Investigation of Jamestown Canyon Virus Ecology in New Hampshire

A thesis

Presented to the Faculty of the Graduate School

of Cornell University

In partial Fulfillment of the Requirements for the Degree of Master of Science

by

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May 2022

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Abstract

Jamestown Canyon virus (JCV) is an emerging arbovirus (*Peribunyavirales: Orthobunyavirus)*, that causes a severe neuro invasive disease in humans. Jamestown Canyon Virus Disease (JCVD) reported cases have been increasing (Pastula et al. 2015), with recent epidemics in 2017-2019 (CDC Arbonet 2022). In nature, JCV is maintained in white-tailed deer (*Odocoileus virginianus)* (Issel 1972) and in mosquitoes. In general, the biology and ecology of mosquito vectors of JCV remains poorly understood. Nationwide, there is a lack of mosquito surveillance across the regions of the USA where JCVD cases have occurred. As human cases continue to occur, many health departments and local vector control agencies remain limited in their ability to conduct crucial JCV surveillance. To address this issue in New Hampshire (NH), a state where JCV is the primary arbovirus of concern (NH arob bulletin 2021), I collaborated with state employees in 2021 to conduct JCV vector surveillance.

I investigated putative JCV vectors in the state from areas where human cases have occurred. I collected mosquitoes, ecological data and with the NH Public Health Lab (PHL), tested over 42,000 mosquitoes for JCV. I also conducted bloodmeal analysis on putative vectors. In total, I collected 12 positive pools of JCV from six mosquito species in the state. I found that snowmelt *Aedes* had the highest infection rates and largely fed on white-tailed deer (88.4%). Other putative vectors, such as *Aedes canadensis, Coquillettidia perturbans* and *Anopheles punctipennis* also fed on white-tailed deer (34%- 68%). My research is the first to evaluate JCV virus and vector ecology in NH, providing crucial insights to the NH PHL.

Biographical Sketch

The author, Joseph Poggi was born to parents Debra and Dean on June 13th, 1996, in Springfield, Massachusetts. He has two older brothers, Chris, a gifted athlete and Nicholas, a self-taught engineer. Joseph was inspired by his connection with nature to pursue a B.S. in Wildlife and Conservation Biology from the University of New Hampshire, where he also competed in Division 1 track and field as an 800m runner and studied the impacts of white-nose syndrome on bats. After graduating in 2018, primed by an interest in disease ecology, Joseph moved to Ithaca in 2019 to start as a laboratory technician in Dr. Laura Harrington's lab. Here he helped to establish the Regional Pesticide Resistance Testing Program under the tutelage of Dr. James Burtis. In 2020, he began the Vector-Borne disease Entomology Master of Science Program funded through the Northeastern Center for Excellence in Vector Biology. This program allowed Joseph to further his passion for disease-ecology through research of mosquito vectors in New Hampshire, where he was raised, and to learn about the science of protecting public health.

Dedication

Dedicated to my loving parents, Deb and Dean, my brothers Chris and Nicholas, and my partner, Renee, who all continually blanket me with comforts of home and belonging. My friends from Ithaca and elsewhere for their good nature, who's music is always playing in the back of my mind, inspiring me and supplying me with joy and purpose beyond science. And importantly, the memory of James Peter Stewart (1995 – 2019) for his love of nature and music. I find constantly, a great reminder of the sweetness and fleeting nature of life in James' absence. Serving as a lesson to enjoy every single day regardless of obstacles standing before me, and to find purpose and peace in connection to nature and the presence of others.

Acknowledgements

There are many people I would like to acknowledge for their guidance, inspiration and support over this adventure. Foremost, Dr. Laura Harrington, Dr. James Burtis and Emily Mader for their constant advice, empathy and mentorship since moving to Ithaca to attend Cornell. I am very grateful to the help and support of Colin Conery, Marco Notarangelo, Abigail Matthewson for their immense contributions to this project in the field and conceptually. For Rebecca Lovell and Denise Bolton and the staff of the NH public health laboratory for assistance with viral testing. And, to the New Jersey Mosquito Control Association for awarding me the 2021 Jobbin's Scholarship which helped fund this project, lending me traps and technical advice. This project was supported by the CDC through the NEVBD agreement.

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Chapter 1: Jamestown Canyon Virus (JCV): A Critical Review of the Literature and a Call for Improved Mosquito Surveillance

Background and Current Knowledge Gaps

Virology and Vector Surveillance

Jamestown Canyon virus belongs to the California serogroup (CSG), which is a paraphyletic group of viruses containing over 150 virus species, further divided into 16 subtypes. Jamestown Canyon virus (JCV) is part of the Melao subtype along with antigenically similar Keystone, Snowshoe hare and LaCrosse viruses. JCV is a negative sense RNA virus, with a small, tripartite genome. JCV is known to be transmitted by several genera of mosquito vectors (Andreadis et al. 2008) which can become infectious after biting an infected reservoir host and/or through vertical transmission. Although our knowledge of JCV vectors and the natural cycle of JCV has increased over time, the published literature on JCV vector ecology remains small despite its importance for guiding effective public health interventions. Outstanding questions remain about the identity and vector competence of putative bridge vectors, vertical transmission rates of putative vectors, and vector control best practices across the virus's wide geographic distribution. JCV is proving to be an increasing, persistent public health threat within the United States (Matkovic et al. 2019) and these knowledge gaps stand as tall barriers to its prevention and control.

JCV surveillance and research are not priorities in local and state vector control agencies due primarily to funding and staffing limitations (NACCHO 2020). However, the addition of JCV monitoring in just seven vector surveillance programs has revealed mosquito and human infections that would have been otherwise overlooked (**Figure 1**) (Matkovic et al. 2019). JCV is

an emerging and important public health threat that needs to be addressed nationwide, as it is a disease system highly susceptible to warming temperatures and increased precipitation.

Symptoms and Detection

Jamestown Canyon Virus Disease (JCVD) is a nationally notifiable mosquito-borne illness caused by an infection of JCV. Public health attention regarding JCVD has been increasing over the last decade, due to a substantial increase in human infections (CDC Arbonet 2022). From 2010 - 2019, there were 135 documented cases of JCVD compared to only 31 from 2000 – 2013 (Pastula et al. 2015). Greater than 70% of documented infections from 2000 - 2019 occurred in Wisconsin and Minnesota, with the majority of these reported cases being neuroinvasive (Rameez et al. 2021, CDC Arbonet 2022). The annual prevalence of the human JCVD has increased from an average of 1.7 (2004 – 2012) to 29.2 cases from 2013 - 2018. More details about Jamestown Canyon Virus Disease in humans has been revealed through recent published case reports (Coleman et al. 2021, Solomon et al. 2021). However, non-severe disease pathology, symptoms, asymptomatic rates (seroprevalence), antibody persistence, and immunity and treatment options remain understudied.

Currently, our knowledge of JCVD symptomology is based on a limited sample size for severe cases, with less than 10 published case reports. In addition, less severe cases are likely not reported due to non-specific clinical presentations (Coleman et al. 2021). The preferred method for JCVD diagnosis is specific antibody testing, such as ELISA for IgM antibodies, followed by plaque reduction neutralization tests (PRNT) (Piantadosi and Kanjilal 2020). Symptoms from JCVD case reports from 2000-2013 commonly included meningoencephalitis,

meningitis, and nonspecific febrile illness symptoms (Pastula et al. 2015). Neuroinvasive cases also typically presented with altered mental status (hallucination), stiffness of the neck, and rarely with seizures (Kumar et al. 2021, Rameez et al. 2021). Additionally, some patients have experienced unusual symptoms such as speech difficulties, migraine-like auras, acute flaccid paralysis, numbness and tingling on one side (Vosoughi et al. 2018, Rameez et al. 2021, CDC Arbonet 2022). While JCVD can cause severe symptoms, these are thought to be rare and typically non-fatal (Piantadosi and Kanjilal 2020); there is no established baseline for infection prevalence to compare the frequency of severe symptoms against. Similarly, a lack of published human serosurveys limit our knowledge in this regard.

Human Serosurveys

Antibody seroprevalence surveys are useful tools for understanding the likelihood of JCVD infection prevalence, and in some communities JCV IgG antibodies in people appear to be common. In Connecticut, between 3.9% and 10.1% of tested blood samples from 1990 and 1995, were found to have IgG antibodies in a serosurvey (Mayo et al. 2001). Patriquin et al. (2018) have found JCV seroprevalence to range from 10% to 45% in some communities in Nova Scotia. Kosoy et al. (2016) found JCV seroprevalence to range from 6.7% to 21.7% in National Park (NP) employees in Rocky Mountain NP, Great Smoky Mountain NP and Grand Teton NP. Grimstad et al. (1986) found that 27% of Michigan residents had antibodies to JCV in the lower peninsula of Michigan. This suggests that IgG antibodies persist long after infection, that JCV is a widespread endemic virus, and that the rate of asymptomatic infection is high. These results clearly indicate a need for funding passive and active surveillance as well as control metrics.

Future seroprevalence work should be conducted in states like Wisconsin, Minnesota, or New Hampshire, where severe symptomatic cases have been relatively well documented (Solomon et al. 2021).

Jamestown Canyon Virus Ecology

Virology

Jamestown Canyon virus (*Bunyavirales: Peribunyaviridae: Orthobunyavirus*) is a negative sense, RNA virus with a tripartite genome (S, M, L segment). The S segment (small, ~1 kb) encodes the nucleocapsid, the M segment (medium, ~4.5 kb) encodes the surface glycoprotein, and the L segment (large, ~7 kb) encodes the RNA-dependent RNA polymerase (Kinsella et al. 2020). As a negative sense RNA virus, JCV depends on this RNA polymerase for replication within infected host cells. A recent study of JCV phylogeny in Connecticut (CT) revealed three distinct clades (Armstrong and Andreadis 2007), however representative sequences from the virus across its US range are lacking. Genetically, JCV is most closely related to Inkoo Virus (INKV) within the Melao subtype, which is an outgroup of the California Encephalitis complex, containing antigenically similar viruses: LaCrosse encephalitis virus (LACV), Tahyna Virus (TAHV) and Snowshoe Hare Virus (SSHV) (Evans and Peterson 2021).

Segment Reassortment

Viruses like *Orthobunyaviruses*, which have segmented genomes, have the ability to change rapidly with segment reassortment (Hughes et al. 2017). Segment reassortment occurs when the S, M, or L segment are swapped between two similar viruses during a cellular coinfection. Segment reassortment may have been an important factor in the evolution of CSG

viruses given the efficiency of experimental genetic crosses at creating different viral phenotypes (Gentsch et al. 1979, Bennett et al. 2012). Given the current association between JCV and over 20 different vector species (Andreadis et al. 2008) and the broad geographic range of white-tailed deer (Innes 2013), it is likely that coinfection and reassortment events may impact virus-vector relationships. Furthermore, recombination events leading to phenotypic changes in virulence or transmissibility may one potential reason for increased human JCV cases over the past decade (Pastula et al. 2015). Other factors leading to increased human cases likely include abiotic environmental conditions such as temperature that may alter influence host-vector contact rates, mosquito densities or frequency of vertical transmission in mosquitoes.

Vertical transmission

JCV can be maintained in mosquito vectors via vertical transmission, in which JCV is passed on from an infected female to her offspring. Transovarial transmission (TOT), or vertical transmission via the oviduct, is one possible mechanism for this to occur (Bergren and Kading 2018). Documented JCV vertical transmission for a few mosquito species is presented in **Table 2**. Transovarial transmission is poorly understood across bunyaviruses (Bergren and Kading 2018) but may be a significant driver of virus overwintering and early season amplification cycles leading to spillover later in the season. Vertical transmission studies for JCV are challenging because of the lack of ability to rear many snowmelt *Aedes* and other vector species in the laboratory.

Most of the foundational work on JCV was conducted in the western and midwestern regions of the US. Berry et al. (1977) were the first to report vertical transmission of JCV, after isolating the virus from field collected *Aedes (Ae.) triseriatus*, that were reared from eggs to adults. JCV isolations have since been made from immature and adult male *Ae. provocans* and *Ae. stimulans* (Boromisa and Grimstad 1986, Boromisa and Grayson 1990) collected from the field in Northern Indiana. Laboratory vertical transmission has also been demonstrated for *Ae. cataphylla, Ae. tahoensis, Ae. squamiger, Ae. washinoi* and *Cs. inornata* (Kramer et al. 1993a), after field collected adults were intrathoracically injected with JCV and offspring were tested by plaque assay. Despite data supporting susceptibility to JCV infection for 30 different species of US mosquitoes (Boromisa and Grimstad 1986, Watts et al. 1986, Boromisa and Grayson 1990, Heard et al. 1991, Kramer et al. 1993a) a consensus on JCV bridge vectors to humans remains elusive.

Animal Serosurveys and Potential Reservoirs

JCV neutralizing antibodies have been found to occur widely in mammals throughout temperate North America through seroprevalence studies (**Appendix 1, Table 7**). Issel et al. (1972, 1973) were the first to report naturally occurring antibodies to JCV in white-tailed deer (*Odocoileus virginianus*). Noticing that these antibodies occurred in high titers, the authors concluded that deer were either consistently reinfected or had persisting antibodies. More recent serosurveys reported JCV antibodies in many other large mammals with varying seroprevalence including, moose (*Alces alces*) and elk (*Cervus canadensis*). JCV antibodies have also been found in horses, cattle, and sheep (Fulhorst et al. 1996, Goff et al. 2012). JCV

antibodies have not been detected in surveys of rodents, mustelids or leporids, though these mammals were initially thought to be reservoirs based on the proposed similarity of JCV to Snowshoe hare virus.

It is important to note that serosurvey results can be misinterpreted. Animal exposure to JCV detected via antibodies (serology) does not mean that the animal serves as an amplifying reservoir for JCV. In fact, many potential hosts remain unevaluated for viremia, which can be studied by experimental injections or from isolating the virus from wild or sentinel animals. To date, white-tailed deer and gray squirrel (*Sciurus caroliniensis)* have been the only hosts studied for viremia. White-tailed deer are the only host that can develop adequate viremias of 4-5 $\log_{10}$ suckling mouse intracerebral lethal dose $(SMICLD)_{50}$ (hereafter, $SMICLD_{50}$) to infect some mosquito species (Watts et al. 1979, Issel et al. 1972). Issel et al. (1972) found that eight of nine yearling white-tailed deer experimentally injected with JCV developed a detectable viremia for 4-5 days (mean = 4.7) ranging from trace to 3.4 $\log_{10}$ $SMICLD_{50}$/ml titers. One of nine deer that did not develop a detectable viremia in this study developed antibodies four days before they appeared in sera of the other deer. It was presumed that this animal had prior, natural, exposure to JCV before the study. Watts and others (1979) inoculated yearling white-tailed deer and gray squirrels with JCV and found a viremia ranging 1.8-5.0 $\log_{10}$ $SMICLD_{50}$/ml in five of six white-tailed deer, lasting for 2-5 days (mean = 2.5). Gray squirrels did not develop a viremia but developed JCV neutralizing antibodies seven days post infection. It is interesting to note that observations of viremic deer do not show apparent signs of illness (Issel et al. 1972). JCV was also isolated from captive white-tailed deer in central Wisconsin (Issel 1973). In a study

of captive, sentinel deer, Grimstad et al. (1987) observed JCV antibody in suckling and five week old fawns, providing evidence for protection by maternal antibody, lasting for up to 22 weeks after birth.

