

# Molecular Identification of Host Feeding Patterns of Snow-Melt Mosquitoes (Diptera: Culicidae): Potential Implications for the Transmission Ecology of Jamestown Canyon Virus

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**ABSTRACT** We collected blood-fed, snow-melt mosquitoes (Culicidae: *Culiseta* and *Aedes*) to describe the feeding patterns of potential mosquito vectors of Jamestown Canyon virus (JCV, Bunyaviridae: *Orthobunyavirus*). JCV is an arthropod-borne, zoonotic virus with deer as the primary amplifying host in western alpine ecosystems. We collected mosquitoes from natural resting areas, fiber pots, and carbon-dioxide baited miniature light traps in the Colorado Rocky Mountains in 2007. We conducted two polymerase chain reactions to amplify and sequence vertebrate DNA extracted from blood-fed mosquitoes, which yielded comparable, but not identical, results. Mammal-specific primers found mule deer (*Odocoileus hemionus*) and elk (*Cervus elaphus canadensis*) as the source of all bloodmeals. To determine if unamplified bloodmeals were from nonmammalian sources, we screened all samples with conserved vertebrate primers, which confirmed the initial polymerase chain reaction results, but also found porcupine (*Erethizon dorsatum*) and human (*Homo sapiens*) as additional bloodmeal sources. We consistently found that mule deer were the primary hosts for mosquitoes in this system. These results suggest that snow-melt mosquitoes, in particular *A. cataphylla*, may be important vectors in western JCV alpine systems and may also act as a bridge vector for JCV from cervid virus reservoirs to humans.

**KEY WORDS** bloodmeal, deer, elk, feeding preference, Jamestown Canyon virus

Molecular analyses on bloodmeals taken from vectors captured in the field can be an important tool to elucidate true host-vector associations. Seroprevalence assays, viral isolation, and laboratory transmission experiments are necessary to provide links between competent vectors and amplification hosts in multi-host, vector-borne pathogen systems. However, these alone are not sufficient, and must be validated through ecological data. Host feeding patterns of vectors are of particular importance for vector-borne diseases.

Jamestown Canyon virus (JCV) (Bunyaviridae: *Orthobunyavirus*) is a mosquito-borne zoonotic pathogen of the California serogroup (Karabatsos 1985). JCV is a multi-host, multi-vector system and is widely distributed throughout temperate North America. Depending on geographic location, white-tailed (*Odocoileus virginianus*), black-tailed (*O. hemionus columbianus*), and mule deer (*O. hemionus hemionus*) have been implicated as primary amplification hosts for JCV (e.g., Issel 1972, Watts 1979, Eldridge 1987). Other free-ranging ungulates like elk (*Cervus el-*

*phus*), bison (*Bison bison*), moose (*Alces alces*), and large domestic animals, may serve as amplification hosts in natural systems (Grimstad 1988) and agricultural settings (Fulhorst 1996).

A variety of mosquito species are competent vectors for JCV; in Connecticut, boreal *Aedes* mosquitoes carried JCV with the highest frequency (Andreadis 2008). However, little is known about the feeding ecology of JCV competent snow-melt mosquitoes (i.e., *A. cataphylla*, *A. communis*, and *A. hexodontus*) in western alpine ecosystems (Kramer 1993). From the few bloodmeal analyses conducted, *Aedes* mosquitoes preferred large mammals, in particular white-tailed deer. Yet, one study was conducted in an agricultural setting, with domestic animals as the source of most bloodmeals (Downe 1960), and the other was conducted in the eastern United States (Molaei et al. 2008). To associate deer and humans with potential JCV mosquito vectors in western alpine systems, we investigated the host-feeding patterns of mosquitoes capable of transmitting JCV.

