Perspective

WHY CAN THE MOSQUITOES AND THE HOUSE FLIES NOT TRANSMIT THE NOVEL CORONA VIRUS (*n*CoV OR SARS-CoV-2)? AN UPDATE OF ARGUMENTS

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INTRODUCTION

For about an year, during most part of 2020, and still continuing through 2021, the whole world has been grappling with an altogether new and highly infectious disease, Covid-19

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(**Co**rona**vi**rus **d**isease-20**19**), caused by a novel coronavirus (*n*CoV or SARS-CoV-2) which is distinct in its genome from the rest of coronaviruses such as, for example, Severe Acute Respiratory Syndrome (SARS-CoV) and Middle East Respiratory Syndrome coronavirus (MERS-CoV). The new coronavirus, already evolved into several new strains across the world, is a respiratory virus which spreads primarily through droplets generated when an infected person coughs or sneezes, or through droplets of saliva or discharge from the nose. To protect yourself, it is advised to clean your hands frequently with a sanitizer or wash them with soap and water for at least 20 seconds and socially distance yourself by at least 2m. Also, avoid close contact with anyone who is coughing and sneezing by continuously wearing a mask and, more effectively, exercising self-isolation. Many countries, including India, have very recently succeeded to develop and deliver vaccines to different strata of population at high risk against Covid-19.

HYPOTHESIS

To date there has been no information or evidence to suggest that there is any other potential route of transmission such as, for example, involving arthropods, albeit a frequently asked question alluding to the possible role of mosquitoes and house flies. As to the mosquitoes, three widely prevalent species, namely, *Aedes aegypti*, *Ae. albopictus* and *Culex quinquefasciatus*, representing the two most significant genera of arbovirus vectors, were subjected to even extreme conditions of intrathoracic challenge with the SARS-CoV-2 virus but the latter was unable to replicate in these mosquitoes.[1] It is, therefore, considered opportune here to place on board all the available information and arguments to deny any role of the mosquitoes and, with a cautionary note, the house flies, at present at least!

Recently, after it had been reported that SARS-CoV-2 could occur in infected human stool and the house fly, *Musca domestica*, was implicated in transmission of the Turkey Coronavirus (TCoV) in the laboratory, speculations were made that SARS-CoV-2 could be also naturally transmitted mechanically through houseflies which are an efficient transporter of very many deadly pathogens.[2,3,4] Houseflies

naturally go in quest of filthy stuff such as human excreta, among others, for its subsistence where upon, in theory at least, mechanical transmission of any pathogen may be possible. Most recently, in the United States of America, innovative experiments were conducted on the house fly to demonstrate with certainty if the insect could at least mechanically transmit the SARS-CoV-2. Not only did the house fly acquire and harbour infectious SARS-CoV-2 in the laboratory, it, after the infectious virus was isolated only from the flies exposed to virus-spiked milk but not from those exposed to virus-spiked medium, also succeeded in mechanically transmitting the SARS-CoV-2 genomic RNA to the surrounding environment up to 24 hr post-exposure.[5] Although this prowess of mechanical transmission of SARS-CoV-2 by the house fly was exhibited under the laboratory conditions, it has nevertheless raised many questions for future risks.

POSSIBLE MECHANISMS AND ARGUMENTATION

On the other hand, mosquitoes, regarded by many as the man's greatest foe on Earth, are well-known carriers for a large number of deadly and/or debilitating human and animal pathogens including protozoans, helminths, bacteria, viruses etc.[6-8] With regard to viruses they mediate transmission of yellow fever, dengue, chikungunya, Zika, West Nile, Japanese encephalitis and various other types of encephalitides. A mosquito can transmit an infection only when she (only females blood-feed) has fed on blood of an infected person. Also, in the mosquito body the pathogen (in this context, a virus) must be able to multiply in her gut, penetrate through its wall into the body-cavity or haemocoel, and then to the salivary glands, by using some path- and site-identifying markers. With the entry of virus into the salivary glands the mosquito now becomes infective and is ready to inoculate the virus into any healthy human being whenever the infected mosquito finds an opportunity to blood-feed. Considering now a quaint situation of the *n*CoV, these and other various conditions must be fulfilled by the virus in order to make the mosquito really infective for its transmission. Here we must acknowledge that there are over 3500 species of mosquitoes in the world but only less than 100 of these are serious vectors (carriers) for some kind of communicable infection to humans. Presuming a SARS-CoV-2 patient is available to the blood-feeding insects such mosquitoes, then we will have five theoretical explanations that would allow them to transmit the infection;