In Central New York, wild deer JCV infection rates as high as 71.3% have been reported, with an average state-wide seroprevalence of 48% (Dupuis et al. 2021). Adult deer occasionally have greater JCV seropositivity than fawns (Issel et al. 1972, Dupuis et al. 2021), likely because of a longer window of virus exposure for adults. Given these observations, yearling deer in the fall, who have just lost maternal antibody, are the cohort most susceptible to be naturally infected with JCV (Grimstad et al. 1987). Observations of chronically infected deer have not been reported. Antibody titers in adult does when quantified with plaque reduction neutralization tests demonstrated persistence of antibodies for over 120 days past infection (Issel 1972). However, more replicated research on the timing of seroconversion in deer is necessary. To address this, serosurveys could be conducted with sentinel deer in the spring and summer in tandem with mosquito sampling and virus testing. These types of studies are more informative than those conducted with hunter-killed deer and avoid pitfalls such as temporal sampling bias from the hunting season occurring generally in the fall, or post-mosquito season selection bias for seropositive animals.

Another potential JCV reservoir host are moose, which may play a role in JCV ecology at the northern limits of deer populations in Canada and the USA (McFarlane et al. 1981, Grimstad et al. 1986). Moose have been previously recognized as potential arboviral reservoirs (Trainer and Hoff 1971), and more work is needed to understand the potential of JCV amplification.

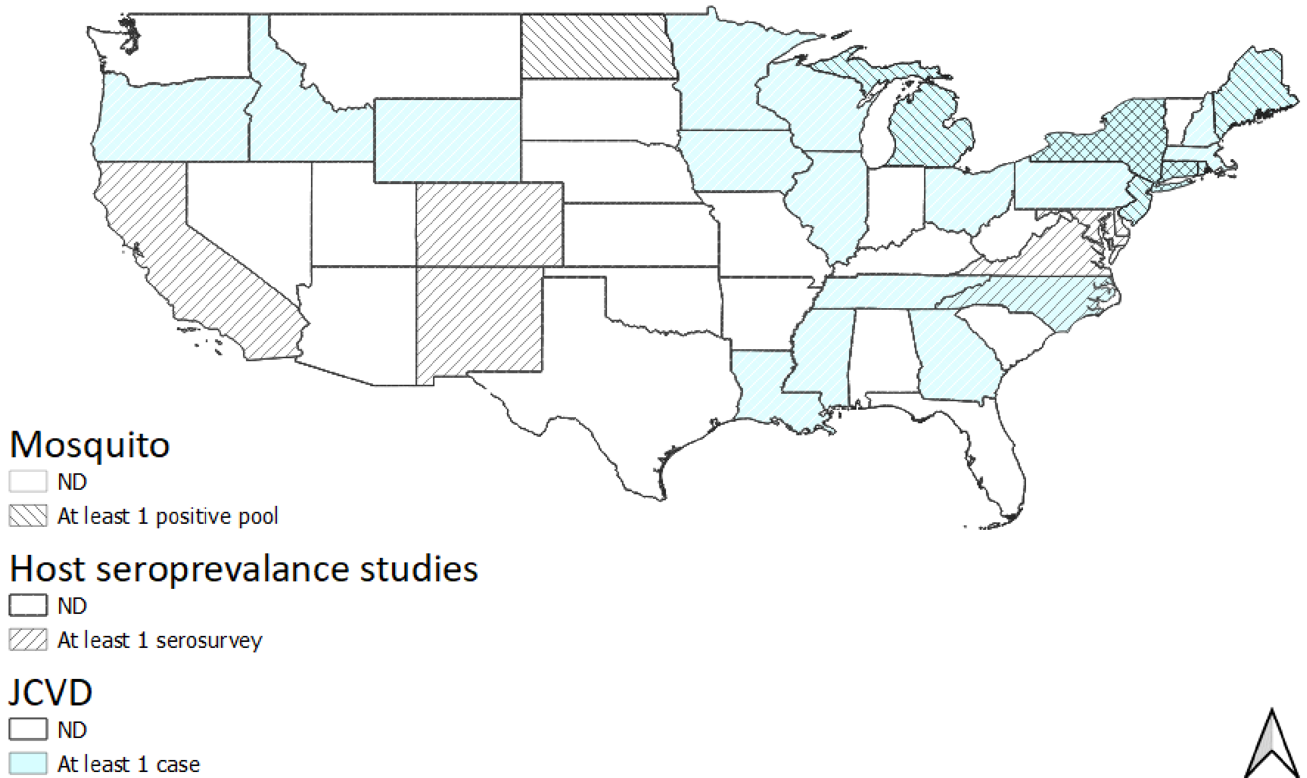

Figure 1. JCV monitoring activities in the United States. ND = No data. JCVD case data: CDC arbonet data request 2022, JCV Mosquito data: CDC arbonet data request 2022, Host seroprevalence data are from studies using PRNT to differentiate JCV specific antibodies.

Additionally, some mammalophilic mosquito species such as *Ae. canadensis* are likely to feed on moose as well as deer (Chapter 2: **Table 6**). Future studies on the potential of white-tailed deer, moose, livestock and horses as potential JCV reservoirs is needed (**Appendix 1: Table 7**).

Table 1. Deer seroprevalence of JCV antibodies in 9 states from 1972 to 2018

Author	Year	State	Sample size	Seroprevalence
Issel et al.	1972	Wisconsin	587	56%

Aguirre et al.	1992	Mexico	5	20%
Hoff et al.	1973	North Dakota	50	50%
Murphy et al.	1989	Wisconsin	41	56%
Neitzel and Grimstad	1991	Minnesota	138	41%
Patriqun et al.	2018	Nova Scotia	82	88%
Zamparo et al.	1997	Connecticut	446	21%
Boromisa and Grimstad	1987	Indiana	1642	38%
Watts et al.	1982	DE, MA, VA	403	18%
Nagayama et al.	2001	North Carolina	135	27%

Putative JCV Vectors

Many mosquito species have been implicated as putative JCV enzootic and bridge vectors (**Table 2**), however more work is necessary to understand individual roles of these mosquito species. JCV isolations have also been made from *Tabanidae* and *Simulioidae* (Defoliart et al. 1969, Villeneuve et al. 2021), though biological transmission, meaning virus replication within the fly, seems unlikely when flies are experimentally infected (Miller et al. 1983). It is likely that mosquitoes capable of vertical transmission pose the largest public health threat, particularly if they have feeding habits that overlap with deer and humans. Many gaps in the primary literature still exist for vertical transmission rates from *Aedes* and *Culiseta* mosquitoes. Currently, the only species for which convincing JCV vertical transmission evidence has been published are *Aedes provocans*, *Aedes stimulans* and *Culiseta inornata* (Boromisa and Grimstad 1986, Boromisa and Grayson 1990, Kramer et al. 1993b).

Our current understanding of JCV transmission dynamics comes from research conducted primarily in Northern US states over the last five decades. These data include yearly

detections of virus from snowmelt mosquitoes (**Table 2**) in the spring and summer, preceded by detections from potentially overwintered *An. punctipennis* collected in late May, in New Hampshire (**Chapter 2: table 5**). Together, these early spring mosquitoes are capable of ramping up the initial amplification cycle between mosquitoes and deer, which has been documented by seroconversion of 90% of captive white-tailed deer from late April – late June in Michigan (Grimstad et al. 1987). This establishes the first of two possible amplification cycles and the likely cause of human cases prior to late-June, aside from those arising from delayed diagnosis.

Human cases have been detected in the US from March – September, peaking in July (Pastula et al. 2015). Cases occurring from July to the end of the mosquito season likely arise from time-lagged, horizontal transmission events from mosquitoes, and likely involve multiple abundant bridge vector species. In June and July, long lived snowmelt *Aedes* overlap with emergence of many abundant species such as *Cq. perturbans*, as well as coastal salt marsh mosquitoes. It seems logical that these overlapping mosquito communities, and the presence of viremic deer hosts could lead to a second amplification cycle fueled by horizontal transmission. Given the abundance of potential bridge vector species such as *Cq. perturbans*, rare transmission events could result a low number of human JCV cases observed each year. However, seroprevalence data in humans to support this theory is lacking. Additionally, the vector competence of mosquito species implicated as putative vectors from yearly JCV detections is unknown of limited. Positive pool data remain just one component of vector

incrimination, a necessary process for identifying the key vectors involved in outbreaks of arboviruses (Weaver 2020), and understanding the influence of environmental factors.

Table 2. Putative JCV vector incrimination data and ecological associations

Vector	Isolations (2011 - 2019)*	Vertical transmission	Horizontal transmission (vector competence)	Ecological associations
Aedes abserratus / punctor	26	unknown	Possible	Snowmelt pools with leaf litter
Aedes aurifer	27	unknown	Unknown	Snowmelt pools with leaf litter
Aedes canadensis	221	unknown	Competent	Snowmelt pools with leaf litter; wetlands; ditches
Aedes cantator	40	unknown	Possible	Saltmarsh with *Spartina patens*
Aedes communis s.l.	1	Yes[a]	Competent	Snowmelt pools with leaf litter at altitude
Aedes excrucians	5	unknown	Unknown	Snowmelt pools with leaf litter
Aedes provocans	19	Yes[b]	Varies	Snowmelt pools with leaf litter
Aedes stimulans	19	Yes[c]	Competent	Snowmelt pools with leaf litter
Aedes taeniorhynchus	8	unknown	Competent	Saltmarsh with emergent vegetation
Anopheles punctipennis	47	unknown	Competent	Wetlands with emergent vegetation
Culiseta inornata	0	Yes[d]	Competent	Snowmelt pools with leaf litter

[*]. CDC arbonet 2022

[a]. Campbell et al. 1991

[b]. Boromisa and Grayson 1990

[c]. Boromisa and Grimstad 1986

[d]. Kramer et al. 1993

[e]. Watts et al. 1986

Vector incrimination steps for identifying JCV bridge vectors include field isolations (Isolations 2011-2019 in **Table 2**), evidence of the ability of the virus to cross three important tissue barriers including the salivary gland barrier (Franz et al. 2015), or evidence of vertical transmission, as well as evidence of host feeding patterns that overlap with both dead-end hosts (humans) and reservoirs (white-tailed deer) in nature (Ledesma and Harrington 2011, Fikrig and Harrington 2021). The true vectors of JCV likely vary regionally due primarily to environmental factors such as temperature and rainfall that can influence emergence timing and dictate the extent of overlap in space and time with white-tailed deer or other potential reservoirs. Genetic factors influencing the virus-vector relationship, and the results of vector competence studies, may also be dictated by these environmental factors. For instance, Kramer et al. (1993) found that California *Aedes tahoensis*, a potential bridge vector of JCV, successfully transmitted a JCV strain (DAV 132) from California *Ae. tahoensis* but did not transmit the prototype (61V2235) strain from Colorado *Cs. inornata* after feeding on an infectious bloodmeal. That study highlights potential geographic differences in JCV that could influence vector competence. In fact, our understanding of JCV bridge vectors lacks evidence required for vector incrimination across the wide geographic range of JCV (**Figure 1**). Below, I review the current literature on JCV vector incrimination and ecological associations for five primary putative mosquito bridge vector species in the USA (**Table 2)**.

Aedes provocans (Walker)

Aedes provocans is a classic snowmelt mosquito that can be readily identified by its cloak of white scales, with distinct patches occurring on the hypostigmal area of the scutum, on

the post-procoxal area of the foreleg and at the base of the costal vein. *Aedes provocans* is a northern species with a range spanning east from Nova Scotia, Canada to northern Washington, USA, and north into the Northwest territories (Darsie and Ward 2005). There is currently limited knowledge on the bloodfeeding habits of this mosquito, though both human and cattle biting have been documented (Gadwaski and Smith 1992, Smith and Gadawski 1994). Though *Ae. provocans* is univoltine, it has been suggested that this species is capable of extending its life by sugar-feeding, and likely takes multiple bloodmeals before producing eggs (Gadwaski and Smith 1992, Smith and Gadawski 1994). Host-seeking *Aedes provocans* appear to be readily captured in CDC traps baited with $C0_2$ as well as $C0_2$ baited tent traps placed over vernal pools (Heard et al. 1990) (**Table 2)**.

From 2011 – 2019, 19 JCV positive pools were reported to CDC from Minnesota and Connecticut, from 16 May to 29 June (CDC Arbonet 2022). Vertical transmission of JCV has been documented for this species, where isolations of JCV have been made from adult males reared from pupae (Boromisa and Grayson 1990). In a vector competence assessment (Boromisa and Grayson 1991), 50% (n=18) of *Ae. provocans* were infectious, by detection of virus in saliva collected via capillary tube, after 14 days at 20C. Daily minimum infection rates (MIR) have been reported to be 1:38 - 1:312 in *Aedes provocans* in Saratoga County, NY. Seasonal MIR of pooled *Ae. provocans* have been reported as high as 1:219 (Boromisa and Grayson 1990) and 1:495 (Heard et al. 1990). Probability-based infection MLE (Maximum likelihood estimate per 1000 pooled mosquitoes) of 4.57 (95% CI 0.26 – 23.21) was reported from a 10 year assessment in Connecticut (Andreadis et al. 2008). Given that *Ae. provocans* is one of the first species to

emerge in the spring, and has the ability to vertically transmit JCV, the emergence of infectious adults likely contributes to deer infections. *Aedes provocans* may play a key role in virus overwintering, as well as infecting humans and deer across its northern range, given its mammalophilic behavior documented in man-baiting studies (Heard et al. 1990).

