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Comment on: Successful propagation of Alkhumra (misnamed as Alkhurma) virus in C6/36 mosquito cells

Currently, Alkhumra virus (ALKV) is phylogenetically recognized as a close relative (or a variant) of Kyasanur Forest disease virus (KFDV), which belongs to the tickborne flavivirus group in the genus *Flavivirus* of the family Flaviviridae. Being a member of this group, KFDV replicates only in vertebrates and in ticks (or in cultures of the cells derived from the respective host group) but not in mosquitoes, as shown by the failure of KFDV replication in three genera of mosquitoes, including *Aedes albopictus*.[1] Like KFDV, ALKV has been isolated from ticks but never from mosquitoes.[2] At the time of writing, no flavivirus has been reported which is transmitted in nature by both mosquitoes and ticks.

Accordingly, if the report of ALKV replication in mosquito cells derived from *A. albopictus* and the speculation of mosquitoes as its natural vector[3] is confirmed virologists would have to make fundamental changes to how they interpret molecular phylogenetic trees, correlate taxonomic position of flaviviruses with host range specificity and establish epidemiological surveillance and control of ALKV infection. However, some concerns and puzzles that are evident in the article by Madani et al.[3] need to be clarified.

The first concern relates to the authenticity of the viral inocula used in the experiment. The infectious plasma and serum specimens used in the report are not considered isolated virus strains by today's standards. As isolated virus strains were not used this poses problems for other researchers in reproducing the methods and results or characterizing the infectious agents used. Confirmation of authenticity is important as a KFDV strain reportedly isolated in China[4] is strongly suspected to be a strain isolated from India that has been used as a reference strain in many laboratories around the world.[5] Second, when a drastically different result of this significance is obtained, it is prudent to arrange confirmation of the authenticity of the infectious agents at a reputable laboratory for an independent identification and to include a previously characterized prototype of ALKV in parallel with the infectious samples for observation of infection not only in mosquitoes or mosquito cells but also in ticks or tick cell culture. Because none of this was done, the validity of the reported result is uncertain. Third, the authors of the report referred to a similarity with past anomalous reports of isolation of some tickborne flaviviruses from mosquitoes in the field or their replication in mosquito cell culture and, conversely, similar puzzling reports of the isolation of some mosquitoborne viruses in ticks or their replication in tick cell culture. However, as far as their role in biological transmission is concerned, each of these unusual examples was contradicted by multiple negative reports in the field; and all odd laboratory results were found not to be reproducible by others.[6] The most likely explanation of these unusual occurrences in the field is transient persistence for these viruses in unnatural vectors as a result of accidental acquisition of the viruses when these vectors feed on infected vertebrate hosts.[6]

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Reply to comment on: Successful propagation of Alkhumra (misnamed as Alkhurma) virus in C6/36 mosquito cells

The reference cited by Kuno described detection of Alkhumra virus (ALKV) RNA and not viral isolation from a single tick that was feeding on a camel.[1] The detection of ALKV RNA or viral isolation from ticks does not confirm the vector competence of ticks for the virus. No biological studies have been published to confirm that ticks or mosquitoes are vectors of ALKV. However, our clinical and epidemiological observations during ALKV outbreaks at various localities in Saudi Arabia

provided evidence that mosquitoes rather than ticks were involved in the transmission of ALKV to patients.[2,3] The successful growth of ALKV in the C6/36 mosquito cell line reported in our paper supports this speculation but does not provide enough evidence for the vector competence of mosquitoes for ALKV.[4] Additionally, the virus has been successfully passaged several times in the C6/36 cell line from which the virus was successfully transferred to other cell lines (T.A. Madani et al., unpublished data). Our results indicated that ALKV in clinical specimens could propagate to a high titre and for several passages in C6/36 cell culture and in mammalian cell lines (T.A. Madani et al., unpublished data). On the other hand, we have demonstrated that ALKV isolated in other cell lines and in baby rat brains also propagated well when passaged in C6/36 cell culture (T.A. Madani et al., unpublished data). Of note is that we have not imported Kyasanur Forest disease virus (KFDV) to Saudi Arabia and never worked with this virus in our laboratory. Our results are therefore authentic and novel as ALKV was successfully isolated in the C6/36 cell line, caused discernible cytopathic effects (CPE), and was identified by indirect immunofluorescence assay and RT-PCR. Our research group is an international team with members from Saudi Arabia (Madani, Azhar, Albar, Abu-Araki), UK (Abuelzein), Germany (Kao) and USA (Ksiazek). Our biosafety level 3 laboratory is a reference laboratory for Saudi Arabia and neighbouring countries and one of WHO's recognized virology laboratories.

As indicated in our discussion, past reports have described the isolation of a number of tickborne flaviviruses from mosquitoes in the field and their replication in mosquito cell culture.[4] Conversely, other reports have described the isolation of a number of mosquito borne viruses from ticks and their replication in tick cell culture.[4] Kuno speculated that this was most likely explained by the persistence of these viruses in unnatural vectors as a result of accidental acquisition of the viruses when these vectors feed on infected vertebrate hosts. If this is so, then the presence of ALKV RNA in a single tick should not be regarded as significant.

Finally, we do have more salient and interesting information about this new virus that we hope to publish in the near future.

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