## Materials and Methods

**Study Design.** Snow-melt mosquitoes were collected from two field sites outside of the Rocky Mountain Biological Laboratory (RMBL) in Gunnison

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**Table 1. Mosquito species and their abundances collected from various trapping methods and effort**

Trap method	Trap hours	Mosquitoes captured
Natural resting areas	59,640 trap hours	<i>A. cataphylla</i> (19), <i>A. communis</i> (1), <i>A. implicatus</i> (17), <i>A. pullatus</i> (1), <i>A. punctor</i> (1), and <i>Cs. impatiens</i> (4)
Fiber peat pots	34,080 trap hours	<i>Cs. impatiens</i> (1)
CDC light traps	4,608 trap hours	<i>A. cataphylla</i> (9), <i>A. implicatus</i> (4)

Trap hours = (# of areas, pots, or traps)\*(# of hours traps sampled per day)\*(# of days sampled in a season).

County, CO, between 15 May and 12 August 2007 (RMBL UTM: N 4312713 E 327700; Washington Gulch UTM: N 4311531 E 325807). Both field sites are between 2902–2987 m elevation and are composed of riparian habitat dominated by bog birch (*Betula glandulosa*), mountain alder (*Alnus tenuifolia*), and willow species (*Salix* spp.) with conifer (*Picea engelmannii*) and aspen (*Populus tremuloides*) stands encroaching at higher elevations. Each field site was stratified by the following habitat types: willow, meadow, and forest. We took a random sample of habitat patches from each field site to ensure two patches from each habitat type per field site were sampled for blood-fed mosquitoes.

**Mosquito Capture.** Mosquitoes were captured from natural resting areas (15–20 per patch) and fiber pots (10 per patch; American Clay Works, Denver, CO, 28 × 28 cm<sup>2</sup> fiber peat pot) with a back-pack aspirator (John W. Hock Company, No. 1412.01, Gainesville, FL) for 2 h after dawn and before dusk each day. Natural resting areas included beneath the bark of trees, natural cavities, and dense shaded vegetation (105 per field site; Service 1976, CM pers. obs.). Fiber pots were set in protected positions: base of emergent vegetation, along fallen trees, and natural cavities (60 pots per field site). All pots were checked for spiders (Service 1976) and moistened daily to elevate their internal humidity (Komar 1995).

Mosquitoes were also collected with carbon-dioxide baited Centers for Disease Control (CDC) miniature light traps (John W. Hock Company, No. 512 fine mesh collection cups, Gainesville, FL; Service 1976). CDC traps were paired 50 m from each other within each patch and checked every 24 h. We trapped each site over an 8 d interval, with patches trapped for two consecutive nights. Traps were then pulled and rotated to the other field site to minimize seasonal or

weather influences. CDC trapping was run in May, June, July, and August. All blood-fed mosquitoes were identified to species level (Darsie and Ward 2005) and stored in a –80°C freezer for bloodmeal analysis.

**Molecular Analyses.** We extracted DNA from the abdomens of blood-fed mosquitoes using the DNeasy Animal Tissue extraction kit from QIAGEN (Valencia, CA). We conducted two polymerase chain reactions (PCRs) using primers identified as conserved in mammals (“mammalian primers”; MammalianF and MammalianR) and vertebrates (“vertebrate primers”; L14816 and H15173). The “mammalian” (Ngo and Kramer 2003) and “vertebrate” (Kocher et al. 1989) primers amplify a 772 and a 358 bp region of the mitochondrial gene cytochrome *b* (*cytB*), respectively. We first screened the samples with the “mammalian” primers to identify any mammalian sources of bloodmeals. We then rescreened with the “vertebrate” primers to determine if unamplified bloodmeals were from nonmammalian sources. Negative and positive controls were always included in PCR reactions, and each primer set was first validated using a range of vertebrate and invertebrate samples (Appendix A [online only] for protocols).

