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Short Communication

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

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Abstract

Flies and other arthropods mechanically transmit multiple pathogens and a recent experimental study demonstrated house flies, *Musca domestica* L. (Diptera: Muscidae), can mechanically transmit SARS-CoV-2. The purpose of this study was to explore the possibility of mechanical transmission of SARS-CoV-2 by domestic insects and their potential as a xenosurveillance tool for detection of the virus. Flies were trapped in homes where at least one confirmed human COVID-19 case(s) resided using sticky and liquid-baited fly traps placed inside and outside the home in the Texas counties of Brazos, Bell, and Montgomery, 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 quantitative reverse transcription PCR (RT-qPCR). Liquid traps were drained, and the collected fluid similarly tested after RNA concentration. We processed the contents of 133 insect traps from 40 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. 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. This study presents evidence that biting and nonbiting flies and cockroaches (Blattodea) are not likely to contribute to mechanical transmission of SARS-CoV-2 or be useful in xenosurveillance for SARS-CoV-2.

Key words: Diptera, SARS-CoV-2, mechanical transmission, xenosurveillance, RT-qPCR

Following the emergence and 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 (Zhu et al. 2020). A complete response to this pandemic requires understanding all modes of transmission and a variety of surveillance tools. SARS-CoV-2 has been shown to be present in aerosols, droplets, and on surfaces (Kwon et al. 2021, van Doremalen 2020). While fomite transmission is less significant than aerosol transmission (Kwon et al. 2021, Goldman 2020), experimental studies have shown that the virus can persist on surfaces for hours or days, in most stable indoor conditions (Kwon et al. 2021, Bueckert et al. 2020). Nonhuman animals, including domestic felines and canines, are also susceptible to infection (Shi et al. 2020, Bosco-Lauth et al. 2020, Halfmann et al. 2020, Gaudreault et al. 2020, Garigliany et al. 2020, Hamer et al. 2021), can have viral RNA on fur and in feces, and can shed infectious virus orally (Hamer et al. 2021). 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 and transmit many pathogens, both biologically and mechanically. Although

SARS-CoV-2 does not typically produce a viremia (Corman et al. 2020b), studies have investigated the potential for biological transmission by mosquitoes (Xia et al. 2020, Huang et al. 2020, Fortuna et al. 2021, Balaraman et al 2021a, b) and biting midges (Balaraman et al. 2021a, b). In these studies, SARS-CoV-2 was detected for less than 24 hr post virus exposure, the virus was unable to replicate in the arthropod, and thus no biological transmission occurred. However, the role of mechanical transmission by insects remains unknown and of concern (Dehghani and Kassiri 2020). Nonbiting flies are capable of mechanical transmission by transferring pathogens via contaminated mouthparts or bodies. This transmission has been documented for hundreds of pathogens (Nayduch et al. 2017), including turkey coronavirus (Calibeo-Hayes et al. 2003). To date, a single experimental study has examined mechanical transmission of SARS-CoV-2 by house flies (*Musca domestica*, L. (Diptera: Muscidae)) (Balaraman et al. 2021a, b) and confirmed that the flies could acquire SARS-CoV-2, which was retained as viral RNA and infectious virus up to 24 hr postexposure, with viral RNA, but not infectious virus, transferred to virus-free surfaces (Balaraman et al. 2021a, b). Although the authors concluded that house flies don't likely play a significant role in SARS-CoV-2 transmission, retention of viral RNA suggests that detection of invertebrate-derived RNA of nonbiting 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 (Grubaugh et al. 2015, Hoffmann et al. 2016, Fauver et al. 2017). Xenosurveillance methods are used to detect pathogens that the arthropod acquired during an infected bloodmeal or from contact with a fomite. In another study, researchers collected flies from a hospital with active COVID-19 cases and detected SARS-CoV-2 RNA (Soltani et al. 2021). However, such a clinical setting with known human cases is likely to have far more virus and viral RNA than a nonclinical setting.

In the current study, we deployed fly traps at households with at least one confirmed human COVID-19 case to investigate SARS-CoV-2 in biting and nonbiting flies, as a complementary effort to a One Health study focused on transmission at the human-animal interface (Hamer et al. 2021).

Methods

Household Recruitment

Household enrollment occurred from June through September, 2020 as previously described (Hamer et al. 2021).

Arthropod Sampling—Sticky Traps

Each home received one to three traps, placed indoors and/or outdoors according to the preference of the home resident. Indoor traps were commonly placed by the front or back door and/or in the kitchen. Outdoor traps were commonly placed outside a front or back door. To capture a variety of insects, multiple commercially available traps were used, including the EZ Trap (Starbar, Schaumburg, IL), Gold Stick Fly Trap (Catchmaster, Bayonne, NJ), and Indoor Fly TrapStik (Rescue!, Spokane, WA). Sticky traps were left for 7 to 19 d with an average of 12.4 d.