(1) *Mechanism One:* A vector mosquito must blood-feed on a SARS-CoV-2 carrier and ingest the virus particles along with the blood in the gut of the mosquito, where several physical and chemical barriers naturally exist (*e.g.*, pharyngeal comb, acid, enzymes etc.), the virus has to overcome all such impediments, attach to site and penetrate through gut wall, and finally be able to migrate to the mosquito's salivary glands. Then the infective mosquito must feed on blood of an uninfected and healthy person so that the virus, already present in the salivary glands, is passed on to its new host to make him or her infected. Arthropod-borne viruses (or arboviruses) like dengue, chikungunya, Zika, Japanese encephalitis etc. involve this kind of mechanism of disease transmission. In case of SARS-CoV-2 such a mechanism has never been reported.

(2) *Mechanism Two:* A vector mosquito begins to blood-feed on a SARS-CoV-2 infected person, but is interrupted in her feeding and forced to give up this host after its successful initial/partial feeding (*i.e.*, adequate blood was drawn along with virus particles to get access to the suction canal in mosquito's mouthparts) for another host which could be either infected or uninfected with the nCoV. Because the mosquito still did not satisfactorily engorge on blood, the urge for full blood-meal pushes her to take on to the new host's blood pronto! In the process of multiple feeding attempts, the virus particles which were left behind stuck on the mouthparts during the 'interrupted' previous feeding(s) get inoculated into the body of the next (presumably) uninfected host as soon as the mosquito's mouthparts penetrate through the latter's skin at the site of feeding. This type of mechanism is rather uncommon in mosquitoes, but equine infectious anemia is transmitted to horses by biting/blood-feeding flies in this manner. Its noteworthy that *Aedes aegypti* and *Ae. albopictus* mosquitoes, the deadly vectors of yellow fever (occurring only in Africa and Central & South Americas), dengue and chikungunya, have a tendency to feed on human host a multiple times! This process is not reported yet in case of SARS-CoV-2.

(3) *Mechanism Three:* In this a mosquito initiates the cycle by beginning to feed on a SARS-CoV-2 carrier (in a manner described above), but on interruption, resumes the partial blood feeding on a different (presumably)

uninfected host. Unfortunately, the mosquito is here knowingly or unknowingly quashed or smeared to death at a wound site on the host's body into where the virus particles can make way in. Theoretically, any mosquito can be subjected to this kind of fate and mechanism of pathogen transmission, but the new uninfected host may actually pick up infection provided by the inoculum of contaminated blood from the crushed mosquito's gut if it had adequate virus particles. This mechanism is a distant thought and a mere conjecture in the situation.

(4) *Mechanism Four:* This mechanism is best called "transovarial" or "vertical transmission", implying that a virus is ingested along with the blood and after crossing over the barrier of the gut wall makes its way, among other body organs, also to the ovaries, to take refuge in the maturing ova. When these ova are mature they are fertilized by the male's sperms already stored in spermathecae and descend to exit to be laid down in some aquatic habitat, along with the virus. From here onward, after the immature of mosquito hatches out of the egg, the life goes through four larval and a pupal, all aquatic, stages for the next 5-7 days. The aerial adult, both males and females, emerging from the pupa may however still continue to harbour the virus in its body or briefly the salivary gland, ready to inoculate virus to a healthy and uninfected person even at the instance of first blood-feeding act. Such a scenario is common amongst the arboviral diseases like Japanese encephalitis, dengue and chikungunya vectors. As to the SARS-CoV-2 no such data are available.

(5) *Mechanism Five:* This is the extreme experiment using mosquito inoculation with SARS-CoV-2. This mechanism was used as an ultimate test of the capacity of SARS-CoV-2 to infect and replicate in three widely prevalent mosquitoes, viz., *Culex quinquefasciatus, Aedes aegypti* and *Ae. albopictus*.[1] Recovery was made of infectious viruses from 13/15 mosquitoes collected within two hours of inoculation. While, no virus was detected in the 277 inoculated mosquitoes after 24 h, suggesting a rapid loss of infectivity and the lack of replication after injection, only 1/48 mosquitoes exhibited infectious viruses in *Ae. albopictus* collected and analysed at 24 h post-inoculation. The quantity of infectious virus in this mosquito corresponded to the amount of inocula, producing infectious

titers at approximately 1.5 logTCID50/ml. No virus was detected in control L-15 medium inoculated mosquitoes. Authors deduced that their experimented mosquito species were refractory to SARS-CoV-2 and unlikely to contribute to viral maintenance and transmission in nature.

