	7/8/2010	*Culex spp.*	150	Positive	Negative	Not Tested	Not Tested
Jackson	7/13/2010	*Culex spp.*	150	Positive	Negative	Not Tested	Not Tested
Kanawha	7/13/2010	*Culex spp.*	41	Positive	Negative	Not Tested	Not Tested
Kanawha	7/14/2010	*Culex spp.*	12	Positive	Negative	Not Tested	Not Tested
Kanawha	7/15/2010	*Culex spp.*	117	Positive	Negative	Not Tested	Not Tested
Kanawha	7/15/2010	*Ochlerotatus japonicus*	8	Positive	Negative	Negative	Negative
Jackson	7/15/2010	*Aedes albopictus*	7	Positive	Negative	Negative	Negative
Jackson	7/15/2010	*Culex spp.*	12	Positive	Negative	Not Tested	Not Tested
Ohio	7/1/2010	*Culex spp.*	65	Positive	Negative	Not Tested	Not Tested
Ohio	6/30/2010	*Ochlerotatus japonicus*	7	Positive	Negative	Negative	Negative
Ohio	7/9/2010	*Culex spp.*	150	Positive	Negative	Not Tested	Not Tested
Kanawha	7/23/2010	*Culex spp.*	13	Positive	Negative	Not Tested	Not Tested
Kanawha	8/3/2010	*Culex spp.*	150	Positive	Negative	Not Tested	Not Tested
Kanawha	8/3/2010	*Culex spp.*	124	Positive	Negative	Not Tested	Not Tested
Kanawha	8/4/2010	*Culex spp.*	150	Positive	Negative	Not Tested	Not Tested
Kanawha	8/5/2010	*Culex spp.*	91	Positive	Negative	Not Tested	Not Tested

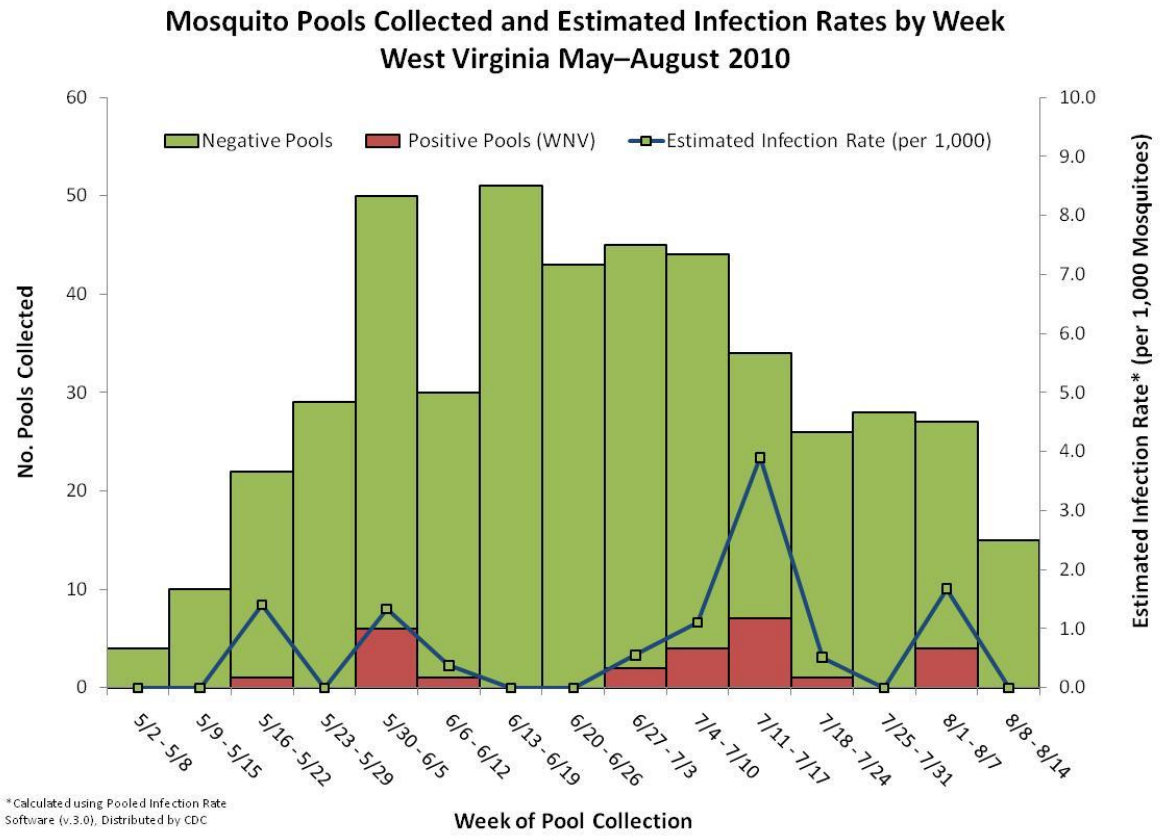


Figure 1. Mosquito pools tested for WNV and pooled infection rates by week during the 2010 surveillance season.

