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Ecology and Epidemiology of Eastern Equine Encephalitis Virus in the Northeastern United States: An Historical Perspective

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Abstract

In the current review, we examine the regional history, ecology, and epidemiology of eastern equine encephalitis virus (EEEV) to investigate the major drivers of disease outbreaks in the northeastern United States. EEEV was first recognized as a public health threat during an outbreak in eastern Massachusetts in 1938, but historical evidence for equine epizootics date back to the 1800s. Since then, sporadic disease outbreaks have reoccurred in the Northeast with increasing frequency and northward expansion of human cases during the last 20 yr. *Culiseta melanura* (Coquillett) (Diptera: Culicidae) serves as the main enzootic vector that drives EEEV transmission among wild birds, but this mosquito species will occasionally feed on mammals. Several species have been implicated as bridge vectors to horses and humans, with *Coquilletstidia perturbans* (Walker) as a leading suspect based on its opportunistic feeding behavior, vector competence, and high infection rates during recent disease outbreaks. A diversity of bird species are reservoir competent, exposed to EEEV, and serve as hosts for *Cs. melanura*, with a few species, including the wood thrush (*Hlocichia mustelina*) and the American robin (*Turdus migratorius*), contributing disproportionately to virus transmission based on available evidence. The major factors responsible for the sustained resurgence of EEEV are considered and may be linked to regional landscape and climate changes that support higher mosquito densities and more intense virus transmission.

Key words: eastern equine encephalitis virus, mosquitoe, vector, northeastern US

Eastern equine encephalitis virus (EEEV) causes a rare, but highly lethal, mosquito-borne illness in humans and horses that represents a growing public health threat in the northeastern United States (Armstrong and Andreadis 2013). The resulting disease of viral encephalitis has a very poor prognosis. The mortality rate of hospitalized patients is approximately one-third and about half of survivors suffer from long-term neurological damage and disability (Ayers and Feemster 1949, Deresiewicz et al. 1997, Lindsey et al. 2020). There are no effective antiviral treatments or commercially available vaccines for use in humans, but a vaccine exists for horses. Therefore, a combination of prevention measures, including mosquito control intervention, public education and outreach, and personal protection measures, that are guided by surveillance data, remain the most effective defense against EEEV infection for the foreseeable future. The effectiveness of these measures requires sustained public investment in surveillance and control programs, and an in-depth understanding of the regional ecology and epidemiology of the virus.

During the past two decades, the number and frequency of human cases have increased in the northeastern United States, culminating into one of the largest EEE outbreaks in history during 2019 (Fig. 1; Lindsey et al. 2020). This outbreak involved 38 human cases nationally, of which 23 occurred in the northeastern region. The factors responsible for this trend of increasing EEE risk are complex and not entirely clear, but likely reflect environmental changes that support higher mosquito densities and more intense virus amplification in the complex transmission cycle. EEEV occurs primarily in forested swamp habitats that are inhabited by the main mosquito vector, *Culiseta melanura* (Coquillett) (Morris 1988). The virus is

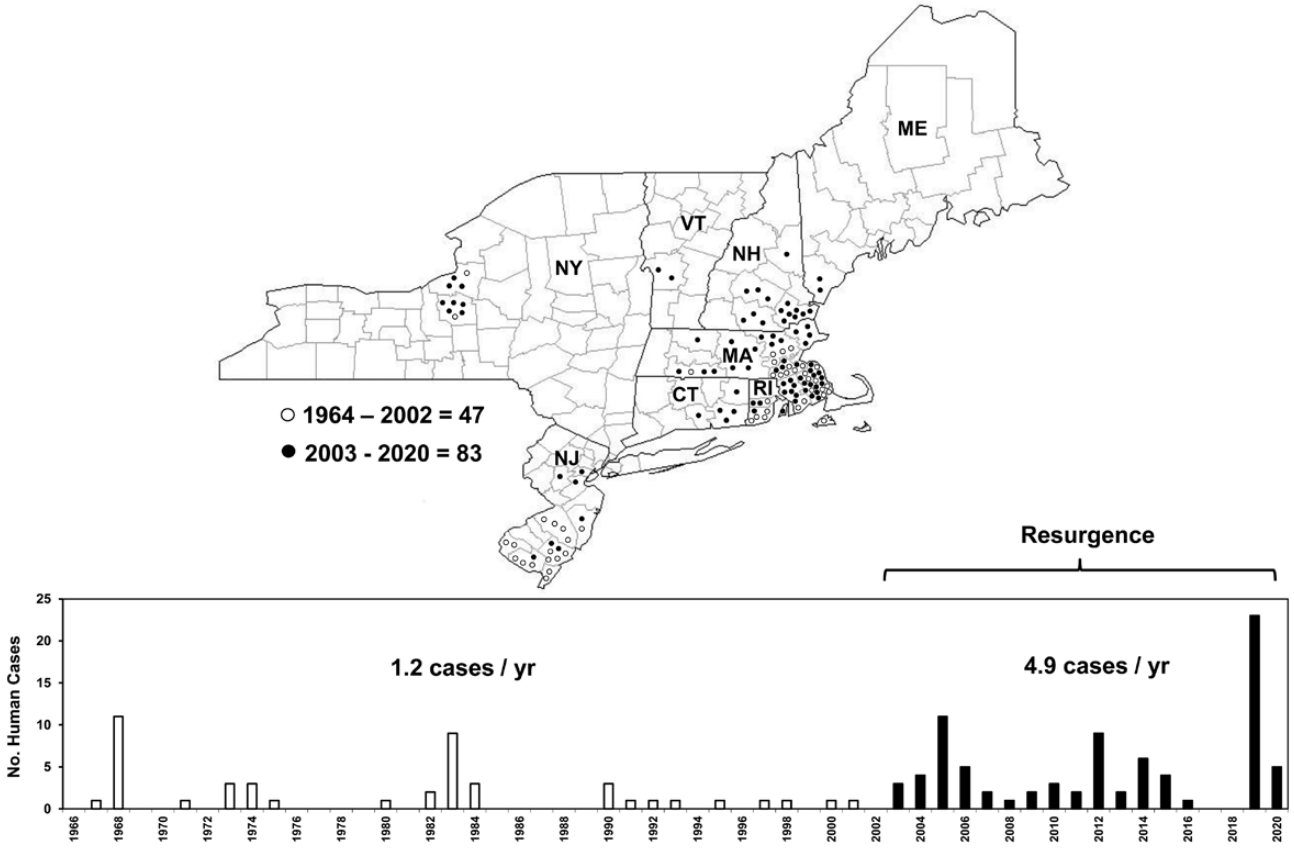

Fig. 1. Geographic distribution and annual number of human cases of eastern equine encephalitis virus in the northeastern United States.

amplified in a bird–mosquito transmission cycle and disease outbreaks occur when the virus overflows into human populations by mosquitoes that feed opportunistically on birds and mammals (Scott and Weaver 1989). Understanding the underlying ecology of this virus and the conditions that lead to disease outbreaks is of intense public health interest and involves untangling the complex web of interactions among the virus, environment, avian hosts, mosquito vectors, and human hosts. The purpose of this article is to review and interpret the literature on the history, ecology, epidemiology, and vector biology of EEEV in the northeastern United States to investigate the major drivers of disease outbreaks. Such an understanding will be essential to countering this threat in the years ahead.

