Blood Meal Analysis and Virus Detection in Mosquitoes Collected from U.S. Air Force Installations, 2017 – 2018

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INTRODUCTION

U.S. Air Force Public Health (PH) personnel routinely conduct entomological surveillance worldwide to assess risks to human health from pests and disease vectors. Collected samples are sent to the Operational Support Entomology Laboratory at Wright-Patterson AFB, OH, for identification and analysis. Samples are normally held at room temperature (RT) after capture and processing, sometimes for weeks or longer, and become dessicated as a result. We wanted to understand the extent to which such holding conditions could affect our ability to conduct downstream analyses, and produce actionable information for PH about local epidemiological and ecological risks from arboviruses. We examined blood fed mosquitoes stored for variable lengths of time to evaluate whether duration of storage at RT (DSRT) affected our ability to identify their host meal source. We also assessed these mosquitoes for the presence of arboviral RNA.

METHODS

Laboratory processing and analysis

Blood fed mosquitoes (n = 183) from 28 installations were identified to genera and species when possible using regionally relevant taxonomic keys, then transferred from RT to -20°C until processed. Mosquitoes were classified by traditional Sella stage[1] (1 – 7) with two additions: trace amounts of blood were scored 1.25; less than fully engorged were scored 1.5. Thoraces with heads and abdomens were dissected and placed in two separate tubes for processing. DNA was extracted from abdomens using the DNeasy Blood & Tissue Kit (Qiagen 69504) with a single 100ul elution. RNA extractions from thoraces and heads were done with the QIAamp Viral RNA Mini Kit (Qiagen 52906) with a single 60ul elution. PCR to amplify blood meal DNA used primers targeting vertebrate mitochondrial cytochrome c oxidase subunit 1 (CO1).[2,3] Amplicon sequencing was done on the SeqStudio Genetic Analyzer [Applied Biosystems (ABI)] using either the BigDye Direct Cycle Sequencing Kit (ABI 4458687) or the BigDye Terminator v3.1 Cycle Sequencing Kit (ABI 4337455). NCBI BLASTn was used to identify host blood meal source using raw sequence data, with search parameters Organism: Chordata and Entrez query CO1. We set our sequence match length at ≥140 nucleotides, and restricted % identity to ≥90%. Mosquito thoraces and heads were assayed for RNA of West Nile, St. Louis encephalitis, eastern equine encephalitis and La Crosse viruses.[4,5,6]

Statistical analyses:

DSRT is defined as the number of days between collection date and sample transfer to -20°C. Sella stage was coded as a ranked categorical variable (1 – 7). We aimed to see if DSRT or Sella stage affected the outcome of PCR and sequencing. A logistic regression model using DSRT and Sella stage as variables was fit to the data to examine if either variable affected whether a blood meal host could be identified (R version 3.6.1, Package 'aod').

RESULTS

Mosquitoes & blood meal hosts	No.
Aedes increpitus	**1**
Northern mockingbird	1
Ae. infirmatus	**1**
White-tailed deer	1
Ae. species	**5**
Desert cottontail	1
Eastern cottontail	1
Mule deer	1
White-tailed deer	2
Ae. species (black legged)	**3**
White-tailed deer	3
Ae. triseriatus	**1**
Human	1
Ae. trivittatus	**2**
Mule deer	1
White-tailed deer	1
Ae. vexans	**7**
Eastern cottontail	1
Human	4
White-tailed deer	2
Anopheles crucians complex	**1**
Eastern cottontail	1
An. quadrimaculatus group	**1**
White-tailed deer	1
Coquillettidia perturbans	**1**
White-tailed deer	1
Culiseta incidens	**3**
Mule deer	2
Red fox	1
Culex erraticus	**8**
Mourning dove	1
Northern mockingbird	1
White-tailed deer	6
Cx. pipiens	**5**
Black-billed magpie	3
Mourning dove	1
Northern mockingbird	1
Cx. pipiens or quinquefasciatus	**1**
Human	1
Cx. quinquefasciatus	**19**
American robin	1
Blue jay	1
House finch	5
House sparrow	1
Human	1
Mourning dove	3
Northern cardinal	1
Northern mockingbird	2
Verdin	1
White-tailed deer	1
White-winged dove	2
Cx. quinquefasciatus or restuans	**3**
American robin	2
House finch	1
Cx. restuans	**1**
American robin	1
Cx. salinarius	**5**
Mule deer	1
White-tailed deer	4
Cx. species	**7**
House sparrow	1
Northern mockingbird	1
White-tailed deer	4
White-winged dove	1
Cx. tarsalis	**3**
House sparrow	1
Mourning dove	1
White-tailed deer	1
Psorophora columbiae	**1**
White-tailed deer	1
Ps. cyanescens	**1**
Human	1

Sequences generated and analyzed

Host blood meal identities were determined from raw sequencing data for 80/183 (43.7%) samples (Table). BLASTn query lengths ranged from 623 – 1191 bp. Median sequence length generated: 811 bp, mode: 820, mean: 828.4. E-values for host matches ranged from 0.0 - 3.0E-46. Sequence length matches against CO1 accessions in NCBI ranged from 146 – 662 bp; 70.0% (56/80) of samples had matching lengths ≥500 bp.

