Journal of Medical Entomology, XX(XX), 2023, 1–18
https://doi.org/10.1093/jme/tjad090
Forum

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La Crosse virus neuroinvasive disease: the kids are not alright

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Subject Editor: Ary Faraji

Received on 23 March 2023; revised on 4 May 2023; accepted on 26 June 2023

La Crosse virus (LACV) is the most common cause of neuroinvasive mosquito-borne disease in children within the United States. Despite more than 50 years of recognized endemicity in the United States, the true burden of LACV disease is grossly underappreciated, and there remain severe knowledge gaps that inhibit public health interventions to reduce morbidity and mortality. Long-standing deficiencies in disease surveillance, clinical diagnostics and therapeutics, actionable entomologic and environmental risk indices, case response capacity, public awareness, and availability of community support groups clearly frame LACV disease as neglected. Here we synthesize salient prior research and contextualize our findings as an assessment of current gaps and opportunities to develop a framework to prevent, detect, and respond to LACV disease. The persistent burdens of LACV disease clearly require renewed public health attention, policy, and action.

Key words: California serogroup, *Aedes triseriatus*, arbovirus, review

Introduction

La Crosse virus (LACV), a member of the California encephalitis serogroup (CAL) in the family *Peribunyaviridae* and genus *Orthobunyavirus*, has long been an important cause of arboviral neuroinvasive disease in the United States. The first isolation of LACV was from a 4-year-old child who died of meningoencephalitis in La Crosse, Wisconsin, in 1960 (Thompson et al. 1965). Following that initial isolation, LACV was recognized as the leading cause of arboviral encephalitis in the United States (Rust et al. 1999). Because of its public health importance, scientists rapidly produced research on LACV and other CAL viruses, leading to an *International Symposium on California Serogroup Viruses* in 1982 to synthesize the findings and provide future recommendations (Calisher and Thompson 1983). A key take away from that symposium was that despite the ample research on various aspects of CAL viruses, there was still a need for applied research that would produce clear public health guidelines for the control and prevention of LACV infections (Reeves et al. 1983). Similarly, the 1993 Centers for Disease Control and Prevention's *Guidelines for Arbovirus Surveillance Programs in the United States* posed several questions (Box 1) to address knowledge gaps for prediction, prevention, and control that remain unanswered (Moore et al. 1993). It speaks to the challenges of vector-borne disease prevention and shifting national public health priorities that today, LACV remains the leading cause of arboviral encephalitis among children in the United States with no well-defined strategy for prevention, detection, or control (Calisher and Thompson 1983, Gaensbauer et al. 2014, Vahey et al. 2021).

La Crosse virus neuroinvasive disease (LACV-ND) is no longer treated as a national priority, as most funding for arboviral research and surveillance focuses on responding to threats from exotic pathogens like West Nile, chikungunya, and Zika viruses and emerging tick-borne viruses (Dye-Braumuller et al. 2022). This scenario creates a harmful feedback loop where LACV-ND may be considered too small a problem to warrant political attention or public health funding, which in turn prevents public health officials from accurately measuring the true burden of disease. Like most vector-borne diseases, LACV infections are grossly underdiagnosed and underreported. Non-neuroinvasive LACV infections are not reportable in most states, and accurate diagnosis and reporting of LACV-ND require clinical awareness and diagnostic persistence to meet the criteria of the case definition. Clinicians in areas with little or no reported LACV-ND are unlikely to consider it as a cause of neuroinvasive disease, and consequently, there are dubious regional and political boundaries in the distribution of LACV-ND. For example, eastern Kentucky rarely reports LACV-ND despite having similar environmental characteristics to neighboring high-risk

Box 1. Persistent questions

Persistent Questions: Questions posed in 1993 that could "improve our understanding of and our ability to predict, prevent, or control epidemic transmission of La Crosse and other California serogroup viruses" (Moore et al. 1993):

- What are the most reliable predictors for human risk?
- What is the influence of rainfall and temperature on *Aedes triseriatus* population density and the amplification of La Crosse virus in a woodlot focus?
- What is the relationship between mosquito population density, vertebrate host density, and La Crosse virus amplification?
- Do the relative densities of amplification hosts and nonamplifier (i.e., large mammals such as deer) influence the status of La Crosse virus in a wooded area?
- What is the potential for *Aedes albopictus* to become involved in the transmission of La Crosse virus?
- What is the geographic distribution of La Crosse, Jamestown Canyon, and other California serogroup viruses in the United States?

Emerging Questions: Additional questions that could improve our abilities to reduce La Crosse virus infections in the Appalachian region.

- How does the abundance of accessory vectors influence the maintenance of La Crosse virus in high-risk areas?
- What mitigatable factors are associated with the long-term focal persistence of La Crosse virus?
- What is the necessary scale of mosquito control interventions to reduce human infections in an area with known transmission?
- How do competitive interactions among *Ae. triseriatus*, *Ae. albopictus*, and *Ae. j. japonicus* augment La Crosse virus transmission risk?
- What is the true burden of human infections in the Appalachian region, and what is the prevalence of unreported infections?
- What are the social and economic impacts of La Crosse virus infections on communities in the Appalachian region?

foci in West Virginia and eastern Tennessee (i.e., the Appalachian Mountains).

The geographic distribution of reported LACV-ND is driven by clinical and community awareness. Historically, most LACV-ND was diagnosed in children residing in midwestern states ranging from Wisconsin to Ohio, but in the 1990s, LACV-ND emerged in Appalachian regions of the southeastern United States (Kappus et al. 1983, Jones et al. 1999). The emergence of LACV-ND in southern Appalachia is often wrongly contextualized as a geographic "spread" of LACV coincident with the introductions of transmission-competent invasive mosquito species (*Aedes albopictus* and *Aedes japonicus japonicus*). However, there was evidence of LACV-ND in western North Carolina (NC) as early as 1964, and 14 cases were reported in western NC from 1964 to 1981 (Kelsey and Smith 1978, Kappus et al. 1983). Thus the "emergence" of LACV disease in Appalachia may be more accurately described as delayed recognition of an endemic problem partly because of the concurrent modernization and standardization of case reporting—the National Electronic Telecommunication System for Surveillance was launched in 1990 alongside new uniform reporting criteria (i.e., case definitions) for arboviral encephalitis from the CDC in collaboration with the Council for State and Territorial Epidemiologists, which contributed to improved reporting of arboviral diseases (Wharton et al. 1990).

Recent decades have seen remarkable consistency in the reported LACV-ND distribution, with a few foci in Ohio, West Virginia, North Carolina, and Tennessee regularly reporting the vast majority of cases (Haddow and Odoi 2009, Gaensbauer et al. 2014, Vahey et al. 2021, Day et al. 2023). Most high-incidence counties are in the Appalachian region, a cultural region with socioeconomically disadvantaged communities that often face severe health disparities relative to the general US population (Haddow and Odoi 2009, Marshall et al. 2017, Day et al. 2023). Many of the communities with consistent LACV-ND risk are predominately rural and lack sufficient public programs for vector-borne disease prevention. Although the distribution of reported cases is persistent and predictable, there is little momentum towards organized public health intervention as LACV-ND continuously affects people—primarily children—in a few small parts of Appalachia with no assurance of effective interventions.

After more than 60 years of recognized endemicity, the true burden of LACV infections and disease remains grossly underappreciated, and there remain severe knowledge gaps that inhibit effective public health interventions. In this forum article, we broadly review the existing body of LACV research in the context of the epidemiologic triad (i.e., "disease triangle") (Fig. 1), highlight key applied knowledge gaps, describe our perspectives on actionable research priorities, and present a vision for prevention, detection, and response of LACV-ND.

LACV Epidemiology: Environmental Risk Factors, Pathogen Dynamics, and Hosts

Transmission Biology and Disease Burden

The biology of LACV transmission is complex (Fig. 2). Native and invasive mosquitoes are known to transmit the virus to amplifying and incidental vertebrate hosts, including humans. Maintenance within vector populations occurs through vertical (i.e., transovarial) and horizontal (i.e., amplification in sciurid hosts and venereal transmission from male to uninfected female mosquito) mechanisms. The virus is genetically heterogeneous with 3 primary lineages found throughout eastern North America, and there appear to be persistent regional genotypes with varying pathogenicity. Environmental factors associated with increased LACV disease in humans include both local (e.g., residential-level) and regional factors. The spatial and temporal distribution of LACV-ND is broadly predictable, with consistent disease incidence in the summer and persistent geospatial clusters in the Appalachian region. To systematically present the complexities of LACV transmission biology, we review relevant salient features using the epidemiological triad (i.e., environment, agent, and hosts) model.

Environment

The risk factors of LACV-ND have been studied extensively in the households of LACV-ND patients. Several observational and formal epidemiological studies have provided strong evidence that household-level environmental factors including artificial containers, proximity to hardwood forests, the presence of tree holes, and abundant *Aedes* mosquito populations are associated with increased LACV-ND risk (Thompson and Gundersen 1983, Hedberg et al.