History

EEE epizootics and epidemics occur sporadically in the northeastern United States, with the first evidence of the disease recorded in Massachusetts (MA) in 1831 (Hanson 1957). During this outbreak, 75 horses died of a neurological disease from midsummer to early fall in the eastern part of the state, which fits with the current disease patterns. Another equine epizootic occurred on Long Island, New York (NY) in 1845 that was also clinically and epidemiologically consistent with EEE (Giltner and Shahan 1939). However, formal recognition of EEE as a distinct clinical entity came much later during a series of disease outbreaks in the 1930s. EEEV was first isolated and implicated in the etiology of equine disease during an epizootic affecting coastal areas of Delaware, Maryland, New Jersey (NJ), and Virginia in 1933 (Giltner and Shahan 1933, Ten Broeck and Merrill 1933). The virus was shown to be serologically distinct from virus strains isolated from horses in California, now known as western equine encephalitis virus (Ten Broeck and Merrill 1933). Soon thereafter, EEEV was established as an agent of human disease when the virus was recovered from patients during a large outbreak in MA in 1938 (Webster and Wright 1938). Hundreds of equine cases and 38 human cases with 25 fatalities were reported from the eastern third of MA (Feemster 1938). In addition, a smaller EEE outbreak occurred in the neighboring states of Connecticut (CT) and Rhode Island (RI) that same year, involving dozens of equine cases and multiple pheasant flocks (Andreadis 1993, Gettman 1993).

During the following decades, EEE outbreaks struck at irregular intervals in certain geographic regions in the northeastern United States. Massachusetts has reported more human cases than any other state except Florida and the most cases during the last 10 yr, with the majority occurring in the southeastern part of the state (Fig. 1). This region supports a vast wetland called the Hockomock Swamp that serves as an important refuge for wild birds and provides excellent habitat for *Cs. melanura* mosquitoes. Southern NJ is also a historical hot-spot of EEE with epizootics recorded during the 1930s followed by a number of epidemics in later decades (Ten Broeck et al. 1935). The largest outbreak occurred in 1959 involving 33 human cases of encephalitis (Goldfield and Sussman 1968). Retrospective serosurveys after the epidemic revealed that many more residents had developed antibodies to the virus and from these data, the authors estimated that 1 in 23 (4.3%) human infections resulted in overt encephalitis (Goldfield et al. 1968b). Another important focus of EEE was later discovered in upstate NY adjacent to Oneida Lake after the first in-state human case was recognized in 1971 (Howard et al. 1994). This site is located much further inland and is more isolated from other foci in the Northeast. Eastern

CT and RI comprise a fourth focal area with sporadic disease outbreaks dating back to 1938 and possibly earlier based on historical accounts (Andreadis 1993, Gettman 1993). Finally, the southern portions of Maine (ME), New Hampshire (NH), and Vermont (VT) represent an emerging expansion front for the virus. The first human cases were recorded in these states from 2005 to 2014 (Centers for Disease Control and Prevention 2006, Saxton-Shaw et al. 2015).

The virus transmission cycle and mechanisms of human and equine infection were the subject of early investigations on EEEV. The virus was suspected to be insect-borne based on circumstantial evidence acquired during the 1933 outbreak in the mid-Atlantic states (Ten Broeck et al. 1935). Equine cases clustered in proximity to wetland habitats following the peak of the mosquito season in late summer. In 1934, laboratory investigations showed that *Aedes sollicitans* (Walker) and *Aedes cantator* (Coquillett) could acquire and transmit EEEV to a susceptible host (Merrill et al. 1934). Recovery of the virus from field-captured mosquitoes, a necessary condition for incriminating the vector, came much later. EEEV was first isolated from *Coquillettidia perturbans* (Walker) in 1949 followed by *Cs. melanura* in 1951 (Howit et al. 1949, Chamberlain et al. 1951a). Numerous virus isolations were made from field-collected mosquitoes since then, with the majority coming from *Cs. melanura*. This led to the growing recognition that *Cs. melanura* served as the primary enzootic vector. This hypothesis was further supported during vector-competence trials in the 1970s, which confirmed that this species could experimentally transmit the virus (Howard and Wallis 1974).

Soon after the discovery of EEEV, birds were implicated as the main amplification hosts for the virus based on epidemiological observations (Ten Broeck et al. 1935). The late summer spread of EEE cases beyond the flight range of mosquitoes suggested that migratory birds served as principle hosts and means for disseminating the virus over long distances. Subsequent studies demonstrated that passerine birds were competent reservoir hosts for EEEV and could readily infect mosquitoes in in the laboratory (Davis 1940). EEEV was initially isolated from domestic pheasants and pigeons in 1938 and then later from wild passerine birds in 1950 (Fothergill et al. 1938, Tyzzer et al. 1938, Kissling et al. 1951).

Enzootic Vectors

Culiseta melanura

EEEV has been isolated or detected in at least 21 different species of mosquitoes in the northeastern United States (ArboNET, Centers for Disease Control and Protection, Atlanta, GA). However, it is well established that *Cs. melanura* is the primary enzootic vector and that it is the key species that largely drives the transmission cycle among wild passeriform birds. This presumption is based on a number of observations and research findings made over several decades that include the following:

(1) The large number and repeated frequency of viral infections detected in field-collected females, especially during outbreak years (Howard et al. 1988, Edman et al. 1993, Andreadis et al. 1998, Oliver et al. 2020, McMillan et al. 2020). For example, from 2004 to 2009, more than 73% of all EEEV positive pools (n = 1,056 representing 21 species) reported to CDC ArboNET for the entire northeast region (CT, MA, ME, NH, NJ, NY, RI) were from *Cs. melanura*. Similarly, during the recent outbreak year of 2019, 45–89% of all EEEV positive mosquito pools (n = 683) tested from CT, MA, NJ, and NY were from *Cs. melanura* (Fig. 2). In CT mosquito/arbovirus surveillance program, where all field-collected mosquitoes are tested for EEEV by viral isolation in Vero cells, 64.4% of all EEEV isolations (n = 534, 19 species) made over the last 24 yr (1996–2019) have been from *Cs. melanura*.

(2) High level of laboratory vector competence. This was first demonstrated by Howard and Wallis (1974) who successfully infected colonized *Cs. melanura* (Farmington, CT) by allowing females to feed on 1-d-old viremic chicks. In their study, the approximate minimal mosquito infective dose was 10^4 baby mouse LD50/0.02 ml, the approximate 50% dose was 10^5, and the approximate 90% dose was 10^6 LD50/0.02 ml. Furthermore, over 85% of infected mosquitoes transmitted EEEV to baby chicks after a 2-wk extrinsic incubation. Vaidyanathan et al. (1997) similarly reported infection rates of 100% in *Cs. melanura* that fed on viremic chicks with titers between 10^5 and 10^9 plaque forming units (PFU)/ml, and a 94% transmission rate after 7 and 14 d based on saliva infection.

