West Virginia Mosquito-Borne Disease Surveillance Report, 2012

I. Introduction

Mosquito-borne diseases, the majority of which are viruses, are transmitted through the bite of infected mosquitoes. Surveillance for these diseases in West Virginia generally focuses on four arboviruses: La Crosse encephalitis virus (LAC), West Nile virus (WNV), St. Louis encephalitis virus (SLE), and Eastern equine encephalitis virus (EEE). Historically, LAC has been the mosquito-borne disease of most concern in WV, with over 40 human cases previously reported in some years. However, during 2012, WNV activity increased throughout the United States causing widespread transmission with intense localized epidemics. WV also detected increased WNV activity as outlined later in this report. For SLE, human cases have not been detected in WV since the 1970s. EEE-infected birds were identified in WV in 2002 and this virus remains a disease of concern due to the high case fatality rate of this virus and its detection in states surrounding WV. Other mosquito-borne diseases, such as malaria and dengue virus, are not endemic to WV but a few travel-associated cases of these diseases are generally reported each year. Maintaining mosquito-borne disease surveillance in WV is also important in detecting novel diseases, such as chickungunya virus.

This surveillance report summarizes the human and non-human cases of mosquito-borne diseases detected in WV during 2012. 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 arbovirus infection. 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 2012, 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, including an environmental assessment of case sites. All reported human cases were classified according to the 2011 national case definition for each mosquito-borne disease (http://wwwn.cdc.gov/NNDSS/script/conditionsummary.aspx?CondID=17). Confirmed and probable arboviral cases were reported to CDC through ArboNet. Surveillance reports were sent bi-weekly to weekly to public health partners from June-November 2012 to provide data feedback on vectorborne

disease activity around the state. 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 MMWR Year 2012. Data were summarized using Microsoft Excel.

Mosquito surveillance

Active adult mosquito sampling occurred from May 23—Sept. 18, 2012. The state public health entomologist and four summer interns conducted regular, weekly mosquito trapping at three counties with high human incidence of LAC (Nicholas, Fayette, Raleigh) and three counties with low LAC human incidence (Kanawha, Wood, Roane) using CDC gravid traps and CO_2 traps. Semi-regular sampling with CDC gravid traps and CO_2 traps was conducted in Mercer, Harrison, Clay, and Putnam 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, retired scientists) around the state collected additional mosquito samples from Cabell, Wayne, Boone, Tyler, Brooke, Braxton, Webster, Jefferson, and Hardy counties. Collaborators sent collected mosquitoes to OLS for arboviral testing.

For testing, mosquitoes were pooled together based upon species, collecting locality, and collecting date with a maximum of 50 specimens per pool. *Culex restuans* and *Culex pipiens* were pooled together due to taxonomic difficulties in differentiating these species from field-collected specimens. 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® QIAmp 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 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 WNV, SLE, LAC, and EEE. *Culex* spp. 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. For more information about the methodology used to calculate MIR see http://www.cdc.gov/ncidod/dvbid/westnile/software.htm.

Active larval mosquito surveillance began May 23 and continued through Oct. 19, 2012. In collaboration with local health departments, larvae were collected during environmental assessments of LAC case sites and community mosquito control. Mosquito larvae were collected near the residence of recent LAC patients and at sites with no recent LAC activity in the human population. Artificial containers (tires, buckets, children wading pools, pans, rain barrels) and natural containers (tree holes) were examined for mosquito larvae. Mosquitoes were differentiated to species in the laboratory. *Aedes japonicus* larvae were retained in cold storage for future studies on transovarian transmission of LACV. No testing was performed on larval specimens.

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 for any equine specimens testing 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 for any dead bird specimens testing positive for an arbovirus.

III. Results

Human Surveillance

Table 1 provides a comparison of human cases of mosquito-borne diseases reported in WV during 2010-2012. During 2012, 14 cases (8 confirmed, 6 probable) of LAC were reported; this represents a 42% decrease from the 24 cases reported in 2011. Eight cases (57%) were male. The mean age of cases was 9 years (range 4-25 years); 13 (93%) cases were <15 years old. Month of illness onset for LAC cases ranged from June 2012 to September 2012. However, 11 (79%) cases had illness onset in July 2012 while no cases had illness onset in August 2012. LAC cases were reported from 9 counties: Boone, Clay, Fayette, Kanawha, Mercer, Monongalia, Nicholas, Raleigh, and Wyoming. Figure 1 shows the geographic distribution of human mosquito-borne disease cases in 2012.

Nine cases of WNV infection were reported during 2012; this represents almost a 5-fold increase in reported WNV cases as compared to 2011. Seven cases (78%) were male. The mean age of cases was 53 years (range 5-87 years); 4 (44%) cases were ≥65 years old. Month of illness onset for cases occurred from July 2012 through September 2012; six (67%) cases had illness onset in August 2012. Cases were reported from six counties (Fig 1.). Five (55%) cases had neuroinvasive disease; the incidence of neuroinvasive WNV cases in WV during 2012 was 0.3 per 100,000 population. The national incidence for neuorinvasive WNV cases during 2012 was 0.9 per 100,000 population. Table 2 shows a comparison of the descriptive epidemiology among human LAC and WNV cases from 2012. In addition to these WNV cases, one presumptive viremic blood donor (PVD) was also reported from Kanawha 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.

Two travel-associated cases of malaria were reported among WV residents from Cabell and Hardy counties. Illness onsets occurred in February 2012 and March 2012. One (50%) case was female. Cases ranged in age from 19 to 35 years. Travel history for malaria cases included Ethiopia and Malaysia. Neither case reported taking malaria chemoprophylaxis.

No human cases of SLE, EEE, or travel-associated dengue virus were reported during 2012.