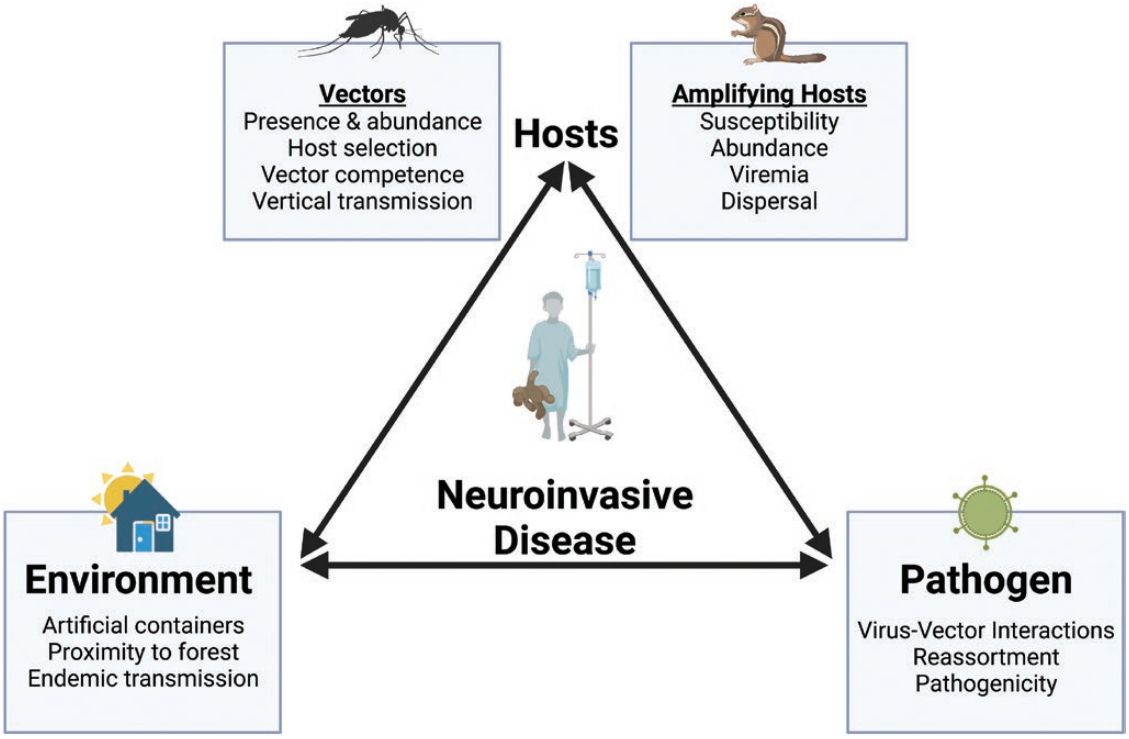

Fig. 1. Epidemiological triad model for La Crosse virus transmission (figure created in collaboration with D. Little using BioRender.com).

1985, Woodruff et al. 1992, Nasci et al. 2000, Erwin et al. 2002). Environmentally linked behavioral factors such as time spent outdoors, nonusage of insect repellants, lack of air conditioning, lack of screened windows, and lack of protective clothing are also associated with LACV-ND (Woodruff et al. 1992, Erwin et al. 2002). Population-level investigations of demographic and socioeconomic risk factors of LACV-ND revealed that areas with low socioeconomic statuses and small population densities have higher risks of LACV-ND (Haddow et al. 2011).

Proximity to previously reported cases is an important risk factor of LACV-ND. In the eastern United States, where most cases are reported, the relative disease risk for people residing in persistent high-risk foci is extremely high. For example, the relative risk of LACV-ND in a high-risk cluster of counties in West Virginia was 70 times higher than in the eastern United States overall from 2003 to 2007 (Day et al. 2023). Persistent geographic risk is also evident at fine spatial scales; from 1966 to 1995, one city and its surrounding suburbs accounted for 40% of all cases in Illinois, and from 2003 to 2007, most cases in Tennessee were reported from a subset of spatially clustered census tracts (Kitron et al. 1997, Haddow and Odoi 2009). At individual households, several instances of spatially associated, noncoincident LACV-ND were identified in North Carolina from 2002 to 2017 (Byrd et al. 2018); multiple LACV-ND cases at a single residence over several years suggest that in some cases, LACV risk remains persistent even at the residential level.

Pathogen

LACV belongs to the *Peribunyaviridae* family and is composed of 3 single-stranded negative-sense RNA segments (S: small [984 nucleotides], M: medium [4,526 nucleotides], L: large [6,980 nucleotides]) (Hughes et al. 2020). The S segment encodes for the nucleocapsid protein, the M segment encodes 2 structural glycoproteins (Gn and Gc), and the L segment encodes for an RNA-directed RNA polymerase/endonuclease protein. Poor RNA replication fidelity, due to the lack of proofreading enzymes, generates LACV quasispecies. In addition to genetic drift, genetic shift (segment reassortment) occurs and is evidenced in nature (Reese et al. 2008). There is also experimental evidence of infection-competent reassortments of LACV with other Orthobunyaviruses (e.g., snowshoe hare virus, Tahyna virus) (Gentsch et al. 1979, Seymour et al. 1983, Chandler et al. 1991).

Within LACV, genetic drift and reassortment lead to distinct genetic lineages that are associated with phenotypic differences, including virulence factors and pathogenesis (Armstrong and Andreadis 2006, Reese et al. 2008, Wilson et al. 2021). Although virulence appears to be under polygenic control, the M segment plays a critical role (Janssen et al. 1986, Bennett et al. 2008). Additional studies have also demonstrated that the NSs on the S segment reduce vertebrate host interferon (Type I) responses to enhance viral pathogenesis (Blakqori et al. 2007). There is also strong phylogenetic evidence for 3 distinct lineages of LACV with lineage I strains linked to increased pathogenesis in vertebrate hosts compared to lineage II and III strains (Huang et al. 1997, Lambert et al. 2015, Eastwood et al. 2020, Wilson et al. 2021).

Borucki et al. (2001) propagated 3 LACV genotypes in cell culture and then fed them to field-collected *Aedes triseriatus*. Dissections followed by genetic sequencing of the virus disseminating through infected mosquitoes indicated high selection pressure from the midgut through dissemination to the salivary glands, where more genotypes were found in the midgut and significantly fewer were isolated from the ovaries and salivary glands. This suggests that while mutations occur, only a few of them are stable enough to be transmitted via bite and most are maintained via transovarial transmission. Thus, the midgut acts as a potent barrier for some genotypes.

Within mosquito hosts, LACV infection is known to alter vector feeding behavior whereby infected female *Ae. triseriatus* tend to probe more frequently, obtain less blood per bite, and refeed more frequently when compared to uninfected siblings (Grimstad et al. 1980, Jackson et al. 2012). Additionally, LACV-infected females

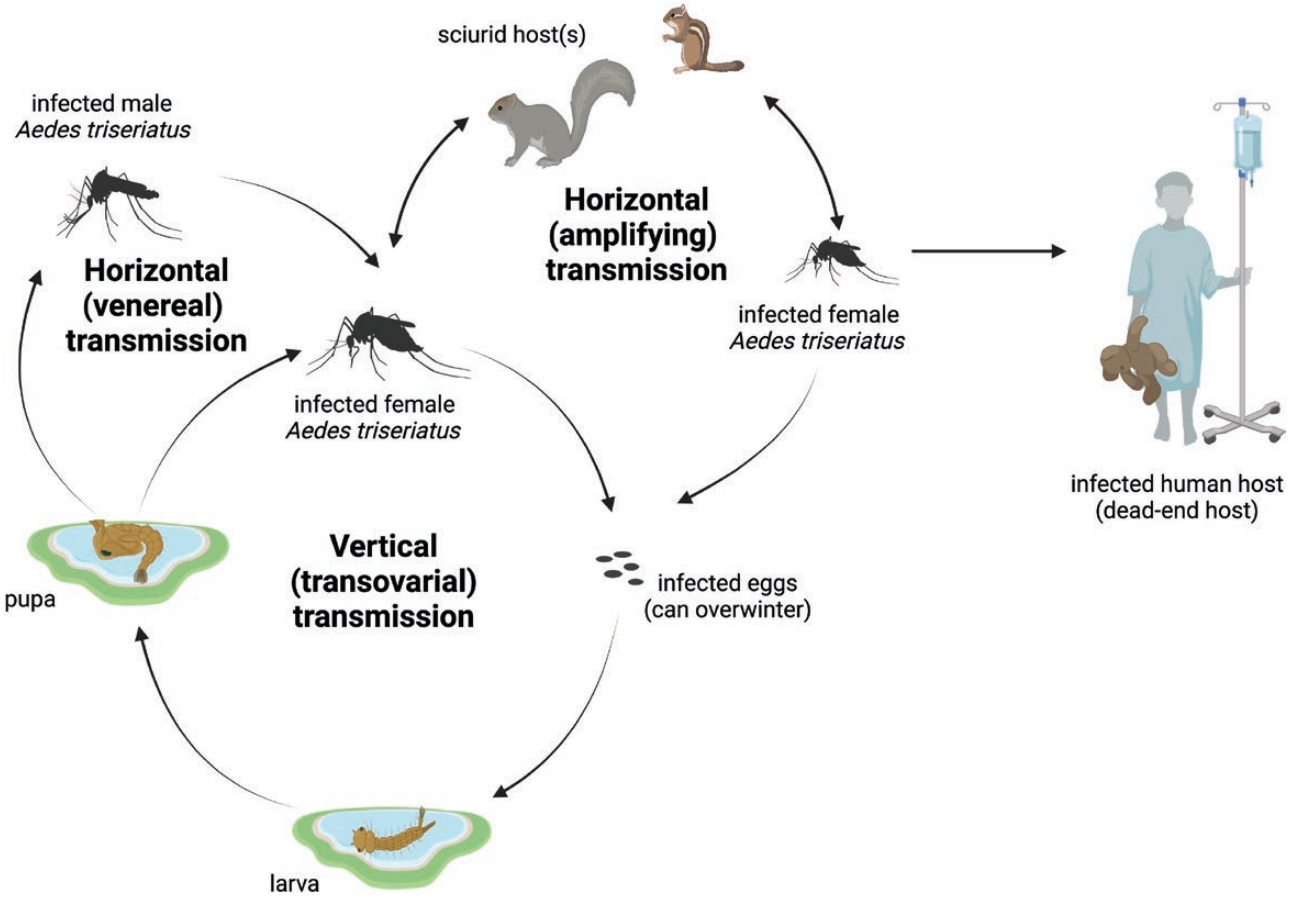

Fig. 2. La Crosse virus transmission cycles (figure created by M. Nordgulen using BioRender.com).

have a significant mating advantage over noninfected females thereby increasing the prevalence of infected females and the virus in nature via vertical transmission to infected progeny (Gabizsch et al. 2006, Reese et al., 2009). Taken together, these behaviors may increase transmission efficacy and vectorial capacity. Costanzo et al. (2014) reported that LACV infection (single prototype LACV strain [human 1960]) did not affect adult female mosquito (*Ae. triseriatus* and *Ae. albopictus*) longevity or fecundity. Conversely, there is evidence that transovarially infected *Ae. triseriatus* eggs experience higher mortality rates during overwintering (McGaw et al. 1998). The mortality rate was nearly 3 times higher when compared with uninfected eggs and was associated with decreases in the filial infection rate. Embryos that were infected also terminated diapause earlier; however, the cumulative mortality rate for infected eggs was 16.7% leading the authors to posit that most infected embryos survive overwintering and transovarial transmission remains an important maintenance mechanism in nature (McGaw et al. 1998).