(3) Rapid dissemination and high virus titers in infected mosquitoes required for efficient transmission. In a series of experiments designed to elucidate the timing and distribution of EEEV to various tissues and organs in adult female *Cs. melanura* (Scott and Burrage 1984, Scott et al. 1984, Scott and Weaver 1989, Weaver et al. 1990), rapid dissemination of infectious virus to the salivary glands was observed within 2–3 d of extrinsic incubation. Consistent with these observations, Armstrong and Andreadis (2010) estimated the infection prevalence and virus titers in 14 naturally infected field-collected mosquito species following an outbreak in CT in 2009 by cell culture, plaque titration, and quantitative RT–PCR, and found that *Cs. melanura* was the only species to support consistently high virus titers (mean = 6.55 $\log_{10}$ PFU/mosquito pool) required for efficient transmission.

(4) Strong feeding preference of adult females for birds. A substantial number of investigations conducted on local populations of *Cs. melanura* from the Northeast have repeatedly and consistently shown that females feed largely on passeriform birds (Magnarelli 1977; Nasci and Edman 1981a; Molaei and Andreadis 2006; Molaei et al. 2006, 2013, 2015, 2016). As many as 65 different avian species have been identified as hosts for *Cs. melanura* from the northeast, among which wood thrush (*Hlocichia mustelina*), American robin (*Turdus migratorius*), tufted titmouse (*Baeolophus bicolor*), and black-capped chickadee (*Poeecile atricapillus*) have been further implicated as key species in certain regions (Fig. 3). Infrequent feeding on mammalian hosts, including humans and horses, has been identified in *Cs. melanura* populations from the northeast, but appears to be quite rare and variable depending on locale. Recent analyses of blood meals from female *Cs. melanura* collected from known foci of EEEV activity in CT, MA, NY, and VT have shown rates of mammalian feeding of 0.3, 1.1, 5.8, and 6.0%, respectively (Molaei and Andreadis 2006; Molaei et al. 2006, 2013, 2015, 2016). These observations are significant because they clearly demonstrate that *Cs. melanura* can contribute to epizootic and epidemic transmission among equines and humans, a topic that has been subject to debate and has important public health implications. These apparent variations in blood feeding behavior among regional populations most likely represent differences in host availability and/or environmental factors. However, genotyping of *Cs. melanura* populations from 10 EEEV foci in the eastern United States ranging from Florida to Canada (Soghigian et al. 2018) has revealed genetic differentiation

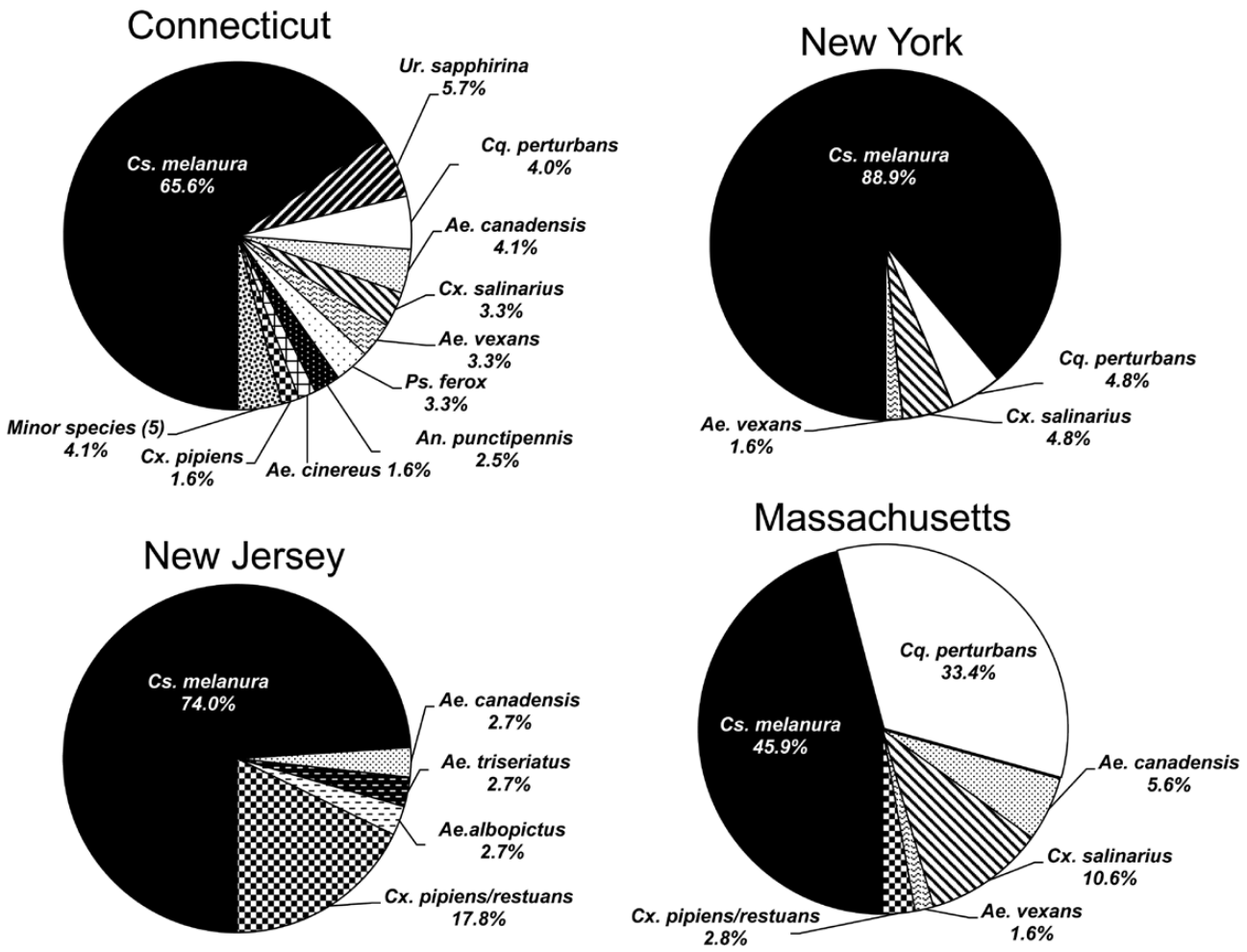

Fig. 2. Species composition of eastern equine encephalitis virus-positive mosquito pools reported by statewide surveillance programs during the 2019 epidemic.

between northern and southern populations and limited fine-scale population structure throughout the northeastern United States, suggesting local differentiation and underlying genetic variations among these populations. What bearing, if any, these differences may have on host feeding behavior remains to be determined.

(5) Consistently, high populations in freshwater hardwood swamps that serve as established EEEV foci. In the northeastern United States, EEEV largely occurs in proximity to inland swamp habitats that support prodigious populations of *Cs. melanura*, and epizootic and epidemic activities in areas adjacent to these swamp sites are almost always associated with above average populations (Wallis et al. 1974, Morris et al. 1980, Howard et al. 1988, Andreadis 1993, Edman et al. 1993, Andreadis et al. 1998). Long-term studies in MA have shown a strong relationship between above average trap collections of *Cs. melanura*, especially early in the season, and subsequent EEEV activity (Edman et al. 1993). Consistent with this, we have observed a significant positive correlation ($P < 0.001$, $r = 0.69$) between the number of adult female *Cs. melanura* collected in CO_2 baited traps and the number of EEEV isolations made from the species in long-term surveillance conducted in CT over the last 24 yr (1996–2019) (Fig. 4).