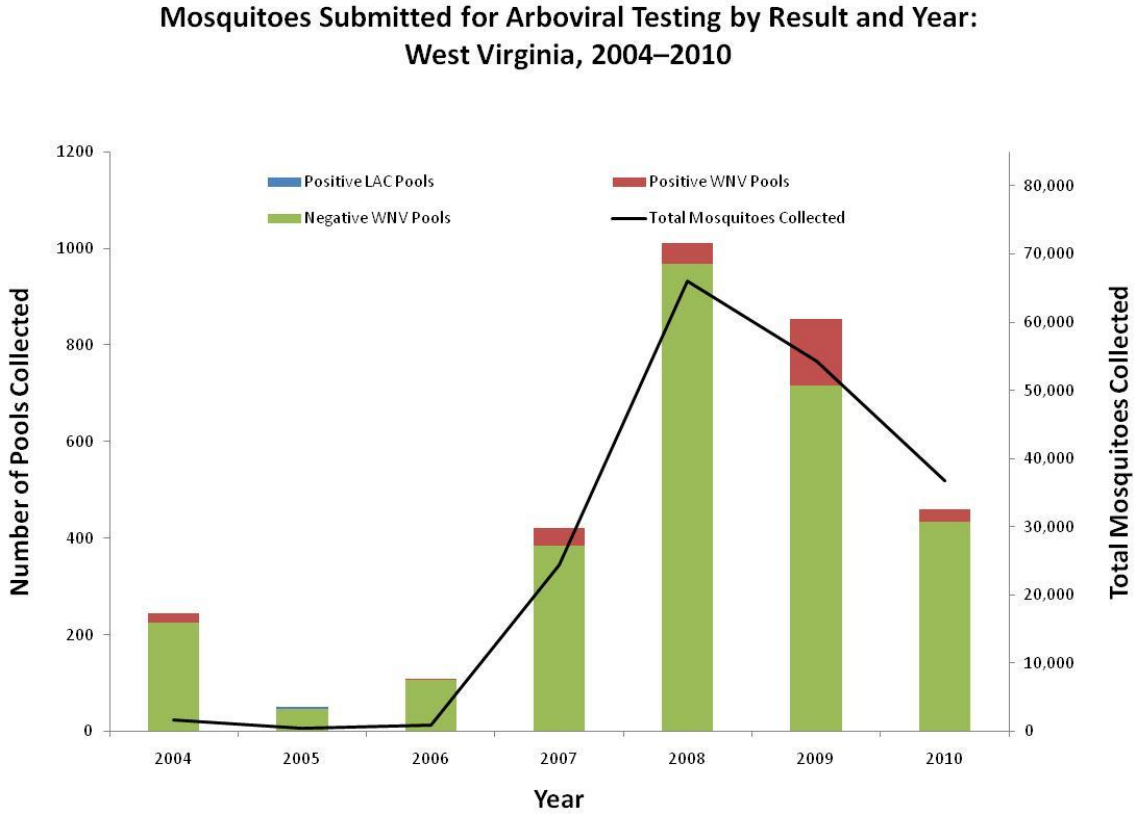


Figure 2. Results of mosquito pools tested for arboviruses during the 2004–2010 surveillance seasons.

Avian surveillance

Local health departments from 8 counties submitted 9 avian specimens for arboviral testing to OLS (Table 2). Specimens represented a variety of avian species. All specimens tested negative for arboviruses. Since 2004, avian submissions have steadily declined by 93% (Figure 3).

Table 2. Results of avian specimens tested for arboviruses during the 2010 surveillance season.

County	Specimen Date	Species	WNV Result	EEE Result	SLE Result
Ohio	3/24/2010	Blackbird	Negative	Negative	Negative
Monongalia	5/26/2010	Sparrow	Negative	Negative	Negative
Kanawha	6/3/2010	Juvenile starling	Negative	Negative	Negative
Kanawha	6/7/2010	Grackle	Negative	Negative	Negative
Hancock	6/10/2010	Crow	Negative	Negative	Negative
Greenbrier	6/11/2010	Unknown	Negative	Negative	Negative
Marshall	7/12/2010	Robin	Negative	Negative	Negative
Wyoming	7/13/2010	Mourning Dove	Negative	Negative	Negative
Hancock	7/27/2010	Robin	Negative	Negative	Negative