**Results**

We collected 57 engorged mosquitoes from natural resting areas, fiber pots, and CDC traps (Table 1) throughout summer 2007 (mosquito species abundances, Appendix B [online only]). We were able to sequence DNA from 32 engorged females using one or both primer sets. Both primer sets amplified vertebrate DNA from 25 of the 57 engorged mosquitoes (43.9%). The “mammalian” primers identified two mammalian hosts, while the “vertebrate” primers identified three mammalian hosts (Table 2). *A. cataphylla*

**Table 2. Mosquito species from which blood-fed females were collected and host species that were identified via BLAST analysis of sequence data amplified using “vertebrate” primers and “mammalian” primers**

Species	Vertebrate primers (n = 25)				Mammalian primers (n = 25)			
	Elk	Mule deer	Porcupine	Human	Elk	Mule deer	Porcupine	Human
<i>Cs. impatiens</i> (2)	0	0	0	0	1	1	0	0
<i>Ae. cataphylla</i> (16)	0	8	1	5	1	10	0	0
<i>Ae. implicatus</i> (13)	0	8	0	2	1	10	0	0
<i>Ae. punctor</i> (1)	0	1	0	0	0	1	0	0
Total (32)	0	17	1	7	3	22	0	0

The majority of blood-meals sequenced originated from mule deer.

Values in parentheses indicate the no. of blood-fed mosquitoes of each species used in the PCR reactions.

Bold values highlight differences between the two primer sets.

All BLAST matches included ≥99% query coverage and >90% identity.

*A. communis* and *A. pullatus* were not included because the primers failed to amplify any vertebrate host DNA.

showed the widest range of hosts, with elk, mule deer, porcupine, and human DNA detected. *A. implicatus* females contained three possible hosts, mule deer, elk, and human. Bloodmeals from *Cs. impatiens* were identified as mule deer or elk, and the lone *A. punctor* contained mule deer DNA (Table 2). Seventeen mule deer positive samples were identified with the "vertebrate" primers and 21 using the "mammalian" primers (including all but one of the 17 amplified with the "vertebrate primers").

There were some discrepancies between the results generated by the two primer sets. Notably, we amplified human, porcupine, and mosquito DNA ( $n = 23$ ) using the conserved "vertebrate" primers, but not with the "mammalian" primers. The amplification of mosquito DNA suggests some nonspecific reaction has occurred; thus, we consider these reaction results as negatives and have excluded them from our summary of host feeding patterns. Overall, the results between the two primer sets are not irreconcilable and are likely because of the degenerate nature of the "vertebrate" primers. Partially digested bloodmeals yield smaller fragments of DNA that may be preferentially amplified by the "vertebrate" primers (Appendix C [online only]).

### Discussion

Despite the difficulty we experienced in capturing blood-fed mosquitoes, and some discrepancy in the results generated from our two primer sets, these preliminary data contribute to describing snow-melt mosquito feeding patterns. The mosquito and vertebrate hosts involved in this study are known vectors and amplifying hosts in the JCV system, and can occur in high abundances throughout many western, high alpine environments (Darsie and Ward 2005, Rue 1997). The majority of bloodmeals taken by *A. cataphylla* (a JCV competent vector) in this study were obtained from mule deer, an amplifying host of JCV (Eldridge 1987, Grimstad 1988). Host feeding patterns of four mosquito species (*Cs. impatiens*, *A. cataphylla*, *A. implicatus*, and *A. punctor*) indicate that these species also may be important vectors involved in the maintenance of arboviruses in cervid reservoirs in alpine systems.

We also found several bloodmeals were taken from humans. We took care when aspirating to avoid collecting mosquitoes that had immediately fed upon us, and we included negative controls to detect contamination in our PCR reactions, of which there was none. Further, the majority of human-source bloodmeals originated from mosquitoes captured in CDC traps (71.4%). Thus, the possibility of capturing mosquitoes that had immediately fed upon the researchers with a back-pack aspirator is small. Nearby rustic campsites (<1.5 km), recreational trails, and the well-staffed RMBL ensure a significant human presence on both field sites during the summer months. Based on these results, we have potentially identified two species of snow-melt mosquito (*A. cataphylla* and *A. implicatus*) that may act as bridge

vectors capable of transmitting zoonotic JCV or other arboviruses from cervid amplification reservoirs to humans in alpine systems.

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