Given the critical role of *Cs. melanura* in the epizootiology of EEEV, as highlighted above, it is appropriate to examine our current knowledge concerning its life cycle, behavioral characteristics, and overall biology. In the northeast, *Cs. melanura* inhabits densely wooded freshwater swamps and sphagnum bogs (Morris et al. 1976, Means 1987). A recent analysis of wetland characteristics and *Cs. melanura* abundance in CT has shown forested deciduous and to a lesser degree, forested evergreen wetlands are most associated with *Cs. melanura* abundance (Skaff et al. 2017). In CT, these swamps are typically dominated by Atlantic white cedar (*Chamaecyparis thyoides*), red maple (*Acer rurum*), yellow birch (*Betula alleghaniensis*), and eastern hemlock (*Tsuga canadensis*) with a well-developed understory of mountain laurel (*Kalmia latifolia*) and extensive *Thudidium delicatulum* and *Sphagnum* spp. moss ground cover (Andreadis et al. 2012). In EEEV foci located in southeastern MA, *Cs. melanura* likewise occurs mainly in Atlantic white cedar and red maple swamps (Komar and Spielman 1994), and in coastal regions of NJ, the mosquito reaches greatest numbers in Atlantic white cedar swamps, while in inland EEEV foci, *Cs. melanura* is associated with swamps comprised mainly of red maple (Crans et al. 1994).

Immature *Cs. melanura* develop in subterranean 'crypts' beneath mats of *Sphagnum* moss with dense fern growth and in deep water filled cavities that form under the roots of uprooted trees (Pierson and Morris 1982, Andreadis et al. 2012; Fig. 5) where water temperatures typically remain below 20°C most of the summer (Mahmood and Crans 1998). The habitat is stable, well shaded, and generally

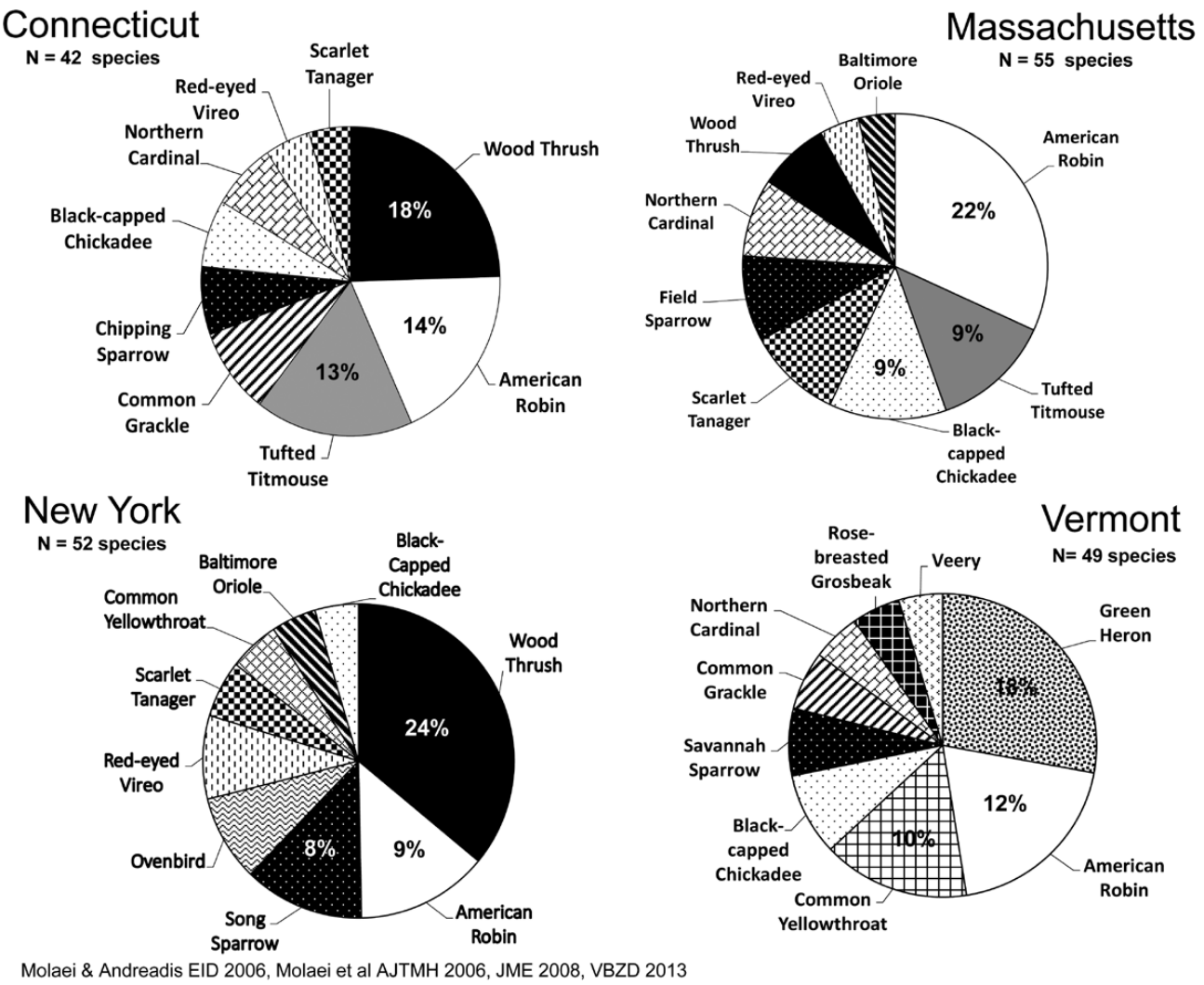

Fig. 3. Avian hosts identified from blood-fed *Culiseta melanura* collected in Connecticut, Massachusetts, New York, and Vermont. The nine most common host species are indicated in pie charts.

contains cool acidic water throughout most of the year. Larval development is notably slow, extending over a period of 2–3 mo. Under controlled conditions, egg hatch to adult emergence takes 8 mo at 10°C, 3 mo at 16°C, and 1 mo at 22°C (Mahmood and Crans 1998). In the northeastern United States, there are typically one overwintering and two to three overlapping summer generations a year (Morris et al. 1976, Mahmood and Crans 1998, Andreadis 2002). *Culiseta melanura* overwinters in the larval stage as a heterogeneous assemblage of second through fourth instars during a period of quiescence induced by low temperature rather than short photoperiod (Maloney and Wallis 1976, Andreadis et al. 2012). Larvae undergo no development during this period and do not appear to be severely affected by any measurable mortality (Andreadis 2012), as they exhibit a marked resistance to cold temperatures (Mahmood and Crans 1998). Laboratory studies (Maloney and Wallis 1976) have shown all four instars can survive in a state of arrested development at least 2 mo at 4°C with little mortality, provided they are cooled gradually. Even under conditions where water temperatures within the crypts result in freezing, *Cs. melanura* larvae are known to avoid freezing by burrowing into mud up to a depth of 15 cm where temperatures do not go below 1.4°C, and then return to open water as spring approaches (Hayes 1961). Detailed investigations conducted in CT (Andreadis et al. 2012) have shown that pupation begins in early April and is characterized by a prolonged period of pupation that encompasses a minimum of 5 wk. This results in a staggered emergence of adults that begins in mid-May, and an overlap of the residual overwintering population with larvae of the first summer generation. It is has been suggested that early season EEEV amplification within a region is directly related to the size, survival, and age structure of overwintering larval population (Mahmood 2002).