Table 1. Summary of Mosquito-Borne Disease Human Cases – West Virginia, 2010-2012

Mosquito-Borne Disease	No. (%) of Human Cases† - 2010	No. (%) of Human Cases† – 2011	No. (%) of Human Cases†- 2012
La Crosse encephalitis virus	8 (62)	26 (74)	14 (56)
West Nile virus*	0 (0)	2* (6)	9* (36)
Malaria	3 (23)	7 (20)	2 (8)
Dengue virus	2 (15)	0 (0)	0 (0)
Eastern equine encephalitis virus	0 (0)	0 (0)	0 (0)
St. Louis encephalitis virus	0 (0)	0 (0)	0 (0)
Total	13 (100)	35 (100)	25 (100)

***Presumptive viremic blood donor not included in case count for West Nile virus**
†Includes only cases classified as confirmed or probable

Table 2. Comparison of descriptive epidemiology among human LAC and WNV cases – West Virginia, 2012

Demographic Information	LAC Cases	WNV Cases
Age (years)		
Mean	9	53
Range	4-25	5-87
Sex distribution (%)		
Males	57	78

Figure 1. Reported month of illness onset among human LAC and WNV cases – West Virginia, 2012

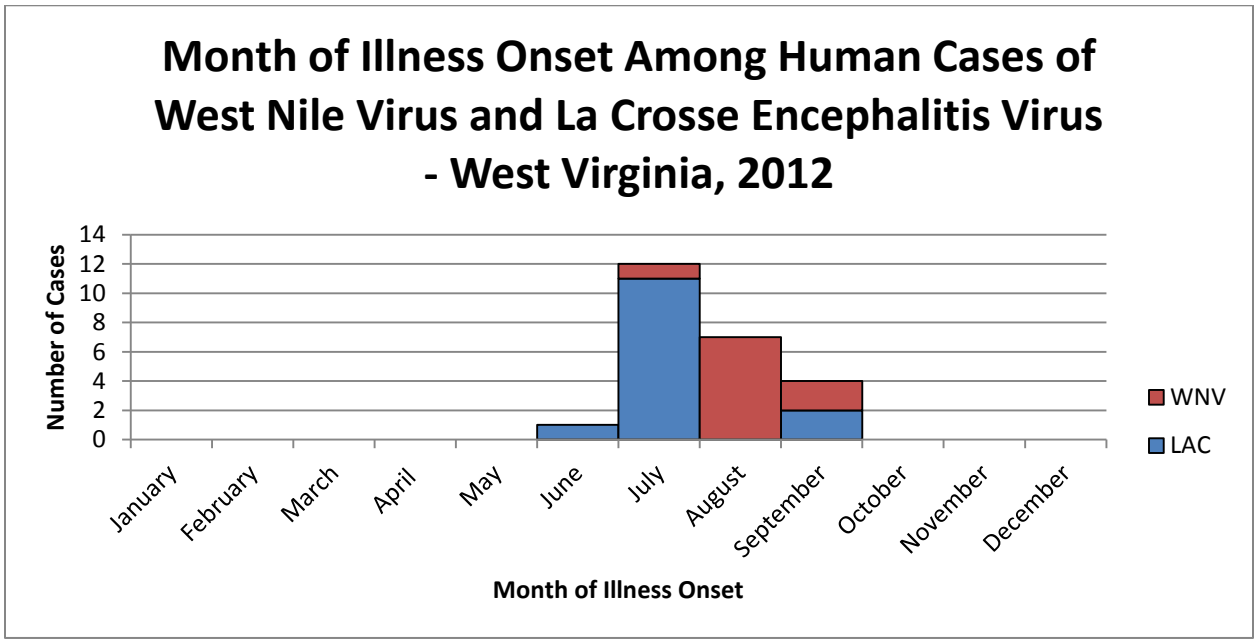
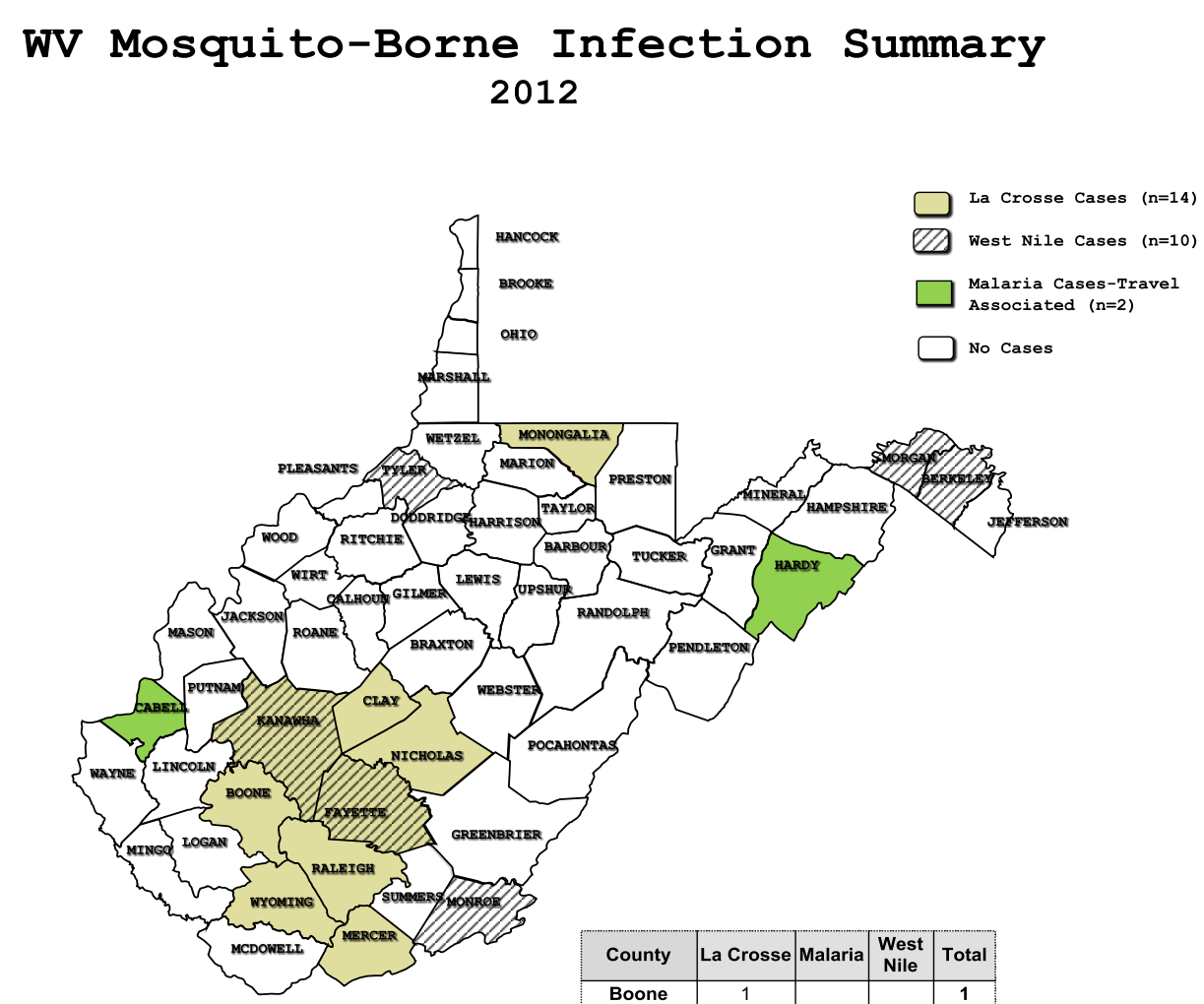