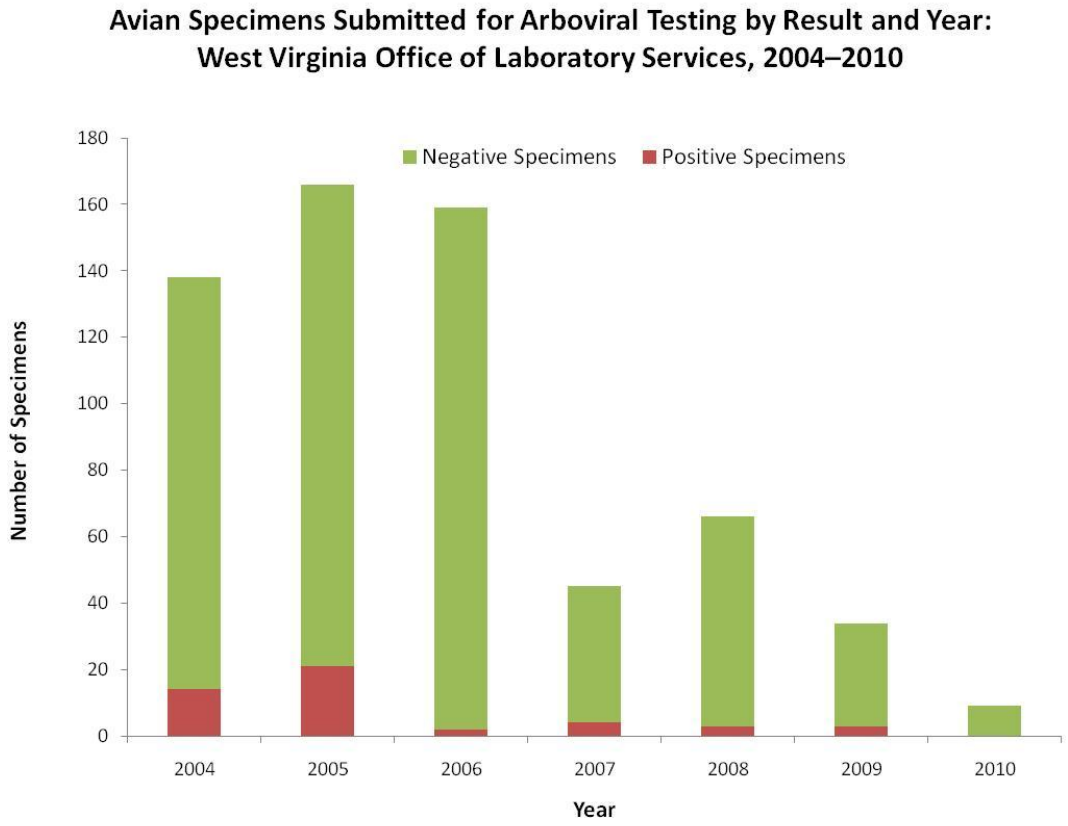


Figure 3. Results of avian specimens tested for arboviruses during the 2004–2010 surveillance seasons.

Equine surveillance

Two veterinary offices from 2 counties submitted 3 equine specimens for arboviral testing to OLS (Table 3). All specimens tested negative. Like avian specimen submissions, equine submissions have also declined, falling 84% from 2004 to 2010 (Figure 4).

Table 3. Results of equine specimens tested for arboviruses during the 2010 surveillance season

County	Specimen Date	WNV Result	EEE Result
Kanawha	8/30/2010	Negative	Negative
Kanawha	8/30/2010	Negative	Negative
Tucker	11/19/2010	Negative	Negative