Adults are active from June through November and eggs laid by the late-fall brood produce larvae that make up the overwintering generation that emerge the following spring (Mahmood and Crans 1998). Host seeking activity by adult females begins shortly after sunset and continues at a relatively constant level throughout the night (Nasci and Edman 1981b). Host seeking ceases with the onset of morning twilight (1 h before sunrise), and flight behavior shifts toward the location of day-time resting sites. This appears to be a well-adapted behavioral pattern, as evening host seeking coincides with the roosting period of their preferred avian hosts (Hayes 1962).

Several studies have been undertaken at EEEV foci in central NY to assess the population dynamics and flight capabilities of adult *Cs. melanura* as is relates to the dispersal of EEEV from swamp foci to

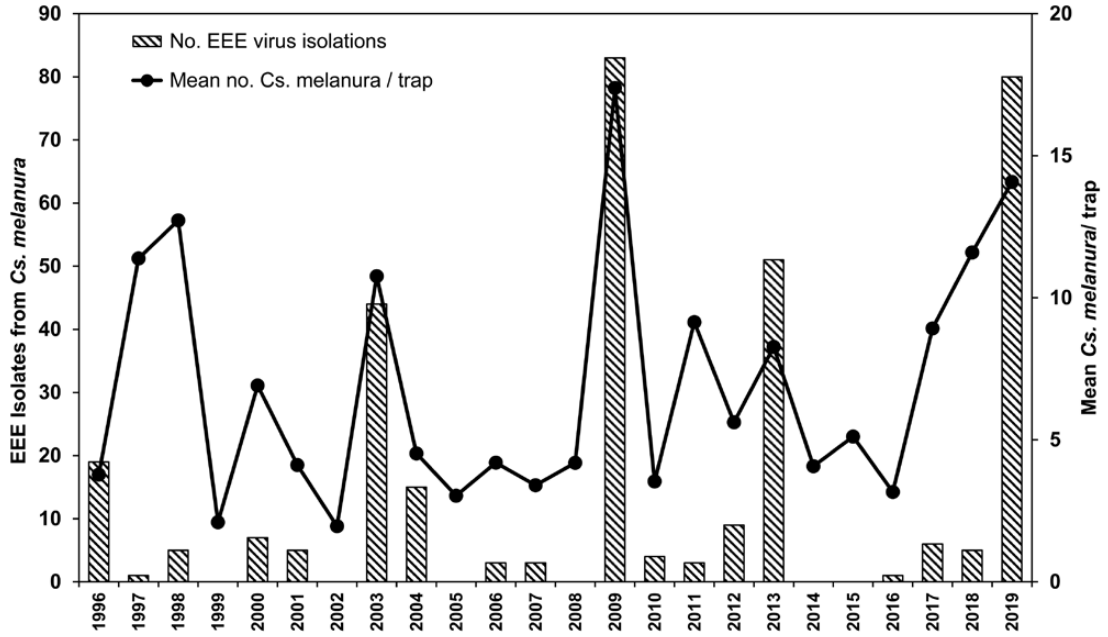

Fig. 4. Annual number of eastern equine encephalitis virus isolations from mosquitoes and mean number of *Culiseta melanura* collected in CO_2-baited CDC light traps during statewide surveillance in Connecticut.

Fig. 5. Example of larval habitat for *Culiseta melanura*. Larvae develop in deep water-filled cavities that form under the roots of swamp trees.

surrounding uplands. In an early study, Morris et al. (1980) demonstrated that adult *Cs. melanura* remain in the swamp environment when populations are low or during dry adverse weather conditions. However, during periods of high populations or warm, wet weather, older females were found to disperse up to 2 km from the larval habitat. Pierson and Morris (1982) further established that in this region of upstate NY, *Cs. melanura* breed primarily near the swamp edge. This led them to conclude that the vector potential of *Cs. melanura* was greatest in areas peripheral to the breeding swamps. This was also seen to strengthen the hypothesis that *Cs. melanura*, though an unlikely epidemic vector, serves as an epizootic vector of EEEV and is responsible for dispersing the virus from the swamp to domestic and peridomestic birds. In an attempt to quantify the flight capabilities of the species, Howard et al. (1989) conducted a mark–recapture study with *Cs. melanura* at the same swamp foci in central NY wherein they found that even at low densities, adults routinely traveled 4 km, and that flights up to 10.8 km could occur. This supported the earlier premise that females are capable of transferring EEEV to upland areas where they could infect local bird species that serve as the virus source for other mosquitoes [i.e., *Aedes canadensis* (Theobald), *Cq. perturbans*], which may transmit virus to human or equine hosts. In a subsequent study, Howard et al. (1996) demonstrated a further role for infected *Cs. melanura* in seeding EEEV into adjacent swamp complexes as well as upland sites, wherein local populations of *Cs. melanura* could amplify the virus in resident bird and mosquito populations and continue to disperse virus into upland and other neighboring swamps consistent with a 'rolling' or expanding epizootic/epidemic.

Culiseta morsitans

Culiseta morsitans (Theobald) has also been implicated as a potentially important, although clearly a secondary enzootic vector of EEEV. The biological and epidemiological potential of the species closely matches that of *Cs. melanura*, especially in central NY (Morris and Zimmerman 1981, Morris 1984, Howard et al. 1988), but it is not nearly as abundant nor widespread throughout other areas of northeast, and it is far less frequently found infected with EEEV even during outbreak years (Edman et al. 1993, Andreadis et al. 1998, ArboNET, Centers for Disease Control and Protection, Atlanta, GA). Like *Cs. melanura*, this species develops in heavily shaded pockets of water in root system hummocks of standing trees and other concealed water-filled depressions in the swamp floor associated with *Sphagnum* moss and dense fern growth (Pierson and Morris 1982). However, *Cs. morsitans* also may be found in open woodland marshes close to the roots and trunks of trees and in open cattail marshes with dense clusters of emergent vegetation (Means 1987). Unlike *Cs. melanura*, which readily utilizes swamp-interior resting and oviposition sites, *Cs. morsitans* exclusively utilizes the swamp edge (Pierson and Morris 1982). The species is long-lived and univoltine with peak adult emergence in mid-June to early July (Morris and Zimmerman 1981, Howard et al. 1988). *Culiseta morsitans* has been found to be locally abundant in EEEV foci in central NY during the June–July virus amplification period and in epidemic foci during July–August (Morris et al. 1976, Morris

and Zimmerman 1981, Howard et al. 1988). Females are strongly ornithophilic, feeding almost exclusively on passeriform birds (Magnarelli 1977; Morris and Zimmerman 1981; Nasci and Edman 1981a; Molaei et al. 2006, 2013, 2015; Shepard et al. 2016), and have only occasionally been found to contain mammalian-derived blood meals (Molaei et al. 2006, 2013). However, it should be noted that in an analysis of blood meals from field-collected *Cs. morsitans* from an endemic focus of EEV in central NY, a moderate proportion (11.5%) of blood meals were from avian and mammalian sources (Molaei et al. 2006). In summary, it seems clear that the strong ornithophilic feeding habits of *Cs. morsitans* largely rule out any significant role as a bridge vector to mammalian hosts. However, it has been suggested that in central NY, *Cs. morsitans* likely contributes to enzootic amplification of EEEV among passeriform birds during late summer dispersal (Morris et al. 1980, Morris and Zimmerman 1981).