Mosquitoes not only serve as LACV vectors but also as sites for virus evolution (Bishop and Beaty 1988, Chandler et al. 1991). Mutations and new strains of LACV can occur when transovarially infected mosquitoes ingest the blood of an infected host, leading to superinfection and potentially segment reassortment (Borucki et al. 1999). Studies on dual infection and reassortment of Bunyaviruses were prevalent in the 1990s; these viruses have been identified as diploid and polyploid and reassortment was more common with interrupted feeding and within the ovaries (Beaty et al. 1983, Borucki et al. 1999). Importantly, Borucki et al. (1999) wrote "*the ability of even a small percentage of transovarially infected mosquitoes to become superinfected and to generate reassortment viruses may be epidemiologically significant*." Mutations in the L segment were linked to altered disease outcomes, and mutations in the G_N glycoprotein are associated with decreased neuroinvasiveness (Bennett et al. 2007).

Hosts—Primary Vector (*Ae. triseriatus*)

Aedes triseriatus, or *Ochlerotatus triseriatus*, is the eastern tree hole mosquito and the primary LACV vector (Fig. 3a). It was incriminated as the primary LACV vector after repeated demonstration of infected mosquitoes at residences of human LACV-ND cases, efficient LACV transmission (i.e., high vector competence), high rates of transovarial transmission, and mammalian feeding behaviors (Pantuwatana et al. 1972, Watts et al. 1972, 1973b, 1975, Beaty and Thompson 1975, Burkot and DeFoliart 1982). *Aedes triseriatus* is the appellative member of the *Ae. (Protomacleaya) triseriatus* group that includes *Ae. triseriatus* (Say, 1823), *Ae. hendersoni* Cockerell, 1918, *Ae. brelandi* Zavortink 1972, and *Ae. zoosophus* Dyar and Knab, 1918 (Zavortink 1972, Taylor 1990, Reinert 2000). *Aedes hendersoni* is a sibling species that is sympatric with *Ae. triseriatus* within areas of endemic LACV transmission (Wilson et al. 2014, Tamini et al. 2021), but *Ae. hendersoni* is not an efficient vector of LACV due to a putative salivary gland barrier (Grimstad et al. 1985, Szumlas et al. 1996c, Nasci et al. 2000, Wilson et al. 2014, Tamini et al. 2021).

Aedes triseriatus is a multivoltine forest-dwelling species native to North America and widely established throughout the eastern United States from Florida to Canada (Darsie and Ward 2005,

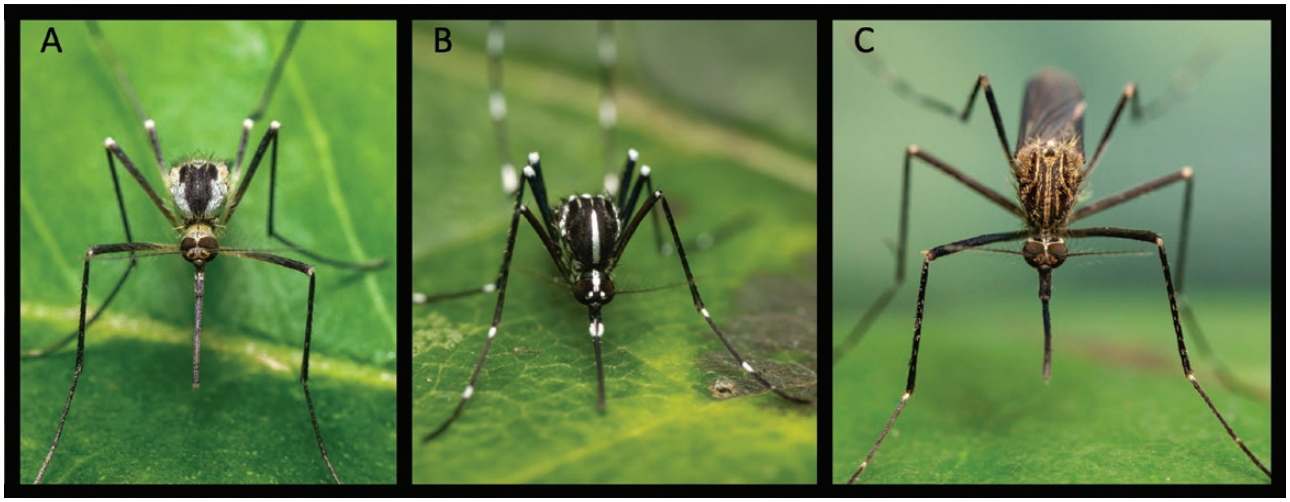

Fig. 3. A)The primary La Crosse virus vector (*Aedes triseriatus*) and invasive secondary vectors B) *Aedes albopictus* and C) *Aedes j. japonicus*. Images used with permission (Anders Lindström).

Koloski et al. 2021). Tree holes are the preferred habitat for oviposition and immature development, but the species also develops in artificial containers like discarded tires (Nasci 1988, Barker et al. 2003). It is predominately associated with hardwood forest habitats (Fig. 4a), but readily disperses into open terrain including domestic environments (Fig. 4b) to oviposit in artificial containers (Mather and DeFoliart 1984, Tamini et al. 2021). Females are diurnal feeders that not only predominately bite sylvan mammals including squirrels, chipmunks, and deer, but also opportunistically feed on dogs and humans (Burkot and DeFoliart 1982, Clark et al. 1985, Lancaster 2005). *Aedes triseriatus* is also known to feed on avian and reptilian or amphibian hosts (Irby and Apperson 1988, Molaei et al. 2008).

The efficiency of LACV vertical transmission varies among geographic strains of *Ae. triseriatus*, which is likely associated with the genetic heritability of transovarial transmission in the species (Miller et al. 1982, Graham et al. 1999). Transovarial transmission is a dominant trait mapped to chromosomes II and III; mosquitoes begin as susceptible to LACV, and their progeny become ~25% homozygous recessive to refraction and then eventually more become ~50% heterozygous or ~25% homozygous to the dominant transovarial transmission alleles (Graham et al. 2003). Knowing the trait is heritable led Graham et al. (2003) to speculate that monitoring the genetic traits of the mosquitoes (e.g., frequency of the recessive alleles) may be a better indicator of disease onset or transmission than monitoring for the virus. Rates for transovarial transmission are often geographically stable (Miller et al. 1978, Paulson and Grimstad 1989, Woodring et al. 1998). Under laboratory conditions in mosquitoes collected from endemic areas, *Ae. triseriatus* can maintain LACV for many generations by transovarial transmission alone, but horizontal transmission between *Ae. triseriatus* and amplifying hosts (i.e., sciurid rodents) is thought to be necessary for long-term LACV maintenance (Miller et al. 1977).

Hosts—Accessory Vectors

Aedes albopictus (Skuse, 1895), or *Stegomyia albopicta*, the Asian tiger mosquito (Fig. 3b), is a multivoltine species that was accidentally introduced to the United States in the mid-1980s through the used tire trade and quickly became established throughout much of the United States (Hawley et al. 1987, Moore and Mitchell 1997, Reinert et al. 2009). It is a competent laboratory vector of LACV, and LACV-infected *Ae. albopictus* have been collected from sites of human infections (Cully et al. 1992, Gerhardt et al. 2001, Westby et al. 2015). *Aedes albopictus* is also capable of transovarial LACV transmission, but at lower rates than *Ae. triseriatus* (Gerhardt et al. 2001, Hughes et al. 2006, Westby et al. 2015). To our knowledge, venereal LACV transmission has not been observed in *Ae. albopictus*.

Aedes albopictus is an ecologically adaptable invasive species that uses a range of natural and artificial habitats for immature development and opportunistically feeds on a wide variety of mammals and birds (Richards et al. 2006, Little et al. 2021, 2022). Because of its association with peridomestic habitats and aggressive human feeding behavior, *Ae. albopictus* may be an important bridge vector that transfers LACV from its natural sylvatic cycle into peridomestic environments (Faraji et al. 2014, Pereira-Dos-Santos et al. 2020). Several studies conducted in Appalachia indicated that *Ae. albopictus* was significantly more abundant at the homes of people diagnosed with LACV-ND compared with noncase residences, suggesting *Ae. albopictus* played a role in the emergence of LACV-ND in the region (Nasci et al. 2000, Erwin et al. 2002, Haddow et al. 2009). However, in some studies, *Ae. triseriatus* remains the dominant LACV vector at case homes (Tamini et al. 2021). Unfortunately, because most research on LACV ecology was conducted before the invasion of *Ae. albopictus*, its overall importance for the maintenance and transmission of LACV remains largely unclear.

