West Virginia Mosquito-Borne Disease Surveillance Report, 2011

I. Introduction

Mosquito-borne diseases, the majority of which are viruses, are transmitted through the bite of infected mosquitoes. Historically, La Crosse encephalitis virus (LAC) has been the mosquito-borne disease of most concern in West Virginia, with over 40 human cases previously reported in some years. Other arboviruses of concern in this state include West Nile virus (WNV), St. Louis encephalitis virus (SLE), with the last human cases reported in the 1970s, and Eastern equine encephalitis virus (EEE). Annually, few human cases of WNV have historically been reported in WV although WNV-positive mosquito pools are detected in the state each year. No human cases of EEE have been reported in WV, however human cases have been reported in surrounding states including Pennsylvania, Maryland, and Virginia. In addition, equine cases of EEE were reported from Ohio in 2010. Malaria, a parasite that infects red blood cells, and dengue virus are not endemic to WV but a few travel-associated cases of these diseases are generally reported each year in WV.

This surveillance report summarizes the human and non-human cases of mosquito-borne diseases detected in West Virginia during 2011. Methods used for surveillance of these diseases are described for humans, mosquitoes, dead birds, and horses.

II. Methods

Human Surveillance

As in previous years, enhanced passive surveillance methods were utilized to help detect human cases of mosquito-borne disease. These methods included 1) a statewide health alert to physicians, 2) a hospital laboratory letter, 3) an email memo to local health departments with important arbovirus information, and 4) a conference call training for local health departments. During 2011, testing of human specimens occurred through hospital laboratories, the Office of Laboratory Services (OLS) and CDC.

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 2011 national case definition for each mosquito-borne disease (http://wwwn.cdc.gov/NNDSS/beta/bConditionList.aspx?Type=0&Yr=2011). Confirmed and probable arboviral cases were reported to CDC through ArboNet. Bi-weekly surveillance reports were sent to public health partners June-October 2011 to provide data feedback on vectorborne disease activity during this time. 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, 2011–December 31, 2011. Data were summarized using Microsoft Excel and ArcGIS v.9.3.

Mosquito surveillance

Active adult mosquito sampling occurred from May 31–October 7, 2011. The state public health entomologist and one summer intern conducted regular, weekly mosquito trapping at sites in Fayette, Kanawha, Nicholas, and Webster counties using CDC gravid traps; CO_2 traps were also used in Fayette, Kanawha, and Webster counties. Daily mosquito samples were returned to OLS in the nets of the mosquito traps and placed in a minus 80 degree Celsius freezer. Volunteers (regional epidemiologists, sanitarians, nurses, students) collected additional adult mosquito samples using gravid traps in Boone and Calhoun counties, CO_2 traps in Cabell and Ohio counties, and both trap types in Wood county. Also, a single mosquito collecting event occurred in Tyler county using hand-capture methods. Collaborators sent collected mosquitoes to OLS for testing. Larval surveillance was initiated in northern counties by collecting samples from natural and artificial containers using mosquito dippers. Larvae were identified to mosquito species by the public health entomologist.

For testing, mosquitoes were pooled together based upon species, collecting locality, and collecting date. However, due to low capture yield, mosquitoes were pooled together based upon species and collecting locality only during June and *Culex restuans* and *Cx. pipiens* were pooled together due to taxonomic difficulties in differentiating these species from field-collected specimens. Mosquitoes were pooled into groups with a maximum of 50 specimens per pool. The pooled groups of mosquitoes were placed into two millimeter Sarstedt micro tubes with two copper beads or glass beads. A buffer solution was added to the micro tubes and the tubes were placed on a mixer mill for 10 minutes. The buffer solution together with the action of the beads, ground the mosquitoes into a slurry, which was centrifuged and extracted. Qiagen® QIAamp RNeasy Mini kit was used to isolate the viral RNA from the mosquito tissue. Real-time reverse transcription PCR was used for arboviral detection. Invitrogen SuperScript® III Platinum One-Step qRT-PCR was used for the PCR amplification. The polymerase chain reactions were run using the ABI 7500FAST. Biosearch Technologies provided the primers and Taqman probes. The CDC provided controls for validation. Pools containing non-*Culex* species were tested for West Nile virus (WNV), St. Louis encephalitis (SLE), La Crosse encephalitis (LAC), and Eastern equine encephalitis (EEE). *Culex* species were only analyzed for WNV and SLE. Mosquito pools positive for an arbovirus were reported to CDC through ArboNet.