Aedes stimulans s.s. (Walk)

Aedes stimulans s.s. is a northern snow melt mosquito that is morphologically difficult to differentiate from *Aedes excrucians* and *Aedes fitchii*, all of which have large bands on the hind tarsomeres, and similarly shaped foreclaws. *Aedes stimulans s.s.* has a range that extends from Newfoundland, Canada, south to Louisiana (Sither et al. 2014) and west to Kansas and Southern Ontario (Darsie and Ward 2005). *Aedes stimulans s.s.* can be found in vernal, leaf lined pools in the early season, emerging in May/June in Minnesota and Connecticut and is readily captured with CO_2 baited CDC traps.

From 2011 – 2019, 38 pools that tested positive for JCV were reported to CDC from Minnesota, Connecticut, and New York, collected from 28 May – 27 July (CDC Arbonet 2022). Field infection studies of *Ae. stimulans* ***s.l.*** have indicated a seasonal MIR of JCV as high as 1:591 in Indiana (Boromisa and Grimstad 1986). This contrasts somewhat with the lower, seasonal infection MLE of 0.21 (0.04 – 0.69 95% CI) per 1000, found in Connecticut (Andreadis et al. 2008), highlighting potential regional variation in infection rates. In Boromisa and Grimstad's 1986 investigation, JCV was also detected in pools of newly emerged males, which indicates that vertical transmission is likely to occur in this species. In a vector competence assessment, 12% (n= 43) of *Ae. stimulans* infected by bloodfeeding were able to transmit the virus to a

suckling mouse, after being challenged with an endemic virus strain isolated from the same species. The transmission rate was 88% (n=24) for *Ae. stimulans* infected by intrathoracic injection (Boromisa and Grimstad 1986). Though this data is limited in extent, *Aedes stimulans* has been shown to prefer mammal hosts through bloodmeal analysis studies (Boromisa and Grimstad 1986, Molaei et al. 2008a) and overlaps with potentially viremic deer in space and time. Given this evidence of vector competence, vertical transmission and mammalophilic host feeding, *Aedes stimulans* is an important JCV vector. *Ae. stimulans* can transmit JCV to both humans and susceptible deer each Spring, and perhaps plays a key role in starting annual virus replication cycles in addition to human health risk.

Aedes canadensis (Theobald)

Aedes canadensis is an easily identifiable mosquito with banded legs apically and basally, and an absence of white scales on the wing (Andreadis et al. 2005, Darsie and Ward 1981). *Aedes canadensis* has a broad geographic range extending from Alaska south to Louisiana, and east to northern Florida and Nova Scotia, Canada. A rare subspecies *Ae. canadensis mathesoni* has been described from limited areas in the Eastern USA (Darsie and Ward 2005). *Ae. canadensis* have more general breeding habitats than snowmelt *Aedes*, with larvae typically found in leaf-lined vernal pools, roadside ditches, and permanent wetlands. Adults can have very large emergence at multiple points throughout the field season. *Ae. canadensis* is an established mammalophilic mosquito (Molaei et al. 2008b, Shepard et al. 2016). Adult females can be readily trapped with CO_2 baited CDC traps set during summer from June to October.

Ae. canadensis. has been previously incriminated as a JCV vector through both vector competence studies (Heard et al. 1991) and by field isolations. From 2011 – 2019, there were 227 pools (cumulative MIR = 1:1602) that tested positive for JCV reported to the CDC from Minnesota, Rhode Island, Connecticut and New York, collected from 28 May – 13 September (CDC Arbonet 2022). Nationwide, *Ae. canadensis* is the species that is the most frequently tested and found positive for JCV, with the largest temporal range of detection. Andreadis et al. (2008) report that *Aedes canadensis* had a seasonal infection MLE of 0.23 (0.16-0.30) per 1000 pooled mosquitoes from 1997 to 2006 in Connecticut, and Kinsella et al. (2020) found similar results in Massachusetts. Overall, it is quite rare to find JCV in a pool of *Ae. canadensis;* however, these data show persistent detections. In a vector competence assessment (Watts et al. 1986), 48% (n=67) *Aedes canadensis* adult females required a relatively high virus dose of 6.0 Log_{10} $SMICLD_{50}$ in order to transmit an infection to a suckling mouse. In this study, <8% (n=52) *Ae. canadensis* females who received lower doses were able to transmit JCV to a mouse, after 7-21 days incubated at 20 C. Given that *Ae. canadensis* are likely multivoltine, emerging throughout the summer months in NH, vector competence assessments conducted at low temperatures such as 20°C may underestimate the true transmission rate under warmer summer field temperatures in New Hampshire (NOAA 2022). Cumulatively and in the absence of vertical transmission data, *Ae. canadensis* are consistently found to be infected, albeit at a low prevalence, and are competent vectors of JCV. *Ae. canadensis* is a key JCV vector with the ability to play a role in both early season and late season transmission of the virus to both deer and humans.

Aedes excrucians (Walker)

Aedes excrucians is a northern mosquito that may be distinguished from *Aedes fitchii* and *Aedes stimulans s.s.* by an irregularly long, sharply bent foreclaw and a sub parallel claw. *Aedes excrucians* has a range similar to *Aedes communis*, spanning south from Alaska, through all of Canada, and south into the United States following the Rocky Mountains. *Aedes excrucians* is a univoltine mosquito that can be found in ephemeral wetlands in the Northern United States (Crans 2016). *Aedes excrucians* lays its eggs in vernal pools lined with organic matter, and often co-occurs with *Aedes communis* (Crans 2016). This mosquito is mammalophilic, and bloodmeal analyses have revealed frequent feeding pattern on cattle, white-tailed deer, and other mammals (Magnarelli 1977, Molaei et al. 2008a, Anderson et al. 2018). Host seeking *Aedes excrucians* can be readily capture in CO_2 baited CDC traps, or tent traps baited with CO_2 placed over vernal pool breeding habitats.

Aedes excrucians JCV virus isolation data from 10 years in Connecticut demonstrate a seasonal MLE of 0.92 (95% CI 0.38 – 1.91) (Andreadis et al. 2008). Frequent isolations were also made in New Hampshire in 2021 (**Chapter 2, table 5**) Vertical transmission of JCV in *Ae. excrucians* remains unevaluated, but consistent isolations, deer bloodfeeding patterns and overlap with deer in space and time certainly implicate horizontal virus transmission for this species. Current evidence suggests that *Aedes excrucians* is most likely to be implicated in JCV transmission during the late spring, into June when population densities are highest and through August where long-lived *Ae. excrucians* are found in low abundance.

Aedes absserratus and Aedes punctor (Kirby)

Aedes absserratus is a morphologically indistinguishable species from *Aedes punctor.* Both are northern mosquitoes with distributions ranging from Ontario to Nova Scotia, Canada, and south from Illinois to New Jersey (Darsie and Ward 1981). *Ae. absserratus/punctor* larvae are of the first to be found in the spring while ice begins to melt (Means 1979). Larvae have an affinity for cool dark places, in the shaded spots of their breeding pools. Common areas to find *Ae. absserratus* include abandoned beaver swamps with sedge tussocks and shaded freshwater bogs with sphagnum moss (Andreadis et al. 2005, Means 1979). *Ae. absserratus/punctor* are also currently thought to be mammalophilic (Molaei et al. 2008b, Murdock et al. 2010, Shepard et al. 2016). Given this niche, *Ae. absserratus/punctor* can be sampled in the beginning of mosquito seasons using CDC traps baited with CO_2, when densities are typically the highest.

Ae. absserratus/punctor is a species consistently found to be infected with JCV, with reported deer bloodfeeding and overlap with deer in space and time. From 2011-2019, there were 27 pools (*Ae. absserratus*) that tested positive for JCV reported to CDC, from CT and NJ, collected from 2 June – 21 June. Walker et al. (1993) reported a seasonal MIR of JCV in this species of 1:3844, and Andreadis et al. (2008) reported an MLE of 0.84 (0.50 – 1.34 95% CI) per 1000 pooled mosquitoes. In a vector competence assessment (Heard et al. 1991), 52% (n= 58) and 12% (n=58) of *Ae. absserratus/punctor* developed a midgut infection and a disseminated infection, respectively, after being challenged by 5.0 Log_{10} $SMILD_{50}$ / 0.025ml of virus. None of these mosquitoes (n=32) were able to transmit the virus to suckling mice after 14 days, at 19C. More vector competence research is needed over the full geographic range of *Ae.*

abserratus/punctor with geographically relevant virus strains. *Ae. abserratus/punctor* likely plays a key role in JCV transmission due to its potential to vertically transmit JCV, yearly isolations, and emergence overlapping with periods of deer susceptibility.

Aedes cantator (Coquillett)

Aedes cantator has a narrow and somewhat sporadic range extending in salt marshes from Quebec and Newfoundland, Canada, south along the coast to southern Virginia (Darsie and Ward 2005). Interestingly, there have been a few collections made inland in Kentucky and Ohio (Darsie and Ward 2005). *Aedes cantator* is a mosquito whose larvae are commonly found in brackish and saltwater wetlands associated with *Spartina patens*. *Aedes cantator* can emerge in large densities in areas near salt marshes in New Jersey and Delaware (Rutgers 2016, Clark et al. 1986) and is multivoltine. This mosquito has been widely incriminated as a JCV vector in coastal states such as Connecticut (Main et al. 1978, Andreadis et al. 2008). *Aedes cantator* primarily feed around dusk but will bite during the day if disturbed (Rutgers 2016). *Aedes cantator* are readily collected in traps baited with CO_2, placed near larval habitats.

Andreadis et al. (2008) report that *Aedes cantator* had a seasonal infection MLE of about 1.16 (0.80 – 1.60 95% CI) in isolations in Connecticut from 1997 to 2006. From 2011 – 2019, 40 pools that tested positive for JCV were reported to CDC, from CT and NJ from 1 June – 14 July (CDC Arbonet 2022). A vector competence assessment (Watts et al. 1986) revealed that 8% (n=71) *Ae. cantator* adult females became infected after 6-29 days at 25 °C. These mosquitoes were orally infected with a high dose of the virus ($10^{5.9}$ PFU/ml, held at 25° C), but the authors did not assess the transmission rate. In the Northeastern US, this mosquito feeds on a variety of

hosts, but prefers mammals such as deer, cattle and horses (Magnarelli 1977, Molaei et al. 2008b) rather than birds or amphibians. Though these results on their own are inconclusive, very little virus needs to escape the midgut to cause salivary gland infections in other virus-vector relationships (Monath 1988). *Ae. cantator* overlaps with the range of white-tailed deer in space and time and emerges in large numbers during the hottest months of the summer, when it is routinely above 30° C. *Ae. cantator* may play role in the JCV transmission cycle if it is able to develop a salivary gland infection and lacks a salivary gland escape barrier, but this, as well as the potential for vertical transmission, has yet to be shown.

Aedes taeniorhynchus (Wiedemann)

Like *Aedes cantator*, *Aedes taeniorhynchus* is a coastal species occurring in salt marsh habitats in the United States. *Ae. taeniorhynchus* larvae develop in brackish wetlands during the summer and can be found in coastal areas in north, central and south America (Agramonte and Connelly 2014). Large emergence of this species occurs after rains when eggs laid in moist soil above the water line are submerged in incoming tides. As a result, this salt marsh mosquito is a large nuisance along the eastern coast (Argamante and Connelly 2014). *Aedes taeniorhynchus* can be readily collected in CDC light traps and NJ light traps that are set near salt marshes and larvae can be collected using dippers.

From 2011 – 2019, eight pools of *Ae. taeniorhynchus* tested positive for JCV and were reported to CDC from CT and NJ, from 15 July – 15 August (CDC Arbonet 2022). Though the number of *Ae. taeniorhynchus* pools tested for JCV each year remains low, this species is often not a priority for inland agencies conducting JCV surveillance, and so its importance may be

understated. A JCV vector competence assessment revealed that 80% (n=17) of *Ae. taeniorhynchus* adult females became infected 5-14 days after feeding on JCV guinea pig blood, as evidenced by viral assay; the transmission rate was not evaluated (Watts et al. 1986). *Ae. taeniorhynchus* are mammalian biters with a preference for dogs (Manrique-Saide et al. 2010) in southern States, and will also readily bite humans (Agramonte and Connelly 2014). This mammalophilic behavior could likely lead to JCV infection in nature if there are viremic deer nearby, and if *Ae. taeniorhynchus* has the ability to develop a salivary gland infection, or vertically transmit JCV, both of which have yet to be evaluated.

Anopheles punctipennis

Anopheles punctipennis is a mosquito easily distinguished from others by two distinct patches of white scales on the apical edge of the costal vein. It has a broad range, stretching south from North Dakota to Mexico and northern Florida, and east to Nova Scotia, and also occurring from Vancouver to about Los Angeles (Darsie and Ward 2005). *An. punctipennis* can be found in steady abundance in wetlands and ponds with abundant emergent vegetation such as sedge, cattails or ferns throughout the mosquito season. *An. punctipennis* overwinters as adults and can be collected on warm days. Past studies on *An. punctipennis* host associations through bloodmeal analysis show that it is mammalophilic (Tuten et al. 2012) and readily feeds on white-tailed deer, in more than 95% of bloodmeals (n = 11, 40,2) (Molaei et al. 2008a, Shepard et al. 2016, Anderson et al. 2018) in Connecticut. In 21 bloodmeals in Canada, *An. punctipennis* fed on mammals and specifically fed on white-tailed deer in 32% of bloodmeals, and humans in 14% (Shahhosseini et al. 2021). These results suggest that *An. punctipennis* is a

generalist, mammalophilic feeder that bites both deer and humans across the northern parts of its range. *Anopheles spp.* are primarily captured in resting boxes, and host seeking *Anopheles spp.* can be caught in both CDC traps baited with CO_2 as well as light traps.

From 2011-2019, 47 pools tested positive for JCV and were reported to CDC from CT, NY, NJ and MI, as early as 2 June and as late as 23 September (CDC Arbonet 2022). In a long-term study (Andreadis et al. 2008), *Anopheles punctipennis* had 13 virus isolations from positive pools, and a seasonal infection MLE of 0.69 (0.38 – 1.15 95% CI) per 1000 pooled mosquitoes. A preliminary vector competence assessment (Heard et al. 1991), revealed that 2.5 % (n=80) of wild, *An. punctipennis* adults, collected in April, were able to transmit a JCV isolate from *An. stimulans* to a capillary tube. This suggests that this species is able to develop a salivary gland infection of JCV and transmit it to a dead end or amplifying host year-round. Though *An. punctipennis* has a lower infection rate than some *Aedes,* it is still consistently found to be infected with JCV and in many places. *An. punctipennis* likely play a key role in maintaining virus transmission during the Winter given their overwintering ecology and observations of seroconversion of sentinel deer before the emergence of Aedine mosquitoes (Grimstad et al. 1987). If adult *An. punctipennis* are overwintering JCV as adults, winter surveillance of this species may be a great indicator of JCV activity in the spring and summer. JCV surveillance for this species should prioritize both early and late season collections of this species, as well as winter collections and insights into vector competence.