Figure 2. Human Cases of Mosquito-Borne Diseases — West Virginia, 2012



County	La Crosse	Malaria	West Nile	Total
Boone	1			1
Berkeley			1	1
Cabell		1		1
Clay	3			3
Fayette	1		1	2
Hardy		1		1
Kanawha	1		5	6
Mercer	2			2
Monongalia	1			1
Monroe			1	1
Morgan			1	1
Nicholas	2			2
Raleigh	1			1
Tyler			1	1
Wyoming	2			2
Total	14	2	10	26

Mosquito Surveillance

A total of 12,864 mosquitoes from 760 mosquito pools were collected and tested for arboviruses. The following mosquito species were identified: 8,834 *Culex pipiens/restuans* specimens (68.7%), 1,487 *Aedes albopictus* (11.6%), 1,373 *Culex erraticus* (10.7%), 489 *Aedes japonicus* (3.8%), 194 *Aedes*

trivittatus (1.5%), 117 *Aedes vexans* (0.9%), 95 *Aedes triseriatus* (0.7%), 86 *Anopheles punctipennis* (0.7%), 79 *Psorophora ferox* (0.6%), 70 *Coquillettidia perturbans* (0.5%), 13 *Anopheles quadrimaculatus* (0.1%), 9 *Aedes* spp. (0.1%), 5 *Anopheles crucians*, 3 *Orthopodomyia signifera*, 3 *Aedes canadensis*, 2 *Uranotaenia sappharina*, 2 *Psorophora* spp., 1 *Aedes cinereus*, and 1 *Anopheles walkeri*. Due to the high species diversity, a large proportion of the pools were non-*Culex* species: 300 pools of *Cx. pipiens/restuans* (39.5%), 137 pools of *Ae. albopictus* (18.0%), 89 pools of *Ae. japonicus* (11.7%), 48 pools of *An. punctipennis* (6.3%), 40 pools of *Cx. erraticus* (5.3%), 37 pools of *Ae. vexans* (4.9%), 34 pools of *Ae. triseriatus* (4.5%), 23 pools of *Ae. trivittatus* (3.0%), 16 pools of *Cq. perturbans* (2.1%), 14 pools of *P. ferox* (1.8%), 5 pools of *An. quadrimaculatus* (0.7%), 5 pools of *Aedes* spp. (0.7%), 3 pools of *An. crucians* (0.4%), 2 pools of *O. signifera* (0.3%), 2 pools of *Psorophora* spp. (0.3%), 1 pool of *Ae. canadensis* (0.1%), 1 pool of *U. sappharina* (0.1%), 1 pool of *Ae. cinereus* (0.1%), and 1 pool of *An. walkeri* (0.1%). Of the 760 mosquito pools tested, 281 (37%) were positive for WNV. LAC, SLE, and EEE were not recovered from any mosquito pools.

Ninety-two (32.7%) of the 281 WNV-positive mosquito pools were *Ae. albopictus* and 55 (19.6%) were *Ae. japonicus*. Only 38 (13.5%) of the WNV positive pools were *Cx. pipiens/restuans*. *Cx. pipiens/restuans* showed a lower MIR (MIR=4.30; 95% C.I.=2.94-5.67) than *Ae. albopictus* (MIR=61.87; 95% C.I.=49.62-74.11), *Ae. japonicus* (MIR=112.47; 95% C.I.= 84.47-140.48), and *Ae. triseriatus* (MIR=168.42;95% C.I.=93.17-243.68) (Table 3). *Culex erraticus* also showed a lower MIR (MIR=2.91; 95% C.I.=0.06-5.76) than *Ae. albopictus*, *Ae. japonicus*, and *Ae. triseriatus* (Table 1). A higher percentage of *Ae. albopictus*, *Ae. japonicus*, and *Ae. triseriatus* pools tested positive for WNV as compared to *Culex* spp. pools (*Cx. pipiens/restuans*, *Cx. erraticus*) (Fig. 2).

Positive WNV pools were found in 18 of the 19 counties where mosquito surveillance occurred; infected mosquitoes were not recovered from Tyler County. The first WNV-positive pools for 2012 were *Ae. albopictus* reared from larvae collected in Cabell County on June 2. The first adult WNV infected mosquitoes were *Ae. japonicus* found in Kanawha County on June 12 and Webster County on June 13. The last WNV-positive mosquito pools were *Ae. albopictus*, *Cx. pipiens/restuans*, and *An. punctipennis* from Cabell County on September 18.

Cx. pipiens/restuans, the proposed primary WNV vector in the eastern United States (Turell et al. 2001; Turell et al. 2005), showed the first MIR >5 in late June 2012 (Fig. 3) . The MIR for *Cx. pipiens/restuans* peaked the week of July 16, when the first human cases of WNV had illness onsets. *Cx. pipiens/restuans* had an earlier occurrence of high infection rates (MIR > 5 per 1000 mosquitoes) in 2012 than the previous four years of low WNV activity (Figs. 4).

Table 3. West Nile virus minimum infection rate (MIR) by mosquito species during 2012 surveillance season – West Virginia.