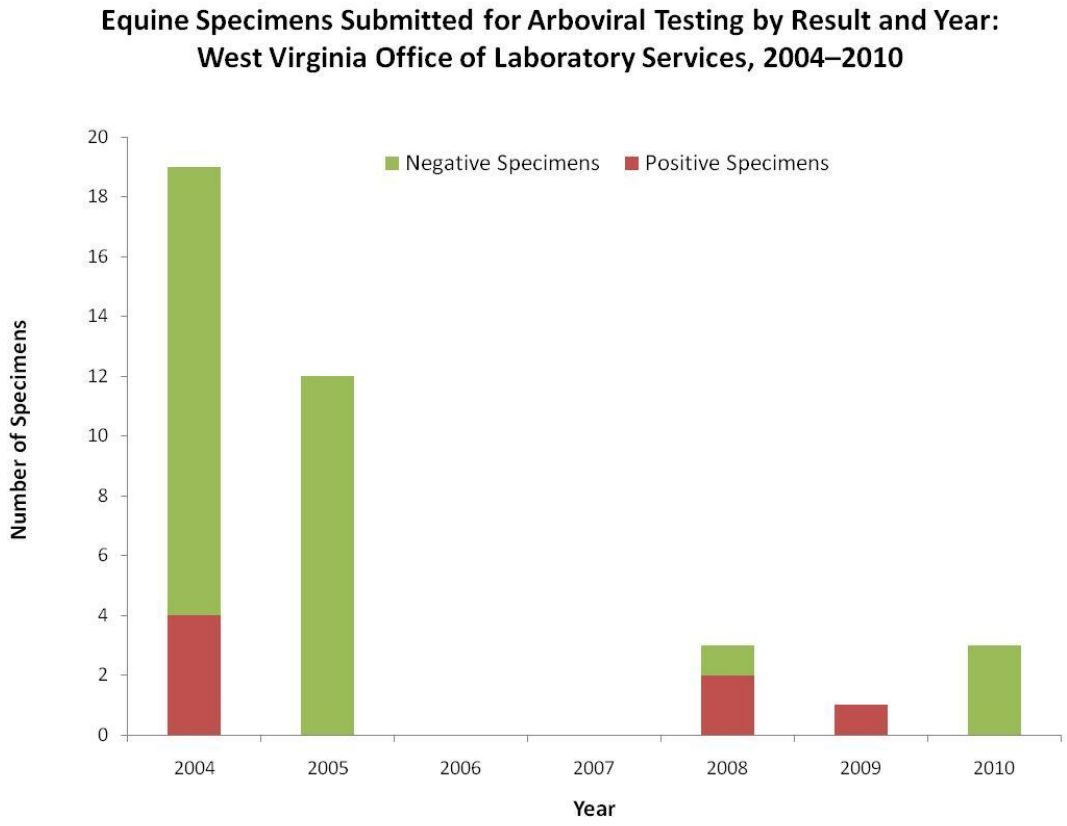


Figure 4. Results of equine specimens tested for arboviruses during the 2004–2010 surveillance seasons.

Human Surveillance

Endemic Arboviruses

Eight human cases of LAC were reported; 4 were classified as confirmed cases and 4 were classified as probable cases. All cases were neuroinvasive. For 2010, the incidence of LAC was 0.4 cases per 100,000 population. Incidence rates of LAC for previous years can be seen in Figure 5.

Date of illness onset for the 8 cases ranged from late June to late October; 50% of cases had illness onset in July. LAC cases were reported from 5 counties: Fayette (1), Kanawha (3), Mercer (2), Mingo (1) and Nicholas (1). One case may have been exposed in Ohio. Median age of cases was 7.5 years (range 4 to 18 years). Fifty percent of cases were female. All cases were hospitalized; no deaths were reported. No human cases of SLE, EEE, or WNV were identified during 2010. Over the past 3 years (since OLS has had a laboratory information management system), human arbovirus submissions have ranged from 35 to 62 specimens per year (Figure 6).

Non-endemic Arboviruses and Other Mosquito-Borne Diseases

In addition to the endemic North American arboviruses, 2 cases of travel-associated dengue fever were reported in 2010. The cases, both confirmed via PCR (and diagnosed within West Virginia at local hospitals), were reported from Wood and Fayette counties. Both cases returned to West Virginia after 1–2 week travel duration in dengue-endemic countries (Philippines and Honduras) and became symptomatic. Both cases were hospitalized due to their illness. No deaths were reported. One of the 2 specimens was forwarded to the CDC Dengue Laboratory, where dengue type 1 was isolated.

Three cases of travel-associated malaria were reported in WV during 2010. Prior to onset, the cases had traveled to either India or Nigeria and none had taken chemoprophylaxis for malaria before traveling to these endemic areas. All cases were reported from Monongalia county and were confirmed by blood smear. One case had a reported *Plasmodium* species of *P. falciparum*. One (33%) hospitalization occurred; no deaths were reported.