Culiseta inornata (Williston)

Culiseta inornata is distinguished from others in the genus by the presence of dark and pale scales on the wings, and legs. *Cs. inornata* has an expansive range, stretching southeast from the Canadian Yukon to Manitoba, and occurring in every state in the US (Darsie and Ward 2005). *Cs. inornata* has been consistently referenced as a key vector of JCV throughout the US (Waldman et al. 1982, Anderson et al. 2015), and was the first species from which JCV was isolated in 1961. *Cs. inornata* collected from the Central Valley of California were singled out as species highly capable of both vertical and horizontal transmission in the lab (Kramer et al. 1993b). This singular vector competence study found a high degree of variation in parental and peroral transmission rates of JCV depending on the strain of JCV used, and the geographic location of where the mosquitoes were collected. Bloodfeeding habits of *Culiseta inornata* in Montana and Florida indicate generalist feeding with preference towards mammal, specifically cattle (Echnan and Webber 1972, Friesen and Johnson 2013). However, blood feeding patterns are not well known across this species' range.

Cs. inornata has been implicated as a priority vector due to its blood feeding behavior, number of field JCV isolates, and its implication in vertical transmission (Heard et al. 1990, Kramer et al. 1993b). *Culiseta inornata* may be commonly found in vernal, ephemeral pools in NJ, and is found in peak densities in May and June (Andreadis et al. 2008). Although this JCV vector has a very wide distribution, appearing in collections from all 50 states, this species appears to be lacking in light trap collections (Andreadis et al. 2008). *Culiseta spp.* are consistently collected in resting boxes, and this is the preferred collection method.

Best Practices for JCV Surveillance in New Hampshire

Active Surveillance

My recommendations are based on surveillance in New Hampshire and nearby locations. Mosquito activity and optimal traps for collection can vary geographically for the same target species. Therefore, JCV surveillance may vary depending on the region surveillance professionals are located in. In general, JCV activity can be detected through collection of putative vectors, virus testing, and serosurveys for both humans and reservoirs. JCV surveillance and detection programs require laboratory space, entomological sampling and taxonomy skills, as well as training in virus detection methods (Mayo et al. 2001, Andreadis et al. 2008, Pastula et al. 2015, Kinsella et al. 2020). Active surveillance for JCV infected mosquitoes should be carried out from larval sampling in April, and adult collections from May through at least July, to cover peak mosquito populations of early and late season vectors (Heard et al. 1990, Andreadis et al. 2008, Anderson et al. 2015). Larval sampling is a helpful tool for understanding where to focus adult sampling when resources are limited. Adult collections are typically best completed near permanent or ephemeral wetlands, such as vernal pools, where an abundance of snowmelt JCV vectors can be captured (**Table 2)**. A diversity of snowmelt vectors can be collected using CO_2 baited CDC traps (lights can be removed to reduce unwanted by-catch), or with emergence traps (Service 1992), which may also be useful for detecting vertically transmitted JCV in unfed mosquitoes. Once mosquitoes are collected, they should be transported with a cold chain and stored at -80° C. Virus detection methods are available in the published literature (Kuno et al. 1996, Kinsella et al. 2020).

Passive Surveillance

Serosurveys can provide spatial prevalence data that can inform active surveillance efforts (Dupuis et al. 2021a). Deer sera can be efficiently and quickly collected from the cavities of hunter-harvested deer, making retrospective analysis of JCV exposure possible through IgG antibody assays. Serology is an extremely valuable public health tool for JCV surveillance, as it can give spatial information about risk. Plaque reduction neutralization tests (PRNT) are the current gold standard for JCV serology, but, cross reactivity by other CSG viruses with overlapping geographic distributions may occur and incorporating differential antibody quantitation may be a useful next step for accurate identification of JCV specific antibodies (Evans and Peterson 2021).

Prevention and Control of Jamestown Canyon Virus

JCV is a rare virus with increasing human incidence in the USA. Infection can cause debilitating and life-threatening disease in humans, therefore effective control and prevention measures are needed. CSG vaccines are currently in development and showing some promise in animal models (Bennett et al. 2012) for CSG prevention. Currently, no human CSG or specific JCV vaccine is commercially available. As a consequence, JCV prevention must rely on public health messaging, individual mosquito bite prevention, and vector control. While JCV prevention strategies are similar to West Nile virus (WNV) and Eastern equine encephalitis virus (EEEV), JCV may differentially affect rural communities to a greater extent than WNV and EEEV. Specifically, rural communities in northern Michigan (K. Johnson personal comm.) and New Hampshire (M. Notarangelo personal comm.), are of high risk, compared to urban corridors at

risk for WNV and coastal communities at risk for EEEV, since these areas often have limited vector surveillance. Additionally, these communities likely do not have access to publicly funded mosquito control. This leaves a gap in mosquito bite prevention communication for rural communities, which are by nature a hard population to reach. To reach rural populations, targeted public health messaging can be employed (Bernhardt 2004).

JCV vector control using public health insecticides remains unevaluated. Conducting early season control efforts to interrupt JCV transmission before amplifying reservoirs become infected may be an effective strategy. In Michigan's metropolitan control district, larvae of snowmelt vectors such as *Ae. stimulans s.l.*, *Ae. provocans* and *Ae. absserratus/punctor* are targeted for treatment with biopesticides as well as insect growth regulators (IGR) such as methoprene. JCV vector control may require extended employment and early recruitment of seasonal field staff in March or April, which provides a barrier for many agencies (NACCHO 2020, Poggi et al. 2020). Efforts to interrupt JCV transmission by controlling vector populations should be evaluated rigorously with entomological (reduction of JCV MIR/MLE) and public health endpoints (reduction of human disease) as well as impacts on non-target species.

Future considerations for JCV

Many challenges will impact of JCV and human health into the future, such as climate change, and virus mutations or shifts. It is likely that large scale climatic changes will influence the northward distribution of all insects, especially winged *Diptera*, such as mosquitoes. These climatic changes, such as increased storm frequency, intensity and warming, can feed back into mosquito and consequently JCV transmission, indirectly. JCV vectors are reliant on ephemeral

wetlands to maintain their lifecycle (**Table 2**). Climate change may reduce appropriate wetland habitat for JCV vectors and it could modify the extrinsic incubation period of vectors as well as the timing of snowmelt mosquito emergence. Though the future is uncertain, continued monitoring of JCV will provide more data from which decisions can be made and uncertainty can be reduced.

Climate and Snow Melt Pool Ecology

Many JCV vectors complete their life cycles in ephemeral wetlands. Larvae emerge from overwintered eggs when pools are filled by melting snow (**Table 2**). Upon eclosion, adult females search for a bloodmeal, mate nearby and oviposit eggs above the water line in the same pool. Vectorial capacity of these mosquitoes is influenced by their likelihood of survival, which for some can be greater than 2 months, and the number of blood meals they take over this lifespan. Increases in winter temperatures, snow fall patterns, and spring temperatures (Evan and Eisenman 2021, Runkle et al. 2022) could influence both the mosquito emergence timing and infection rates. At just 1° C degree of warming, vernal pool mosquitoes may emerge sooner, with higher survival rates in the spring due to earlier snow melt. In the northeast, increased winter temperatures and number of days greater than 21 °C can increase the likelihood of survival of overwintering adult *Anopheles punctipennis*, and increased winter precipitation in New Hampshire could influence the number of vernal pools available to support snowmelt mosquitoes.

For multivoltine vectors *Ae. sticticus* and *Ae. canadensis*, increased precipitation could yield longer lasting pools, especially in shaded habitats. Which may increase the likelihood of

epidemics given that these species are both human biting and may vertically transmit the virus in some locations, though more research on the latter is needed. Bergren et al. (2018) and Kramer et al. (1993) reported that filial infection rates are temperature dependent in a laboratory setting, with the implication that warmer temperatures produce more infectious mosquitoes per generation. Vertical transmission rates within many putative JCV vectors (*Ae. abserratus, Cs. inornata*) has not been evaluated in a laboratory. In general, we do not currently know enough about specific virus-vector interactions to make concrete assessments of the impact of climate change. More laboratory and long-term field research is necessary, with collections of vectors, virus testing and measuring environmental variables longitudinally at the same sites.

Future research should address improved strategies for rearing snowmelt mosquitoes. While this work may be prone to failure to do our lack of understanding of species-specific biology, large rearing tents with available hosts may inform laboratory work. These questions remain as tall barriers to further research of the effect of temperature on vertical transmission rates, an important knowledge gap for understanding directional impacts of climate change. Long-term field studies and temperature models can be used to understand the long-term impacts of climate on JCV transmission under many scenarios and include expanding / invasive vectors such as *Aedes albopictus*. Isolations of JCV from *Aedes albopictus* have been made in NJ, compounding the potential public health impact of this mosquito species. Container breeding mosquitoes could influence JCV in urban areas and be indirectly impacted by climate change through range expansion.

Conclusions

JCV is an emerging California serogroup (CSG) (Melao subtype) virus that has the potential to impact public health across the Northern United States, and temperate zones worldwide (Kato et al. 2020). JCV is transmitted by many species of biting flies, but primarily by the snow melt *Aedes* mosquitoes. Ungulates such as white-tailed deer serve as reservoirs for JCV, but many other potential hosts remain unevaluated.

JCV is increasingly known to infect humans, and as an intervention, many states including MA, CT, NJ, NY, WI and MI now have routine surveillance programs targeting this virus. These programs detect JCV passively through antibody screening of human or animal sera. These passive methods can be supplemented with active JCV surveillance methods that prioritize trapping potential mosquito vectors that are attracted to light, but primarily CO_2 and other lures. Collaborations between state and county vector surveillance programs and academia can support investigations of blood feeding habits for primary vectors and assess transmission routes and ultimately human risk factors and control targets. Adequate active JCV surveillance is reliant on correct trap placement and timing. To maximize mosquito collections, traps should be placed nearby ephemeral woodland pools in proximity to permanent marsh/swamps in the larval habitats of priority putative vectors. In order to capture early season vector activity, JCV active mosquito surveillance should begin no later than 1 June in Northern states and by 15 May in NJ and Southern states to catch emergence of *Aedes provocans*.

At the time of writing, there are no standardized risk thresholds or published investigations of JCV vector control methods. Though standard practices for mosquito control in the Northern United States exist, any attempt to mitigate virus risk should be conducted with a well thought out experimental design including, at a minimum, negative controls and impacts to non-target species. Although severe human infections by California serogroup viruses including JCV are rare (Hughes et al. 2017), they can still present a significant cost to both patients and healthcare providers (Solomon et al. 2021). Vertical transmission rates and vector competence remain to be evaluated for many priority vectors and could be impacted by global warming events. JCV remains an understudied virus that has the potential to impact public health and should be a priority for surveillance programs within the United States.

Chapter 2: Investigation of the Ecology of Jamestown Canyon Virus *(Peribunyaviridae: Orthobunyavirus)* in New Hampshire

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Introduction

Jamestown Canyon virus (JCV) is a rare mosquito-borne virus first identified in the USA in 1961 (CDC Arbocat 1984). JCV can cause serious disease in humans and incidence has increased over the past two decades as monitoring has increased (Rosenberg et al. 2018). The discovery of widespread human seroprevalence in Connecticut and Nova Scotia suggests that illness from JCV is underreported in the Northeastern hemisphere (Mayo et al. 2001, Patriquin et al. 2018) and this matches evidence of high exposure in wildlife, such as white-tailed deer (*Odocoileus virginianus)* (Dupuis et al. 2021).

In New Hampshire (NH), JCV has gradually replaced Eastern Equine Encephalitis (EEE) as the leading arboviral human infection (NH arbovirus bulletin 2021). Given these findings, it is likely that mosquito-borne transmission of JCV occurs frequently in NH, potentially by a range of mosquito species (Andreadis et al. 2008). Given the lack of formal JCV surveillance in NH, we conducted a survey of mosquito vectors in the state guided by three research questions: 1), Which mosquito species are the primary vectors of JCV in New Hampshire? 2) Which species are important bridge vectors of JCV? 3) What are the host-seeking females of these species attracted to, and can capture be enhanced by light, CO_2 or octenol (1-octen-3-ol)? To address these questions, I collected host seeking and blood fed mosquitoes using several trap methods, from 12 May through 10 August 2021. Mosquito pools were submitted for JCV testing. Blood fed mosquitoes were matched to blood hosts to assess potential JCV bridge vectors. Overall, my study identified potential JCV vectors in NH based on viral field detections and increased blood

host patterns information for key species. My study also provides guidance for field surveillance programs that target JCV vectors.

Materials and Methods

Field Sites

South central New Hampshire has been recognized as a focus for JCV disease risk within the state (Pers. Comm. Matthewson 2020). I selected eight state-owned locations to conduct mosquito trapping (**Figure 2).** Sites were selected based on the presence of vernal pools, shaded crypts, semi-permanent to permanent wetlands, and the presence of putative vectors (**Ch. 1. Table 2**) in larval samples conducted around 20 April 2021.