Mosquito Species	MIR per 1,000 mosquitoes	MIR Lower Limits	MIR Upper Limits	No. of Pools	No. of Positive Pools	Total No. of Mosquitoes
Psorophora spp.	1000.00	1000.00	1000.00	2	2	2
Anopheles walkeri	1000.00	1000.00	1000.00	1	1	1
Uranotaenia sappharina	500.00	0.00	1192.95	1	1	2
Aedes spp.	333.33	25.35	641.31	5	3	9
Orthopodomyia signifera	333.33	0.00	866.77	2	1	3
Anopheles punctipennis	313.95	215.87	412.04	48	27	86
Anopheles quadrimaculatus	230.77	1.74	459.80	5	3	13
Anopheles crucians	200.00	0.00	550.61	3	1	5
Aedes triseriatus	168.42	93.17	243.68	34	16	95
Coquillettidia perturbans	128.57	50.16	206.98	16	9	70
Aedes vexans	119.66	60.85	178.47	37	14	117
Aedes japonicus	112.47	84.47	140.48	89	55	489
Psorophora ferox	88.61	25.94	151.27	14	7	79
Aedes albopictus	61.87	49.62	74.11	137	92	1487
Aedes trivittatus	36.08	9.84	62.33	23	7	194
Culex pipiens/restuans	4.30	2.94	5.67	300	38	8834
Culex erraticus	2.91	0.06	5.76	40	4	1373
Aedes canadensis	0.00	#N/A	#N/A	1	0	3
Aedes cinereus	0.00	#N/A	#N/A	1	0	1

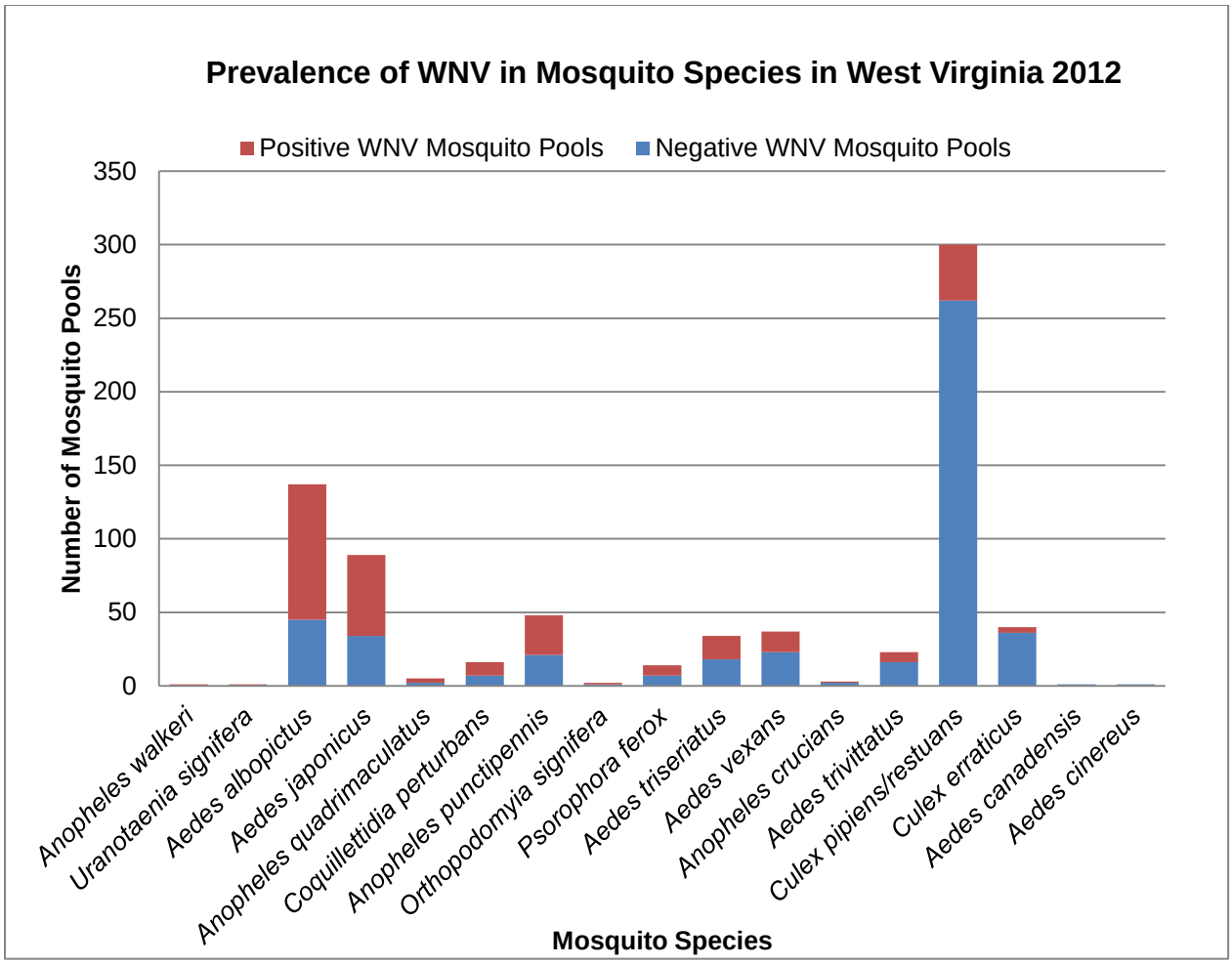


Fig. 2. Prevalence of WNV in mosquito species in West Virginia during the 2012 surveillance season. Species are arranged from species with high percentage of mosquito pools WNV positive (ex. *Anopheles walkeri*) to species showing no WNV infection (*ex. Ae. cinereus*).

