No Evidence of SARS-CoV-2 Among Flies or Cockroaches in COVID-19 Positive Households

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Short report

Keywords: severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), No evidence, flies or cockroaches, COVID-19, positive households

Posted Date: November 1st, 2021

DOI: https://doi.org/10.21203/rs.3.rs-1005788/v1
Abstract

Background:

The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has resulted in a pandemic of coronavirus disease (COVID-19), which continues to cause infections and mortality worldwide. SARS-CoV-2 is transmitted primarily via the respiratory route and has experimentally been found to be stable on surfaces for multiple days. Flies (Diptera) and other arthropods mechanically transmit several pathogens, including turkey coronavirus. A previous experimental study demonstrated house flies, *Musca domestica*, can mechanically transmit SARS-CoV-2, but the ability of flies in general to acquire and deposit this virus in natural settings has not been explored. The purpose of this study was to explore the possibility of mechanical transmission of SARS-CoV-2 by peridomestic insects and their potential as a xenosurveillance tool for detection of the virus.

Methods:

In order to optimize chances of viral detection, flies were trapped in homes where at least one confirmed human COVID-19 case(s) resided. Sticky and liquid baited fly traps were deployed inside and outside of the homes of SARS-CoV-2 human cases in Brazos, Bell, and Montgomery Counties, from June to September 2020. Flies from sticky traps were identified, pooled by taxa, homogenized, and tested for the presence of SARS-CoV-2 RNA using qRT-PCR. Liquid traps were drained, and the collected fluid similarly tested after RNA concentration. Experimental viral detection pipeline and viral inactivation were confirmed in a Biosafety Level 3 lab. As part of a separate ongoing study, companion animals in the home were sampled and tested for SARS-CoV-2 on the same day of insect trap deployment.

Results:

We processed the contents of 133 insect traps from 44 homes, which contained over 1,345 individual insects of 11 different Diptera families and Blattodea.

These individuals were grouped into 243 pools, and all tested negative for SARS-CoV-2 RNA. Dead flies exposed to SARS-CoV-2 in a BSL3 lab were processed using the same methods and viral RNA was detected by RT-PCR. Fourteen traps in seven homes were deployed on the day that cat or dog samples tested positive for SARS-CoV-2 RNA by nasal, oral, body, or rectal samples.

Conclusions:

This study presents evidence that biting and non-biting flies are not likely to contribute to mechanical transmission of SARS-CoV-2 or be useful in xenosurveillance for SARS-CoV-2.
Following the emergence and global spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the World Health Organization declared a pandemic of coronavirus disease 2019 (COVID-19) in March 2020 [1]. COVID-19 has led to historic challenges to human health and many other aspects of society. A complete response to this ongoing pandemic requires an understanding of all possible modes of transmission and the use of a variety of surveillance tools.

SARS-CoV-2 has been shown to be present in aerosols, droplets, and on surfaces [2, 3]. While fomite transmission is likely less significant than aerosol transmission [2, 4], experimental studies have shown that the virus can persist on surfaces for hours or days, with stability being greatest in indoor conditions with low relative temperature and humidity [2, 5]. Additionally, SARS-CoV-2 RNA has been detected in the feces of infected humans [6, 7]. While studies have largely focused on human disease and direct transmission, other animals, including domestic felines and canines, are also susceptible to infection [8–13], can have viral RNA on fur and in feces [13] and can shed infectious virus orally [13]. These data indicate that in the home of an infected case, SARS-CoV-2 RNA and potentially infectious virus, may be found on surfaces, droplets, aerosols, and in fecal matter from both humans and pets.