Aedes japonicus japonicus (Theobald, 1901), or *Hulecoeteomyia japonica*, commonly called the Asian rock pool mosquito or Asian bush mosquito (Fig. 3c), is a multivoltine species that was identified in the United States in 1998, likely following multiple accidental introductions through the used tire trade (Peyton et al. 1999, Reinert 2000, Reinert et al. 2009, Kaufman and Fonseca 2014). The species is now widely distributed throughout the eastern United States and some western states (Kaufman and Fonseca 2014). *Aedes j. japonicus* is a competent laboratory vector of LACV (Sardelis et al. 2002), and there are 2 published records detecting LACV from field-collected specimens, including LACV-infected immatures, indicating the species is capable of transovarial LACV transmission (Harris et al. 2015b, Westby et al. 2015). To our knowledge, venereal transmission of LACV has not been studied in this species. *Aedes j. japonicus* oviposits in a wide variety of container habitats including rock pools, tree holes, and artificial containers and adult females feed on a range of mammals and birds (Andreadis et al. 2001, Scott and Crans 2003,

Apperson et al. 2004, Bevins 2007, Molaei et al. 2009, Kaufman et al. 2012, Kaufman and Fonseca 2014). Less is known about the role of *Ae. j. japonicus* in LACV transmission than *Ae. triseriatus* and *Ae. albopictus*, likely due in part to the difficulty of maintaining laboratory colonies of *Ae. j. japonicus*, but also because most major research on LACV ecology was conducted before the invasion of *Ae. j. japonicus* (Williges et al. 2008).

Aedes canadensis (Theobald, 1901), or *Oc. canadensis*, is a native freshwater univoltine woodland species well known as an aggressive human biter (Carpenter and LaCasse 1955, Reinert 2000). The species not only feeds predominantly on mammals but also feeds on avian and reptilian hosts (Molaei et al. 2008). It is a competent laboratory LACV vector and has been found naturally infected with the virus (Berry et al. 1986); one study within a LACV-endemic area of West Virginia reported LACV infection rates in *Ae. canadensis* at rates similar to *Ae. triseriatus* (Nasci et al. 2000). In the same study, host-seeking adults were collected by CO_2-augmented human landing collections. Throughout the study, investigators collected approximately 77% more *Ae. canadensis* (n = 1,863) when compared with *Ae. triseriatus* (n = 1,055). The abundance of *Ae. canadensis* in this study does not appear to be typical when compared with other studies investigating host-seeking adults at residences within LACV-endemic areas (Szumlas et al. 1996a, 1996c, Tamini et al. 2021).

Additional LACV transmission-competent mosquito species have been identified (Watts et al. 1973a, Freier and Beier 1984, Harris et al. 2015a). However, there is little evidence supporting their incrimination as vectors within endemic areas. Nearly 30% of *Ae. vexans* (Meigen, 1830) were capable of transmitting LACV to suckling mice (Watts et al. 1973a). *Aedes vexans* are commonly observed within endemic areas and at LACV-ND case residences, but often at much lower abundances relative to the primary and accessory vectors described above (Szumlas et al. 1996c, Nasci et al. 2000, Tamini et al. 2021). *Aedes atropalpus* (Coquillett, 1902), or *Oc. atropalpus*, was susceptible to oral infection with LACV and efficiently transmitted the virus to suckling mice at rates similar to *Ae. triseriatus* (Freier and Beier 1984, Reinert 2000). The same study also demonstrated transovarial transmission of LACV to F1 *Ae. atropalpus* adults with filial infection rates similar to *Ae. triseriatus*. *Aedes atropalpus* is an autogenous species with low relative abundance at LACV-ND case residences and is therefore not considered an important accessory vector (Bowen et al. 1994, Szumlas et al. 1996c, Nasci et al. 2000, Tamini et al. 2021).

Hosts—Amplifying Mammals

Naturally occurring LACV infections have been identified in at least 15 animal species, but only a few species meet the criteria to be considered important amplifying hosts—i.e., produce sufficient viremias to infect vectors and are regularly bitten by vectors in endemic areas (Harding et al. 2018). Eastern chipmunks (*Tamias striatus* L.) and gray squirrels (*Sciurus carolinensis* Gmelin) are sciurid mammals (Rodentia: Sciuridiae) that are consistently associated with natural LACV transmission cycles and are considered important amplifying hosts (Moulton and Thompson 1971, Gauld et al. 1974, 1975, Berry et al. 1975, Balfour et al. 1976, Ksiazek and Yuill 1977, Cully et al. 1992). Other mammals including the red fox, *Vulpes vulpes* L, and the groundhog, *Marmota monax* L, are potentially important amplifying hosts that produce sufficient virmieas to infect *Ae. triseriatus* but are understudied relative to chipmunks and gray squirrels (Amundson and Yuill 1981, Amundson et al. 1985).

The first effort to identify LACV amplifying hosts was a serosurvey of small mammals on a farm with previously documented LACV-ND, which reported high antibody rates in the eastern chipmunk (53% seroprevalence) and the eastern gray squirrel (39% seroprevalence) (Moulton and Thompson 1971). A laboratory study later confirmed that chipmunks and gray squirrels often produce sufficient viremias to infect susceptible *Ae. triseriatus* for periods of 1–3 days (Pantuwatana et al. 1972). Isolations of LACV from the blood of naturally infected chipmunks were also reported from previously known seronegative chipmunks that were recaptured (Gauld et al. 1975). The annual maturation of spring and summer juvenile chipmunks provides a susceptible host population, and the maturation of spring juveniles closely coincides with the peak biting activity of *Ae. triseriatus* (Gauld et al. 1974), but the high prevalence of LACV-neutralizing antibodies in adult chipmunks also poses a significant immune population that will not contribute to LACV amplification, which may significantly diminish LACV infection rates in mosquito populations (DeFoliart 1983).

Other wildlife species, including the eastern cottontail, *Sylvilagus floridanus* (Allen), and white-tailed deer, *Odocoileus virginianus* (Zimmermann), are naturally exposed to LACV, but are not considered important amplifying hosts because of either low seroprevalence or lack of evidence for high (infectious) viremias (Issel et al. 1972, Dressler et al. 1988, Osorio et al. 1996).

Hosts—Human

Humans are incidental and "dead-end" hosts whereby infection with LACV does not result in viremias sufficient to infect blood-feeding mosquitoes. The neurotropic nature of LACV, and lack of detectable viremias, pose clinical and diagnostic challenges that limit the ability to rapidly detect LACV infection early in the disease process. Consequently, reporting and research on the clinical characteristics of LACV infections are mostly limited to severe cases, typically neuroinvasive, which represent a minute subset of all infections. There are 30–90 LACV-ND cases reported annually in the United States, of which more than 90% result in hospitalization, but serologic evidence indicates most LACV infections are asymptomatic and unreported (Szumlas et al. 1996b, Rust et al. 1999, Vahey et al. 2021, CDC 2023b). There is no preventive vaccine or specific treatment for LACV-ND, so treatment for hospitalized patients is limited to the management of symptoms (CDC 2023c).

Approximately 90% of reported LACV-ND occurs in children younger than 18 years and manifests as encephalitis, meningitis, meningoencephalitis, acute flaccid paralysis, or other signs of neurological dysfunction. Symptoms of LACV-ND include varying combinations of headache, fever, vomiting, altered mental status, and seizures (McJunkin et al. 2001, Miller et al. 2012, Gaensbauer et al. 2014, Vahey et al. 2021, Boutzoukas et al. 2023). A patient's condition upon arrival at the hospital is predictive of disease severity. Younger patients, patients with altered mental statuses, and patients with seizures at the time of admission are more likely to have longer hospitalization periods and greater disease severity than other patients (Miller et al. 2012, Boutzoukas et al. 2023). Severe cases typically require admission to pediatric intensive care units and often require mechanical ventilation and intubation (McJunkin et al. 2001, de los Reyes et al. 2008, Miller et al. 2012, Boutzoukas et al. 2023). Early during the disease course, LACV-ND cases are often presumptively treated for herpes simplex encephalitis and/or bacterial meningitis before receipt of laboratory test results. Presumptive treatment results in unnecessary chemotherapeutic exposures that are ineffective for LACV-ND (McJunkin et al. 2001). Length of hospitalization is usually less than 1 week and fatalities are rare (approximately 1–2% of cases), but severe cases of LACV-ND regularly result in long-term neurological sequelae (McJunkin et al. 2001, de los Reyes et al. 2008, Miller et al. 2012, Boutzoukas et al. 2023).

The long-term impacts of LACV-ND are underrecognized but likely represent the most negative impact (burden) of the disease for most patients. The largest and most recent study of pediatric LACV-ND patients revealed that up to 54% of patients responded abnormally to neurological assessments after a minimum of 1 month from hospital discharge, regardless of disease severity (Boutzoukas et al. 2023). Thirty-five percent of those patients required specialized education plans, and 17% were receiving mental health services or psychotherapy. A plethora of long-term sequelae have been reported, including reduced IQ scores, reduced pediatric quality of life (PedsQL) scores, speech problems, memory loss, and recurring seizures (McJunkin et al. 2001, de los Reyes et al. 2008, Boutzoukas et al. 2023).

The CDC case definition for LACV-ND (arboviral disease, neuroinvasive California serogroup virus disease) includes a combination of laboratory and clinical criteria. Clinical criteria require a patient to exhibit either meningitis, acute flaccid paralysis, encephalitis, or another sign of acute central or peripheral neurologic dysfunction (CDC 2023c). Laboratory criteria require either the isolation of the virus or demonstration of LACV-specific antigen or nucleic acid in tissue, a 4-fold change in LACV-specific antibody titers in paired sera, LACV-specific IgM antibodies and LACV-specific neutralizing antibodies in serum, or LACV-specific IgM antibodies in cerebrospinal fluid or serum (CDC 2023c). There are antigenic similarities and antibody cross-reactivity within the CAL group of arboviruses that complicates serodiagnosis. This is primarily a concern for differentiating LACV and Jamestown Canyon virus (JCV) infections. A plaque-reduction neutralization test (PRNT) separately challenges serial dilutions of sera against a static amount of virus to measure the loss of plaque neutralization and assign etiology to the virus that neutralizes at a lower serum concentration. Serological studies have detected LACV and JCV antibodies in park employees at the Great Smoky Mountains National Park on the eastern TN/western NC border, suggesting that both CAL group arboviruses could be endemic in the region (Adjemian et al. 2012). Thus, cross-reactivity of JCV and LACV complicates serological diagnoses, and lack of regional or state laboratory capacity to conduct PRNTs delays definitive diagnosis and hinders reporting of confirmed cases.