Pooled infection rates were examined for each species each week using the CDC-developed Microsoft Excel add-in "Pooled Infection Rate." The minimum infection rate (MIR) is the ratio of virus positive mosquito pools to the total number of mosquitoes in the sample. The associated maximum likelihood estimator (MLE) is the proportion of infected mosquitoes that best fits the number of positive mosquito pools (of a set size). Both the MIR and MLE helped to provide an estimate of the infection prevalence among mosquitoes tested. For more information about the methodology used to calculate MIR and MLE, see http://www.cdc.gov/ncidod/dvbid/westnile/software.htm .

Horse Surveillance

Veterinarians suspecting arboviral infection in a horse 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.

Dead Bird Surveillance

Local health department personnel submitted oral swabs from dead birds to OLS for testing of WNV, SLE, and EEE at the Southeastern Cooperative Wildlife Disease Study. A report was submitted to CDC through ArboNet if any dead bird specimen tested positive for an arbovirus.

III. Results

Human Surveillance

Table 1 provides a summary of human cases of mosquito-borne diseases reported in WV during 2011. Twenty-six cases (24 confirmed, 2 probable) of LAC were reported during 2011; this represents > 3-fold increase from the eight cases reported in 2010. Onset dates for cases ranged from June 2011 to September 2011. Sixteen cases (61.5%) were male. The mean age of cases was 10 years (range 3-46 years); 25 (96%) cases were <15 years old. LAC cases were reported from 15 counties; only 5 counties reported LAC cases during 2010. Figure 1 shows the geographic distribution of human mosquito-borne disease cases in 2011.

Two cases of WNV infection were reported during 2011. One case (50%) was female; both cases were adults, aged $\geq$ 75 years. The cases were reported from Wood and Pendleton counties. Onset dates for the cases occurred in August and September 2011. In addition, to these WNV cases, one presumptive viremic blood donor (PVD) was also reported from Wood county. This patient did not report any clinical symptoms of WNV but tested positive for the virus when their donated blood was screened by a blood bank.

Seven travel-associated cases of malaria were reported among WV residents. The onset dates ranged from January 2011 through July 2011. Four (57.1%) cases were male. Cases ranged in age from 1 day old to 68 years old. The one-day old patient is believed to have been congenitally infected as the mother was also reported as a case of malaria. Travel history for malaria cases included Nigeria, Pakistan, Uganda, Ghana, and Cameroon. One case (14.3%) reported taking malaria chemoprophylaxis.

No human cases of SLE, EEE, or travel-associated dengue virus were reported during 2011.

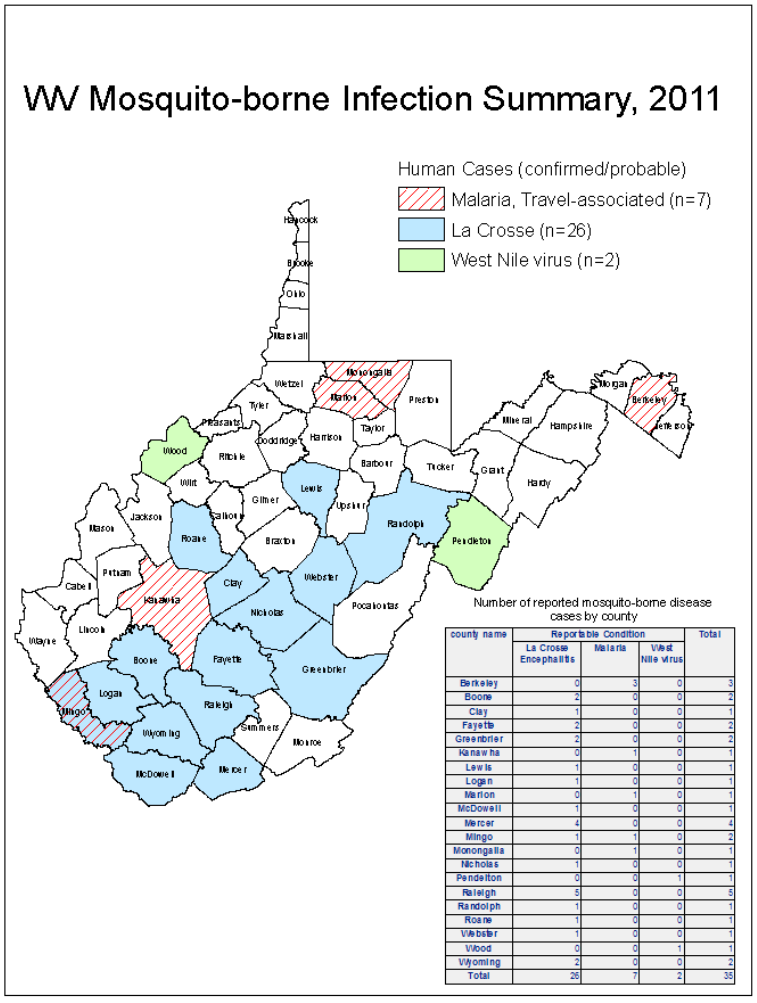
Table 1. Summary of Mosquito-Borne Disease Human Cases – West Virginia, 2010-2011