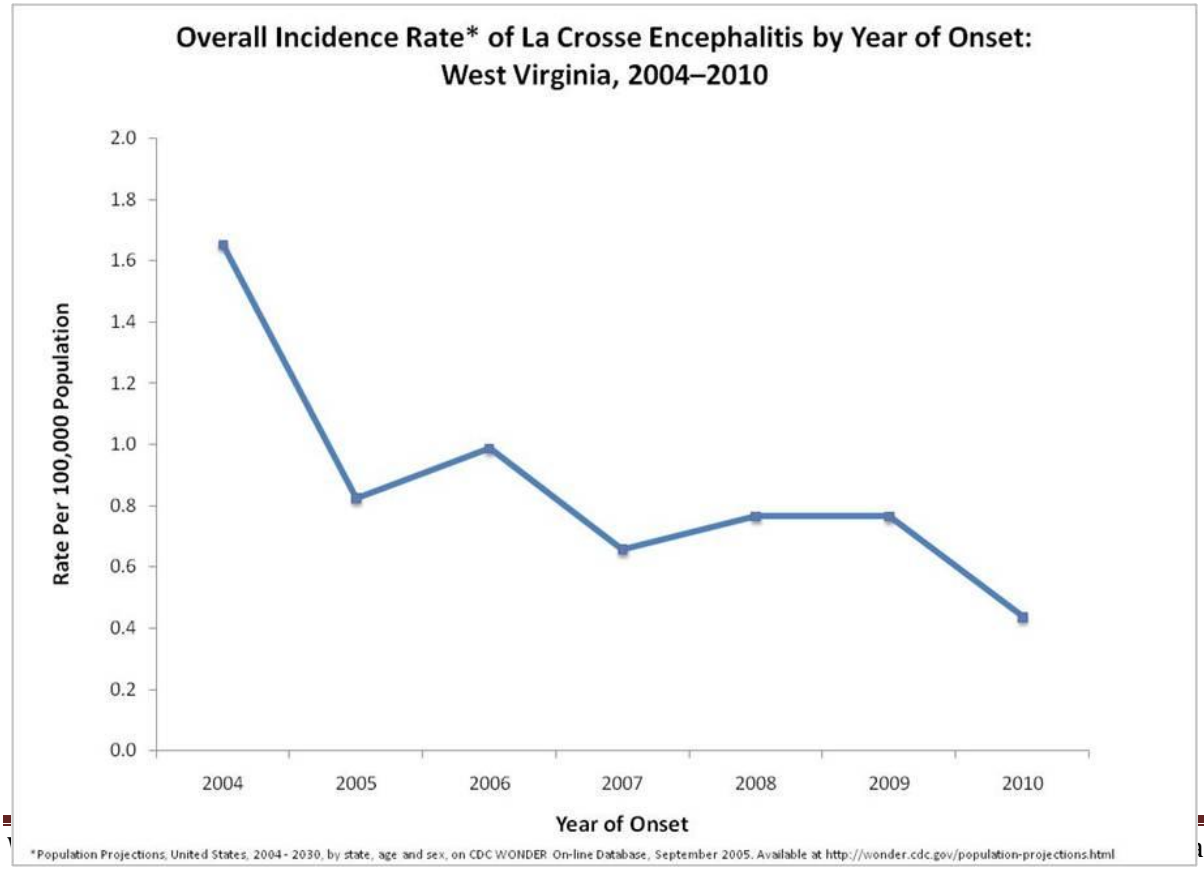


Figure 5. Overall incidence rates of La Crosse Encephalitis per 100,000 persons from 2004–2010.

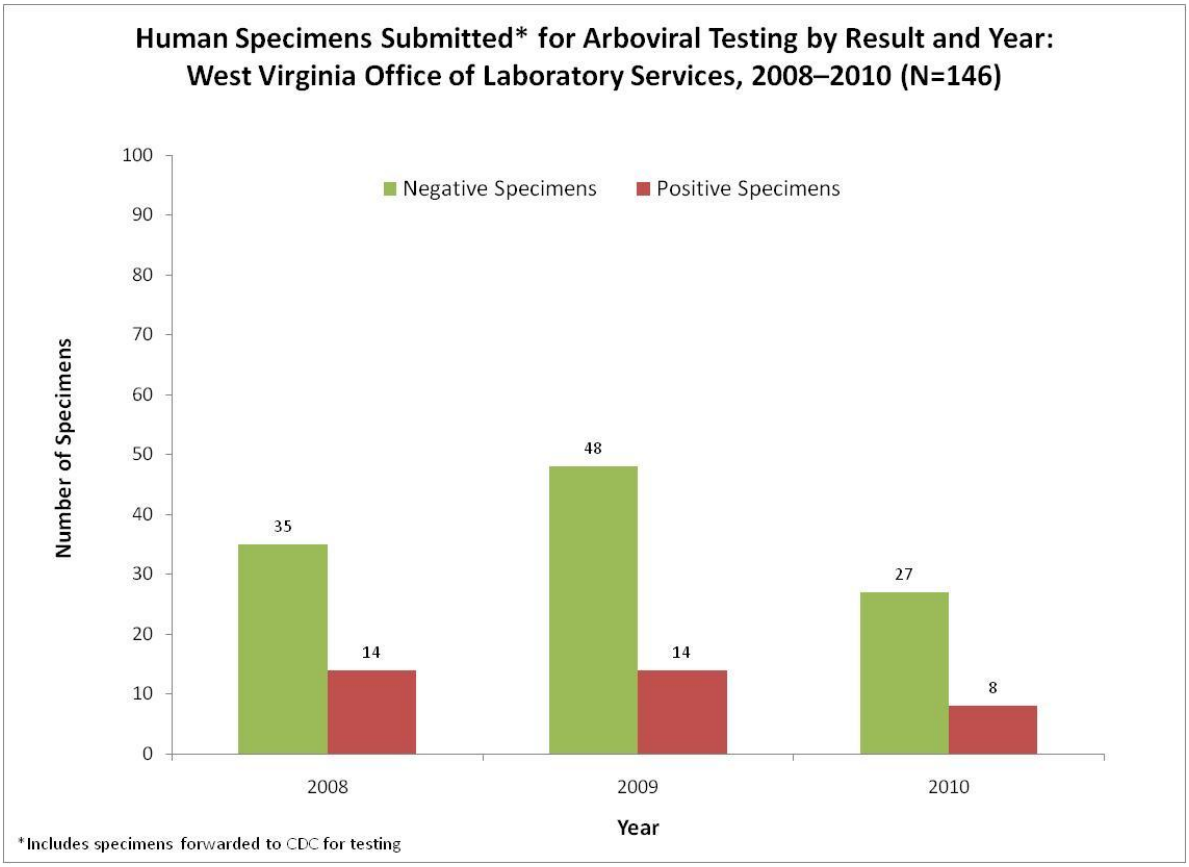


Figure 6. Results of human specimens tested for arboviruses during the 2008–2010 surveillance seasons.