Knowledge Gaps that Limit the Ability to Promote Effective Public Health Response

Despite robust historical knowledge from early laboratory studies, field observations, and epidemiological investigations that are partially reviewed above, the reality is that LACV exposure and transmission risk has likely changed since the introduction of *Ae. albopictus* and *Ae. j. japonicus*. Interactions between conspecific and heterospecific species, particularly in larval habitats, remain poorly resolved. Variability in LACV susceptibility and transmission efficacy for each vector is a result of complex genetic and dynamic environmental factors, and the potential impacts of climate change on LACV transmission are unknown. Limited knowledge of environmentally persistent LACV lineages and their interactions with vertical-horizontal transmission cycles limit our ability to predict transmission risk at a meaningful scale. We also do not have actionable thresholds or proven strategies for vector control to interrupt LACV transmission cycles, and the social and economic burden of LACV-ND on affected communities is poorly characterized. Collectively, these knowledge gaps impede the development of a well-defined and evidence-based strategy for control or prevention. To address these gaps, future evaluation and research efforts must be performed in areas where LACV-ND has been prevalent during the past 2 decades—principally in the Appalachian region. In this section, we provide an overview of existing research and knowledge gaps for high-priority research areas.

Vector Interactions

Each of the 3 major LACV vector species (*Aedes triseriatus*, *Ae. albopictus*, and *Ae. j. japonicus*) co-occur in larval habitats within much of the endemic range of LACV-ND, creating the potential for resource competition that could result in species' displacement or exclusion (Bevins 2007, Leisnham and Juliano 2012). In controlled settings, *Ae. albopictus* is a superior competitor to *Ae. triseriatus* and *Ae. j. japonicus*, while competition between *Ae. triseriatus* and *Ae. j. japonicus* only has weak effects on either species (Livdahl and Willey 1991, Hardstone and Andreadis 2012). There is no evidence of competitive exclusion of *Ae. triseriatus* in its native range, but there is evidence of displacement of *Ae. triseriatus* from artificial container habitats that are dominated by *Ae. j. japonicus* or *Ae. albopictus* (Joy and Sullivan 2005, Bevins 2007, Burger and Davis 2008, Andreadis and Wolfe 2010, Rochlin et al. 2013). *Aedes triseriatus* remains well-established in natural containers, particularly in forested environments, possibly because of competitive advantages in habitats with natural detritus or its superior ability to evade natural predators compared to invasive species (Joy and Sullivan 2005, Yee et al. 2007, 2012, Juliano et al. 2019, Westby and Medley 2020). These observations indicate that natural environments (i.e., sylvan habitat, hardwood forests) may serve as a refuge for *Ae. triseriatus* from unfavorable anthropogenic habitats that are dominated by *Ae. albopictus* and *Ae. j. japonicus*, but the importance of those habitat delineations for LACV transmission is unclear. It seems possible that the displacement of *Ae. triseriatus* from urban habitats and its persistence in hardwood forests may allow for targeted vector control in natural habitats to interrupt LACV transmission cycles, but there is insufficient evidence to confidently draw that conclusion (Nasci et al. 2000, Haddow et al. 2011, Bewick et al. 2016).

In addition to species displacement or exclusion, competitive interactions among vectors also have indirect effects on the capacity of each species to transmit LACV. Resource competition among mosquito larvae results in nutritional deprivation which has consequences for the physiology of mosquitoes that survive larval competition and emerge as adults. For *Ae. triseriatus*, resource deprivation as larvae results in smaller adults with greater vector competence than adults that emerged from resource-rich environments (Grimstad and Haramis 1984, Grimstad and Walker 1991, Paulson and Hawley 1991). Larval competition between *Ae. triseriatus* and invasive vectors are therefore hypothesized to produce *Ae. triseriatus* with elevated competence for LACV transmission, which could be an indirect pathway for the invasive species to increase LACV-ND risk (Bevins 2008).

One study found that although larval competition between *Ae. triseriatus* and *Ae. albopictus* was detrimental to *Ae. triseriatus* survival rates, the *Ae. triseriatus* larvae that survived to adulthood were larger than those that survived from single-species treatments (Bevins 2008). Contrary to expectations, the larger *Ae. triseriatus* adults also had higher LACV infection rates than smaller counterparts from single-species treatments, driven by the fact that *Ae. triseriatus* were more likely to successfully blood feed than small individuals. It is not known why *Ae. triseriatus* emerging from competition with *Ae. albopictus* were larger than individuals that emerged from intraspecific treatments (Bevins 2008). These contradictory findings illustrate the complexity of relationships between larval developmental conditions and LACV competence, and it is presently impossible to extrapolate the results of existing research to the natural

environment. Future research in this area should be paired with observations from endemic areas to determine whether these effects are present under natural conditions.

Comparative LACV Vector Competence

Most research characterizing *Ae. triseriatus* as the primary LACV vector was completed prior to the introductions of *Ae. albopictus* and *Ae. j. japonicus*, and the roles of the invasive vectors in LACV transmission and maintenance remain unclear. Early research indicated *Ae. triseriatus* has higher rates of disseminated infections and transovarial transmission than *Ae. albopictus* and *Ae. j. japonicus* (Grimstad et al. 1989, Sardelis et al. 2002, Hughes et al. 2006), but the most recent comparative study found that *Ae. j. japonicus* had the highest effective vector competence (i.e., the rate of change of vector competence over time), followed by *Ae. albopictus* and then *Ae. triseriatus* (Bara et al. 2016). There were important methodological differences between Bara et al. (2016) and previous studies which contributed to the contrasting results; most notably, previous studies tested mosquitoes for LACV infection 14 or 21 days after exposure, but Bara et al. (2016) tested mosquitoes after incubation periods of 3–11 days (Grimstad et al. 1989, Sardelis et al. 2002, Hughes et al. 2006). That discrepancy is epidemiologically meaningful because the results indicate that the invasive species become infectious earlier than *Ae. triseriatus*, and therefore they can transmit the virus earlier and longer from a daily survival perspective, increasing overall LACV vectorial capacity. To better understand the risk posed by each species, future research should comparatively measure infection rates and vectorial capacity of the vectors under a range of natural conditions in the endemic area.

LACV Maintenance

The maintenance of LACV over long periods is not understood. In theory, vertical transmission—which is not 100% efficient—is supplemented by horizontal transmission between mosquitoes and amplifying hosts and venereal transmission. Although each of those mechanisms exists in nature, field studies have failed to prove that they are sufficient for the maintenance of LACV, as inefficiencies of each transmission mechanism under natural conditions are thought to drastically reduce the potential for LACV to be sustained over time (DeFoliart 1983, Bewick et al. 2016). This knowledge gap affects appropriate vector control response; if LACV maintenance relies heavily on horizontal transmission between mosquitoes and amplifying hosts, the reduction of mosquito populations below a certain threshold should interrupt transmission cycles. However, if transovarial and venereal transmission within mosquito populations is sufficient to maintain LACV, control of the virus may depend on eradicating all transovarially infected eggs in an area.

LACV Maintenance—Vertical Transmission Within Vector Populations

Long-term persistence of LACV is thought to depend on transovarial transmission within mosquito populations, particularly *Ae. triseriatus* (DeFoliart 1983). At minimum, transovarial transmission is critical because infected diapause eggs are the only overwintering reservoir for LACV (Beaty and Thompson 1975). Transovarial transmission also presents a serious challenge for LACV control because delayed hatching of *Ae. triseriatus* eggs allow infected mosquitoes to emerge after extended dormant periods, which would allow LACV to persist in larval habitats even if infected adults were totally eliminated (Beaty and Thompson 1975).

Vertical transmission rates in field populations of *Ae. triseriatus* are too low to explain long-term LACV maintenance in nature, yet focal endemicity of LACV in small areas has been well documented (Moulton and Thompson 1971, Beaty and Thompson 1975, Lisitza et al. 1977, Landry et al. 1988, Kitron et al. 1997, Byrd et al. 2018). A possible explanation for long-term focal persistence is that some *Ae. triseriatus* populations may achieve stabilized infections with LACV. Reese et al. (2010) describe "super-infected" *Ae. triseriatus* from a single site that may represent stabilized infections, which may allow that single-mosquito population to maintain the virus over long periods primarily through transovarial transmission, but this requires further investigation as the molecular bases and frequency of this phenomenon are unknown (Reese et al. 2010).

Aedes albopictus and *Ae. j. japonicus* are often dominant in artificial container habitats, and their rates of transovarial transmission and success as overwintering LACV reservoirs may have important consequences for human exposure. Additionally, *Ae. j. japonicus* emerges early in the mosquito season and colonizes riverine rock pools that are not used by other LACV vectors, so overwintering in *Ae. j. japonicus* may influence the seasonality and focal distribution of LACV maintenance and transmission (Kaufman and Fonseca 2014, Reuss et al. 2018, Day et al. 2020). We are aware of only one laboratory investigation of transovarial transmission in *Ae. albopictus*, which found that transovarial and filial transmission rates (52% and 18%, respectively) were significantly less than in *Ae. triseriatus* (71% and 46%, respectively) (Hughes et al. 2006). Field-collected *Ae. albopictus* larvae and eggs infected with LACV proved that transovarial transmission also occurs naturally in this species, but there is currently no evidence of LACV overwintering in diapause *Ae. albopictus* eggs (Gerhardt et al. 2001, Westby et al. 2015). Like *Ae. albopictus*, vertically infected *Ae. j. japonicus* have been collected in the field, but we are not aware of any laboratory studies of transovarial LACV transmission in the species (Harris et al. 2015b, Westby et al. 2015).