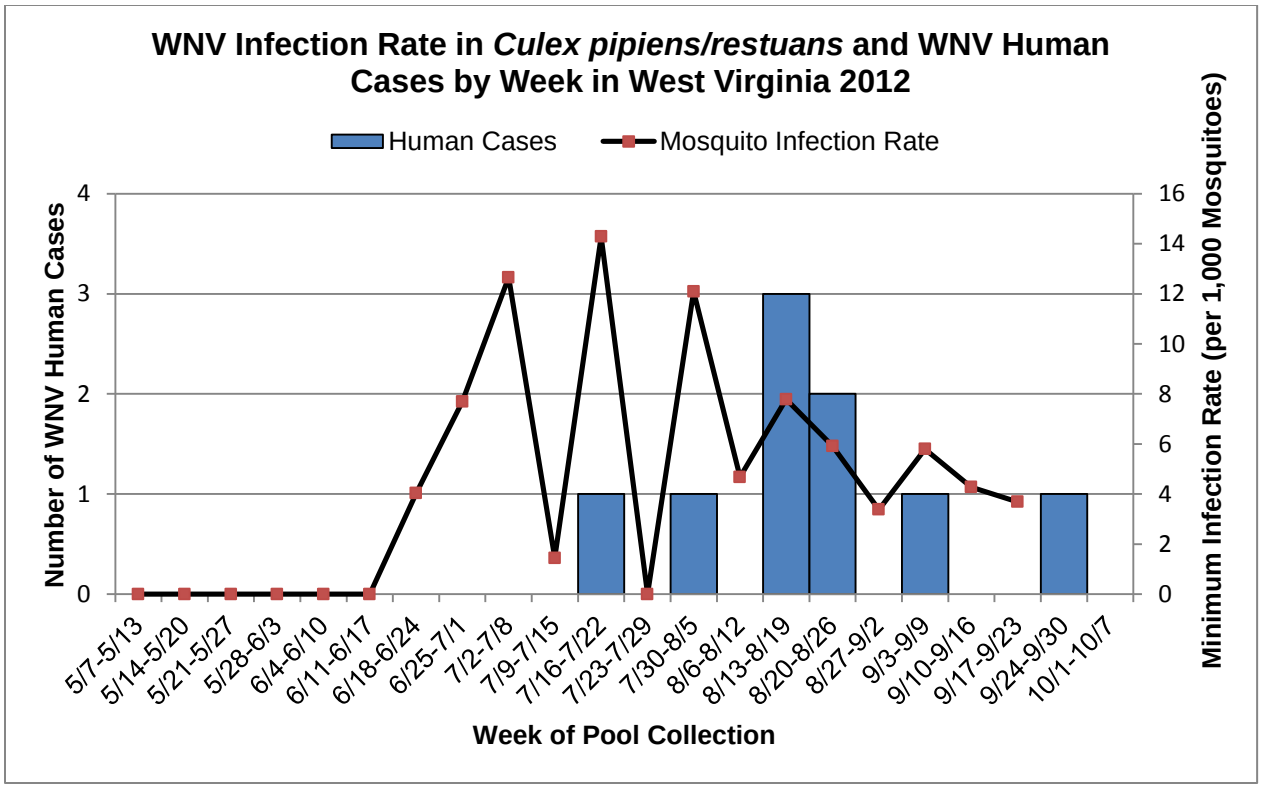


Fig. 3. WNV infection rate in *Culex pipiens/restuans* and WNV human cases by calendar week in West Virginia 2012.

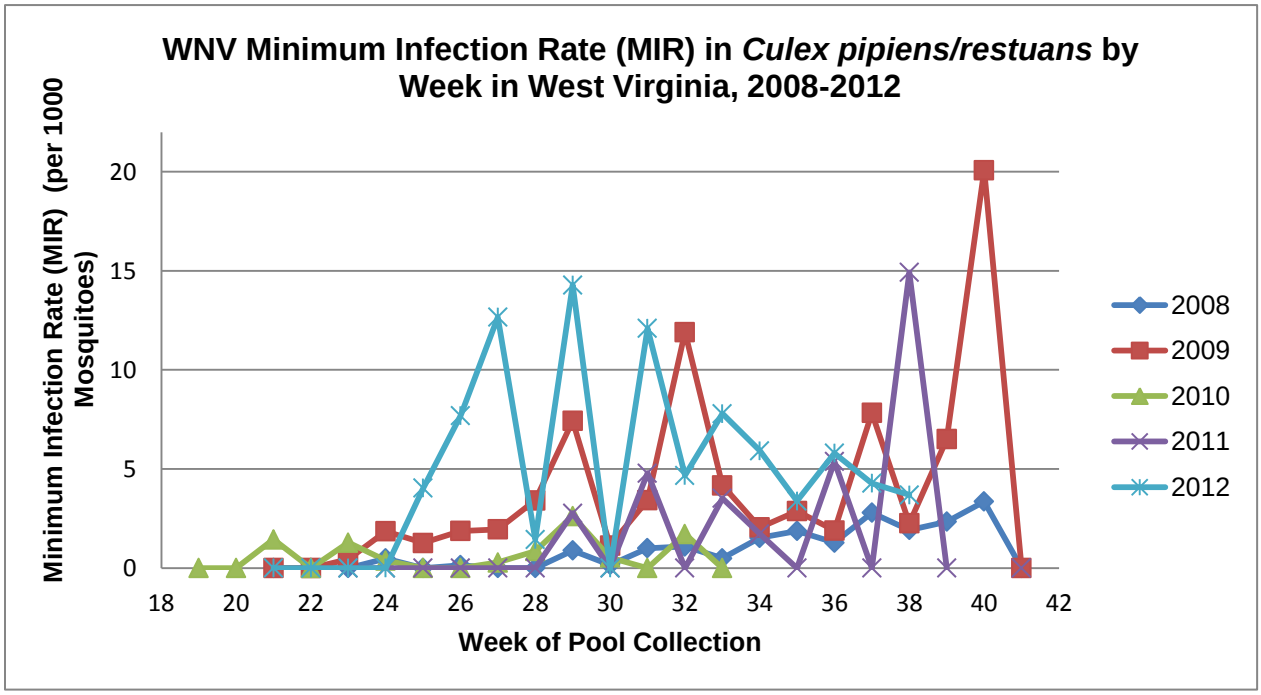


Fig. 4. WNV minimum infection rate (MIR) in *Culex pipiens/restuans* by week in West Virginia, 2008-2012.