Discussion

During 2010, 8 human cases of LAC were detected. For travel-associated cases of mosquito-borne diseases, 2 cases of dengue and 3 cases of malaria were reported (Figure 8). In addition, 26 WNV-positive mosquito pools were identified. No positive cases of arboviral infection were identified among equine or avian specimens. LAC was the only endemic arbovirus reported to infect humans during 2010, indicating this virus continues to be the major disease of concern for human illness in WV. This data also represents the seventh consecutive year arboviruses have been detected in mosquito pools and the fifth consecutive year WNV has been detected in mosquito pools. Therefore, WNV has likely become established within WV mosquito populations. To date, at least 6 species of mosquitoes trapped in WV have tested positive for WNV, including *Culex* spp., *Ochlerotatus triseriatus* (Eastern treehole mosquito), *Aedes albopictus* (Asian tiger mosquito), *Ochlerotatus japonicas* (Asian rock pool mosquito), and *Aedes vexans* (Inland floodwater mosquito). Small submission numbers for avian and equine specimens create difficulty in drawing conclusions about arboviral activity in these species.

Many factors may explain the reason for the maintenance of WNV in mosquito populations yet few recognized human cases. These include individual- and community-level preventative measures, development of immunity in dead-end and reservoir host species, changes to public health reporting activities, changes in the population of host species, and variations in climate.[1,2]

In addition, overall rates of reported human LAC infection have decreased since 2004 (Figure 5). Given the unique transmission cycle of LAC virus, similar reasons may at least partially explain the continued decline of reported human LAC infections; however, insufficient data have been collected on mosquitoes infected with LAC virus in WV to draw conclusions. Ground squirrels are an important amplifying reservoir for LAC virus in nature and any significant reductions in the population of ground

squirrels could result in fewer LAC-infected mosquitos, and thus fewer human LAC infections[3,4] Although data is lacking to determine if there have been changes within the population dynamics of WV ground squirrels, particularly in southern West Virginia where most human LAC infections are reported, this is one possible consideration to understanding the decrease in human cases.

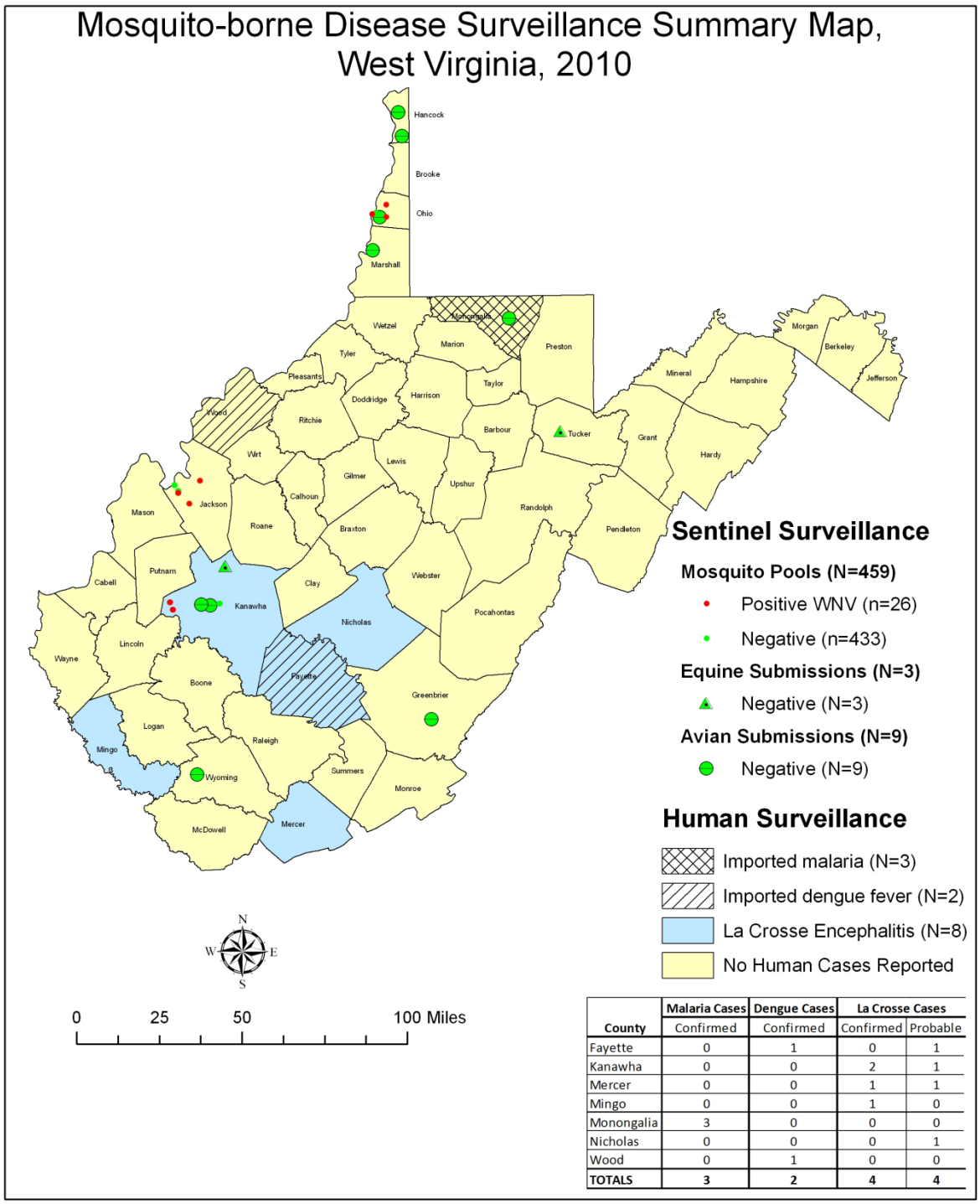
A sharp decrease in the number of submitted avian and equine specimens has been observed over the past several years. Several reasons may explain these declines including decreased interest by local health departments and veterinary offices in arboviral surveillance, decreased public interest in arboviral diseases, decreased resources for local health departments to collect and/or accept specimens, or actual decreases in ill or deceased avian and equine hosts. Mosquito pool numbers were also lower this year than in previous years, mainly due to an abbreviated mosquito trapping season.