LACV Maintenance—Horizontal Venereal Transmission Between Vectors

Male *Ae. triseriatus* that are infected via transovarial transmission can horizontally transmit LACV to uninfected females during mating (i.e., venereal transmission), which can then transmit the virus horizontally to amplifying hosts and vertically to their offspring (Patrican and DeFoliart 1987). This transmission mechanism is hypothesized to supplement transovarial transmission for LACV maintenance within mosquito populations, but its contribution to infection rates of mosquitoes under natural conditions is not known. Venereal infection rates of up to 50% have been reported under laboratory conditions, but infection rates vary depending on multiple factors such as the timing of a female's bloodmeal and the presence of LACV-neutralizing antibodies in a bloodmeal (Thompson and Beaty 1978, Thompson 1983). We are unaware of any research on venereal LACV transmission by accessory vectors. Determining the importance of venereal transmission to LACV maintenance will require an understanding of male infection rates, the rate of venereal transmission from infected males to uninfected females, and the transmission rates of females that become infected, all under natural conditions.

LACV Maintenance—Horizontal Amplification by Vertebrate Hosts

Horizontal transmission between mosquitoes and amplifying hosts is also theorized to account for the inefficiency of transovarial

transmission, but some researchers have questioned whether this mechanism is adequate to support long-term LACV maintenance (Watts et al. 1973b, Miller et al. 1977, 1979, Burkot and DeFoliart 1982).

DeFoliart (1983) described a "series of attritions" that reduce the success of horizontal transmission:

I. Wastage of vector bites on nonamplifier species, which is common among all the 3 important LACV vectors because they all regularly feed on hosts that are not important LACV amplifiers (Burkot and DeFoliart 1982, Cebrian-Camison et al. 2020).
II. Wastage of infective vector bites on immune amplifier species (i.e., biting animals that already developed neutralizing antibodies), which is probably common given the high seroprevalences of amplifier hosts (Moulton and Thompson 1971, Gauld et al. 1974, Balfour et al. 1976, Amundson and Yuill 1981).
III. Amplifier hosts and vectors that do not become infected upon exposure. Not all amplifying hosts develop sufficient viremia to infect large proportions of mosquitoes, and even when viremia is high, not all mosquitoes become infected (Patrican et al. 1985a, 1985b).
IV. High mortality of orally infected mosquitoes before the second gonotrophic cycle. Orally infected *Ae. triseriatus* rarely achieve transovarial transmission in the first gonotrophic cycle, but in natural populations, a small percentage of mosquitoes are expected to survive to the second cycle (Watts et al. 1973b, Miller et al. 1979).

That series of attritions is thought to reduce the rate of horizontal transmission below the necessary threshold for LACV maintenance (DeFoliart 1983, Defoliart et al. 1986). Unfortunately, attempts to elucidate the unknown factors in vertical and horizontal transmission that would explain long-term LACV maintenance were never conclusive (Landry et al. 1988), and research interest in this subject appears to have faded in recent decades. Notably, these discussions focus primarily on LACV maintenance and not on human disease risk. We are not aware of any study that has identified spatiotemporal associations amplifying host infections with human cases, and it seems likely that increased vector infection rates after exposures to infected amplifying hosts would increase human disease risk even if it does not support viral maintenance.

Dispersal (Geographic Spread) by Vector or Host

The rate and mechanism of LACV dispersal have considerable implications for the efficacy of control efforts. If the virus readily disperses throughout the endemic area, then localized control efforts will not produce sustained effects. However, if LACV transmission occurs in isolated patches with little pathogen dispersal, targeted control may achieve local eradication that produces a long-term reduction in human disease risk.

Arboviruses are dispersed by the movement of infected vectors or hosts. *Aedes triseriatus* and *Ae. albopictus* are weak dispersers that fly distances of less than 1,000 m from their larval habitat in the absence of human-mediated (anthropogenic) dispersal, and *Ae. j. japonicus* is only a slightly better disperser, which means that they are not likely to transport LACV over long distances (Verdonschot and Besse-Lototskaya 2014, Schmidt et al. 2017). There is some potential for dispersal via the movement of artificial containers holding LACV-positive eggs, but this has not been investigated to our knowledge. An alternative method for LACV dispersal would be host-mediated dispersal, that is, the movement of infected amplifying hosts to new areas with susceptible vector populations. This is common for other anthroponotic arboviruses like dengue, which have long-range dispersal through infected humans, or West Nile virus, which infects birds that may fly extended distances while viremic (Owen et al. 2006, Carver et al. 2009).

For a host to act as a vehicle for LACV dispersal, it must disperse during the viremic period, which is typically only 1–3 days (Pantuwatana et al. 1972). Adult gray squirrels can disperse distances as far as 14.5 km over long time periods, but most reported dispersal ranges are much shorter (e.g., <1,000 m) (Goheen et al. 2003, Perlut 2020). Chipmunks disperse infrequently; in one study, only 3% of chipmunks dispersed during a 55-yr study period (Denomme-Brown et al. 2021). The best candidate for host-mediated LACV dispersal seems to be the red fox, which travels as far as 25 km/day (Storm et al. 1976). Unfortunately, the red fox is understudied relative to chipmunks and gray squirrels, so little is known about its infection rates or its likelihood to transport LACV.

Domestic Animals and LACV

Domestic animals have been investigated as potential amplification hosts for LACV, but no domestic species has been indicated as an important amplifier. Canines and pigs developed viremias sufficient to infect large proportions of susceptible mosquitoes (Godsey et al. 1988), but in one study on a farm with endemic LACV transmission, only a small percentage of any domestic species (range 1–5%) had LACV antibodies, indicating that natural exposure to the virus was rare (Godsey et al. 1988). A more recent serosurvey of canines in Virginia documented approximately 5% of dogs had antibodies to LACV (Troyano 2009). Considering that the dogs were not sampled based on proximity to known LACV transmission, the findings of Troyano (2009) indicate that canine exposure may be more frequent than expected.

We are not aware of any reports of livestock becoming ill from LACV infections, but canines can develop severe and fatal neuroinvasive disease from LACV infections. Godsey et al. (1988) inoculated 4 puppies with LACV, and 3 of them died after presenting clear disease of the central nervous system. Naturally occurring fatal LACV infections have also been reported in canines. In 1988 and 1991, illness and death occurred in litters of puppies from south Georgia which was attributed to LACV (Black et al. 1994). In 1999, an adult dog died of viral neuroinvasive disease in Florida, which was also attributed to LACV infection (Tatum et al. 1999). In the latter case, 2 other dogs that visited the residence also developed seizures, but recovered and were not tested for LACV infections.

LACV is not considered to be a pathogen of veterinary concern and no veterinary testing is done for the virus, so any associated disease is unlikely to be attributed to LACV (Black et al. 1994, Tatum et al. 1999). Increased surveillance for LACV in canines with neuroinvasive disease may reveal that LACV causes more veterinary diseases than previously recognized. Exposure of canines and other domestic animals may also be an indicator of local human disease risk, and the roles of peridomestic nonamplifying animal abundance (domestic and sylvan) as a potential environmental "sink" for LACV and/or host availability for LACV vectors have not been explored in a meaningful manner. Additional serosurveys of domesticated animals in endemic areas are warranted.

Control of LACV Vectors

There is no published research to identify the most effective approach to mosquito population management in the context of LACV control, and there are no actionable risk indices for vector abundance

or infection rates to inform the timing of mosquito control. There is an urgent need for actionable research that uses epidemiologically meaningful response variables to demonstrate efficacy, such as mosquito infection rate, host seropositivity, or reduction in vector–human contact, and considers the environmental context of LACV-endemic areas. Because of transovarial and venereal transmission, effective methods will require an integrated mosquito management approach that controls vectors at all life stages. Source reduction, mosquito-disseminated insect growth regulators, and insect barrier sprays should all be assessed for their efficacy in reducing LACV transmission risk.

Socioeconomic Impacts of LACV Disease

To date, there are only 2 studies analyzing the social and economic impacts of LACV-ND, both of which focused on western North Carolina (Utz et al. 2003, 2005). A 2003 study of North Carolina LACV-ND patients or their parents/guardians revealed that the cost of cases with lifelong sequelae ranged from $48,775 to $3,090,798 (or equivalent to $79,304–$5,025,402 in 2023) (Utz et al. 2003). Parents of affected children were found to lose an average of 47.9 workdays and to travel an average of 1,717 miles for visits to medical facilities (Utz et al. 2003). Those financial costs are compounded with severe social tolls, as parents and guardians of LACV-ND patients suffer severe emotional impacts while caring for children who sometimes see long delays in proper diagnosis. Individuals with lifelong neurological sequelae may lose between 17.46% and 92% of future productive life years, and children are especially vulnerable to diminished quality of life due to issues with self-esteem, social life, and educational delays (Utz et al. 2005).

Areas with the highest LACV-ND risk are comprised of predominately white, rural communities in Appalachia with low socioeconomic statuses (Haddow et al. 2011). Impoverished white communities in Appalachia were identified as one of 8 "Americas" with substantial health disparities and a hidden burden of neglected diseases. *Aedes*-borne diseases often disproportionately affect areas with low socioeconomic statuses, and although LACV-ND is not classified as a neglected disease of poverty, disease incidence and socioeconomic impacts on poor communities in rural Appalachia are likely undermeasured and underrecognized (Murray et al. 2005, Hotez 2008, Whiteman et al. 2020). However, it is important to recognize that although population-level studies have identified socioeconomic risk factors of LACV-ND, those findings do not suffice as evidence that low-income individuals have elevated risk compared to high-income individuals living in the same area. Anyone residing in a high-risk area is at risk of LACV exposure, but the areas with highest risk tend to have lower socioeconomic metrics overall. Additional studies verifying the socioeconomic burdens of LACV-ND are necessary to understand the true burden of disease on individuals and communities.