JCV Vector Surveillance Methods

Mosquito collections

A schematic representation of my study design is provided in **Appendix 1, figure 6.** CDC CO_2 baited light traps were deployed in forested areas within 20m of vernal pools or wetlands at each site. Routine mosquito surveillance was conducted for 23 weeks from 6 May to 14 October 2021. CO_2 baited CDC miniature light traps (John Hock Company model 512) with the lights removed were set at sites 1-8 from 22 May to 22 August 2021. New Jersey light traps (John Hock Company model 1112.5), powered by a 12V deep cycle battery (SLI24D24XD 12V 80ah) connected to a sine wave inverter (Jupiter 56496) with the photo-switch covered, were rotated with CDC traps at sites 1, 4 and 5 during July and August. Resting boxes were set at sites 1-8 from 22 June – 11 August. Mosquitoes were collected from resting boxes using an aspirator

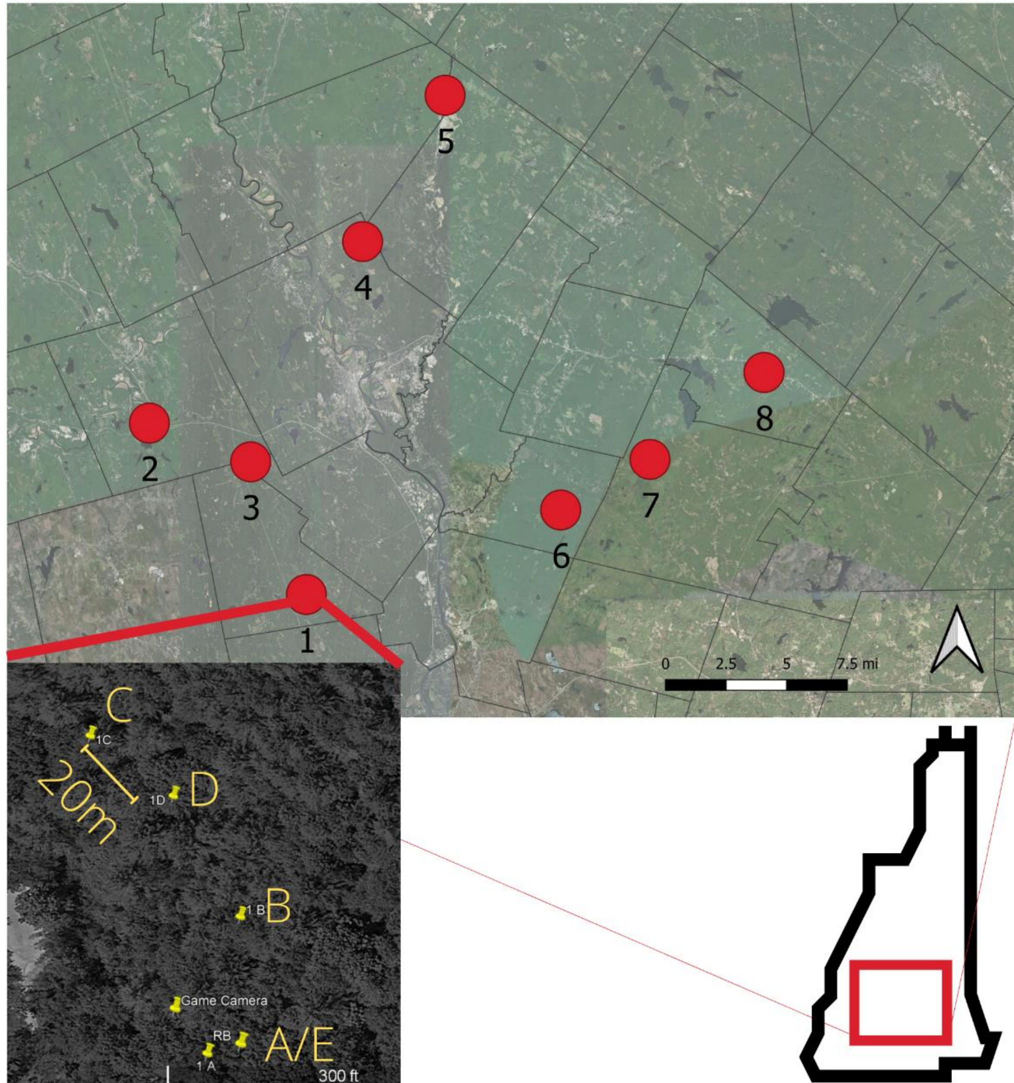

Figure 2. (Main) Location of sites within south-central NH. (Corner) Location of subsites within site 1. CDC traps and NJ light traps rotated between subsites 1A and 1B. Paired octenol traps rotated between subsites 1C and 1D. More details in Appendix 1, Figure 6

(John W. Hock model 1419) while CDC and NJ traps were checked. Sites 4, 5, 7, and 8 were not trapped until 20 May due to person power limitations. All mosquito collections were recorded along with trap type, date, location, and set and check time.

Testing trap enhancement with octenol for JCV bridge vectors

Known JCV hosts, such as white-tailed deer, exhale octenol. To determine if enhancing traps with octenol would increase capture of large mammal seeking mosquitoes, I compared CDC traps supplemented with ~1.8kg of $C0_2$ and 1.5ml octenol (1-Octen-3-ol, Sigma Aldrich W280518) and traps with no octenol (~1.8kg of $C0_2$ only). Octenol was deployed in a 1.7ml microcentrifuge tube taped upright to the trap with parafilm and a short wick (Kline et al. 1990). Traps were placed 30m apart and equidistant from relevant habitat features at C and D subsites. Traps were rotated among subsites for two consecutive nights. The experiment was repeated at 3 field sites (Sites 1, 3, 6), in mid-June, and during August (**Appendix 1, figure 6**).

Ecological data collection methods and host availability survey

At five sites (sites 3, 4, 5, 8) across a north to south gradient, data loggers (HOBO Pro V2; U23-001) were deployed to record relative humidity and temperature at half hour intervals. Precipitation data was downloaded from the National Oceanic and Atmospheric Administration's NOWData (ACIS 2022). Game cameras (Moultrie M-880, #MCG-12691, Calera, AL, USA) were placed at sites 1-7 on deer trails to measure deer availability for mosquito feeding. Based on the risk of vandalism, and the size and variability of the forested habitats surrounding trap sites, some sites (2, 3, 5, 6, 7) had two cameras while other sites (1, 4) had one camera placed within 100m of the 'A' trap site.

Mosquito identification

After collection, mosquitoes were transported on dry ice and stored briefly at -20° C or -80° C in filter paper lined petri dishes. Mosquitoes were identified to species using published keys (Darsie and Ward 1981, Andreadis et al. 2005). From May to August 2021, all pooled

collections were identified to species and submitted for virus testing. Non-pooled mosquito collections were transported to Cornell University on dry ice and re-identified both morphologically and with PCR (see methods below). Subsets of pooled collections were identified using PCR and compared to morphological identifications. Specimens of good quality were pinned and submitted to the Cornell University Insect Collection as vouchers.

Virus testing

Of over 20 mosquito vectors associated with JCV (Andreadis et al. 2008), 13 species were chosen from my collections for virus testing (**Appendix 1, table 10).** These species were selected based on our A-priori understanding of JCV transmission from previous studies in the Northern US (Boromisa and Grayson 1990, Heard et al. 1990, Andreadis et al. 2005). Un-engorged mosquitoes were pooled in groups of 1-50 and transferred to sterile 2 ml tubes, labelled, and stored at -20° C until submission for virus testing at the New Hampshire Public Health Lab (NH Health and Human Services, 29 Hazen Dr. Concord, NH). RNA was extracted from pooled samples using an automated KingFisher Flex instrument (ThermoFisher Scientific Waltham, MA 02541) and MagMAX™ Viral nucleic acid kit (ThermoFisher Scientific Waltham, MA 02541). Quantitative PCR was prepared using a qScript One-Step qRT-CR Kit, Low ROX™ and TouchMix (Quanta Biosciences Beverly, MA 01915). PCR reactions included JCV174-Forward + JCV269c Reverse primers, and a JCV231c-Probe (5’ FAM-TCCGCTCCGGTTTACGAGCG-BHQ1 3’). PCR cycling conditions included 50° C for 30 minutes, 95° C for 10 minutes and 45 cycles of 95° C for 15 seconds, 60° C for 60 seconds. Positive pools were confirmed by repeating the qPCR assay.

Bloodmeal analysis

Engorged mosquitoes were examined with a dissecting microscope and degree of engorgement was noted by evidence of red or black blood in the abdomen. These mosquitoes were placed individually into sterile 1.7 ml microcentrifuge tubes and stored at -20° C for transport back to Cornell University. Abdomens of engorged mosquitoes were removed using flame-sterilized forceps and placed in a new sterile microcentrifuge tube.

DNA was extracted using a PureGene kit (Gentra Systems, Minneapolis, MN) following the manufacturer's instructions. Briefly, abdomens were homogenized with a cell lysis buffer and incubated in a protein precipitate solution. After precipitating protein, supernatant was transferred to a new, sterile tube, and DNA was washed in 70% ethanol. Finally, 50ul nuclease free water was added to each tube.

The *cytochrome c oxidase I* (COI) region of vertebrate mitochondrial DNA was targeted for DNA amplification using two different primer sets (**Table 3**) (Townzen et al. 2008, Reeves et al. 2018). The primers from Reeves et al. (2018), VertCOI_7194 and ModRepCOI, target a 395 base pair amplicon in the *cytochrome c oxidase I* (COI) region of vertebrate mitochondrial DNA. Primers described and used by Townzen et al. (2008) and Ledesma (2014) COI long, and COI short amplify a short (324 bp) and long (663bp) segment of the COI region. Each PCR reaction was a 20ul volume with nuclease free H_20, primers and Taqred Master mix (Genesee Scientific Corp., San Diego, CA).

DNA was amplified under the following conditions: for the VertCOI_9174/ModRepCOI primers, thermal cycling conditions were: 94°C for 3 min (minutes) followed by 40 cycles of

94°C for 40s (seconds), 51.2°C for 30s, 72°C for 1 min, followed by an extension step at 72°C for 7 min. For the COI long and short primers, thermal cycling conditions were 95°C 5 min, 40 cycles of 95°C for 30s, 50°C for 50s, 72°C for 1 min, followed by an extension step at 72°C for 5 min. All PCR products were visualized on a 1% agarose with gelRED, run at 100V and visualized on a BioRad Gel Doc XRS. PCR products that yielded bands at the correct amplicon were cleaned with ExoSap-IT and submitted for sequencing at Cornell Biotechnology Resources Center. Amplicon sequences were matched against NCBI databases, using ≥ 90% identity, and an E value of $< 10^{-40}$ as a threshold for matching identification.

Mosquito PCR-based identification

DNA extraction, PCR, sequencing, and matching was performed as described above on mosquitoes that were damaged or not identifiable morphologically to species, as well as a selected subset of mosquito species that were also tested for virus. Mosquito COI primers (**Table 3**) were selected that have been used previously to identify mosquito species accurately using the COI barcode of life (Kumar et al. 2007, Chan et al. 2014). Each PCR reaction contained 22ul nuclease free H_20, 25ul Apex taq RED MasterMix (Genesee Scientific Corp., San Diego, CA), and 1ul each of 10uM forward and reverse primer (**Table 3**). PCR cycling conditions used a reverse-touchdown method as follows: 95°C for 5 min, then 5x cycles of 95°C for 30s, 45°C for 30s, 72°C for 1 min, then 35 cycles of 95°C 30s, 51°C for 40s, 72°C for 1 min, to finish an extension step at 72°C for 5 min. PCR products that yielded bands at the correct amplicon were cleaned with ExoSap-IT and submitted for sequencing at Cornell Biotechnology Resources Center.

Table 3. Primer sets used in all PCR reactions

Primer name	3' - 5' - forward	5' - 3' reverse	Amplicon
VertCOI_7194 + ModRepCOI	CGMATRAAYAAYATRAGCTTCTGA	TTCDGGRTGNCCRAARAATCA	395 bp
COI Long (BM)	AACCACAAAGACATTGGCAC	AAGAATCAGAATARGTGTTG	663 bp
COI Short (BM)	GCAGGAACAGGWTGAACCG	AATCAGAAYAGGTGTTGGTATAG	324 bp
COI (ID)	GGATTTGGAAATTGATTAGTTCCTT	AAAAATTTTAATTCCAGTTGGAACAGC	738 bp
JCV174-Forward + JCV269c Reverse	CAGTCTGTCAGCCGTTAGGA	AATTTCCACCTGCCACTCTC	-----

Data analysis

After identification, mosquitoes were enumerated and entered into an excel data sheet by collection date, location, and trap type. Maximum likelihood estimates (MLE) were calculated (Biggerstaff 2008) for virus positive pools. All statistical and descriptive analyses were conducted with MS excel™ & R Studio, version 3.6 (R Core Team 2021), using the ggplot2 (Wickham 2016), GLMTMMB (Brooks et al. 2017), bbmle (Bolker and R Core Team 2021) and emeans (Length et al. 2021) packages.

Octenol supplementation of CDC traps comparisons were conducted at three sites (1,3,6), over 24 paired trap nights in June and August. The effect of octenol supplementation, site and month on mosquitoes collected were assessed with seven generalized linear model forms (**R code provided in Supplement**), and the model with the lowest AIC value was selected

(**Appendix 1, Table 9**). Final models were assessed with ANOVA, dispersion test, histogram of residuals, and a qqplot using simulated residuals from the package DHARMa() (Hartig 2022). In addition the collection months were analyzed separately to explore the effect of octenol supplementation on *Anopheles punctipennis* and snowmelt *Aedes* collections separately. *An. punctipennis* abundance fit a general Poisson distribution (glmmTMB::genpois()), all other species abundance fit a negative binomial distribution (glmmTMB::nbinom2()) with the exception of *Ae. sticticus* (glmmTMB::nbinom1()). GLM Models compared abundance of species in traps by site, octenol, month and trapping time (GLM, Abundance ~ Site + Octenol + Month + offset(trap run time)). The offset() term was included to allow for a predictive response standardized by trap run time, to account for slight variations between hour traps were run each night, using the emeans() package.

Differences between NJ light traps and CDC traps baited with CO_2 were analyzed for three genera and 13 species of mosquitoes using a paired approach. Only data from paired trap nights at sites 1,4 and 5, where NJ traps and CDC traps were used in tandem and swapped between collection nights from 7 July to 10 August was included. These data were assessed visually using ggplot2() and checked for normality in differences using a Shapiro-Wilk test. A paired Wilcoxon ranked sign test, or a paired t-test was used to assess differences for each species, depending on if the differences between trap types were normally distributed.