There are at least two limitations to arboviral surveillance in WV. First, the number of avian and equine specimens received for testing is too small to draw proper conclusions regarding arboviruses potentially affecting these species. Second, although mosquito sampling resulted in the collection of several hundred pools, there were only 12 trapping sites in 3 counties (Kanawha, Jackson and Ohio). At least 5 unique, defined level-III ecoregions exist in West Virginia (Figure 9), and all mosquito sampling in 2010 took place in only 1 ecoregion (Southern Unglaciated Allegheny Plateau Section)[5]. Ecoregions are characterized in part by climate, vegetation, wildlife present, hydrology, and other factors which may influence the diversity of species and relative number of mosquitos present.

The 2010 mosquito-borne disease surveillance data indicates continued LAC activity affecting humans and continued WNV activity in mosquitoes despite lack of human illness. Although this surveillance data may appear to indicate a decrease in arboviral activity, these diseases are unpredictable and surveillance efforts should continue to monitor changes. In addition, education and outreach efforts should continue to inform the public about appropriate prevention measures, including measures to take during travel (Box 1). Enhanced passive surveillance through outreach to local health departments and healthcare providers will continue in 2011. Due to limited trapping sites and decreasing submission numbers, evaluation of current surveillance efforts for mosquitoes, avians, and equines is advisable. Options to expand mosquito sampling into additional areas and ecoregions of WV should be assessed. Outreach to veterinarians may be important in increasing the number of avian and equine submissions. Local health departments and other public health partners, such as the WV Department of Agriculture and the WV Department of Natural Resources, should also be included in future planning and surveillance activities.