Insects are ubiquitous in many homes globally and transmit several pathogens, both biologically by blood-feeding and mechanically through contact with contaminated surfaces. Although SARS-CoV-2 does not typically produce a viremia in infected people [14], several studies have investigated the potential for biological transmission by mosquitoes (Diptera: Culicidae) [15–18] and biting midges (Diptera: Ceratopogonidae) [18]. In these studies, SARS-CoV-2 was detected for less than 24 hours post infection, the virus is unable to replicate in the arthropod, and thus no biological transmission occurs. However, the role of mechanical transmission by insects remains unknown and of concern [19]. Non-biting flies are capable of mechanical transmission of other pathogens, which involves the transfer of infectious virus by contaminated mouthparts or bodies. This mode of transmission has been documented for hundreds of different pathogens [20], including turkey coronavirus [21]. Because of this, possible SARS-CoV-2 transmission by arthropods through the mechanical mode of transmission remains an important area of research. To date, a single study has examined the possibility for mechanical transmission of SARS-CoV-2 by house flies (Musca domestica, L. (Diptera: Muscidae)) [22] and confirmed that the flies were able to acquire SARS-CoV-2, which was retained as both viral RNA and infectious virus up to 24 hours post exposure, with viral RNA, but not infectious virus, transferred to virus-free surfaces [22]. Although the authors concluded that house flies are not likely to play a significant role in SARS-CoV-2 transmission, the retention of viral RNA suggests that detection of invertebrate-derived RNA of non-biting flies could be used to detect SARS-CoV-2 circulation. This concept, termed xenosurveillance, builds on the recent work using mosquitoes and other flies as sampling devices of human pathogens [23–25]. Xenosurveillance methods are used to detect pathogens that the arthropod acquired during an infected bloodmeal or from contact with a contaminated surface.

In the current study, we deployed multiple fly traps both indoors and outdoors in households with at least one human COVID-19 case to investigate SARS-CoV-2 in biting and non-biting flies, as a complementary effort in a One Health study focused on transmission at the human-animal interface [13].
Methods

Household recruitment

Household enrollment occurred from June through September, 2020 as previously described [14]. Briefly, individuals who tested positive for SARS-CoV-2 were underwent case and contact investigation according to local health department protocols. Households with pets (dogs or cats) that agreed to participate in an animal testing study were also invited to participate in the insect xenosurveillance study, and consenting households had fly traps deployed and collected by the study team. This study was determined to not involve research with human subjects (IRB2020-0762).

Arthropod sampling – sticky traps

Each home received one to three traps, placed indoors and outdoors according due to owner preference. Indoor traps were commonly placed by the back door, in the kitchen, and/or by the front door. Outdoor traps were commonly placed on the back patio and outside of the front door. Multiple commercially available traps were used to capture a wide variety of insects. These included the EZ Trap (Starbar, Schaumburg, IL, USA), Gold Stick Fly Trap (Catchmaster®, Bayonne, NJ, USA), and Indoor Fly TrapStik (Rescue!, Spokane, WA, USA). Sticky traps were left for a range of 7 days to 19 days with an average of 12.4 days before collection from the home.

Traps were collected from homes and stored at 4°C for no more than 3 days before processing. Flies and other insects were individually identified to the family taxonomic level using a morphological identification keys [26, 27]. Each arthropod taxa from a single trap was placed in separate 2 mL microcentrifuge tubes (Eppendorf, Hamburg, Germany) containing 1 mL of viral transport media (VTM; made following CDC SOP#: DSR-052-02) with a 2.8 mm stainless steel grinding ball (OPS Diagnostics, Lebanon, NJ, USA). Samples were homogenized using a Tissue Lyser II (Qiagen, Hilden, Germany). Homogenates were centrifuged at 2500G for 30 min at 4C (Beckman Coulter, Allegra X-15R, Brea, CA, USA), and the supernatants aliquoted for RNA extraction and qRT-PCR, as previously described [12]. A sample of the glue was also obtained and tested from each trap as control to determine if viral RNA in the house had contaminated the glue surface.

Arthropod sampling – liquid traps

A subset of households also received liquid baited traps as an alternative method for xenosurveillance. The bait for these traps was used at 25% the recommended concentration since they were being deployed for a short time. These liquid-baited traps included Reusable Fly Trap (Rescue!, Spokane, WA, USA) and Fly Jar (Catchmaster®, Bayonne, NJ, USA). Liquid traps were left for a range of 5 to 12 days with an average of 8.6 days before collection from the home.

After liquid traps were collected from homes, the liquid from the traps was collected into 50 mL centrifuge tubes and processed similar to protocols for testing wastewater for SARS-CoV-2 [26]. The flies remaining in the trap were rinsed with 50 mL of VTM, which was collected. Samples were spun down at 2500G for
30 min at 4°C in a centrifuge (Beckman Coulter, Allegra X-15R, Brea, CA, USA) to remove solids. For RNA concentration, supernatant was passed through a 5 µM syringe filtration (Pall, Acrodisc, New York, NY, USA) and then concentrated using a Vivaspin 20 mL centrifugal concentrator (Sartorius, Vivaspin, Gottingen, Germany) according to manufacturer's protocols.