Mosquito-Borne Disease	No. (%) of Human Cases† – 2011	No. (%) of Human Cases† - 2010
La Crosse encephalitis virus	26 (74)	8 (62)
West Nile virus*	2 (6)	0 (0)
Malaria	7 (20)	3 (23)
Dengue virus	0 (0)	2 (15)
Eastern equine encephalitis virus	0 (0)	0 (0)
St. Louis encephalitis virus	0 (0)	0 (0)
Total	35 (100)	13 (100)

***Presumptive viremic blood donor not included in case count for West Nile virus**

†Includes only cases classified as confirmed or probable

Fig. 1. Human Cases of Mosquito-Borne Diseases, West Virginia, 2011



county name	Reportable Condition			Total
	La Crosse Encephalitis	Malaria	West Nile virus	
Berkeley	0	3	0	3
Boone	2	0	0	2
Clay	1	0	0	1
Fayette	2	0	0	2
Greenbrier	2	0	0	2
Kanawha	0	1	0	1
Lewis	1	0	0	1
Logan	1	0	0	1
Marion	0	1	0	1
McDowell	1	0	0	1
Mercer	4	0	0	4
Mingo	1	1	0	2
Monongalia	0	1	0	1
Nicholas	1	0	0	1
Pendleton	0	0	1	1
Raleigh	5	0	0	5
Randolph	1	0	0	1
Roane	1	0	0	1
Webster	1	0	0	1
Wood	0	0	1	1
Wyoming	2	0	0	2
Total	26	7	2	35

Mosquito Surveillance

A total of 4,939 mosquitoes from 259 pools were collected and tested for arboviruses. The following mosquito species were identified: 3,726 *Culex pipiens/restuans* specimens (75.5%), 471 *Aedes albopictus* (9.5%), 365 *Aedes japonicus* (7.4%), 160 *Aedes trivittatus* (3.2%), 111 *Aedes triseriatus* (2.2%), 61 *Aedes vexans* (1.2%), 21 *Anopheles punctipennis* (0.4%), 17 *Aedes* spp. (0.3%), 6 *Aedes sollicitans* (0.1%), and 1 *Toxorhynchites rutilus septentrionalis*. Due to the high species diversity, a large proportion of the pools were non-*Culex* species: 112 pools *of Cx. pipiens/restuans* (43.2%), 44 pools of *Ae. albopictus* (17.0%), 38 pools of *Ae. japonicus* (14.7%), 34 pools of *Ae. triseriatus* (13.1%), 9 pools of *An. punctipennis* (3.5%), 6 pools of *Ae. vexans* (2.3%), 6 pools of *Ae.* spp. (2.3%), 5 pools of *Ae. trivittatus* (1.9%), 4 pools of *Ae. sollicitans* (1.5%), 1 pool of *T. rutilus septentrionalis* (0.4%). Of the 259 mosquito pools tested, 27 (10.4%) were positive for WNV and 2 (0.8%) were positive for LAC. SLE and EEE were not recovered from any samples. Table 2 lists the positive mosquito pools for WNV and LAC identified during 2011.

Nine (33.3%) of the 27 WNV positive pools were *Ae. albopictus;* seven (25.9%) were *Ae. triseriatus*, three (11.1%) were *Ae. japonicus*, and two (7.4%) were *Ae*. spp.. Only six (22.2%) of the WNV positive pools were *Cx. pipiens/restuans* (Table 2). *Culex pipiens/restuans* showed a lower MIR (MIR=1.61; 95% C.I. = 0.32-2.90) than *Ae. albopictus* (MIR=19.11; 95% C.I. = 6.74-31.47) and *Ae. triseriatus* (MIR=63.06; 95% C.I. = 17.84-108.28) during the entire surveillance season (Table 3). The MLE estimator for infection rate (1) in *Cx. pipiens/restuans* (MLE = 1.64; 95% C.I. = 0.68-3.41) was also statistically lower than *Ae. albopictus* (MLE=18.69; 95% C.I. = 10.6-31.91) and *Ae. triseriatus* (MLE=63.21; 95% C.I. = 31.23-115.33) (Table 3). Weekly infection rates across species appeared to rise in August and peak in September (Figs. 2 and 3).