Results

Mosquito collections by trapping method

Cumulatively, 50,520 adult female mosquitoes were collected, representing over 30

mosquito species and five genera. *Cq. perturbans* and *Ae. canadensis* were, by far, the most abundant mosquitoes. Sampling effort was as follows: a total of 177 successful trap nights for CDC traps, 28 trap nights from NJ traps, and 56 collections were made from resting boxes. On

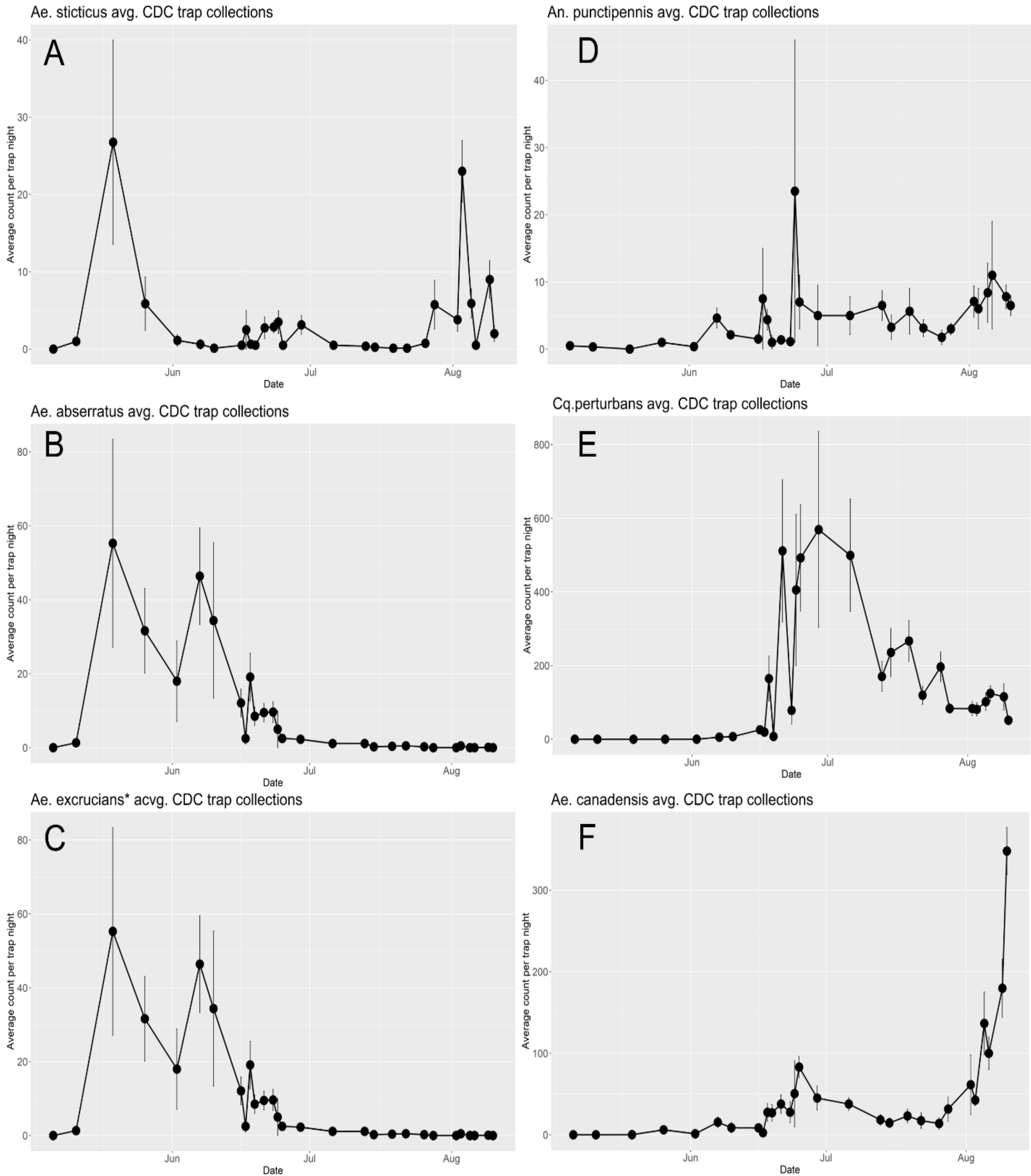

Figure 3. Average numbers of mosquitoes collected in traps (abundances) across CDC trap collections. Error bars represent standard error. A) Aedes sticticus, B) Aedes absberratus, C) Aedes excrucians, includes fitchii, stimulans, D) Anopheles punctipennis, E) Coquillettidia perturbans, F) Aedes canadensis*

average, CDC traps were run from 14:15 hrs to 10:00 hrs the following day, for an average run

time of 19.5 hours per trap night. Based on averaged collections from CDC traps, peak abundance of both *Ae. sticticus* and *Ae. abserratus* occurred around 20 May, though these species were collected beginning on 12 May when temperature and relative humidity were both at their lowest (**Appendix 1, figure 8**). Peak abundance of *Ae. excrucians* occurred on approximately 10 June, but this species was first collected on 20 May (**Figure 3**). Collection data for *Ae. canadensis* and *Ae. sticticus* (**Figure 3,4**) suggest they may have multivoltine cycles in NH, in contrast to what is commonly reported in the literature. Octenol supplementation did not enhance the capture of snow melt *Aedes* mosquitoes in any of the three field sites where it was deployed, nor the abundance of putative JCV vectors (GLM, df= 24, $P > 0.05$; **Appendix 1, table 9**). However, octenol slightly enhanced the capture of *Anopheles punctipennis* in CDC traps in June and August together (GLM, $p = 0.06$) (**Appendix 1, figure 7**). Site level effects (e.g. Trap placement) were greater predictors of collection numbers than effect of octenol for *Ae. canadensis* (ANOVA, $F= 9.9$, $p =0.001$), *Cq. perturbans* (ANOVA, $F = 9.185$ $P= 0.001$) and *Ae. abserratus* (ANOVA, $F = 3.938$, $P = 0.03$).

In paired collections of CDC and NJ traps (n=25, respectively), CDC traps captured more mosquitoes (n=7755) than NJ traps under direct comparisons. In July and August, CDC traps caught significantly more mosquitoes, including Aedes *excrucians/fitchii/stimulans*, *Aedes sticticus* and *Aedes abserratus* (Wilcoxon test, $P < 0.05$) than NJ traps. In general, CDC collections captured more *Cq. perturbans* (n=4980) and *Ae. canadensis* (n=1160 than NJ traps (Wilcoxon test, $P < 0.05$). CDC traps also captured significantly more *Ae. trivittatus* and *Psorophora ferox* than NJ traps (Wilcoxon test, $P < 0.05$). NJ traps (n=1486) caught more

mosquito species than CDC traps, including Culex *territans* and *Uranotaenia sappharina* (Wilcoxon test, P < 0.05). NJ traps also captured significantly more *Culiseta species* (including *Cs. melanura* and *Cs. morsitans*), *Aedes aurifer/cinereus* than CDC traps (Wilcoxon test, P < 0.05). Collection numbers of *Anopheles punctipennis* (Paired t-test, P = 0.541), *Aedes vexans* (Wilcoxon test, P = 0.104), *Culex pipiens/restuans/salinarius* (Wilcoxon test, P = 0.343) were not significantly influenced by trap type.

A total of 270 mosquitoes were collected from resting boxes from 56 collections. During July and August, resting boxes were efficient at collecting *An. punctipennis* (28.5%), *An. quadrimaculatus* (21.9%) and *Cs. melanura (11.2%)*. A significant percentage of blood fed mosquitoes were captured in resting boxes (25% of total).

Virus testing results

Virus detections from submitted pools occurred throughout the field season at four out of eight field sites (**Table 4**). All positive pools came from CDC trap collections, with five pools derived from the octenol experiment. To conceptualize JCV virus infection rates, vector indices were calculated from the maximum likelihood estimate per 1000, multiplied by the average abundance per trap night (**Figure 4**). Error bars represent a 95% confidence interval derived from the MLE infection rate, which considers the number of pools submitted for each species. *Aedes sticticus, Aedes* excrucians, and *Aedes abserratus* all had higher vector indices in May and

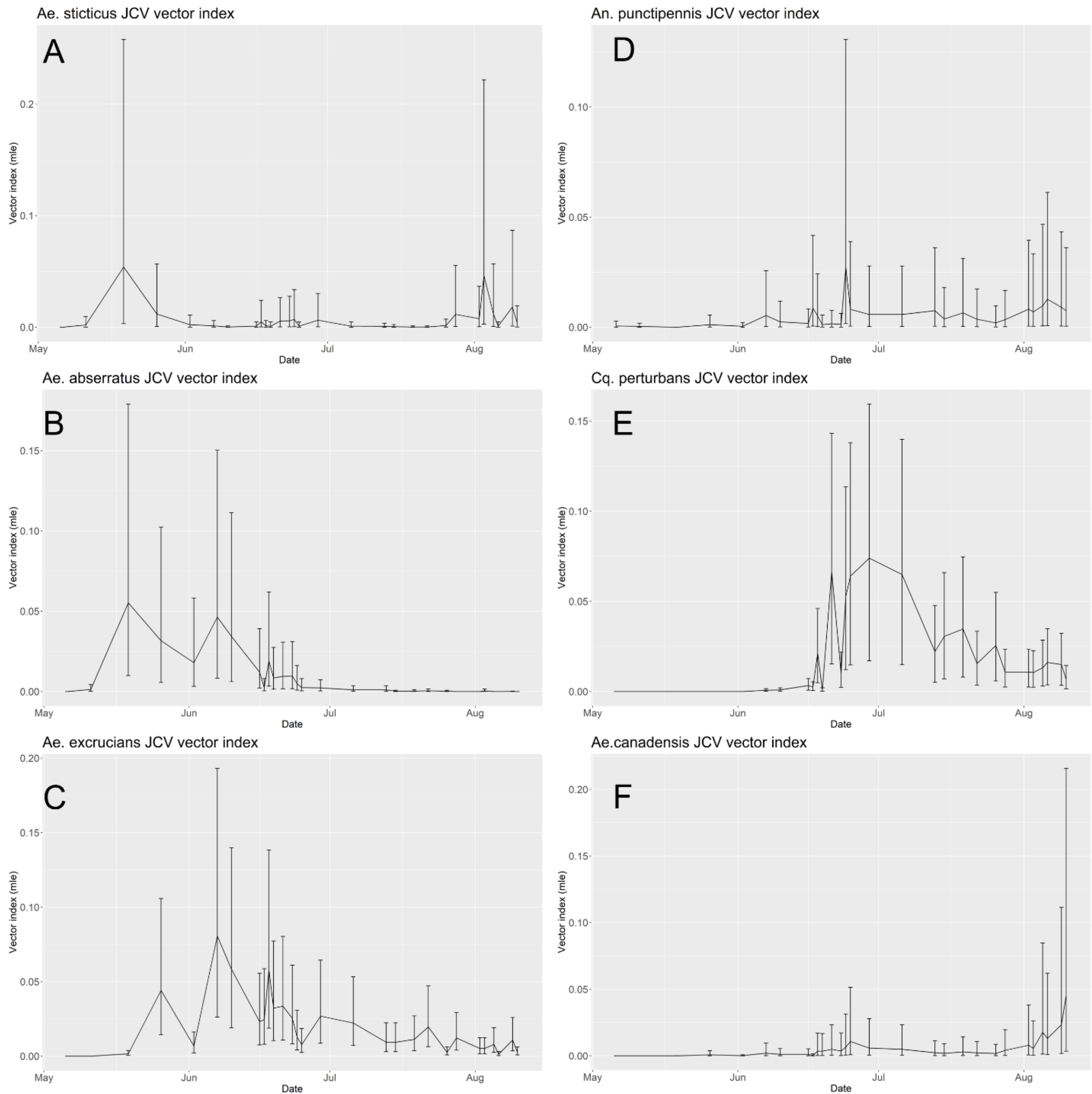

*Figure 4. Vector indices per trap night, calculated from the avg/trap night * (mle/1000). Error bars represent a 95% CI for the MLE estimate. A) Aedes sticticus, B) Aedes absserratus, C) Aedes excrucians*, includes fitchii, stimulans, D) Anopheles punctipennis, E) Coquillettidia perturbans, F) Aedes canadensis*

early June, while *Cq. perturbans, Aedes canadensis,* and *An. punctipennis* had higher vector

indices later in the season. A positive pool of one *An. punctipennis* (BIDC331) collected on 27 May represented the first virus detection of the season. *Ae. excrucians/stimulans/fitchii* yielded positive pools throughout the season on 8 June (BIDC107), on 7 July (BIDC781), on 20 July (BIDC1050) and on 10 August (BIDC1640). Two positive pools were collected from *Ae. abserratus* on 11 June (BIDC185) and 18 June (BIDC1566). A positive pool of three *Ae. sticticus* (BIDC204) was collected on 17 June. *Cq. perturbans* yielded three positive pools, shortly after emergence on 18 June (BIDC1570), followed by 20 July (BIDC1053), and 10 August (BIDC1642). *Ae. canadensis* yielded one positive pool 10 August (BIDC1647) concurrent with peak densities of this species. Five JCV positive pools were clustered between 8 June and 18 June. Site 1 yielded four total JCV positive pools, from multiple species from 17 June to 7 July. Site 5 also yielded four total JCV positive pools, from 27 May to 20 July.

Jamestown Canyon virus was isolated from 6 mosquito species across 3 genera in 12 positive pools; NH DHHS reported an additional 2 positive JCV pools in 2021 from *Ae. canadensis* that were not included in this study. Vectors that likely contributed to transmission of JCV in south central NH during 2021 were *Ae. excrucians/fitchii/stimulans*, *Ae. sticticus*, *Anopheles punctipenni*s, *Ae. abserratus*, *Ae. canadensis*, and *Coquillettidia perturbans* **(Table 4, 5)**. *Ae. excrucians/fitchii/stimulans* and *Ae. sticticus* had the highest JCV infection rates (MIR and MLE).

*Table 4. JCV Positive pools, * = Aedes fitchii/stimulans included*

ID	Mosquito	Pool size	Date	Town	Site	Trap
BIDC331	*An. punctipennis*	1	5/27/2021	Canterbury	5	CDC

BIDC107	*Ae. excrucians**	5	6/8/2021	Bow	3	CDC
BIDC185	*Ae. abserratus*	50	6/11/2021	Canterbury	5	CDC
BIDC204	*Ae. sticticus*	3	6/17/2021	Dunbarton	1	CDC
BIDC1570	*Cq. perturbans*	27	6/18/2021	Dunbarton	1	CDC
BIDC1566	*Ae. abserratus*	4	6/18/2021	Dunbarton	1	CDC
BIDC781	*Ae. excrucians**	25	7/7/2021	Dunbarton	1	CDC
BIDC1053	*Cq. perturbans*	50	7/20/2021	Canterbury	5	CDC
BIDC1050	*Ae. excrucians**	17	7/20/2021	Canterbury	5	CDC
BIDC1642	*Cq. perturbans*	50	8/10/2021	Allenstown	6	CDC
BIDC1640	*Ae. excrucians**	7	8/10/2021	Allenstown	6	CDC
BIDC1647	*Ae. canadensis*	50	8/10/2021	Allenstown	6	CDC

*Table 5. Cumulative JCV infection rates by putative vector mosquito species. * = Includes Ae. fitchii/Ae. stimulans*

Mosquito	**Pools**	**Positive Pools**	**Total tested**	**MLE (95% CI)**	**MIR**
Ae. abserratus	119	2	2026	1	1:1013
				(0.18 - 3.24)	
Ae. canadensis	289	1	7875	0.13	1:7875
				(0.01 - 0.62)	
Ae. excrucians*	199	4	1563	2.58	1:391
				(0.84 - 6.18)	
Ae. sticticus	74	1	485	2.02	1:485
				(0.12 - 9.64)	
An. punctipennis	167	1	871	1.16	1:871
				(0.07 - 5.56)	
Cq. perturbans	682	3	28796	0.1	1:9599
				(0.03-0.28)	

Bloodmeal Analysis and Host Availability

DNA was successfully amplified from 95 out of 178 (53%) putative JCV vector mosquitoes with apparent full or partial bloodmeals (**Table 6**). Snowmelt species, *Ae. excrucians/fitchii/stimulans* (n=12), *Ae. sticticus* (n=5) and *Ae. abserratus* (n=7), exhibited

mammalophilic feeding patterns, with the majority of bloodmeals from white-tailed deer (88.4%). Additionally, one raccoon (*Procyon lotor*) bloodmeal was identified from *Ae. abserratus* and two human (*Homo sapiens)* bloodmeals were identified from *Ae. sticticus* and *Ae. excrucians/fitchii/stimulans*, respectively.