Now, in view of the above possible mechanisms of transmission by a mosquito, we must test the antithesis as to why can mosquitoes not transmit the SARS-CoV-2? Let's argue carefully. A pathogen has to keep alive in mosquito's body if it were to cause infection to another human host on transfer following a subsequent blood meal. On the other hand, if the mosquito digested the pathogen/virus, the transmission cycle is terminated and the virus cannot be passed to the next host. Interestingly, several pathogens exercise varied *modus operandi* to avoid being treated as food for the mosquitoes. While some pathogens are simply refractory enough to the digestive enzymes inside the mosquito's stomach and pass out intact, others target the receptive gut wall and bore their way out of the stomach as fast as possible to evade the deleterious effects of digestive enzymes that would otherwise decimate their very existence. For example, malaria parasites survive inside mosquitoes for 9-12 days and actually go through a series of necessary life stages during that period. Encephalitis virus particles survive for 10-25 days inside a mosquito and replicate enormously during the incubation period. Similarly, a lymphatic filariasis worm (larval stage) also takes a little over one week's time to stay in the body of mosquito before getting ready to be ejected for a transfer to a (human) host. In contrast to these many studies have shown that the Human Immunodeficiency Virus (HIV) seemingly serve as a 'food' to the mosquito and is digested along with blood meal, within a couple of days, destroying completely all virus particles. With respect to HIV even intrathoracic inoculation to mosquitoes was carried out, with an objective to bypass any gut barriers, but the virus still did not show any sign of reproduction. Therefore, as far as SARS-CoV-2 is concerned, where no such studies have so far been carried out, it can be safely surmised, keeping in mind the pathology of Covid-19, that it is unlikely that this virus finds a compatible environment in the mosquito's body and tends to multiply at all. Conclusively, unless and otherwise discovered, the SARS-CoV-2 currently stands no chance to effect any transmission through mosquitoes.

An alternative argument likely emerging here could also be tested. Mosquitoes might not ingest enough SARS-CoV-2 particles to effect Covid-19, just like the

evidence available for another highly infectious multi-routed viral disease, Acquired Immunodeficiency Virus Syndrome (AIDS). Succinctly all arboviral diseases which have the potential to be transferred from one individual to the next via contaminated mouthparts of the mosquito must circulate at very high levels in the peripheral blood stream of their human host. Transfer by mouthpart contamination requires invariably sufficient virus particles to initiate a new infection, though the exact quantum may vary from one disease to another. For example, AIDS virus circulates at very low levels in the peripheral blood; far below than any known mosquito-borne disease. The infected individuals rarely circulate more than 10 units of HIV, and 70-80% of HIV-infected persons have undetectable virus particles in their blood. Statistically, a mosquito interrupted while feeding on a HIV-infected person, circulating 1000 units of HIV, has a 1:10 million probability of inoculating a single unit of HIV from contaminated mosquito mouthparts. In the same way, if a fully fed mosquito containing HIV-infected blood will also not approach the levels needed to initiate infection. It appears, therefore, that mechanical transmission of SARS-CoV-2 by the contaminated mosquitoes is but a far outcry and hypothetical!

DISCUSSION

In conclusion, over 500 viruses are transmitted by arthropods but, in spite of the recovery of coronaviruses or coronavirus-like agents from various arthropods, no virus in the family Coronaviridae has been ever isolated from mosquitoes[2-9]. The sole exception, however, is the report in which the application of xenosurveillance in detecting human bacteria, parasites, and viruses in mosquito bloodmeals is highlighted, and mosquitoes are related to the epidemic coronaviruses.[10] On the other hand, as far as house flies are concerned, the concrete evidence slowly emerging in support of their role as a potential mechanical transmitter of the SARS-CoV-2, indeed calls for more intensive investigations into the behaviour of the insect. Also, keeping in mind their prowess to keep the genomic RNA for about a day, it immediately warrants to explore further whether the house flies could somehow threaten to mediate transmission in nature with serious public health implications?

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