Bridge Vectors

The list of 'potential bridge' vectors of EEEV in the northeastern United States includes at least a dozen species that exhibit feeding patterns that include both avian and mammalian hosts. However, the following species are considered the most likely vectors based on the number and frequency of virus isolations from field-collected females, laboratory vector competence and both host and habitat associations: *Ae. canadensis*, *Ae. sollicitans*, *Aedes vexans* (Meigen), *Anopheles punctipennis* (Say), *Anopheles quadrimaculatus* (Say), *Cq. perturbans*, and *Culex salinarius* (Coquillett) (Crans and Schulze 1986, Crans et al. 1986, Vaidyanathan et al. 1997, Moncayo and Edman 1999, Molaei et al. 2008). Within this group, there appears to be differences across various geographic environs within the region.

Along the NJ coast where virus outbreaks have historically occurred, the salt marsh mosquito, *Ae. sollicitans* has been implicated as the most probable vector of EEEV (Hayes et al. 1962; Crans 1977; Crans et al. 1986, 1990). Although the species is strongly oriented toward mammalian hosts with only occasional feeding on birds (Crans et al. 1990, Molaei et al. 2008), it is a highly efficient laboratory vector (Chamberlain et al. 1954b) and has been shown to acquire EEEV during epizootic periods when local populations of *Cs. melanura* are undergoing marked population increases (Crans et al. 1986) and when human and equine outbreaks have occurred in the past (Goldfield et al. 1966, 1968a; Goldfield and Sussman 1970).

In the toad Harbor-Big Bay and Cicero swamp EEEV foci in upstate central NY, *Ae. canadensis* and, to a lesser degree, *Cq. perturbans* have been incriminated as the most likely bridge vectors to humans and horses. This conclusion is based on the multiple isolations of EEEV from *Ae. canadensis* and high population densities of both species during a major outbreak in 1983 that involved one human and nine equine cases (Howard et al. 1988). EEEV has also been frequently detected in *Ae. canadensis* throughout the northeast, including CT, MA, NJ, NH, NY, and RI (see Fig. 2; Morris et al. 1975, Edman et al. 1993, Andreadis et al. 1998, Armstrong and Andreadis 2010, ArboNET, Centers for Disease Control and Prevention, Atlanta, GA). *Aedes canadensis* is a moderately competent vector (Vaidyanathan et al. 1997) and virus titers considered minimal for transmission (3.2 $\log_{10}$ PFU) have been documented in field-collected specimens (Armstrong and Andreadis 2010). Although females feed mainly on mammals, including horses and humans, avian blood meals have been identified (Magnarelli 1977, Molaei et al. 2008).

In CT, EEEV has been isolated from 21 different species of mosquitoes collected statewide over a 24 yr. period (1996 to 2019). Among potential bridge vectors, the highest proportion of viral isolates (n = 534) after *Cs. melanura* have come from *Ae. canadensis* (6.9%), *Aedes cinereus* Meigen (3.7%), *Ae. vexans* (3.5%), *Uranotaenia sapphirina* (Osten Sacken) (3.6%), and *Cx. salinarius* (3.0%). *Aedes canadensis* is the most frequently trapped mosquito in CT and is found in a variety of habitats including freshwater hardwood EEEV swamp foci located in the southeastern corner of the state. Adult populations peak late June to early July but extend well into fall (Andreadis et al. 2005), particularly if a second hatch occurs with late season periods of heavy rainfall. Despite the frequent isolation of EEEV from field-collected females, the relative contribution of *Ae. canadensis* to epizootic/epidemic transmission in CT may be limited due to the detection of relatively low virus titers (<3.0 $\log_{10}$ PFU/ml) in field-collected mosquito pools of the species (Nasci and Mitchell 1996, Armstrong and Andreadis 2010).

EEEV-infected *Ae. cinereus* collected in CT have been found to harbor viral titers (3.5 $\log_{10}$ PFU/ml) sufficient for transmission (Armstrong and Andreadis 2010). However, the ability of this species to transmit EEEV has not been evaluated in the laboratory and thus, its contribution as a bridge vector is at best uncertain. Females show a strong preference for mammalian hosts, but the species does feed on birds as well (Magnarelli 1977, Nasci and Edman 1981a, Molaei et al. 2008). Adults are abundant from June through October and are frequently found in habitats that support concurrent populations of *Cs. melanura* (Andreadis et al. 2005).

Aedes vexans is a multivoltine floodwater species that develops in a wide variety of temporary freshwater pools and depressions in open woodland areas and flooded fields (Andreadis et al. 2005). It has been frequently cited as a suspect bridge vector of EEEV in the northeastern United States (Hayes et al. 1962, Nasci and Edman 1981a, Edman et al. 1993, Komar and Spielman 1994), and was the first mosquito from which EEEV had been isolated in CT (Wallis et al. 1960). Its incrimination as a possible bridge vector largely stems from its production of large, mid- to late season broods following heavy rains that often coincide with EEEV outbreaks (Edman et al. 1993) and its aggressive human biting behavior. However, laboratory vector competence trials have rated this species as an inefficient vector (Chamberlain et al. 1954c, Vaidyanathan et al. 1997) and low virus titers (<3.0 $\log_{10}$ PFU/ml) have been found in naturally infected females from two independent studies (Nasci and Mitchell 1996, Armstrong and Andreadis 2010), therein suggesting a negligible role for the species.

The role of *Ur. sapphirina* is enigmatic. Although a fair number of EEEV isolations have been made from this species in CT, especially during epizootic outbreaks (Fig. 2), its vector competence has not been evaluated and extremely low virus titers insufficient for transmission have been found in infected females (mean titer = 1 $\log_{10}$ PFU/ml; Armstrong and Andreadis 2010). Larvae are most frequently found in permanent and semipermanent ponds and swamps with abundant emergent and floating vegetation such cattails and duckweed (Means 1987, Andreadis et al. 2005), but in Suffolk County, Long Island, NY, larvae have often been collected in open sphagnum bogs around the trunks of trees and in cavities beneath blankets of moss in association with *Cs. melanura* (Means 1987). The species is multivoltine and although its preferred hosts are largely unknown; an analysis of a limited number of engorged females collected in CT has revealed a mixture of avian, reptilian, and mammalian (including human) blood meals (Molaei et al. 2008). Interestingly, *Ur. sapphirina* was found to feed readily on annelid

worms in Florida, but it is unknown whether northeastern populations share this feeding behavior (Reeves et al. 2018).

Culex salinarius is one of the most frequently captured *Culex* species in coastal regions of the northeastern United States and is recognized as an important bridge vector of West Nile virus in this region (Andreadis et al. 2001, 2004; Andreadis 2012; Rochlin et al. 2019). Larvae develop in brackish and freshwater wetlands, often associated with *Phragmites*. The species is multivoltine, and in CT, adults are locally abundant in August and September when EEEV activity is at its height (Andreadis et al. 2001, 2004, 2005). It is a highly competent laboratory vector (Vaidyanathan et al. 1997), but low virus titers, insufficient for transmission, have been found in field-collected infected females (n = 3 mosquito pools, mean titer = 1.3 $\log_{10}$ PFU/ml; Armstrong and Andreadis 2010). *Culex salinarius* exhibits the most catholic feeding behavior of any species that has been incriminated as a likely bridge vector in this region. Local populations feed indiscriminately on birds and mammals and readily bite humans (Crans 1964, Means 1987, Apperson et al. 2002, 2004, Molaei et al. 2006). Despite the apparent low virus titers found in infected females, the frequent isolations of EEEV from this species especially during outbreak years, in concert with its local abundance, broad feeding habits, and demonstrated vector competence make *Cx. salinarius* a likely bridge vector to humans and horses in northeastern United States.