Traps were collected from homes and stored at 4°C for up to 3 d before processing. Flies and other insects were individually identified using morphological identification keys (Ross and Arnett 2000, Triplehorn et al. 2004). Each arthropod taxa from a single trap was placed in separate 2 mL microcentrifuge tubes (Eppdendorf, 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). The number of arthropods per tube depended on the size of the arthropod (total biomass) to allow for sufficient space for homogenization; max of 1 cockroach, 5 large flies (e.g. *M. domestica*), and 15 small flies (e.g. Drosophilidae) per tube. Samples were homogenized using a Tissue Lyser II (Qiagen, Hilden, Germany). Homogenates were centrifuged at 2,500 X *g* for 30 min at 4°C (Beckman Coulter, Allegra X-15R, Brea, CA), and supernatants aliquoted for RNA extraction and quantitative reverse transcription PCR (RT- qPCR), as previously described (Hamer et al. 2021). A sample of glue was also tested from each trap as a negative control.

Arthropod Sampling—Liquid Traps

A subset of households also received liquid traps, baited at 25% the recommended concentration. These traps included Reusable Fly Trap (Rescue!, Spokane, WA) and Fly Jar (Catchmaster, Bayonne, NJ). Liquid traps were left for 5 to 12 d with an average of 8.6 d.

After liquid traps were collected from homes, the liquid was transferred to 50 mL centrifuge tubes and processed similar to protocols for testing wastewater for SARS-CoV-2 (Nemudryi et al. 2020). The flies remaining in the trap were rinsed with 50 mL of VTM, which was collected and combined with the liquid from the trap. Liquid samples were centrifuged at 2,500 X *g* for 30 min at 4°C (Beckman Coulter, Allegra X-15R, Brea, CA). For RNA concentration, supernatant was passed through a 5 µM syringe filtration (Pall, Acrodisc, New York, NY) and then concentrated using a Vivaspin 20 mL centrifugal concentrator (Sartorius, Vivaspin, Gottingen, Germany) according to manufacturer's protocols. Following the VTM rinse, the remaining arthropods were sampled by adding three approximately 1mL quantities placed separately into 2 mL tubes and processed following the methods for sticky trap samples.

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). RNA was screened by RT-qPCR for two SARS-CoV-2 genes, RNA-dependent RNA Polymerase (RDRP) and Envelope (E), as previously described (Corman et al. 2020a, Konrad et al. 2020), and used for the identification of positive pets in households where COVID-19 positive cases resided (Hamer et al. 2021).

Protocol Validation

We validated our protocol to ensure SARS-CoV-2 RNA could be detected using our insect processing, pooling, and RT-qPCR protocol. To achieve this, *Lucilia sericata* Meigen (Diptera: Calliphoridae) from a colony were killed by freezing at –20°C 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 serially diluted viral concentrations containing from 10^5 to 10 plaque-forming units (pfu)/mL. Following exposure, a single SARS-CoV-2-exposed fly was immediately added to a pool of 4 other flies in VTM which was homogenized and tested by RT-qPCR as described above. For each viral concentration, 5 replicates were tested alongside unexposed fly pools as negative controls. Viral inactivation of SARS-CoV-2 samples leaving the BSL3 was validated internally as described in Supp. Material.

Results

Arthropod Collections

A total of 235 traps were deployed in 81 homes. One hundred and thirty-three traps from 44 homes were recovered and traps from 40 homes contained arthropods. Some traps were not recovered due to lack of response to follow-up calls.

Indoor sticky traps captured 8 different Diptera families and Blattodea (Supp. 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 (Supp. Table 1). The most widespread taxa included Calliphoridae detected at 11 homes and Muscidae at 8 homes. Indoor sticky traps were deployed an average of 9.6 d post-COVID-19 diagnosis of the human case. 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 (Hamer et al. 2021) (Supp. 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 (Supp. Table 2). The three most common taxa collected outdoors were Calliphoridae with 485 individuals, Sarcophagidae with 218 individuals, and Muscidae with 58 individuals (Supp. Table 2). The most widespread taxa included Calliphoridae detected at 23 homes and Muscidae and Sarcophagidae at 15 homes. In eight traps from seven households, the outdoor sticky traps were deployed on the same day the animals tested positive for SARS-CoV-2 by nasal, oral, body, or rectal samples (Supp. Table 2).

The EZ Trap captured 1.58 flies per day indoors, followed by Gold stick (0.945), followed by Trapstick (0.012) (Table 1). The EZ Trap captured 2.24 flies per day outdoors, followed by Trapstick (0.50), followed by Gold stick (0.24) (Table 2).

Liquid fly traps contained up to hundreds of individual flies which were not counted or identified due to 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 liquid and arthropod pools from 28 liquid traps were tested for SARS-CoV-2 RNA. None of these pools or samples tested positive for SARS-CoV-2 RNA.

