SARS-CoV-2 and mosquitoes: an extreme challenge

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Abstract

The research addresses public speculation that SARS-CoV-2 might be transmitted by mosquitoes. The World Health Organization has stated “To date there has been no information nor evidence to suggest that the new coronavirus could be transmitted by mosquitoes”. Here we provide the first experimental data to the capacity of SARS-CoV-2 to infect and be transmitted by mosquitoes. Using three species of mosquitoes from both genera involved in transmission of most arboviruses that infect humans we demonstrate that SARS-CoV-2 virus is unable to replicate in mosquitoes and therefore cannot be transmitted to people.

Introduction

The question has been asked as to whether or not SARS-CoV–2, the causative agent of COVID–19, can infect and be transmitted by mosquitoes. The WHO has definitively stated that mosquitoes cannot transmit the virus\(^3\), and in interviews, various experts have unanimously and definitively also dispelled the suggestion that SARS-CoV–2 could be transmitted by mosquitoes. The presumption may be based on various observations and facts extrapolated from other coronaviruses. For example, neither the closely related SARS-CoV nor MERS produce the level of virus in the blood that for typical arthropod-borne viruses such as dengue and yellow fever viruses, would be regarded as high enough to infect mosquitoes. Recent studies with infected humans and non-human primates infected with SARS-CoV–2, found no detectable virus in peripheral blood\(^4,5\). Lack of viremia is also suggested by the fact that neither SARS-CoV nor MERS infections have resulted from blood transfusions or organ transplantations. Despite the lack of detectable viremia, experiments to determine the potential role of mosquitoes in SARS-CoV–2 transmission, are necessary because previous experiments have demonstrated that mosquitoes may become infected with viruses even when exposed to levels of infectious virus that are below the level of detection\(^6–8\).

To be a biological vector of viruses, mosquitoes must take up sufficient virus to infect midgut epithelial cells, and the virus must then disseminate to infect other organs in the hemocoel, notably the salivary glands. Overcoming the midgut infection and escape barriers is essential for a virus to be transmissible by mosquitoes. If these barriers are bypassed by direct inoculation of virus into the hemocoel, then even non-susceptible mosquitoes can be infected. Inoculation can accomplish short-term infection of insects that could never be naturally infected because they do not feed on blood. These include not only non-hematophagous mosquitoes such as \textit{Toxorhynchites spp}, but also male mosquitoes and even beetles and butterflies\(^9,10\).

Like the over 500 viruses that are transmitted by arthropods\(^11\), with the exception of African swine fever virus, coronaviruses have an RNA genome. In spite of the recovery of coronavirus or coronavirus-like agents from various arthropods\(^12,13\), no virus in the family has been isolated from mosquitoes. To date, only one report related to epidemic coronaviruses and mosquitoes has been published\(^14\). This study evaluated the potential use of mosquitoes for surveillance, included feeding of MERS virus to \textit{Anopheles}
*gambiae* mosquitoes. Residual viral RNA, probably in the remains of the bloodmeal in the midgut, was detected up to 1 day post-feeding. Similarly, positive PCR detection was observed for *Bacillus anthracis*, *Trypanosoma brucei gambiensis*, and Zika virus, none of which infect or are transmitted by *An. gambiae*. Levels of detected RNA were equal to or below the input level, indicating a lack of replication.

## Results And Discussion

In this study, the susceptibility of three mosquito species, *Ae. aegypti*, *Ae. albopictus* and *Cx. quinquefasciatus*, were determined through the intrathoracic inoculation with SARS-CoV-2. Infectious viruses could only be recovered from mosquitoes collected within two hours of inoculation. Quantities of infectious viruses corresponded to the amount of inocula, producing infectious titers at approximately 1.5 logTCID$_{50}$/ml and suggesting the lack of replication after injection. Waning of infectious titers was rapid and led to the lack of infectious viruses isolated from the majority of mosquitoes obtained between one and fourteen days post challenge suggests that mosquitoes in the *Aedes* and *Culex* genera are refractory to SARS-CoV-2 and unlikely to contribute to viral maintenance and transmission in nature (Table 1.).