Ae. canadensis (n= 16) exhibited slightly broader mammalophilic blood feeding habits compared to the snowmelt *Aedes*, with bloodmeals matched to white-tailed deer (68.8%), moose (*Alces alces*, 25%), and one gray squirrel *(Sciurus carolinensis*). One avian blood meal was matched from *Ae. canadensis* to a woodcock (*Scolopax minor*). Anopheles *punctipennis* (n= 8) fed on mammals, with bloodmeals matched to white-tailed deer (50%), human (25%) and eastern chipmunk (*Tamias striatus,* 25%). *Cq. perturbans* (n= 44) had the broadest host feeding patterns with bloodmeals matched to mammals (94%) and birds (6%). Mammalian bloodmeals were identified as human (47%), white-tailed deer (34%), river otter (*Lontra canadensis*, 4.5%), moose (4.5%), and one bloodmeal from red fox (*Vulpes vulpes*) and eastern chipmunk, respectively. The three *Cq. perturbans* avian bloodmeals were derived from barred owl (*Strix varia*), black-capped chickadee (*Poecile atricapillus*) and broad winged hawk (*Buteo platypterus*). A total of 137 bloodmeals were also collected from non-putative vector species but were not matched to hosts due to time constraints.

*Table 6. Bloodmeals identified from putative vectors (n = 95), matched to NCBI database, * = includes fitchii/stimulans*

Host species	*Ae. abserratus*	*Ae. sticticus*	*Ae. excrucians**	*Cq. perturbans*	*Ae. canadensis*	*An. punctipennis*
Gray squirrel					1	

Human		1	1	21		2
Moose				1	4	
River otter				2		
White tailed deer	6	4	11	15	11	4
Raccoon	1					
Chipmunk				1		2
Red fox				1		
Barred owl				1		
Black-capped chickadee				1		
Broad winged hawk				1		
Woodcock					1	

Host availability, measured by the game camera survey, yielded observations of ten mammal species and three species of bird, which is likely an underestimate of available hosts given the available habitats (DeGraaf and Yamasaki 2001). The number of days that cameras were active and the number of observations taken by cameras varied by site but was not significantly different between sites (Kruskal-Wallis test, p value > 0.05). Deer and human photos were taken at each site (**Figure 5**), which was not surprising given that camera placement was biased towards deer, and all game cameras were near trails or publicly used areas. Sites with at least one JCV positive pool (1, 3, 5, 6) varied in the number of deer captured on camera but were not consistently abundant in deer. Humans were also captured at each site, and particularly at site 5 where large numbers of people were observed and suspected to be geocaching. Human activity at each site could be related to risk of an infectious mosquito bite, and in fact one human case was detected nearby site 5 after the conclusion of the field season.

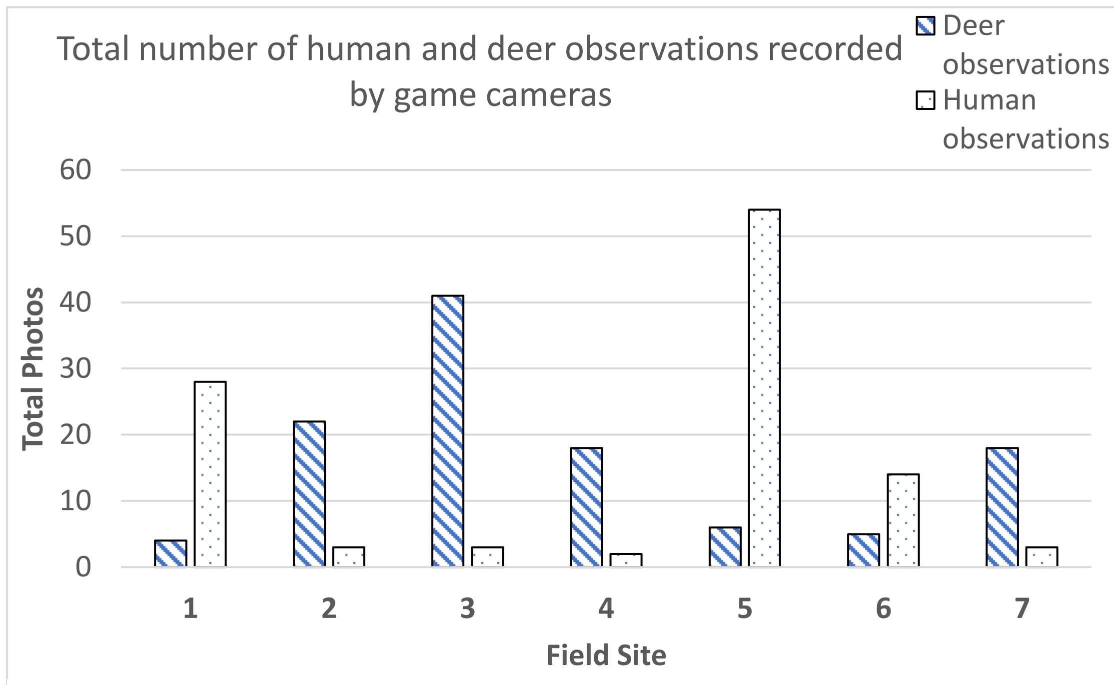

Figure 5. Numbers of human and deer observations at 7 sites from game cameras. Sites 2,3,5,6,7 all had two cameras, while sites 1 had one camera.

Discussion

Overall findings

My study represents the first assessment for JCV mosquito vector ecology in NH, including mosquito phenology, comparison of collection methods, virus detection, and bloodmeal analysis. Based on the most likely mosquito epizootic and enzootic species reported from other locations, I focused on snowmelt *Aedes, Cq. perturbans, Ae. canadensis* and *An. punctipennis* for my study in NH. Snowmelt *Aedes* adults were the first host-seeking adult vectors to be collected in May, followed by high numbers of *Cq. perturbans* and *Ae. canadensis* in June. *Anopheles punctipennis* was first detected in CDC traps and resting boxes in June. CDC traps baited with CO_2, regardless of the presence of octenol, were the best trapping tool for

surveillance of putative JCV vectors, with the exception of *An. punctipennis* which were readily collected using resting boxes. A total of 12 JCV positive pools was detected from my collections, ranging from 27 May to 10 August, with the greatest frequency of positive pools in June. All six of the putative vector species I evaluated fed on white-tailed deer, implicating their potential role in enzootic transmission of JCV. My study provides context regarding geographical variation in JCV vector ecology (Pastula et al. 2015) and establishes the first JCV blood feeding data for potential vectors in New Hampshire.

Virus isolations

JCV was isolated from six out of thirteen mosquito species tested for virus. JCV was not isolated from the previously incriminated *Ae. provocans*, of which I tested 286. I may have collected too few samples of some species to be able to detect the virus, such as collections of *Aedes communis* and *Aedes provocans.* MIRs for *Ae. provocans* range from 1:219 – 1:419, and 1:17 – 1:612, varying seasonally (Boromisa and Grayson 1990, Andreadis et al. 2008). These species may still be important in New Hampshire and continued monitoring is needed to fully understand their role in JCV transmission.

Among the many aspects of JCV ecology is the overwintering mechanism. The JCV isolation from *An. punctipennis* in late May suggests a mechanism for JCV overwintering in NH. *Anopheles punctipennis* overwinter as adults and may be able to sustain an infection until activity resumes in the spring. Thus, it is possible that infected *An. punctipennis* may disseminate JCV to reservoir hosts before the emergence of snowmelt *Aedes*, as suggested previously (Defoliart et al. 1969, Issel 1973). Future work should address JCV in overwintering

An. punctipennis, as well as their vector competence. Ideally these experiments should be conducted with a locally isolated virus, such as the BIDC331 isolate collected in this study. The clustering of five JCV positive pools in June suggests that month may be a crucial virus amplification period and time for human risk in central NH.

Blood feeding Habits of JCV vectors

All putative JCV mosquito vectors I tested fed on white-tailed deer, the only amplifying reservoir of JCV that has been identified to-date (Issel 1972, Watts et al. 1979). However, studies on reservoir hosts have been limited in scope. Enzootic JCV transmission could occur regularly in NH between deer and mosquitoes, particularly in June, when activity of putative vector populations overlap with each other and with white-tailed deer activity. This could then potentially lead to epizootic spillover from infected deer to humans if sufficient human biting vectors (e.g. *Cq. perturbans)* become infected. A study on penned deer in Michigan revealed that timing of deer JCV seroconversion broadly overlapped with peak JCV vector mosquito densities (Grimstad et al. 1987). Sites with detections of JCV did not have consistently high deer abundance, which is in agreement with relationship between JCV and deer in CT (Andreadis et al. 2008). However, the presence of humans at sites with JCV activity (sites 1 and 5) suggests a high likelihood of exposure to infectious mosquitoes, particularly at site 5, where people (geocachers) were very abundant.

JCV outbreaks appear to be fueled, in part, by snowmelt *Aedes* mosquitoes. These species, including *Ae. provocans*, may become vertically infected with JCV with potential to be maintained in the egg stage over winter and be infectious when biting in the spring. In

addition, JCV may overwinter in infected adult *Anopheles punctipennis.* During June, newly infected deer could be exposed to high densities of biting *Ae. canadensis* and *Coquillettidia perturbans*. A small proportion of these mid-summer vectors that bite viremic deer will become infectious and go on to bite humans during the late mosquito season (July or August) in NH. This potential JCV transmission cycle is consistent with the onset of JCV cases in NH, starting in May until the conclusion of the mosquito season. This is earlier than other more well-studied arboviruses such as West Nile virus.

Trap comparisons

I did not see an enhancement in *Aedes* snow melt mosquito species with the addition of octenol as a supplemental lure. However, significantly more An. punctipennis were captured in octenol supplemented traps. My analysis was limited by being split between two months. Other researchers have reported enhanced collections of *Cq. perturbans* with octenol in the Florida everglades (Kline et al. 1990), or when combined with ammonium bicarbonate to enhance capture of *An. punctipennis* in CT (Eastwood et al. 2020). NJ traps collected a wider range of species than CDC traps baited with CO_2, however, they caught far fewer host seeking mosquitoes. Many putative JCV vectors are readily attracted to CO_2 more than light, but *An. punctipennis* did not appear to have a strong pattern of attraction to either. Future research should compare these two methods along with other lures such as ammonium bicarbonate to increase captures of early season *An. punctipennis*, as an approach for monitoring early season JCV virus risk.

There were several limitations to my study. Mosquito identification was difficult to conduct with the high number of mosquitoes that were collected, and person power was limited. Morphological identification errors were most commonly made between *Ae. provocans/Ae. communis* as well as *Ae. sticticus*. I was able to review my morphological identifications after transport of blood fed mosquitoes back to Cornell University, correcting the low number of errors. However, there is a chance that some species in positive pools submitted to the NH DPH were misidentified. In addition, the sampling effort was limited to May – August 2021. There are at least three additional months when mosquitoes are active in NH and could be sampled. Furthermore, the NJ traps and resting boxes were not operated until late June, which limited my ability to compare capture of early season mosquitoes.

Other limitations include my assessment of host availability and environmental data collection. Since my game camera survey was biased towards deer, and lacked replication within sites, I do not have a true understanding of the presence of other potential hosts. I was unable to monitor the relative humidity and temperature at three out of eight sites. Although I selected sites where I would optimally capture JCV putative vectors, selection was non-random, which may have introduced some bias into my analysis, particularly when comparing sites where I did not detect JCV to those where I did. I only conducted the study for one year. In addition, my testing results are biased to the thirteen species of putative JCV vectors I collected. Future studies should attempt to overcome these limitations, with multi-year evaluations of mosquito abundance and blood feeding patterns as well as virus surveillance across the full season on vector activity in NH.

My study represents the first mosquito survey in south central NH since 1952 (Blickle 1952) and expands knowledge of regional variation in JCV vector ecology in the United States. I described the first mosquito species pools to test positive for JCV following 18 human disease cases, including one fatality, described in NH since 2013. In 2021, there were a total of five human cases reported in NH from 28 June to 27 October. One case was reported from within 1km of site 5 in Canterbury, where JCV was detected in four mosquito pools from 27 May – 20 and where human activity was quite high. My study provided essential data to the NH Public Health Laboratory, a resource-limited agency, to pursue additional support for JCV research and mitigation funding.

Future research within NH should assess overwintering and early season virus amplification and laboratory work important for vector incrimination. Remaining questions include the potential for JCV to overwinter in *An. punctipennis* adult mosquitoes in human made cellars, sheds, as well as thermally protected evergreen forest areas. Vector competence and vertical transmission assessments are needed for all putative vectors within NH. Seroconversion and isolation of JCV from white-tailed deer and potentially moose are needed in tandem with continued bloodmeal analysis from putative vectors, perhaps integrating emerging xenosurveillance strategies (Grubaugh et al. 2015). Furthermore, isolating and genotyping the JCV I collected from mosquitoes in NH may yield insights into the virulence of JCV in NH. This virus strain should be compared to isolations from other states and mosquito species to further our understanding of regional differences in JCV phylogeny (Armstrong and Andreadis 2007). Taken together, my study provides evidence of Jamestown Canyon virus

activity within mosquito vectors that will aid in future prevention of human disease through the control of mosquitoes, informed by an understanding of JCV vector ecology.

Funding

This research was supported in part by Centers for Disease Control and Prevention cooperative agreement: 1U01CK000509-01. This content is solely the responsibility of the authors and does not necessarily represent the official views of the Centers for Disease Control and Prevention.