Studies have been conducted in MA to assess the relative contribution of several suspect bridge vectors of EEEV in established foci located in the southeastern region of the state. Based on estimates of laboratory vector competence, frequency of virus isolations from field-collected mosquitoes, host seeking, and mixed blood-feeding behavior coinciding with human disease, Vaidyanathan et al. (1997) ranked the following species from most to least probable bridge vectors: *Cx. salinarius*, *An. quadrimaculatus*, *Ae. canadensis*, *Cq. perturbans*, *Ae. vexans*, and *An. punctipennis*. Incorporating flight range, population abundance, and both spatial and temporal overlap at EEEV foci of know transmission during epidemic months, Moncayo and Edman (1999) further implicated *Cq. perturbans*, *Ae. canadensis*, and *Cx. salinarius* as more likely bridge vectors of EEEV in MA than either *Ae. vexans*, *An. Punctipennis*, or *An. quadrimaculatus*.

Among these species, *Cq. perturbans* is now recognized as the leading suspect for transmission to humans and horses. This is largely based on recent surveillance activities in which the virus has been consistently detected in *Cq. perturbans* populations from EEEV foci, especially in outbreak years that involve human cases. This was underscored during the 2019 outbreak that included 12 human cases in the state, where over one-third of all EEEV-positive mosquito pools were from *Cq. perturbans* (Fig. 2). Equally significant were the observations made most recently in 2020 (five human cases), wherein an impressive 68% (n = 66) of all EEEV detections were reported from *Cq. perturbans* (MA Department of Public Health, https://www.mass.gov/info-details/massachusetts-arbovirus-results-summary#mosquito-detection-of-eee/wnv-). Moreover, the first detections of EEEV from *Cq. perturbans* were made as early as 1 July, several weeks prior to the onset of the first human case (20 July), and continued to be made through mid-August. It perhaps notable that only 30% of all EEEV-positive pools documented during the outbreak were from *Cs. melanura*.

Coquillettidia perturbans is moderately competent for EEEV transmission (Vaidyanathan et al. 1997) and infected field-collected females have been shown to contain ample virus titers (>3.0 $\log_{10}$ PFU/ml) sufficient for transmission (Nasci and Mitchell 1996). *Coquillettidia perturbans* is an abundant and commonly trapped species that develops in permanent bodies of water with muddy substrates and abundant emergent vegetation such as cattails that frequently abut swamp habitats that support *Cs. melanura* (Edman et al. 1993). Host-seeking females emerge as a single generation that peak in early July and then decline by mid-August when EEEV typically begins to amplify in most years (Edman et al. 1993, Andreadis et al. 2005). However, Moncayo and Edman (1999) reported that *Cq. perturbans* continue to be the most abundant and consistent species in certain sites in southeastern MA during the time of EEEV transmission in mid- to late August. Females are aggressive human biters and are mostly mammalophilic, but will also feed on a variety of birds, amphibians, and, to a lesser degree, reptiles (Crans 1964; Means 1968, 1987; Nasci and Edman 1981a; Apperson et al. 2004; Molaei et al. 2008; Shepard et al. 2016).

Avian Hosts

Passerine birds have been identified as the main amplification hosts that are necessary for supporting EEEV transmission in nature. This knowledge is based on decades of research showing that passerines develop sufficient viremias to infect susceptible mosquitoes, serve as the main hosts of *Cs. melanura* mosquitoes, and are frequently infected in nature. In the following discussion, we review the evidence implicating birds in the natural history of EEEV and the contribution of different species in supporting virus transmission.

Relatively few bird species have been evaluated for reservoir competence, which remains a knowledge gap in the literature. These experiments are difficult to perform because they require high levels of animal biocontainment and specialized training to capture, maintain, infect, and bleed wild birds. Moreover, EEEV is listed as a select agent, which creates additional regulatory oversight and hurdles when conducting research with this virus. Nevertheless, a handful of studies have been published to report that virtually all passerine bird species tested supported sufficient viremias (>10^3 PFU/ml) required to infect susceptible mosquitoes (Davis 1940, Kissling et al. 1954, Komar et al. 1999, Owen et al. 2011). Species tested included house sparrow (*Passer domesticus*), brown-headed cowbird (*Molothrus ater*), northern cardinal (*Cardinalis cardinalis*), common grackle (*Quiscalus quiscula*), gray catbird (*Dumetella carolinensis*), European starling (*Sturnus vulgaris*), red-winged blackbird (*Agelaius phoeniceus*), American robin, and song sparrow (*Melospiza melodia*), but sample sizes were small (n < 3) for some of these species. European starling was notable because they developed a fulminant EEEV infection that resulted in a higher viremia and mortality than for the other bird species evaluated. Other species tested included domestic pigeon (*Columba livia*), mourning dove (*Zenaida macroura*), great egret (*Ardea alba*), glossy ibis (*Plegadis falcinellus*), white ibis (*Eudocimus albus*), and snowy egret (*Egretta thula*) (Davis 1940, Kissling et al. 1954, McLean et al. 1995, Komar et al. 1999). All of these species developed viremias after experimental infection, but their titers tended to be lower than that of their passerine counterparts.

Avian species that are frequently fed upon by the main vector could serve as effective amplifying hosts for EEEV. To measure vector–host contact rates, a number of studies have analyzed blood-fed *Cs. melanura* from enzootic sites by PCR and sequencing techniques to identify vertebrate hosts. This approach represents a significant advance in our understanding of mosquito blood-feeding patterns. Earlier studies had relied on serological techniques using broadly reactive antisera but these techniques have insufficient resolution to identify avian blood meals to the species level (Magnarelli

1977, Nasci and Edman 1981a). Molecular analyses of *Cs. melanura* blood meals showed that this species feeds on a wide diversity of bird species (42–55 species) with certain orders, families, and species identified as common hosts (Fig. 3; Molaei and Andreadis 2006; Molaei et al. 2006, 2013, 2015, 2016). Passeriformes comprised the majority of blood meals (77.7–97.5%) in *Cs. melanura* populations sampled from CT, NY, MA, and VT. Members of the family Turdidae (thrushes), specifically wood thrush and American robin, were identified as the most common hosts for populations of *Cs. melanura* from CT, NY, and MA and *Cs. morsitans* from central NY. At local scales, the wood thrush was found to have the highest feeding index for *Cs. melanura* when compared with other species and was predicted to disproportionately increase virus transmission in mathematical models (Molaei et al. 2016). One notable exception to these trends was in VT where the green heron (*Butorides virescens*) served as the dominant host early in the season followed by other species during August–September, including American robin and common yellowthroat (*Geothlypis trichas*) (Molaei et al. 2015). Other commonly utilized hosts include the tufted titmouse, black-capped chickadee, song sparrow, and common grackle but with considerable variation among locations. Mosquito feeding patterns appear to be strongly influenced by the local abundance of available hosts within swamp foci.