**Viral screening**

A 400 µL aliquot of homogenized fly tissue or concentrated RNA suspension was extracted using a MagMAX CORE Nucleic Acid Purification Kit on a 96-well Kingfisher Flex System (ThermoFisher Scientific, Waltham, MA, USA). The extraction included one negative control for every 10 samples. RNA was screened by qRT-PCR for two SARS-CoV-2 genes, RDRP and E, as described previously [13]. All primers, probes, and positive controls were provided by IDT (Integrated DNA Technologies, Coralville, IA, USA) and negative controls were used on all reactions.

**Protocol validation**

*Lucilia sericata*, (Meigen) (Diptera: Calliphoridae) from a colony in the lab of an author (JKT) were killed by freezing at -20ºC and then exposed to SARS-CoV-2 by dipping the tips of the legs of an individual fly into minimum essential medium with Earle's balanced salt solution containing SARS-CoV-2 in a Biosafety Level 3 Laboratory (BSL3). Flies were exposed to one of a serially diluted viral concentration containing from 10^5 to 10 pfu/mL. Following exposure, a single SARS-CoV-2-exposed fly was added to a pool of 5 total flies in VTM which was homogenized and tested by qRT-PCR as described above. Viral inactivation of SARS-CoV-2 samples leaving the Biosafety Level 3 Laboratory (BSL3) was validated internally as described in Additional file 1 Table S1.

**Results**

**Arthropod Collections**

A total of 235 traps were deployed in 81 homes with at least one SARS-CoV-2 human case. Out of the traps deployed, 133 traps from 44 homes were recovered for testing. Traps not recovered were because of the lack of a response to follow-up calls to arrange for trap pick-up.

The indoor sticky traps captured 8 different Diptera families and Blattodea (Table 1). Indoor sticky traps had an average of 9.4 flies (range 0-177) with an average of 1.6 unique taxa (range 1-4) from positive traps. The three most common families collected indoors were Phoridae with 180 individuals, Calliphoridae with 85 individuals, and Drosophilidae with 81 individuals (Table 1). The indoor sticky traps were deployed for an average of 12.4 days with the date of deployment starting an average of 9.6 days post COVID-19 diagnosis of the human in the household. For 6 traps from 4 households, the indoor sticky traps were deployed on the same day that 6 animals in these households tested positive for SARS-CoV-2 [14] (Table 1). Six animals tested positive by nasal swab, two by oral swab, four by body fur swab, and four by rectal swab.
Outdoor sticky traps had an average of 10.7 flies (range 0-317), with an average of 1.8 unique taxa in the positive traps (range 1-6). The outdoor sticky traps collected 11 different Diptera families and Blattodea (Table 2). The three most common taxa collected outdoors were Calliphoridae with 485 individuals, Sarcophagidae with 218 individuals, and Muscidae with 58 individuals (Table 2). In eight traps from seven households, the outdoor sticky traps were deployed on the same day the animals were sampled and tested positive for SARS-CoV-2 by nasal, oral, body, or rectal samples (Table 2).

The EZ Trap captured 1.58 flies per day indoors, followed by Gold stick (0.945), followed by Trapstick (0.012) (Additional file 2: Table S1). The EZ Trap captured 2.24 flies per day outdoors, followed by Trapstick (0.50), followed by Gold stick (0.24) (Additional file 3: Table S1).

The liquid fly traps contained up to hundreds of individual flies which were not counted or identified due to the degradation of the specimens.

**Arthropod testing for SARS-CoV-2**

During this experiment, 243 arthropod pools (1,345 individuals) from 133 sticky traps along with samples from 28 liquid traps were tested for presence of SARS-CoV-2 RNA. None of these pools or samples tested positive for SARS-CoV-2 RNA by RT-PCR.

**Protocol validation**

We validated that our SARS-CoV-2 testing protocol would work on dead *Lucilia sericata* (Meigen) (Diptera: Calliphoridae) with exposure to SARS-CoV-2 in the lab. Following exposure of the fly’s legs to the viral dilutions and homogenization with a pool of four non-exposed flies, RNA extraction and RT-PCR was performed. This protocol verifies that pooled flies with 1 individual exposed to $2 \times 10^2$ pfu/mL would be detected by our RNA extraction and testing platform (see Additional file 1: Table S2).