Positive WNV pools were found in seven of the ten counties where mosquito collecting took place. The first WNV positive pools for 2011 were *Ae. triseriatus* and *Ae. japonicus* collected from Kanawha County on June 20. The last WNV positive pools were *Ae. albopictus* from Kanawha County and *Ae. triseriatus* from Boone County on September 25. The first LAC positive pool in 2011 was *Ae. triseriatus* from Kanawha County on June 22. The second (and last) LAC positive pool was *Ae. albopictus* from Fayette County on July 12. Figure 4 shows the distribution of WNV- and LAC-positive mosquito pools from 2011.

Based upon larval surveillance, *Aedes triseriatus* larvae were found in the following counties: Braxton, Mercer, Nicholas, Webster, Tucker, and Wood. Larvae of *Ae. japonicus* were recovered from Harrison, Kanawha, Lewis, Mercer, Monongalia, Nicholas, Taylor, Tucker, Upshur, Webster, and Wood counties. *Aedes albopictus* larvae were found in the following counties: Barbour, Braxton, Kanawha, Monongalia, Nicholas, Tucker, and Wood.

Table 2. Mosquito pools testing positive for WNV and LAC during the 2011 surveillance season.

County	Collection Date	Species	Pool Size	WNV Result	SLE Result	EEE Result	LAC Result
Kanawha	6/20/2011	*Aedes triseriatus*	7	Positive	Negative	Negative	Negative
Kanawha	6/20/2011	*Aedes japonicus*	1	Positive	Negative	Negative	Negative
Fayette	7/12/2011	*Culex* spp.	50	Positive	Negative	Not Tested	Not Tested
Fayette	7/26/2011	*Culex* spp.	50	Positive	Negative	Not Tested	Not Tested
Ohio	8/10/2011	*Culex* spp.	1	Positive	Negative	Not Tested	Not Tested
Fayette	8/16/2011	*Aedes* spp.	2	Positive	Negative	Negative	Negative
Cabell	8/17/2011	*Aedes albopictus*	2	Positive	Negative	Negative	Negative
Cabell	8/17/2011	*Aedes albopictus*	1	Positive	Negative	Negative	Negative
Calhoun	8/21/2011	*Culex* spp.	1	Positive	Negative	Not Tested	Not Tested
Calhoun	8/21/2011	*Aedes* spp.	1	Positive	Negative	Negative	Negative
Nicholas	8/22/2011	*Aedes albopictus*	8	Positive	Negative	Negative	Negative
Fayette	8/23/2011	*Aedes japonicus*	6	Positive	Negative	Negative	Negative
Fayette	8/23/2011	*Aedes albopictus*	3	Positive	Negative	Negative	Negative
Fayette	8/23/2011	*Aedes triseriatus*	2	Positive	Negative	Negative	Negative
Fayette	8/30/2011	*Culex* spp.	50	Positive	Negative	Not Tested	Not Tested
Fayette	8/30/2011	*Aedes triseriatus*	2	Positive	Negative	Negative	Negative
Kanawha	9/7/2011	*Aedes albopictus*	3	Positive	Negative	Negative	Negative
Fayette	9/7/2011	*Aedes albopictus*	1	Positive	Negative	Negative	Negative
Fayette	9/13/2011	*Culex* spp.	51	Positive	Negative	Not Tested	Not Tested
Fayette	9/13/2011	*Aedes japonicus*	1	Positive	Negative	Negative	Negative
Fayette	9/13/2011	*Aedes triseriatus*	1	Positive	Negative	Negative	Negative
Fayette	9/13/2011	*Aedes albopictus*	4	Positive	Negative	Negative	Negative
Kanawha	9/13/2011	*Aedes albopictus*	1	Positive	Negative	Negative	Negative
Fayette	9/20/2011	*Aedes triseriatus*	1	Positive	Negative	Negative	Negative
Boone	9/23/2011	*Aedes triseriatus*	1	Positive	Negative	Negative	Negative
Kanawha	9/25/2011	*Aedes albopictus*	10	Positive	Negative	Negative	Negative
Boone	9/25/2011	*Aedes triseriatus*	1	Positive	Negative	Negative	Negative
Kanawha	6/23/11	*Aedes triseriatus*	2	Negative	Negative	Negative	Positive
Fayette	7/12/11	*Aedes albopictus*	2	Negative	Negative	Negative	Positive

Table 3. West Nile virus minimum infection rate (MIR) and maximum likelihood estimated infection rate (MLE) by mosquito species during 2011 surveillance season.