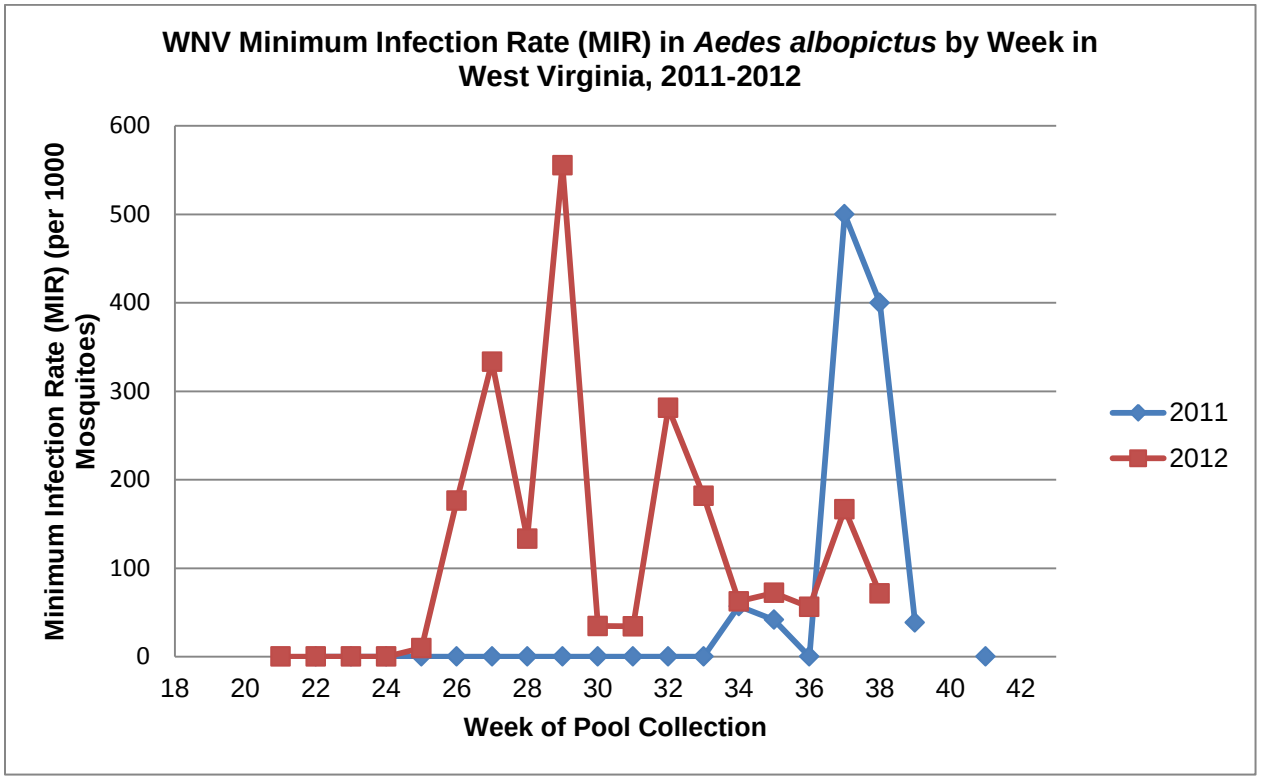


Fig. 5. WNV minimum infection rate (MIR) in *Aedes albopictus* in West Virginia, 2011-2012.

Cx. pipiens/restuans showed significantly higher WNV infection in 2012 (MIR=4.30; 95% C.I.=2.94-5.67) than in 2011 (MIR=1.61; 95% C.I.=0.32-2.90). Even with the wide variation in WNV infection rates throughout the state, *Cx. pipiens/restuans* showed higher WNV infection during weeks 29 (middle of July) and 33 (early August) in 2012 than in 2011. WNV infection rates in adult *Cx. pipiens/restuans* active from the middle of August through September were similar between 2011 and 2012. Although there was higher infection among *Cx. pipiens/restuans* in 2012, the population of *Cx. pipiens/restuans* was half the size it was during 2011.

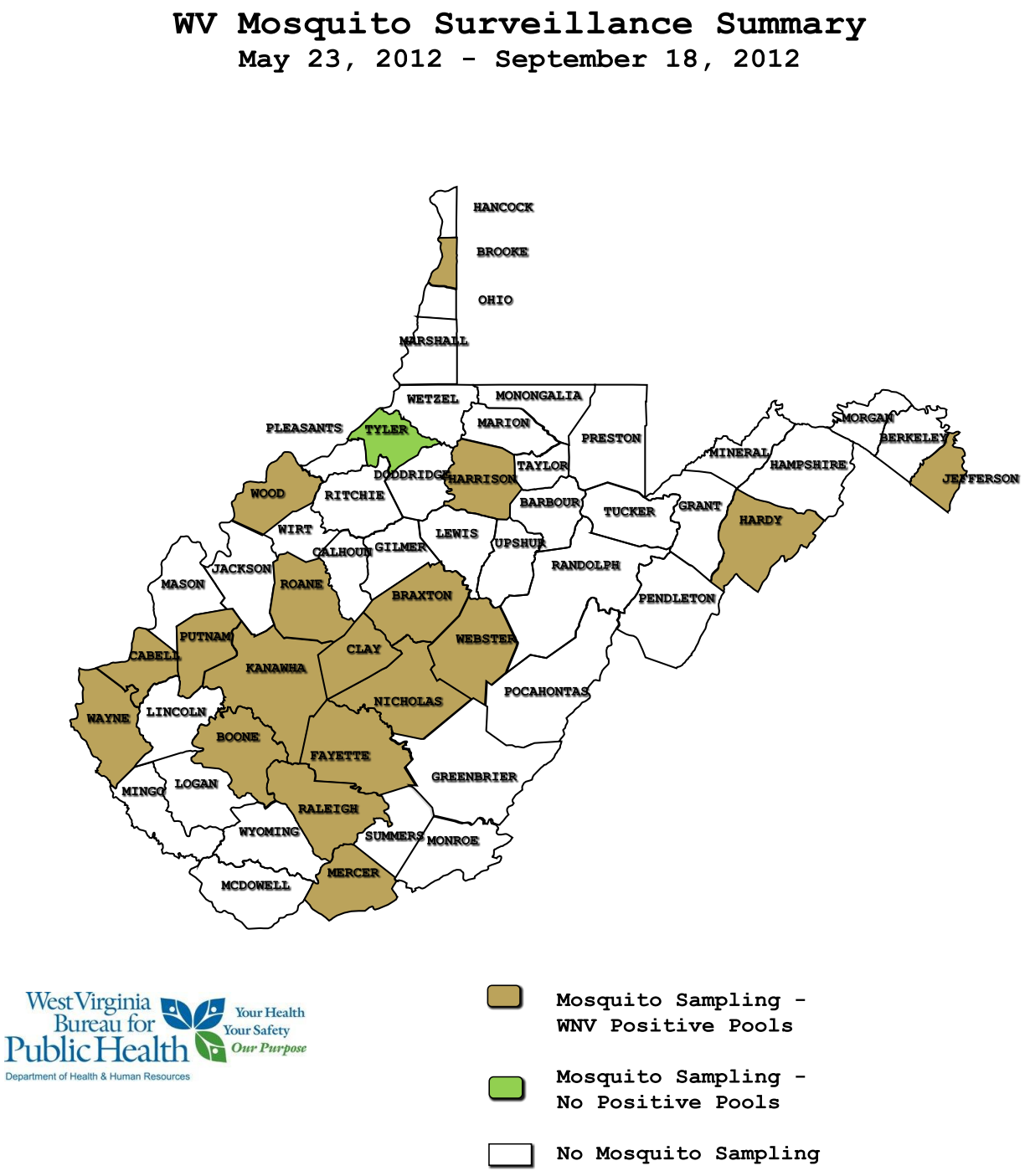
Aedes albopictus, a competent vector for WNV (Turell et al. 2001; Turell et al. 2005), followed similar trends as *Cx. pipiens/restuans* (Fig. 5). *Aedes albopictus* showed significantly higher infection in 2012 (MIR=61.87; 95% C.I.=49.62-74.11) than in 2011 (MIR=19.11; 95% C.I.=6.74-31.47). *Aedes albopictus* had higher WNV infection throughout July (weeks 27-32) and early August (week 33). Infection rates in *Ae. albopictus* active from the middle of August through September were comparable between 2011 and 2012.