A Framework for Prevention, Detection, and Response to Reduce LACV-ND in Appalachia

Control and prevention of LACV-ND is possible and has been accomplished in the past. The most well-documented example is La Crosse County, WI, where a mosquito control program was established in 1978 after 2 fatalities and multiple morbidities were attributed to LACV (Parry 1983). The program focused on reducing *Ae. triseriatus* abundance in areas with known LACV transmission, facilitating clinical education programs on LACV-ND symptoms and treatment, and providing community education programs on *Ae. triseriatus* ecology to encourage source reduction (Parry 1983). Those efforts successfully reduced LACV-ND incidence in La Crosse County, while areas without mosquito control programs maintained a consistent rate of disease (Parry 1983, Thompson and Gunderson 1983). That program and others like it have persisted in the upper Midwest, and LACV-ND incidence in that region declined substantially in recent decades (Vahey et al 2021, Day et al 2023).

To reduce LACV-ND in Appalachia, we propose the development of a prevention, detection, and response framework for LACV consistent with the CDC's overall approach to epidemic infectious diseases (CDC 2023a). At present, analogous efforts are limited within LACV-endemic regions, where existing infrastructure is generally limited to a single city/county and is often episodic. Potential prevention approaches should include community outreach and engagement to raise awareness of risk factors and how to mitigate them along with targeted vector control. Detection should include traditional vector surveillance combined with model-based risk predictions, improved clinical diagnosis, and rapid reporting. Response should entail the timely deployment of technical experts to areas with the reported disease to apply interventions that prevent future disease events and ongoing efforts to minimize persistent risk in those areas. While some of these efforts were used during a Tennessee LACV-ND outbreak in 2001, they are no longer used in the endemic area because of limited public health infrastructure, which may include competing priorities, minimal staff and resources, and lack of interest or awareness (Jones et al. 1999, Erwin et al. 2002).

Collaboration

Prevention, detection, and response to LACV-ND in Appalachia will require collaboration among universities, state public health departments, local mosquito abatement and health departments, and other community stakeholders. Each entity brings unique expertise and resources for disease control, and effective disease reduction in this region will require each to work in tandem.

Collaboration—Universities

Universities in LACV-endemic regions have tenured faculty experts who have led research in the area for a considerable time. In many areas, university faculty are the only local experts on LACV because employment turnover of health directors, communicable disease nurses, environmental health specialists, and other local public health personnel happens more frequently than tenured faculty. With funding support from federal agencies, universities are well positioned to conduct longitudinal surveillance and research in pursuit of novel methods for LACV detection and control in endemic areas.

The value of universities in LACV-ND response and detection is already demonstrable. Residentially linked coincident (synchronous) and asynchronous LACV-ND cases in North Carolina (NC) were only recognized because a university faculty member was conducting environmental assessments at case residences for more than a decade (Byrd et al. 2018). In 2022, there were only 2 LACV-ND cases from NC reported to CDC's ArboNet, representing a greater than 85% reduction in the 20-yr annual median number of cases. Inquiries into this precipitous drop to the state health department and large regional hospital revealed new hospital-based public health epidemiologists with no prior experience with La Crosse encephalitis, a change in laboratory testing providers, and a change in reporting terminology. The university faculty member was alerted to these potential reporting confounders early when local health

department staff reached out for assistance interpreting the newly formatted arboviral testing results from the hospital.

Collaboration—State Public Health Departments

State public health departments should provide capacity for arboviral testing and training support for local vector control agencies. Additionally, state public health departments are best positioned to conduct epidemiological surveillance of LACV to improve response and detection. They can lead efforts to conduct serosurveys of humans and animals at hospitals and veterinary clinics, and they have timely access to reported cases of disease. Effective collaboration among agencies will require state health departments to engage in clear and open communication with universities and local agencies. In NC, TN, and WV, active surveillance efforts and CDC "Epi-Aid" or technical assistance requests from state health departments have resulted in the detection of endemic LACV-ND cases that would not previously have been recognized (Kindle et al. 1988, Jones et al. 1999, Erwin et al. 2002, Johnson 2006).

Collaboration—Local Mosquito Control and Public Health Departments

Local mosquito abatement districts and health departments should employ individuals with medical entomology training to use evidence-based methods for LACV surveillance, disease prevention, and case response. These entities are the frontline for LACV control, and they require substantially more guidance and funding support than has been previously provided. Universities can contribute to local efforts by developing research-based methods for LACV control and training new entomologists, environmental health specialists, and other public health professionals with knowledge of the local area. Specifically, students can conduct undergraduate capstone projects and complete intensive internship experiences with direct public health relevance. Federal and State agencies should lead efforts to establish local mosquito control infrastructure and provide funding support to reduce vector-borne disease risk.

Collaboration—Community Stakeholders

An effective program for reducing LACV, or any other vector-borne disease in Appalachia, will require the support and inclusion of local communities. The focal nature of LACV-ND necessitates action to reduce human exposure to mosquitoes in people's homes. For public health officials to conduct prevention and case response, they will require permission and cooperation from families and homeowners to operate on their properties. In most cases, sustained risk reduction will require humans to engage in responsible behaviors like source reduction and wearing repellent. It is difficult to garner that level of public engagement, especially under the current circumstances of political divisiveness that diminishes public trust in public health. Instead of working unilaterally, government agencies and universities should work with local community leaders, such as churches and schools. Community leaders know best how to communicate with the local populace and are likely to have more reach, trust, and specialized expertise in their communities than a government entity.

Capacity

In general, Appalachia lacks the public health infrastructure for vector surveillance and control. Where programs do exist, they are often the responsibilities of nonspecialist programs, which often means that time, training, and funding are insufficient for effective disease prevention. The solution for this is not obvious; ideally, local funding could be appropriated for vector surveillance and control, but this is unrealistic in rural communities with limited tax revenue. If LACV-ND and other vector-borne diseases are to be controlled in Appalachia, there must be some external (i.e., federal and state) funding that allows local agencies to create programs. It may be unnecessary to create unique programs for every rural community, but instead there could be state-funded regional control programs that serve rural endemic areas (e.g., western North Carolina or eastern Tennessee). These regional programs could be patterned after the CDC Regional Centers of Excellence of Vector-Borne Diseases or developed as a component of existing centers.

Prevention—Community Outreach and Engagement

In areas with persistent LACV-ND, families are often unaware of this endemic problem until they experience it personally. In the context of a focally persistent mosquito-borne disease that can be prevented by personal protective behaviors, the lack of community awareness in endemic areas represents a public health failure. We propose that engagement with community stakeholders to raise awareness of the disease and behavioral risk factors should be a core component of a program for LACV-ND prevention. Ideally, community engagement efforts would consist of collaborative efforts between universities and local public health agencies, which can work together to identify the most effective outreach methods for specific communities (e.g., classroom visits, community science projects). For resource efficiency, these efforts could be directly targeted at communities identified as having persistent high risk, such as schools in high-risk areas (Day and Trout Fryxell 2022). Sustained funding, long-term health and education commitments, and additional collaboration with local public health agencies will be required for community engagement programs to have large-scale effects on disease prevention.

Prevention—Risk Assessment

In the absence of large-scale, area-wide control throughout Appalachia, prevention of LACV-ND will depend on targeted efforts in areas that are known to have elevated risk. This should, at minimum, be based on an analysis of the historical and recent distributions of reported cases, which would uncover hotspots with persistent transmission. Underdiagnosis and new human habitations of natural areas will continue to create new risk for LACV exposure, so retrospective studies will not be entirely sufficient. Traditional mosquito surveillance and arbovirus testing would also be valuable to identify risk, but robust mosquito surveillance throughout rural Appalachia that would be actionable from a control perspective is likely infeasible. As an alternative, longitudinal field-based ecological studies that identify the environmental drivers associated with the presence of LACV transmission should be conducted to create models that can provide spatial-temporal predictions of LACV infection risk. Such models could guide resource-limited programs to apply their resources where they are most needed.

Prevention—Vector Control Tools

Although source reduction has been used successfully for LACV-ND control in the upper Midwest, we are not aware of any robust scientific studies that prove the efficacy of any methods for LACV control. This gap leaves us unable to provide evidence-based guidance for control and prevention in high-risk areas. Short-term adult control methods are not likely to have a sustained effect because of transovarial transmission, which would allow LACV to persist in immature mosquitoes even if all adults in the area were eradicated. A targeted effort to minimize transovarial transmission of LACV within *Ae. triseriatus* is plausible, which should begin with larval