Mosquito Species	MIR per 1,000 mosquitoes	MIR Lower-Upper Limits	MLE per 1,000 mosquitoes	MLE Lower-Upper Limits	No. of Pools	No. of Positive Pools	Total No. of Mosquitoes
Aedes albopictus	19.11	6.7-31.5	18.69	10.6-31.9	44	9	471
Aedes sollicitans	0.00	#N/A	0.00	0.0-341.7	4	0	6
Aedes spp.	117.65	0.0-270.8	106.51	24.3-298.4	6	2	17
Aedes vexans	0.00	#N/A	0.00	0.0-36.3	6	0	61
Anopheles punctipennis	0.00	#N/A	0.00	0.0-128.9	9	0	21
Culex pipiens/restuans	1.61	0.3-2.9	1.64	0.7-3.4	112	6	3726
Aedes japonicus	8.22	0.0-17.5	8.06	2.2-21.2	38	3	365
Aedes triseriatus	63.06	17.8-108.3	63.21	31.2-115.3	34	7	111
Aedes trivittatus	0.00	#N/A	0.00	0.0-16.9	5	0	160
Toxorhynchites rutilus septentrionalis	0.00	#N/A	0.00	0.0-793.5	1	0	1

Fig. 2. Mosquito collecting activity/seasonal phenology and minimum infection rates during 2011 surveillance season.

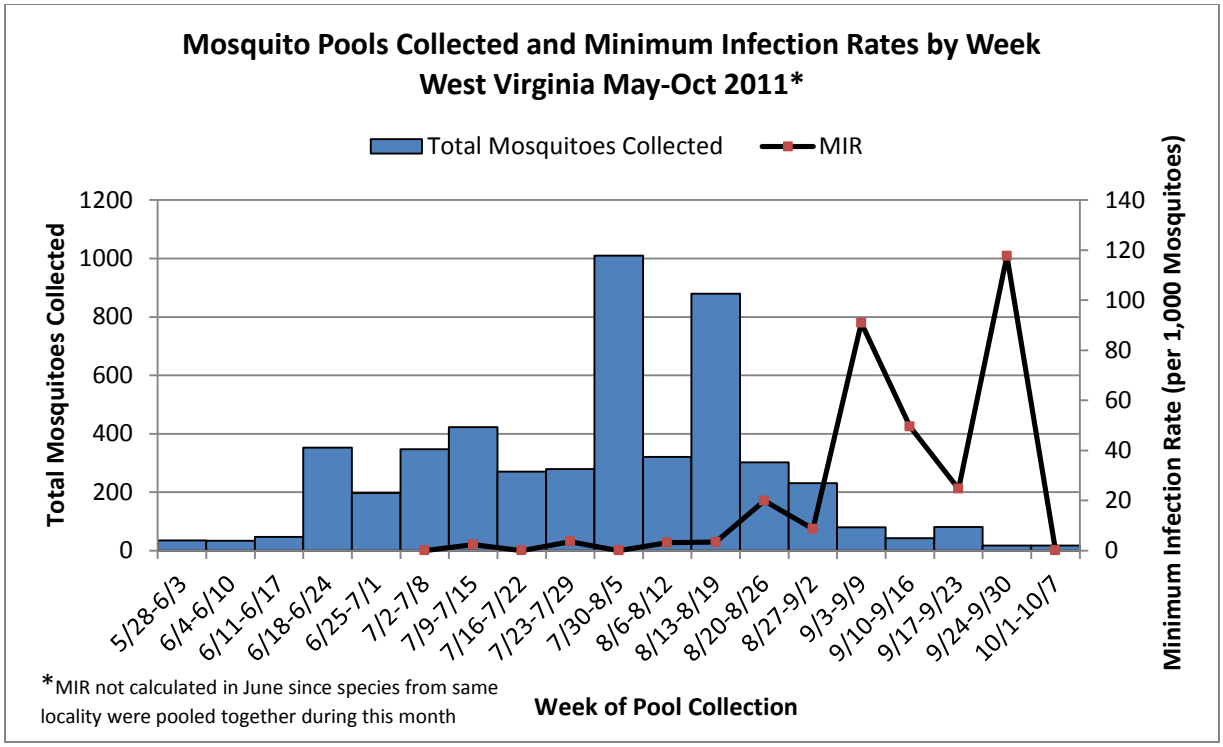


Fig. 3. Mosquito collecting activity/seasonal phenology and estimated infection rate during 2011 surveillance season.