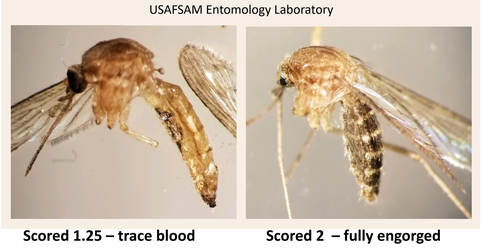
Blood meal host identities

AF installation mosquito blood meals were from 16 different vertebrate species (Table). Birds as a group accounted for the most blood meals, 34 (42.5%), mainly obtained from *Culex* mosquito species. Deer accounted for 33/80 (41.3%) samples (28 white-tailed deer, 5 mule deer). The next most common single blood meal source was humans, 8/80 (10%).

DSRT and Sella stage effect on outcome

Sample DSRT ranged from 7 to 295 days, median DSRT was 15 days, and mean DSRT was 32.7 days. DSRT was significant as a variable in the logistic regression model ($p = 0.005$), with increased DSRT affecting the probability that a blood meal host could be identified. No Sella stage, however, showed a significant relationship with outcome (ability to identify host blood meal source). [All $p > 0.12$].

Sella staging of dessicated mosquitoes



Scored 1.25 – trace blood | Scored 2 – fully engorged

Arboviral testing

No arboviral RNA was detected in any mosquito thoraces/heads.

CONCLUSIONS & DISCUSSION

- Blood meal host identity could be determined from our blood fed mosquitoes stored at RT for up to 295 days.
- The majority of sequences matching host identities were ≥500 bp, suggesting that DNA degradation occurring in samples does not universally limit blood meal analyses.
- White-tailed deer were the dominant blood meal host species for mosquitoes among the 28 Air Force installations represented.
- We were able to determine host blood meal source for 19/38 (50%) samples that contained only trace amounts of blood. Of those 19, 14 seem to have been interrupted feedings on deer.
- Public Health personnel have confirmation of human exposures to biting mosquitoes at some locations – this type of information can reinforce surveillance site choice, or inform alternate choices.
- *Aedes vexans* took 4/8 human blood meals. *Aedes triseriatus*, the primary enzootic vector of La Crosse virus, also had a human blood meal. *Culex pipiens complex* and *Cx. quinquefasciatus* (WNV and SLEV vectors) took 2 human blood meals, and the nuisance biter *Ps. cyanescens* had one.
- *Culex* species mosquitoes on installations appear, as expected, to feed preferentially on birds, though arbovirus positive mosquito pools are rarely detected during routine surveillance. Birds most commonly fed upon were house finch, mourning dove and northern mockingbird (n=6 each).
- The finding that samples with host meal sources identified had greater DSRT than those without a host determination is somewhat biologically implausible, and we consider that additional unmeasured variables are likely contributing to this outcome. The small sample size is also a factor.
- Sella stage as an indicator of blood meal digestion progress[3,7], and hence blood meal DNA quality, has no significant relationship with host identity outcomes for our samples. This finding is inconsistent with the literature.[3,7] It is likely that misclassification of Sella stages 2 – 4, along with added category 1.5, occurred due to desiccation and discoloration of our samples (see photos on left).
- Given the small sample size and possibility that host seeking mosquitoes analyzed were nulliparous, it is not surprising that no arboviruses were detected in the samples. As well, arboviral RNA degrades over time.
- Future plans: analyze remaining samples; compare additional primer sets to current primers; review Sella staging potential misclassification.

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8. Thanks to J. Feller in our lab for running our stats in R.

DISCLOSURE INFORMATION

I have no financial relationships to disclose. I will not discuss off-label use and/or investigational use in this poster. The views expressed in this poster are those of the authors and do not necessarily reflect the official policy or position of the Air Force, the Department of Defense, or the U.S. Government. References to trade names do not constitute endorsement of any products.

AMCA Annual Meeting 2021 • Submission #SA-21-121674 • Cleared 28 Jan 2021. Case #AFRL-2021-0209.