### Table 1. Recovery rates of SARS-CoV-2 in mosquitoes receiving intrathoracic injection.

<table>
<thead>
<tr>
<th>Species</th>
<th>Inoculum</th>
<th>0*</th>
<th>1</th>
<th>3</th>
<th>7</th>
<th>10</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ae. aegypti</em></td>
<td>SARS-CoV-2</td>
<td>83.3% (5/6)</td>
<td>0.0% (0/17)</td>
<td>0.0% (0/5)</td>
<td>0.0% (0/24)</td>
<td>0.0% (0/26)</td>
<td>0.0% (0/27)</td>
</tr>
<tr>
<td></td>
<td>Mock**</td>
<td>0.0% (0/6)</td>
<td>0.0% (0/3)</td>
<td>0.0% (0/5)</td>
<td>0.0% (0/5)</td>
<td>0.0% (0/6)</td>
<td>0.0% (0/5)</td>
</tr>
<tr>
<td><em>Ae. albopictus</em></td>
<td>SARS-CoV-2</td>
<td>83.3% (5/6)</td>
<td>0.0% (0/4)</td>
<td>0.0% (0/2)</td>
<td>0.0% (0/4)</td>
<td>0.0% (0/2)</td>
<td>0.0% (0/5)</td>
</tr>
<tr>
<td></td>
<td>Mock**</td>
<td>0.0% (0/6)</td>
<td>0.0% (0/4)</td>
<td>0.0% (0/2)</td>
<td>0.0% (0/4)</td>
<td>0.0% (0/2)</td>
<td>0.0% (0/5)</td>
</tr>
<tr>
<td><em>Cx. quinquefasciatus</em></td>
<td>SARS-CoV-2</td>
<td>100.0% (3/3)</td>
<td>0.0% (0/1)</td>
<td>N/A</td>
<td>0.0% (0/1)</td>
<td>N/A</td>
<td>0.0% (0/1)</td>
</tr>
<tr>
<td></td>
<td>Mock**</td>
<td>0.0% (0/3)</td>
<td>0.0% (0/17)</td>
<td>N/A</td>
<td>0.0% (0/17)</td>
<td>N/A</td>
<td>0.0% (0/28)</td>
</tr>
</tbody>
</table>

* Mosquitoes obtained at day 0 post infection were collected within two hours from the time of intrathoracic injection.

** All mock control groups of mosquitoes received Leibovitz’s L-15 media.

## Methods

**Virus:** SARS-CoV-2 virus WA1/2020 strain was obtained from BEI Resources (Catalog # NR-52281). Virus was propagated in Vero76 cells at the approximate multiplicity of infection of 0.01. Infectious titers of viral stocks used for intrathoracic injection were approximately 5.5 logTCID$_{50}$/ml.

**Mosquitoes:** The colonized *Aedes aegypti* strain Rex D, Higgs white eye was originally obtained from Puerto Rico$^{15}$, *Ae. albopictus* generation F11 originated from New Jersey, and *Culex quinquefasciatus* F43 were from Florida$^{16,17}$. All mosquitoes were reared as previously described and have proven to be susceptible to several arboviruses.
Viral challenge of mosquitoes: For intrathoracic inoculation, mosquitoes were cold-anaesthetized on ice, transferred to a secure glove box, and then inoculated with approximately 0.5µl of viral stock. It was anticipated that each mosquito received approximately 2.0 logTCID₅₀/ml of infectious viruses. L-15 medium was inoculated as a negative control. The results were compiled from two experiments using *Ae. aegypti* and *Ae. albopictus* and one single experiment using for *Cx. quinquefasciatus*. Experimentally challenged mosquitoes were maintained and sampled under conditions as previously described¹⁸.

**Declarations**

**Data Availability Statement:** The data that support the finding of this study are available in the table of this manuscript.

**Acknowledgements** (optional). This work was performed in the ACL-3 insectary at Kansas State University’s Biosecurity Research Institute.

**Author contributions.** S.H., D.L.V. and Y.S.H conceived the study. Y.S.H., A.B., H.M.S., and S.H. executed the experimental plans. D.L.V. provided financial support and reviewed the manuscript. S.M.H prepared approvals for the research, coordinated experiments, and prepared manuscript.

**Competing interests.** The authors declare no competing interests. The funder has not role in the design and execution of experimental plans or the interpretation of data.

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