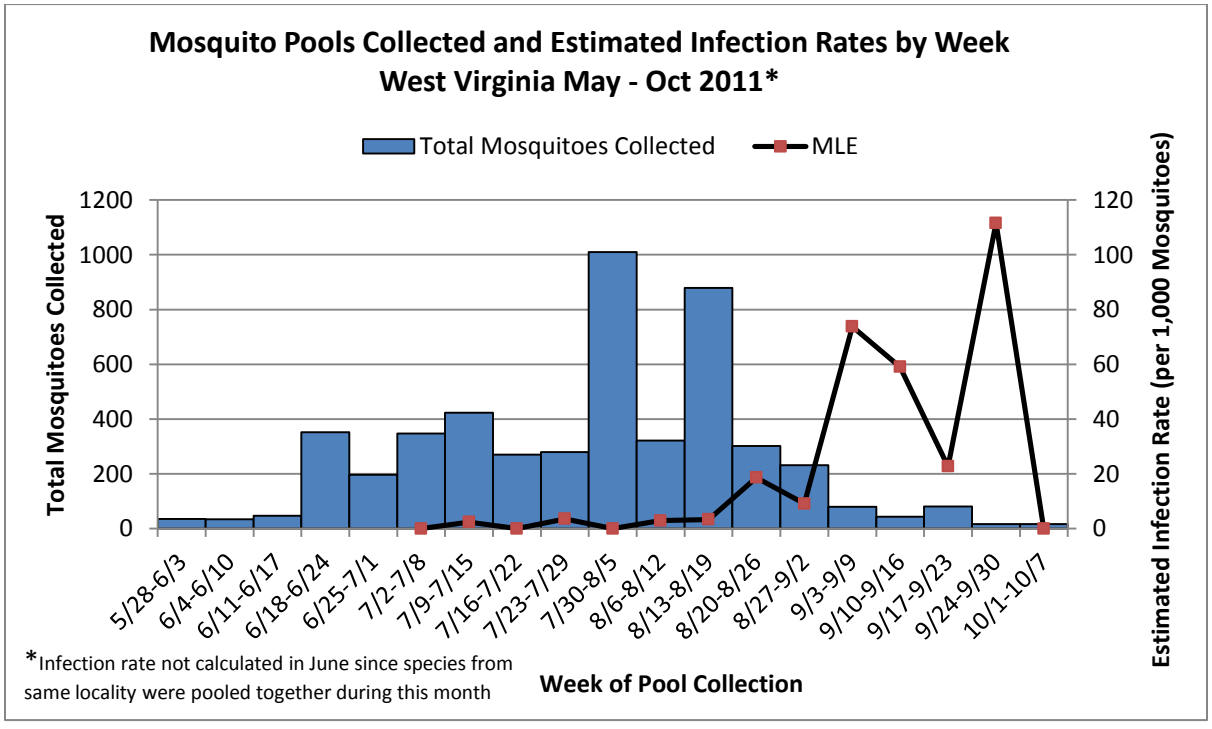
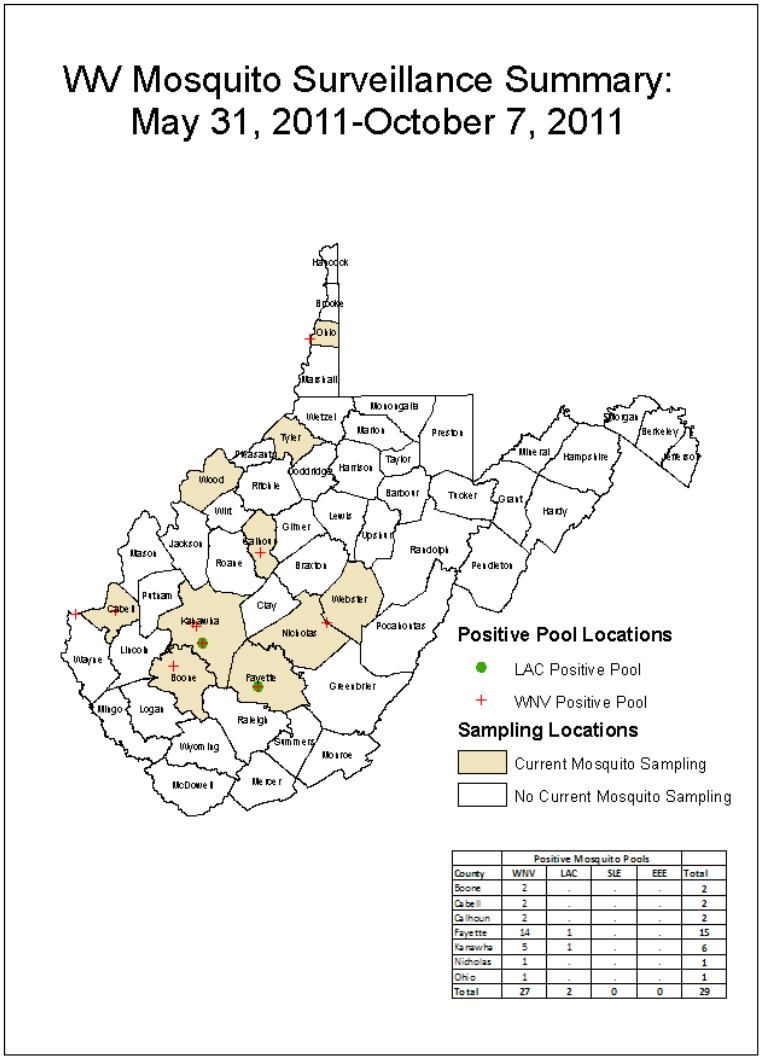