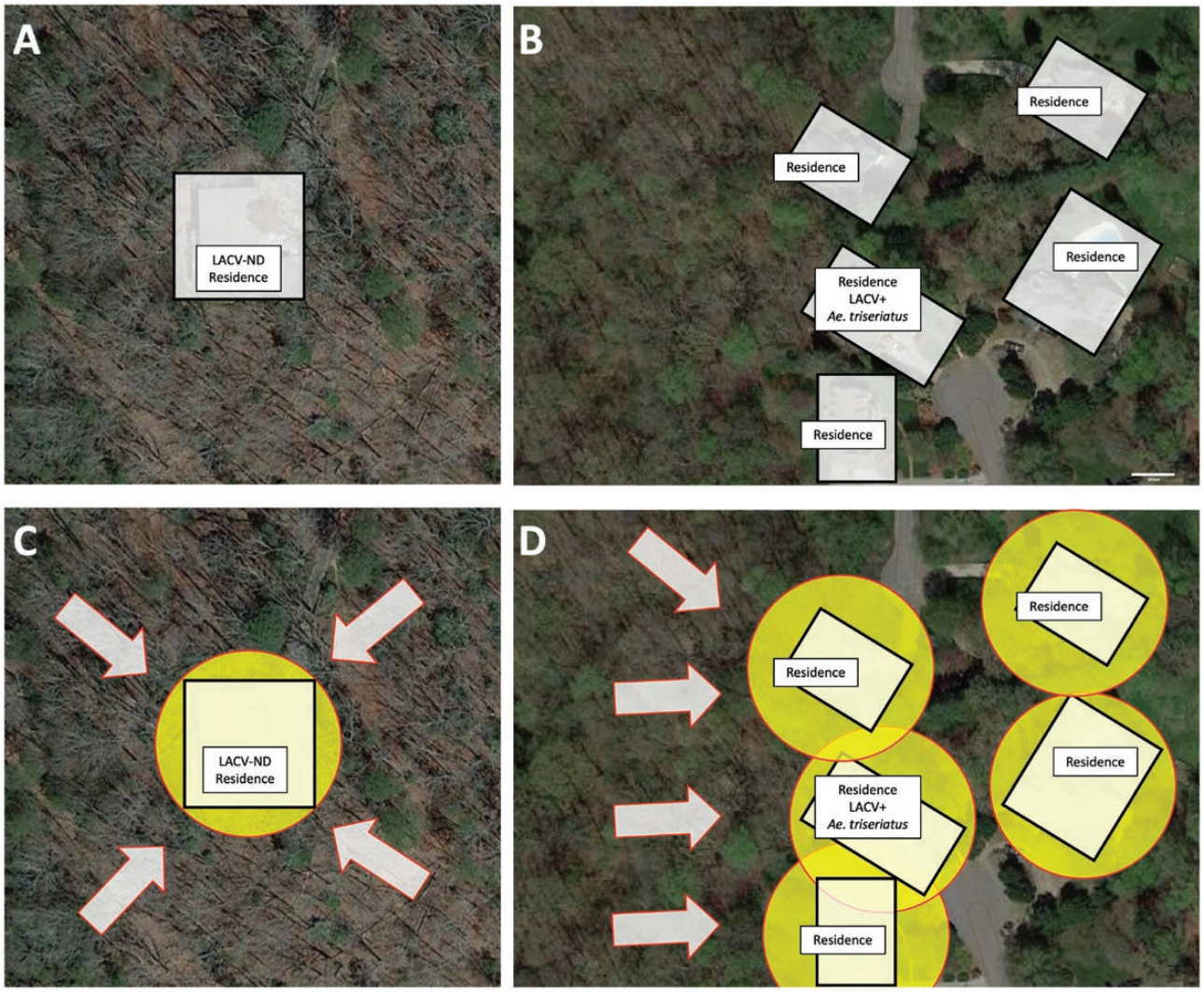

Fig. 4. Typical LACV-ND residences in the southern Appalachian region are commonly defined by limited clearing around the house nested in dense forest (sylvan) habitat (abundant *Aedes triseriatus*). A) An example of a LACV-ND case residence where the isolation, limited driveway approach, and close proximity to dense forest provide multiple challenges for area-wide and integrated vector control. B) An example of a residence with LACV-positive *Ae. triseriatus* 600 m from a second (LACV-ND case) residence (not shown). The close proximity to dense forest, coupled with additional nearby residences, provide different risks and challenges for a community-level integrated vector control. In both settings (C and D), the immediate peridomestic environment (demonstrated within circles) risk is greatly influenced by residential-level factors (e.g., containers, house condition, and shading) but also from the risk of sylvan *Ae. triseriatus* (arrows) from tree holes. Images used with permission by homeowners (parents) and slightly augmented to reduce the likelihood of recognition.

habitat reduction but could also include mosquito-disseminated insect growth regulators to achieve larval control in cryptic habitats. There is thus a need for research on vector management methods (e.g., barrier sprays, source reduction, In2care traps, pyriproxyfen-treated *Ae. albopictus*, and a combination of approaches) at different scales to identify the best tools for prevention and response. These studies can and should be focused at peridomestic sites both in rural communities with single homes (Fig. 4c) largely surrounded by acres of forest and in neighborhoods where multiple residences are at risk (Fig. 4d). Recommendations must be based on field trials within endemic areas with epidemiologically meaningful response variables such as vector exposure (biting) rates, vector LACV infection rates, or seropositivity in animals or humans. The cost and practicality of vector control methods should be carefully considered for the Appalachian region and typical control sites, and applied research studies at sites with known LACV transmission should be conducted to demonstrate the efficacy of LACV control methods to support evidence-based guidance to local agencies.

Detection

Detection of LACV-ND presently relies on state health departments reporting confirmed and probable LACV-ND cases to local health departments. Limited and delayed (e.g., acute/convalescent serology) diagnostic methods are a serious impediment to case recognition, timely clinical care, case reporting, and any subsequent public health measures. Additional challenges complicating LACV-ND detection includes physician knowledge of LACV-ND and laboratory confirmation with an assay that is cross-reactive with other CAL group arboviruses. This is probably best exemplified with the lack of diagnosed cases in eastern Kentucky counties that border highly endemic counties in other states (Day et al. 2023). Non-neuroinvasive LACV disease is very poorly recognized but should be identified in order to evaluate and mitigate the entomological/environmental residential risk. More resources should be focused on improving and evaluating detection methods for the vector and virus.

Limitations in entomologic and arboviral surveillance for LACV have prevented the establishment of vector indices in the context of transmission risk to humans. Entomologic survey methods have included ovitraps, large-bore aspirators, CO_2-baited CDC light traps, BG-Sentinel traps, and gravid traps (Nasci et al. 2000, Barker et al. 2003, Tamini et al. 2021). In addition to differences in the personnel effort required for each method, there are species-specific and gonotrophic biases to most of these methods. Species-specific differences in oviposition behavior and olfactory cues for LACV are poorly defined and likely hinder the ability to develop a "standardized" LACV vector surveillance approach for both the primary and accessory vectors with a single method. In our experience, identifying LACV-infected mosquitoes at case residencies has been challenging, perhaps due to limited sampling effort, LACV resistance developing in the mosquitoes, the lack of species-specific trap design for LACV-positive mosquitoes (specifically, *Ae. triseriatus*), or other factors.

Response

Prior approaches to LACV-ND from both clinical and public health perspectives included statements such as "well, the cat's already out of the bag," implying that there was nothing else to do once a child became ill. We have built a body of evidence documenting that LACV transmission risk is both regionally focal and likely persists at LACV-ND case sites. In this context, we consider effective case response to at least include entomologic/environmental risk assessments and risk mitigation at the residence of the affected individual, ideally with a case investigation to identify other areas of potential transmission (e.g., a public park or school) where transmission risk may be elevated. At sites with potential transmission, disease risk can be reduced through the rapid removal of mosquito larval habitats like discarded tires, application of adulticides, and awareness campaigns encouraging the use of mosquito repellents. If conducted in a timely manner—as soon LACV infection is suspected—then a targeted response should be an effective way to prevent additional human infections. At a minimum, healthcare providers should relay that enhanced personal protection measures should occur at the house while waiting for confirmation of LACV-ND. In our experience, siblings often remain at home without the use of obvious mosquito protection (e.g., use of repellents, wearing protective clothing, etc.), while the LACV-ND child recovers in the hospital.

The nature of the case responses should be guided by evidence-based studies from government agencies or academia, and timeliness will rely on rapid communications between clinicians, public health departments, and mosquito control agencies. At the time of writing this article, evidence-based environmental management and vector control does not occur in or near LACV-ND case sites as part of a formal public health response plan. Working with the CDC and local health departments, communities can establish community response teams that respond to LACV-ND cases and help mitigate community problems. Communities of practice can include varied stakeholders with the skillsets necessary to help affected families within a community.

Summary and Conclusions

Although much is known about the biology of LACV transmission, well-defined prevention and control strategies remain enigmatic. Limited public health infrastructure, lack of political will and funding, co-occurring health burdens, and health disparities help perpetuate LACV-ND as a neglected vector-borne disease in Appalachia. Inconsistent disease surveillance efforts, limited laboratory diagnostics, and lack of LACV-specific chemotherapeutics continue to hinder the clinical care of LACV-ND cases. Vaccine development does not appear to be pragmatic solution at the present time. Economic impediments to development, vaccine reluctance upon approval, and other barriers limit the effectiveness of a LACV vaccine as a primary prevention strategy. At present, prevention measures must instead be focused on reducing human exposure. Community awareness of LACV is unacceptably low within endemic regions and the lack of actionable entomologic and environmental risk indices and limited case–response capacity (i.e., environmental assessments and vector control modalities) allow LACV transmission to continue unconstrained. This is especially problematic given the existing ecological and epidemiological support substantiating the peridomestic risk of LACV transmission. At a minimum, there is a clear duty to act once a LACV-ND case has been identified to reduce the LACV risk for other family members—especially pediatric siblings. The development of a clear *Prevent—Detect—Respond* framework for LACV must be prioritized.

As this framework is being constructed, it also is critically important that evaluations, interventions, and capacity building be focused in endemic areas of Appalachia, where most LACV-ND risk has been persistently reported in recent decades (Day et al. 2023). Every year we anticipate LACV-ND in our region and in our communities. Every year we are called to LACV-ND case residences, and each time we see: the kids are not alright.

Acknowledgments

The authors thank Dakhota Little and Mary Nordgulen for their assistance and creativity in designing figures. Figures 1 and 2 were created using Biorender.com (Fig. 1: Agreement Number: UT25BLWQH0, D. Little; Fig. 2: Agreement Number: FW25BLMFM4, M. Nordgulen). Anders Lindström provided the color macrophotographs used in Fig. 3. We also thank Ary Faraji for the invitation to this forum article and 2 anonymous reviewers for providing helpful comments. Importantly, we wish to acknowledge, and advocate for, the families of children affected by LACV-ND.

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