**Discussion**

COVID-19 is an ongoing pandemic that continues to be a problem for many countries. Fully understanding all modes of transmission and surveillance tools continues to be a research priority. The prevalence of biting and non-biting flies in and around the domestic environment make them an important aspect of potential transmission as well as a potential xenosurveillance tool. These flies and other arthropods such as cockroaches potentially come into contact with aerosolized viral particles, droplets, fomites, and virus in the feces of infected pets. These arthropods are therefore of significant interest as both potential mechanical vectors and targets for xenosurveillance strategies. Efforts to use wastewater screening as a SARS-CoV-2 surveillance tool [28] have highlighted the importance of alternative surveillance strategies during this pandemic.

During a period of active virus transmission in the community, our study recovered insects from 133 sticky traps and 28 liquid traps in 44 homes of known human COVID-19 cases. A subset of these homes had dogs or cats with documented shedding of SARS-CoV-2. Following testing of flies and cockroaches
that were captured, our study found no evidence of SARS-CoV-2 RNA in the insect pools or trap liquid samples. While *M. domestica* was recently found to be capable of experimental acquisition of virus and deposition of viral RNA in a laboratory setting [22], our results indicate that this is unlikely to occur in natural high-risk settings in which humans, and in some cases household pets, were shedding virus in the home.

One of the strengths of our study is the broad sampling of biting and non-biting flies in households with at least one laboratory-confirmed COVID-19 case, including homes with infected pets. In addition to many households harboring pets with infected respiratory or rectal swabs, many pets also had body (fur) swabs, which is likely indicative of a contaminated environment that may serve to also contaminate insects that are in and around the household. Limitations of our study include not knowing the stability of viral RNA on flies left at ambient temperatures for multiple days on the sticky traps or in liquid traps, some of which were exposed to UV light. Studies have shown that SARS-CoV-2 can be viable for days on some surfaces at room temperature [5], indicating that the fly traps deployed indoor would have been the most likely to result in a positive fly pool. However, the indoor traps captured fewer flies than the outdoor traps, where the virus would have been less viable. Additionally, our traps were deployed an average of 8.2 days following the human diagnosis of COVID-19, and therefore we were unable to confirm the human case was still positive or shedding viral RNA. However, in four households with indoor traps and three additional households with outdoor traps the companion animals sampled at the time of trap deployment were positive for SARS-CoV-2 RNA. In one of these households, the animals were resampled two weeks later and respiratory and body fur samples were positive [13], implying that viral RNA was in the environment while flies were being trapped.

In conclusion, we found no arthropods with residual SARS-CoV-2 RNA in or around the homes where humans, and sometimes their animals, have tested positive for SARS-CoV-2. This suggests a low likelihood that arthropods contribute to the transmission of SARS-CoV-2. Thus, their utility in xenosurveillance is not herein substantiated.

**Declarations**

**Acknowledgements**

We appreciate the participation of Texas homeowners. We thank Santos Navarrette, Robert Lampkin, the Brazos County Health Department, and Texas A&M University case investigators for their assistance in facilitating communication with COVID-19 cases. We thank Central Life Sciences, Sterling International, Inc. Rescue!, and AP&G Catchmaster® for providing the fly traps used in this study. We thank Samantha Sawyer for training in Diptera morphological identification.

**Authors’ contributions**

CMR, SAH, APC, and GLH conceptualized and designed the work; RSBF facilitated access to homes with positive COVID-19 cases; IBZ, EBD, LDA, and SAH deployed traps in homes; SLS, and JKT guided fly
trapping and processing; CMR, WT, and HG conducted lab work; CMR wrote the first draft of the manuscript; All authors contributed to reviewing and editing the manuscript and approved the final draft.

Funding

This research was funded by Texas A&M AgriLife Research and the Centers for Disease Control and Prevention RFP 75D 301-20-R-68167. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention or the USDA.

Availability of data and materials

Data from this study are provided in Tables and Appendix.

Ethics approval and consent to participate

The Texas A&M University Institutional Review Board determined that this study did not involve research with human subjects (IRB2020-0762).

Consent for publication

Not applicable.

Competing interests

The authors declare no conflict of interest.

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Tables

Due to technical limitations, table 1 and 2 xlsx are only available as a download in the Supplemental Files section.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- FlyCovidAdditionalFile1.docx
- Additionalfile2TableS1.xlsx
- Additionalfile3TableS1.xlsx
- FlyCoViDgraphicalabstract.png
- Table1.xlsx
• Table2.xlsx