Fig. 4. Distribution of Mosquito Pools Positive for WNV and LAC, West Virginia, 2011



	Positive Mosquito Pools				
County	WNV	LAC	SLE	EEE	Total
Boone	2	.	.	.	2
Cabell	2	.	.	.	2
Calhoun	2	.	.	.	2
Fayette	14	1	.	.	15
Kanawha	5	1	.	.	6
Nicholas	1	.	.	.	1
Ohio	1	.	.	.	1
Total	27	2	0	0	29

Horse Surveillance

One horse specimen from Cabell County was submitted for testing during 2011. This specimen tested negative for WNV and EEE.

Dead Bird Surveillance

Six dead bird specimens were submitted for testing during 2011; no specimens tested positive for SLE, WNV, or EEE. Specimens were submitted from Wyoming, Monongalia, Clay, Hancock, Marshall, and Tucker counties between June 2011 and August 2011. Bird species submitted included robin, sparrow, finch, hawk, bronzed cow bird, and redheaded woodpecker.

IV. Discussion

LAC remains the mosquito-borne virus of most concern in WV with the number of human LAC cases increasing during 2011 as compared to 2010. In addition, the number of counties reporting LAC cases also increased, although most of these counties have previously reported LAC cases. And while the 26 LAC cases from 2011 are not as high as case numbers reported from the mid-1990s (upwards of 70 cases per year during that time period), the sharp increase is concerning and serves as a reminder that the virus is still circulating throughout the state. Descriptive data from the 2011 LAC cases also shows that children <15 years of age remain at highest risk for infection.

An increase in LAC mammal reservoirs could explain this increase in human LAC cases as Peters et al. (2) reported a high population of gray squirrels, (a well-documented LAC reservoir), in West Virginia during 2011. The good mast (food from trees or shrubs) conditions in 2010 resulted in excellent overwintering survival and numerous healthy litters of gray squirrels for 2011 (2).

WNV activity also increased during 2011 as compared to 2010, however the number of reported cases is not unusual for WV. The descriptive data for these cases indicates that elderly persons continue to be at highest risk for WNV infection. For the first time, a PVD was reported during 2011; PVDs can serve as potential transmission sources if their donated blood is allowed to enter the blood supply. Fortunately, screening for WNV has become routine at blood banks, mitigating this risk.

During 2011, WV also saw an increase in the number of travel-associated malaria cases as compared to 2010 (7 cases versus 3 cases). Unlike 2010, no cases of travel-associated dengue virus were reported in WV during 2011. All of the malaria cases reported travel to areas of the world that are endemic for this disease, however only one case reported taking chemoprophylaxis for malaria. With an estimated 3.3 billion airline passengers expected to travel by 2014 (3), exposure to pathogens not typically found in WV will continue to be a risk and travelers should educate themselves about possible disease risks. CDC's website for travelers' health is a good resource to help find this information (http://wwwnc.cdc.gov/travel/). Travelers can look up the country they will be traveling to and review important health information related to that country.

The number of WNV positive mosquito pools reported during 2011 is similar to the number reported from 2010 and again, is an indicator that WNV continues to circulate throughout WV. Two LAC-positive mosquito pools were identified during 2011 as compared to no positive LAC mosquito pools in 2010.