Serosurveys of wild bird populations in the northeastern United States show that a wide diversity of bird species are exposed to EEEV, reinforcing findings from mosquito bloodmeal analyses. In southern NJ, EEEV antibody was detected in 47 bird species, with highest seroprevalence in the blue jay (*Cyanocitta cristata*), wood thrush, and tufted titmouse, in addition to other species classed as permanent residents or summer residents (Crans et al. 1994). Viremic birds and seroconversions were documented as early as May before the detection of EEEV in mosquitoes. Based on these findings, the authors hypothesized that a cryptic cycle develops in birds in early spring perhaps by recrudescence of latent virus in previously infected birds. This possibility requires further validation. Owen et al. (2011) could not document persistent infections and viral recrudesce in experimentally infected gray catbirds, except for the detection of viral RNA from a single cloacal sample. In a study by Howard et al. (2004), wild birds inhabiting the toad harbor swamp in central NY were captured from 1986 to 1990 and screened for the presence of EEEV antibodies and live virus. Eighty species were captured with gray catbird, song sparrow, and veery (*Catharus fuscescens*) representing 55% of species bled and 61% of all seropositive birds. EEEV was isolated from 0.7% of tested birds, of which song sparrows had the highest number of recorded isolates from an individual species. Many of the same bird species were identified as seropositive in an earlier study from the same location (1978–1980), with seroprevalence rates highest among mid- to large-sized species, including veery, gray catbird, and wood thrush (Emord and Morris 1984). In southeastern MA, the wood thrush and swamp sparrow (*Melospiza georgiana*) had the highest prevalence of EEEV antibody (Main et al. 1988). The authors also found that the duration of neutralizing antibodies was ephemeral for some species, such as black-capped chickadees, and long-lasting for others, such as gray catbirds and swamp sparrows, based on analysis of recaptures. Finally, in southern ME, Elias et al. (2017) documented increasing seroprevalence among locally hatched songbirds from midseason to late season, suggesting a key role for this age cohort in supporting virus amplification. Taken together, these studies indicate that multiple avian species become infected and likely participate in the enzootic cycling of EEEV.

Resurgence and Expansion

EEEV activity and human risk of infection are clearly increasing in the northeastern United States starting during the early 2000s (Fig. 1). The average annual number of reported human cases between 2003 and 2020 has increased fourfold versus 1964–2002 period after EEE became a reportable disease. Human cases have expanded northward into northern New England for the first time when the disease struck NH in 2005 followed by VT in 2012 and ME in 2014 (Armstrong and Andreadis 2013, Lindsey et al. 2018). Other evidence for northward expansion is supported by the recent detection of virus in mosquitoes, antibodies from wildlife (moose and deer), and veterinary cases (emus and horses) from this region (Lubelczyk et al. 2013, Saxton-Shaw et al. 2015, Kenney et al. 2020). Disease outbreaks are also increasing in frequency in established foci in southern New England and NY in recent decades. These increases are unlikely to be explained by increased awareness and case reporting. EEE is not a new disease and has been under surveillance for decades prior to the introduction of West Nile virus in 1999. Hospitalized cases of EEE are likely to be reported given the severity of the illness and media attention during disease outbreaks. Other indicators of increased risk come from mosquito surveillance programs. EEEV has been detected in mosquitoes more consistently in NY State since surveillance began in 1971 (Oliver et al. 2018). Likewise, the MA Department of Public Health has reported increased numbers of EEE positive mosquitoes, including mammalian-biting species, after a long period of quiescence during the 1990s (ArboNET, Centers for Disease Control and Prevention, Atlanta, GA; Hachiya et al. 2007).

The viral source of these outbreaks is not fully understood, but may derive from newly introduced virus strains or from locally overwintering virus. Phylogenetic analyses indicate that EEEV strains persist in the northeastern United States for a period of 1–5 yr before going locally extinct (Armstrong et al. 2008, Young et al. 2008, Tan et al. 2018). New virus strains are also periodically introduced into this region to initiate new cycles of transmission and appear to originate from source populations in Florida where transmission occurs year round (Bigler et al. 1976). Migratory birds remain the most plausible means for transporting the virus over long distances from southern locations, although dispersal of virus-infected mosquitoes via human-mediated transport or wind-aided dispersal is also a possibility (Service 1997). The mechanism(s) for virus overwintering remains a long-standing enigma and could include viral persistence and recrudescence in resident birds as previously discussed or by vertically infected, overwintering mosquitoes (Watts et al. 1987, Reisen 1990). To date, there is no conclusive evidence to support either of these possibilities, but the phylogenetic data strongly supports the existence of viral overwintering in northern foci.

The underlying conditions responsible for the regional resurgence of EEEV are unknown and undoubtedly complex, but most likely reflect ongoing changes to the environment. Komar and Spielman (1994) proposed that landscape changes have created a more permissive environment for the main mosquito vector and avian hosts within swamp habitats. *Culiseta melanura* requires the availability of mature swamp trees with buttressed root systems that provide water pockets for larval development. The regeneration of these critical habitats after centuries of exploitation for lumber, firewood, and agriculture may have increased overall breeding habitat for *Cs. melanura*. Meanwhile, the expansion of suburban and exurban developments have placed more humans at greater risk for EEEV infection. For example, in CT, developed land and associated turf/grass categories have increased faster than any other land use category during the last 30 yr, with new

developments concentrated in forested and agricultural parts of the state rather than near existing developed areas (Arnold et al. 2020). These landscape changes are also expected to affect the composition of the avian fauna and may have allowed the American Robin to proliferate to become one of the most abundant bird species in the eastern United States (Chase and Walsh 2006). Robins forage in suburban environments and form massive nighttime roosts in and around swamps in late summer. They serve as important hosts for *Cs. melanura* in addition to bridge vectors, which could potentially increase the risk for epidemic transmission (Molaei and Andreadis 2006; Molaei et al. 2008, 2013).

Weather conditions may also directly impact mosquito development and abundance that could affect the timing and magnitude of EEE outbreaks. Historically, EEE activity occurs in multiyear cycles with epidemics occurring after periods of excessive rainfall starting during the proceeding year (Grady et al. 1978, Mermel 2020). High rainfall and accumulated ground water levels in swamps will likely increase the amount of habitat for larval overwintering and development of *Cs. melanura*. Mosquito behavior, physiology, and development are also highly sensitive to temperature. Larval development, frequency of blood feeding, and rate of virus replication in mosquitoes (extrinsic incubation period) will all accelerate with increasing temperature up to a thermal optimum that leads to increased virus transmission intensity (Mills et al. 2010, West et al. 2020). Climate projections for the Northeast indicate a trend toward milder winters, hotter summers, and more frequent dry periods punctuated by heavy rainstorms increasing the risk of flooding (Horton et al. 2014). The impact of these changes on the complex EEEV ecosystem remains uncertain, but the greatest effects will most likely occur at the temperature extremes of its endemic range. The northward expansion of EEEV into regions where the virus was previously rare or unknown fits that prediction, but clearly, more research is needed to better understand this phenomenon. This could include work on understanding the thermal range limits of *Cs. melanura*, the impact of precipitation and temperature on key mosquito species and vector–host interactions, and modeling climate scenarios on vectorial capacity. This is an urgent priority given that CO_2-driven climate change is already well underway with unknown consequences to this system (WMO 2020).

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