References

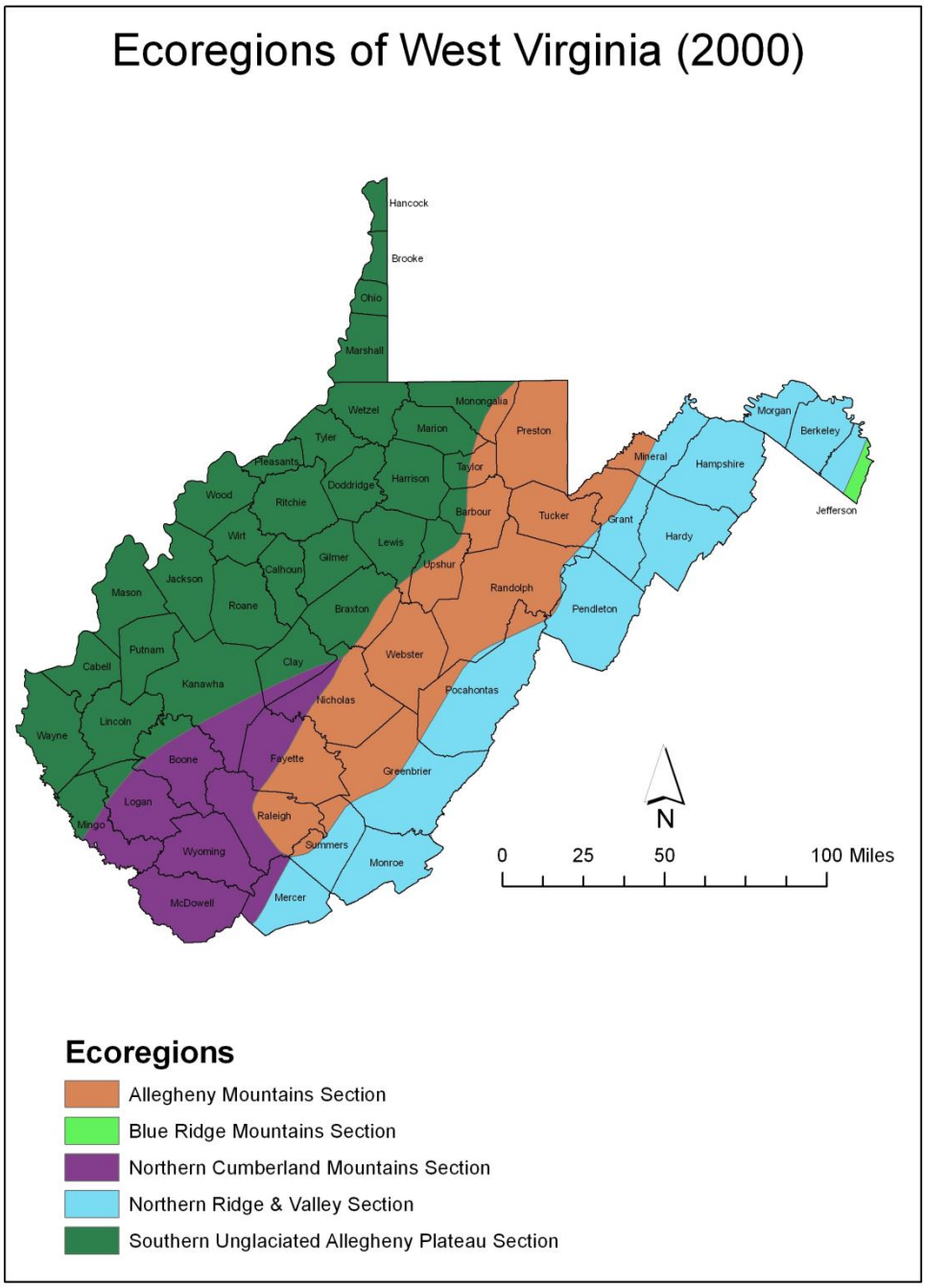
1. Komar N. West Nile virus: epidemiology and ecology in North America. Adv Virus Res 2003;61:185--234.
2. Hayes EB, Komar N, Nasci RS, Montgomery SP, O'Leary DR, Campbell GL. Epidemiology and transmission dynamics of West Nile virus disease. Emerg Infect Dis 2005;11:1167--73.
3. Rust RS, Thompson WH, Matthew CG, et al. La Crosse and other forms of California encephalitis. *J Child Neurol* 1999 14:1–14.
4. McJunkin JE, Khan RR, and Tsai TF. California-La Crosse encephalitis. *Emerg Infect Dis* 1998 12:83–93.
5. US EPA. Western ecology division: ecoregions of North America. Available at: http://www.epa.gov/wed/pages/ecoregions/na_eco.htm Accessed 19 Jan 2011.



County	Malaria Cases Confirmed	Dengue Cases Confirmed	La Crosse Cases Confirmed	La Crosse Cases Probable
Fayette	0	1	0	1
Kanawha	0	0	2	1
Mercer	0	0	1	1
Mingo	0	0	1	0
Monongalia	3	0	0	0
Nicholas	0	0	0	1
Wood	0	1	0	0
TOTALS	**3**	**2**	**4**	**4**

Map created: 3/18/2011 (jml)

Figure 8. Mosquito-borne disease surveillance summary map for West Virginia in 2010.



Map created 2/17/11
Map based on files made available by the US Forest Service in 2000.

Figure 9. Map depicting the five defined ecoregions in West Virginia.

Box 1. Prevention Tips for Mosquito-Borne Diseases

Reducing exposure to mosquito bites is the best defense against getting infected with a mosquito-borne disease. The following tips can be used to prevent and control mosquito-borne diseases.

- Use insect repellant that contains DEET, picaridin, IR_{3535} or oil of lemon eucalyptus on exposed skin and clothing when outdoors. Always follow package directions. Apply sparingly to children, avoiding hands and face, and wash them with soap and water when they come indoors.
    - Permethrin is a repellant that can be applied to clothing and provide protection through multiple washes. Do not apply permethrin-containing repellants directly to skin.
- Wear protective clothing such as long sleeves, pants, and socks when weather permits.
- Be aware of peak mosquito hours.
    - For many mosquitoes, peak hours are between dusk and dawn or evening and early morning.
    - For the mosquitoes that transmits La Crosse encephalitis virus (*Ochlerotatus triseriatus, Ochlerotatus japonicus, Aedes albopictus)*, peak hours are actually during the daytime (dawn until dusk).
- Install and repair window screens as needed to keep mosquitoes out of homes.
- Keep mosquitoes from laying eggs near your home by removing areas of standing water.
    - Empty standing water from flower pots, buckets, barrels, and tires.
    - Change the water in pet dishes regularly.
    - Replace water in bird baths weekly.
    - Drill holes in tire swings so the water drains out.
    - Empty children's wading pools and store on their side when not in use.
    - Empty standing water from canoes and boats.