Based on data collected during environmental assessments of 2012 LAC case sites, *Ae. triseriatus* was found in 5 of 23 mosquito breeding containers near case sites, *Ae. albopictus* was found in 7 of 23 mosquito breeding containers near case sites, and *Ae. japonicus* was found in 17 of 23 mosquito breeding containers near case sites. When compared to areas without human LAC activity, the

distribution of LAC-competent vectors was similar; 12 of 56 mosquito breeding containers had *Ae. triseriatus*, 17 of 56 mosquito breeding containers had *Ae. albopictus*, and 42 of 56 mosquito breeding containers had *Ae. japonicus*.

Anopheles crucians was discovered for the first time in WV this year. *Anopheles crucians* was first recovered on Sept. 5-6 in an urban environment in Wood County using a CO_2 trap. A second *An. crucians* was collected on Sept. 6-7 in wetland habitat in Nicholas County using the same collecting method. The specimen from Nicholas County was positive for WNV.

Figure 6. Counties with mosquito sampling and positive mosquito pools — West Virginia, 2012



Horse Surveillance

One horse specimen from Nicholas County was submitted for testing during 2012. This specimen tested negative for WNV and EEE.

Dead Bird Surveillance

Eighteen dead bird specimens were submitted for testing during 2012. Specimens were submitted between May 2012 and October 2012 from nine counties: Hancock, Jackson, Jefferson, Kanawha, Marshall, Nicholas, Pendleton, Wirt, and Wyoming. Bird species submitted included robin, sparrow, crow, blue jay, veery, and yellow-bellied sapsucker (woodpecker). One specimen, a blue jay found in September 2012 from Wirt county, tested positive for WNV. No specimens tested positive for SLE or EEE.

IV. Discussion

LAC continues to be the mosquito-borne disease of most concern in West Virginia; however 2012 proved to be a record year for the number of human WNV cases. For LAC, a decrease in the number of human cases was noted in 2012 as compared to 2011, and no mosquito pools tested positive for this virus. Analysis of human LAC cases verified epidemiologic trends that have been noted previously in WV, particularly children <15 years of age continue to be the age group at highest risk for infection and most cases occur in the south-central portion of the state.

Among human WNV cases, adults (particularly those aged ≥65 years) appear to be at highest risk of infection. The geographic distribution of human WNV cases varied from the northern and eastern panhandles to the south-central part of the state. Kanawha county had the highest number of WNV cases, which may be attributable to the higher human population in this area.

Two travel-associated cases of malaria were reported among WV residents in 2012. Neither case reported taking malaria chemoprophylaxis despite traveling to malaria-endemic areas (Ethiopia and Malaysia). Travelers should educate themselves about possible disease risks before leaving the United States. 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.

Among mosquitoes, early, seasonal peaks in WNV infection were detected and likely influenced the increased number of WNV human cases. The onset date of the first WNV human case was July 19, two weeks after the initial peak of WNV activity in adult *Cx. pipiens/restuans* and within the 2-14 day WNV incubation period in human patients (American Academy of Pediatrics, 2012). Human cases continued during the summer and early fall when infected adult mosquitoes were active. Other possible explanations for the increase in human WNV cases seem unlikely given the 2012 mosquito data. For example, earlier emergence of WNV in the mosquito population and high mosquito abundance were not documented in 2012 as compared to previous years. During the 2012 season, the first WNV-positive adult mosquitoes (*Ae. japonicus*) were active on June 12. In both 2011 and 2010, the first WNV-positive

mosquitoes were also detected in early June. Also during 2012, a decrease in mosquito numbers was noted; comparing mosquito numbers to 2011, there were twice as many *Cx. pipiens/restuans* and *Ae. japonicus* in Fayette County during 2011 than in 2012.

High temperatures during 2012 could have been responsible for the high infection rate in mosquitoes and this has previously been associated with increases in WNV activity (Soverow et al. 2009; Chuang & Wimberly 2012). Higher environmental temperatures caused an increase in WNV infection among the *Cx. pipiens*/*restuans* population in northeast Illinois during 2005 and 2006 (Ruiz et al. 2010) and *Culex* spp. in Los Angeles County in 2007 (Liu & Weng 2012) and resulted in higher human incidence of WNV in both places. Laboratory studies have also shown the percentage of *Culex* spp. mosquitoes with infection, dissemination of virus throughout the body cavity, and capacity to transmit through the salivary glands increases as a function of temperature increase (Dohm, Guinn & Turrell 2002; Richards et al. 2007; Kilpatrick et al. 2008) and the accumulation of high temperatures over time (Reisen, Fang & Martinz 2006). According to the National Climatic Data Center (2013), the annual temperature in WV during 2012 was much higher than normal and 2012 had the highest annual ranked temperature over the past decade.

