ABSTRACT BOOK

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ADAPTATION OF HAZARA ORTHONAIROVIRUS FOLLOWING FEW PASSAGES IN HYALOMMA-DERIVED TICK CELLS

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Introduction and Aim of the Study: Crimean-Congo Hemorrhagic Fever Virus (CCHFV) is a tick-borne pathogen that presents a serious threat to human health, causing a severe hemorrhagic fever with high mortality rate. Ticks of the genus *Hyalomma*, are the principal vectors of CCHFV, harboring the virus persistently and acting as its natural reservoir. However, due to biosafety concerns, limited knowledge is available regarding the virus-vector interaction. Cycling between hosts likely contributes to the virus's evolutionary flexibility, however cross-species transmission imposes selective constraints, influencing viral adaptation. This study explores the evolutionary progression of Hazara virus (HAZV), used as a model for CCHFV, across different host cell lines, aiming to elucidate how pressure within the host environment drives viral adaptation and impacts infectivity.

Methods used: We used HAZV grown in human SW13 cells to infect the *Hyalomma* tick cell line HAE/CTVM8. On days 30, 60 and 90 we collected samples and passaged virus onto new tick and SW13 cells, evaluated after a further 30 and 6 days respectively. Genotypic and phenotypic changes were assessed, respectively, by NGS sequencing of cell pellets and supernate and by evaluating viral infection in tick and SW13 cells.

Results and Conclusions: Through analysis of HAZV propagation in HAE/CTVM8 cells, we determined the emergence of mutations within all three viral genome segments. Notably, passage of HAZV within this cell line appears to lead to greater stabilization of two mutations, one synonymous and one nonsynonymous, within the gene encoding the RdRp and one nonsynonymous mutation in the glycoprotein precursor (GPC). HAZV adapted to tick cells showed a host-dependent effect on viral infection efficiency, with higher infectivity in HAE/CTVM8 compared to mammalian cells. Conversely HAZV from SW13, used as a control, showed the opposite trend.
Mutations appeared in HAZV during the first 30 days and increased slowly up to 30-40% by day 90. With subsequent passage in tick cells, mutations in the RdRp and GPC increased from 15-30% to 60-80% and 20% to 50% respectively, suggesting a host-driven adaptation. Viral replication kinetics indicated that tick-adapted HAZV replicates better in tick cells, suggesting a potential role of the selected mutations in viral adaptation to the invertebrate host. Although genetic changes in passaged HAZV were minimal they seemed to lead to increased relative fitness and replicative ability of the virus in the homologous HAE/CTVM8 cell line. Further experiments will be performed after isolation of mutated viruses.
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