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Appendix 1. Supplemental Tables and Figures

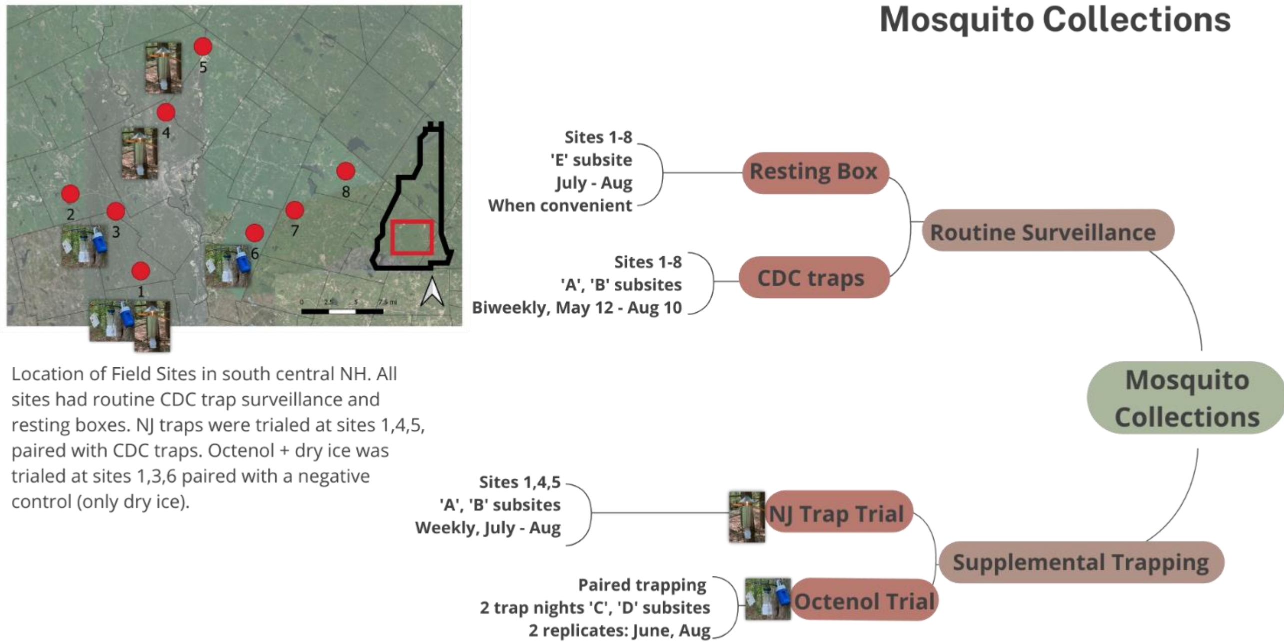

Figure 6. Appendix 1. Schematic of unbalanced study design. Sites with supplemental NJ traps or octenol trials are highlighted on the map. All sites had CDC traps and resting boxes.

Table 7. Seroprevalence of JCV antibodies in 11 studies using PRNT (Greatest accuracy for differentiating JCV from other CSG), with a sample size greater than 10. LI = Long Island, HV = Hudson Valley, CNY = Central New York, WNY = Western New York

Author	**Year publ.**	**State**	**Host**	**Sample size**	**JCV seroprevalence**
Watts et al.	1982	DE,MA,VA	Goat	16	0%
Goff et al.	2012	Newfoundland	Hare	20	0%
Fulhorst et al.	1996	California	Brush rabbits	21	0%
Fulhorst et al.	1996	California	Ground Squirrels	29	0%
Miller et al.	2000	California	Swift/Kit fox	37	0%

Fulhorst et al.	1996	California	Woodrats	39	0%
Fulhorst et al.	1996	California	Deer mice	42	0%
Watts et al.	1982	DE,MA,VA	Reptiles	227	0%
Watts et al.	1982	DE,MA,VA	Gray Squirrel	275	0%
Fulhorst et al.	1996	California	Dogs	91	1%
Goff et al.	2012	Newfoundland	Mink	202	5%
Miller et al.	2000	Colorado	Swift/Kit fox	39	5%
Miller et al.	2000	New Mexico	Swift/Kit fox	18	6%
Fulhorst et al.	1996	California	Cattle	155	9%
Nelson et al.	2004	California	Horse	425	16%
Watts et al.	1982	DE,MA,VA	White-tailed deer	403	18%
Watts et al.	1982	DE,MA,VA	Horse	16	19%
Dupuis et al.	2021	New York (LI)	White-tailed deer	58	19%
Zamparo et al.	1997	Connecticut	White-tailed deer	446	21%
Nagayama et al.	2001	North Carolina	White-tailed deer	135	27%
Fulhorst et al.	1996	California	Horse	306	28%
Watts et al.	1982	DE,MA,VA	Sika deer	47	34%
Watts et al.	1982	DE,MA,VA	Cottontail rabbit	21	38%
Goff et al.	2012	Newfoundland	Sheep	119	45%
Goff et al.	2012	Newfoundland	Cattle	253	47%
Aguirre et al.	1992	Mexico	Blacktail jackrabbit	10	50%
Dupuis et al.	2021	New York (WNY)	White-tailed deer	141	50%
Dupuis et al.	2021	New York (HV)	White-tailed deer	113	52%
Goff et al.	2012	Newfoundland	Horse	51	64%
Dupuis et al.	2021	New York (CNY)	White-tailed deer	178	72%
Patriquin et al.	2018	Nova Scotia	White-tailed deer	82	88%

Table 8. Paired NJ and CDC trap nights (n=25, respectively) at sites 1,4,5.

Species	**CDC trap with CO_2**	**NJ light trap**
Aedes abserratus	21	0
Aedes aurifer/cinereus	37	264
Aedes canadensis	1160	97
*Aedes excrucians**	270	8
Aedes provocans	6	0
Aedes sticticus	98	6
Aedes triseriatus	20	2

Aedes trivittatus	638	42
Aedes unknown	64	41
Aedes vexans	81	164
Anopheles punctipennis	105	61
Anopheles quadrimaculatus	9	7
Anopheles walkerii	9	8
Coquillettidia perturbans	4980	590
Culex pipiens/restuans	25	33
Culex salinarius	34	58
Culex territans	0	13
Culex unknown	10	7
Culiseta spp.	3	22
Psorophora ferox	184	21
Uranotaenia sappharina	0	38

Table 9. Results from generalized linear models used to evaluate the effect of octenol on putative vector abundance.

Predictand	Family	AIC	Dispersion (>1 = over dispersed)	Octenol effect (a=0.05)
Ae. canadensis	Neg. binomial	243.1	2.4	0.78
Cq. perturbans	Neg. binomial	275.8	1.35	0.6
An. punctipennis	Poisson	164.8	11.1	0.1
Ae. sticticus	Neg. binomial	125	0.873	0.31
Ae. excrucians	Neg. binomial	138.7	1.43	0.16
Ae. abserratus	Neg. binomial	83	2.67	0.53
Snowmelt *Aedes*	Neg. binomial	183.5	1.8	0.14

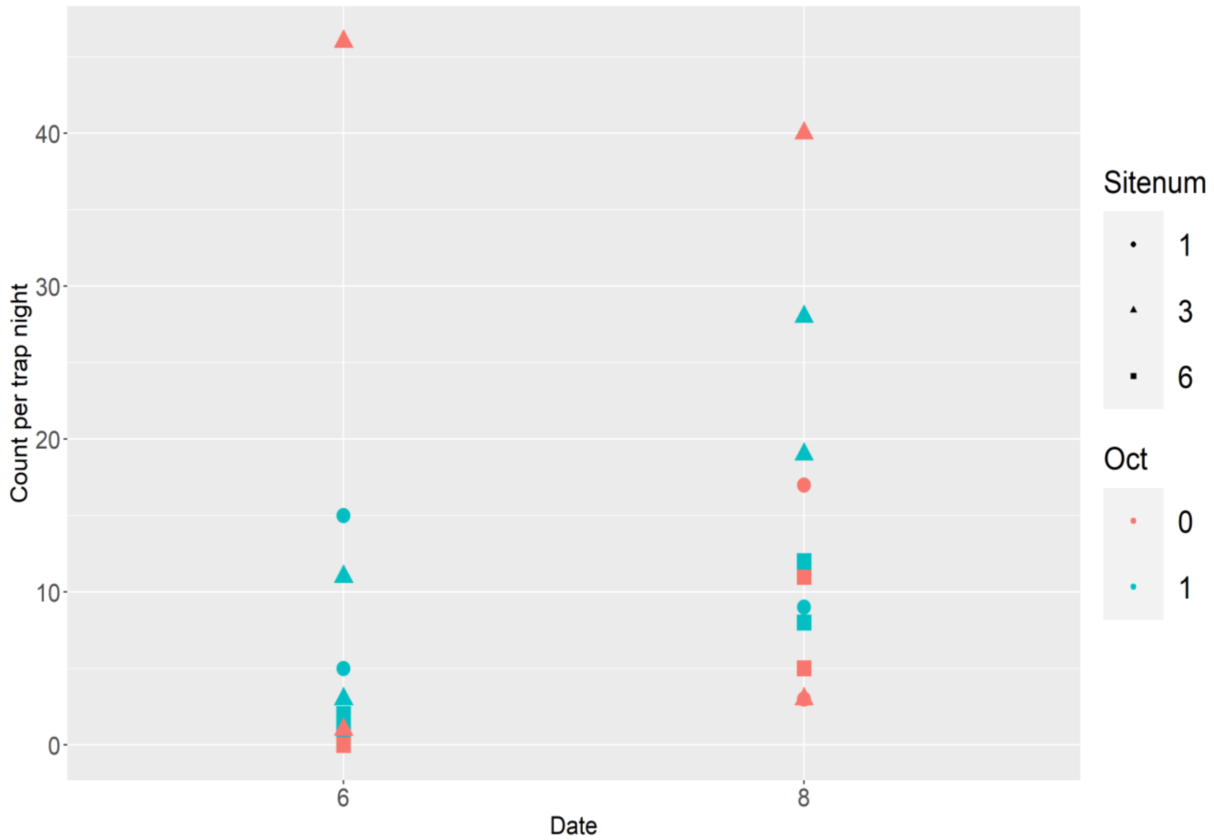

Figure 7. Distribution of Anopheles punctipennis abundances from octenol experiment, n = 24. Circle = site 1, triangle = site 3, square = site 6. Red = no octenol, blue = octenol. Octenol .somewhat improved somewhat improved capture of An. punctipennis (p = 0.1, a = 0.05)

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Table 10. Targeted species for JCV testing in NH

Target JCV vectors	
1	*Ae. canadensis*
2	*Ae. abserratus*
3	*Ae. fitchii*
4	*Ae. stimulans*
5	*Ae. cantator*
6	*Ae. aurifer*
7	*Ae. sticticus*
8	*Ae. excrucians*
9	*Ae. provocans*
10	*Ae. communis*
11	*An. quadrimaculatus*
12	*An. punctipennis*
13	*Cs. morsitans*
14	*Cq. perturbans*
15	*Ae. intrudens*

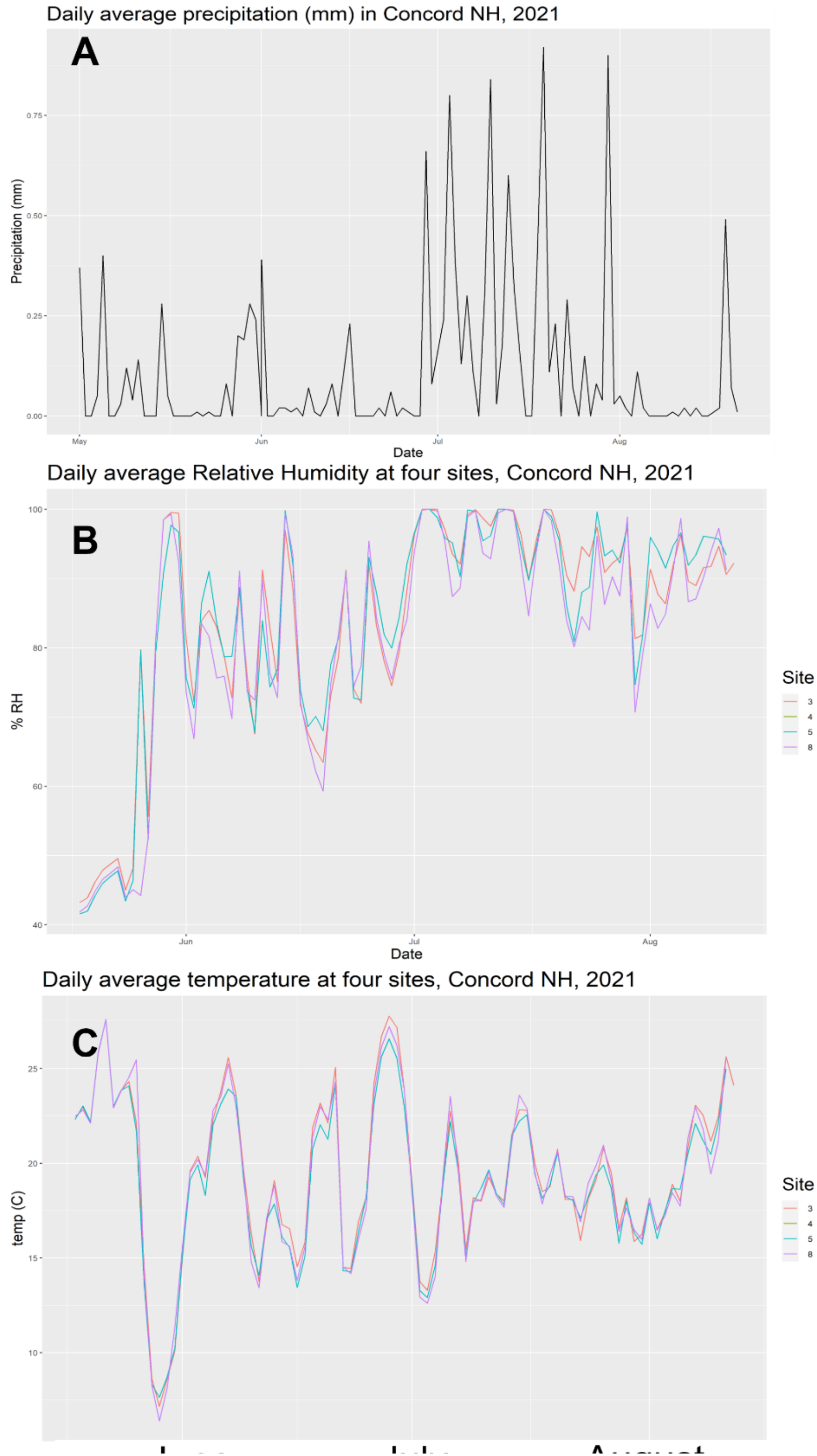

Figure 8. Environmental data for the 2021 mosquito season. A) daily average precipitation (mm) from NOAA for the Concord area, B) daily average relative humidity at sites 3,4,5,8, C) daily average temperature at sites 3,4,5,8