Although *Cx. pipiens* plays a significant role in WNV epidemiology, other mosquito species may be involved with the transmission cycle. All of the mosquito species that tested positive for WNV during 2012 are known to be susceptible to WNV in nature (Centers for Disease Control & Prevention 2009; Kutz, Wade & Pagac 2003; Cupp et al. 2007; Kulasekera et al. 2001; Bernard et al. 2001; Andreadis, Anderson & Vossbrinck 2001; Nasci et al. 2001; Farajollah et al. 2005). Species susceptible to WNV infection in nature and capable of transmitting WNV to humans include *Ae. albopictus* (Turell et al. 2001), *Ae. japonicus* (Turrell et al. 2001; Sardelis & Turrell 2001), and *Ae. triseriatus* (Turell et al. 2005; Erickson et al. 2006; Platt et al. 2007). Although other surveillance programs have recorded lower WNV infection rates in *Ae. japonicus* and *Ae. triseriatus* than *Culex* spp. (Kulasekera et al. 2001; Bernard et al. 2001), these other mosquito species should not be dismissed. For example, the 2012 WV mosquito surveillance data showed that *Ae. albopictus*, *Ae. japonicus*, and *Ae. triseriatus* had higher MIRs than *Culex pipiens*/*restuans* and *Cx. erraticus*. Also, monthly human WNV cases were highly correlated to infection rates in *Ae. albopictus* (and *Cx. pipiens quinquefasciatus*) in Harris County, Texas from 2000-2006 (Dennett et al. 2007).

Based on the 2012 mosquito surveillance data, high infection rates in *Cx. pipiens/restuans* (MIR > 5 per 1,000 mosquitoes) sustained over two weeks may be indicative of future WNV human outbreaks in WV. This phenomenon has been noted in other places including New York where an MIR > 5 per 1,000 *Cx. pipiens* resulted in high human incidence of WNV in 2000 (Kulasekera et al. 2001; Bernard et al. 2001). In California, mosquito control programs implement epidemic emergency responses if the WNV MIR > 5 per 1,000 *Culex* spp. (California 2005). Infection among other local mosquito species also demonstrates infection in the community; however, there is not enough historical data to relate other mosquito species infection rate to future human disease incidence. For surveillance and future management purposes, infection rates in other mosquito species may prove more useful than *Culex* spp. because some mosquito species develop infection earlier in the season in WV. However, for 2013, an MIR of >5 per 1,000 mosquitoes in Culex spp. will be used as an indicator for implementing stronger control

measures and will be outlined in the 2013 WV Mosquito Surveillance Plan (see http://www.dhhr.wv.gov/oeps/disease/Zoonosis/Mosquito/Documents/Mosquito-Surveillance-Plan.pdf) .

It is not completely understood what has been responsible for the high human incidence of LAC in West Virginia during the past 25 years. The role of the native mosquito vector, *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 (Wright & DeFoliart 1970; Pantuwatana et al. 1972; Watts et al. 1972) and is capable of transovarial transmission, which maintains LAC in nature independent of mammalian reservoirs (Watts et al. 1973; Hughes et al. 2006). For the other two invasive mosquito species (*Ae. albopictus*, *Ae. japonicus*), however, their role in LAC epidemiology in West Virginia is less clear. LAC has been isolated from *Ae. albopictus* active in Tennessee during 1999 (Gerhardt et al. 2001), Texas during 2009 (Lambert et al. 2010) and last year in West Virginia. In eastern Tennessee, human case sites had significantly more *Ae. albopictus* than collecting sites without human disease incidence (Erwin et al. 2002) and *Ae. albopictus* was more prevalent than *Ae. triseriatus* at LAC confirmed case sites (Haddow et al. 2009). The situation with *Ae. albopictus* and LAC human incidence is not the same in West Virginia. In West Virginia during 2012, counties with the highest *Ae. albopictus* burden did not show the highest LAC incidence and *Ae. triseriatus* was equally evident at sites near LAC human incidence and sites without disease. *Ae. japonicus* has been shown to acquire and transmit LAC under laboratory conditions (Sardelis, Turell & Andre 2002); however, LAC has not been isolated from field-collected mosquitoes. In West Virginia during 2012, counties with the highest *Ae. japonicus* burden did not show the highest LAC incidence and there was no relationship between the presence of *Ae. japonicus* at a collecting locality and incidence of LAC.

The discovery of *An. crucians* in West Virginia is not surprising as it in states bordering WV (Darsie & Ward 2005). Finding *An. crucians* in WV demonstrates another value to mosquito monitoring programs. Mosquito surveillance could uncover new human disease-transmitting mosquitoes previously unrecorded in WV but established in surrounding states (ex. *Culiseta melanura*). Also, mosquito monitoring programs can discover exotic species capable of transmitting non-endemic diseases (ex. *Aedes aegypti*, a competent vector for dengue fever and chikungunya). Invasive exotic species may be managed if the localized sources of mosquito infestation are properly managed.

The low number of horse specimens did not add useful information to the surveillance of mosquito-borne diseases during 2012. The number of dead bird specimens, however, increased from the past few years and provided a good representation of birds from around the state. One WNV-positive bird was identified. This is the first WNV-positive bird detected in WV since 2009 and indicates that increased surveillance among dead birds may provide supplemental information related to WNV activity. The positive bird from 2012 was found in a county where no other environmental surveillance had been conducted and thus, provided useful information that there was WNV activity in that county.

Based on the 2012 mosquito-borne disease surveillance data, the following recommendations can be made. LAC educational campaigns should be targeted to young children and their parents. A new LAC

pamphlet has been drafted for 2013 and is available for use (http://www.dhhr.wv.gov/oeps/disease/Zoonosis/Mosquito/Documents/arbovirus/lacrosseencephalitis/La-Crosse-Encephalitis-Pamphlet.pdf). 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, work should continue in managing data during the season such that timely surveillance feedback can be provided in instances of increased mosquito arboviral activity. Although, horse and dead bird surveillance are still recommended by CDC, WV should continue to evaluate methods for improving these aspects of mosquito-borne disease surveillance. New national guidelines for WNV surveillance may be released during 2013 and WV should use these guidelines to assess current surveillance activities, once available.

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