All of the mosquito species that tested positive for WNV during 2011 are known to be susceptible to WNV infection in nature (4-11) and many are also capable of transmitting WNV to humans. These species include *Ae. albopictus* (12), *Ae. triseriatus* (13, 14), *Ae. japonicus* (12, 15), *Cx. pipiens* (12, 16-19), and *Cx. restuans* (18-20). Other surveillance programs have recorded low WNV infection rates in *Ae. albopictus* or *Ae. triseriatus*, which generally are not higher than *Cx. pipiens* and *Cx. restuans* infection rates (7, 8) and we recognize the significant role of *Cx. restuans* and *Cx. pipiens* in the maintenance and transmission of WNV. However, other mosquito species should not be dismissed. For example, the WV 2011 mosquito surveillance data showed that *Ae. albopictus* and *Ae. triseratus* had higher MIRs and MLEs than *Culex* spp. throughout the season. And, *Ae. albopictus* (not *Culex* spp.) was found at a human

case site in Wood County this year. *Aedes albopictus* is an opportunistic feeder capable of feeding on viremic birds, infected mammals, and susceptible humans (21, 22).

During the 2011 mosquito surveillance season, *Ae. albopictus* and *Ae. triseriatus* were found to be naturally infected with LAC. The role of *Ae. triseriatus* in LAC epidemiology has been well documented as studies have shown this mosquito species plays a role in the acquisition and transmission of LAC amongst the squirrel and chipmunk populations (23-25) and is capable of transovarial transmission, which maintains LAC in nature independent of mammalian reservoirs (26, 27). For *Ae. albopictus,* however, its role in LAC epidemiology is less clear. One study showed that human LAC case sites had significantly more *Ae. albopictus* than collecting sites without human disease incidence in eastern Tennessee (28). Additionally, the increase in human LAC infections in eastern Tennessee/western North Carolina coincided with the accidental introduction of the invasive *Ae. albopictus* to the area (29). In WV, mosquito surveillance data from 2005-2006 showed both *Ae. albopictus* and *Ae. triseriatus* at sites of human LAC cases and this season's data showed only one LAC positive pool of *Ae. albopictus* despite 44 being tested throughout the season. Also, counties with the highest *Ae. albopictus* burden did not show the highest LAC incidence. For example, during the 2011 season, Kanawha County had over four times the number of *Ae. albopictus* of Fayette County despite reporting no human LAC cases and Fayette county reported two human LAC cases. Conversely, Fayette County had a substantially greater *Ae. triseriatus* burden than Kanawha County. In addition, more *Ae. albopictus* were collected from Cabell County than any other locality but no human LAC cases were reported from this county.

To summarize the 2011 mosquito-borne surveillance data, a sharp increase in the number of human LAC cases was noted; the numbers of human WNV cases and travel-associated malaria cases were similar to surveillance data from previous years. The number of specimens from dead birds and horses continued to be low during 2011, making it difficult to garner much useful data from these surveillance methods. Mosquito surveillance data indicates that WNV continues to circulate in various mosquito species throughout the state despite reports of few human cases. A different composition of mosquito species testing positive for WNV was detected during 2011 as compared to previous years and questions remain on the role of *Aedes* spp. mosquitoes with regard to WNV epidemiology. The mosquito species testing positive for LAC in 2011 included *Ae. triseratus*, a known vector for LAC, and *Ae. albopictus*, who's role in LAC epidemiology remains less clear. Also during 2011, it should be noted that routine mosquito surveillance expanded to include more counties and provided additional surveillance data. However, an important limitation found with mosquito surveillance is that little to no correlation is noted between the location of WNV- and LAC- positive mosquito pools in relation to human cases. Despite this, mosquito surveillance remains an important tool for providing a better understanding of mosquito vector composition and the diseases they can carry, along with monitoring for invasive species.

Based on the 2011 mosquito-borne disease surveillance data the following recommendations can be made. Local health departments should target LAC educational messages to young children and their parents. Additionally, community campaigns to remove tires and clean-up trash-ridden areas may also be effective in helping to reduce breeding sites for LAC-carrying mosquitoes and reduce the number of LAC cases. Similarly, WNV educational messages should be targeted to elderly persons. For mosquito surveillance, more studies are needed on vector composition and mosquito infection rates near sites of

human cases. In addition, the effectiveness of continuing to test specimens from dead birds and horses will need to be evaluated in the coming years.

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