Protocol Validation

Validation of the protocol showed that pooled flies with 1 individual exposed to 2 x 10^2 pfu/mL would be detected by our RNA extraction and testing protocol (Supp. Table S1).

Discussion

During active SARS-CoV-2 transmission in the community, we collected insects from 133 sticky traps and 28 liquid traps in 40 homes of confirmed human COVID-19 cases. A subset of these homes had pets with documented shedding of SARS-CoV-2. Our study found no evidence of SARS-CoV-2 RNA in any insect sample tested. While *M. domestica* was recently found to be capable of experimental acquisition of virus and deposition of viral RNA in a laboratory setting (Balaraman et al. 2021a, b) and viral RNA was detected on flies collected from a hospital with active cases (Soltani et al. 2021), our

Table 1. Summary of insects collected on sticky traps deployed outside of homes with at least one human COVID-19 case

Trap type	Number of traps Deployed	Total days deployed	Blattodea	Calliphoridae		Dolichopodidae	Drosophilidae	Muscidae	Phoridae	Sarcophagidae	Miscellaneous**	Total	Flies captured per trap day
				Cochliomyia	Other			*M. domestica*		*Sarcophaga*			
EZ	32	276	75	7	69	20	37	29	178	15	7	437	1.6
Trapstik	16	170	0	0	0	0	0	0	2	0	0	2	0.012
Gold Stick	4	55	0	0	1	6	0	44	0	0	1	52	0.95
Total	52	501	75	7	70	26	37	73	180	15	8	491	

**Miscellaneous includes taxa for which less than 5 individual insects were collected: Lucilia, *Phormia regina*, Muscidae, Psychodidae

Table 2. Summary of insects collected on sticky traps deployed inside homes with at least one human COVID-19 case

Trap type	Number of traps deployed	Total days deployed	Calliphoridae				Dolichopodidae	Drosophilidae	Muscidae		Sarcophagidae	Miscellaneous**	Total	Flies captured per trap day
			Cochliomyia	*Lucilia*	*Phormia regina*	Other			*M. domestica*	other	*Sarcophaga*			
EZ	40	341	97	41	13	292	43	26	34	15	198	6	765	2.2
Trapstik	29	123	0	0	31	0	0	14	0	3	14	0	62	0.50
Gold Stick	12	149	0	0	0	11	2	0	0	6	13	4	36	0.24
Total	81	613	97	41	44	303	45	40	34	24	225	1	863	

**Miscellaneous includes taxa for which less than 5 individual insects were collected: Asilidae, Blattodea, Ulidiidae (*Ceroxys*), Phoridae, Syrphidae, Tabanidae

results indicate that this is unlikely to occur in household settings in which humans, and in some cases pets, were shedding virus in the home.

One of the strengths of our study is the broad sampling of 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 positive body (fur) swabs, which is likely indicative of a contaminated environment that may serve to also contaminate insects in and around the household; accordingly, these high risk settings afforded a prime opportunity for evaluation of fly xenosurveillance.

Limitations of our study include not knowing the stability of viral RNA on flies left at ambient temperatures on the sticky traps or in liquid traps, some of which were exposed to UV light, which can rapidly inactivate virus. Studies have shown that SARS-CoV-2 can be viable for days on some surfaces at room temperature (Goldman 2020, Bueckert et al. 2020), indicating that the indoor insects are likely exposed to virus from surfaces. However, the indoor traps captured fewer flies than the outdoor traps. Additionally, our traps were deployed an average of 8.2 d following the human diagnosis of COVID-19 and 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 again positive (Hamer et al. 2021). Furthermore, in our recent study using sticky traps for indoor and outdoor collections, we detected viral RNA of an insect-specific virus in 30% (40 of 138) of *Aedes aegypti* mosquito pools (Martin et al. 2020). Most of these mosquitoes were dead when collected at 7 d intervals confirming the utility of a sticky trap for recovering viral RNA in insects using the same protocols and equipment as the current study. However, insect-specific viruses are replicating in mosquitoes, while evidence suggests SARS-CoV-2 is unable to replicate in insect cells (Xia et al. 2020, Balaraman et al 2021a, b), likely reducing the potential for molecular detection or use for xenosurveillance.

In conclusion, we found no insects with SARS-CoV-2 RNA in or around the homes where humans, and sometimes animals, tested positive for SARS-CoV-2. This study suggests a low likelihood that insects contribute to the transmission of SARS-CoV-2. The lack of detection of SARS-CoV-2 viral RNA in these fly samples may reflect either the lack of contamination of flies by infected humans, animals or surfaces in the household, and/or degradation of viral RNA in flies that may have been contaminated. Either scenario suggests that insects are of low utility as a SARS-CoV-2 xenosurveillance tool in high-risk domestic settings.

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Supplementary Data

Supplementary data are available at *Journal of Medical Entomology* online.

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